POLSKIE TOWARZYSTWO
TECHNOLOGÓW ŻYWNOŚCI
WYDAWCA ODDZIAŁ MAŁOPOLSKI

ŻYWNOŚĆ TECHNOLOGIA JAKOŚĆ

Suplement

Nr 4(17) Kraków 1998 Rok 5

Materiały
VIII International Starch Convention
Kraków, 16–19 czerwca 1998 r.

Redakcja naukowa materiałów: prof. dr hab. Piotr Tomasik

Wydanie dofinansowane przez Komitet Badań Naukowych

POLSKIE TOWARZYSTWO
TECHNOLOGÓW ŻYWNOŚCI
WYDAWCA ODDZIAŁ MAŁOPOLSKI

ŻYWNOŚĆ TECHNOLOGIA JAKOŚĆ

Suplement

Nr 4(17) Kraków 1998 Rok 5

WYDAWCA:

POLSKIE TOWARZYSTWO TECHNOLOGÓW ŻYWNOŚCI
Oddział Małopolski

Wydawanie publikacji dofinansowane przez Komitet Badań Naukowych

ISSN 1425-6959

ADRES REDAKCJI:

31-425 KRAKÓW, AL. 29 LISTOPADA 46

SKŁAD I DRUK:

Wydawnictwo „Akapit", Kraków
tel./fax (012) 266-92-69

SPIS TREŚCI / CONTENTS

Zamieszczone artykuły są recenzowane

Czasopismo jest referowane przez: AGRO-LIBREX, Chemical Abstracts Service i IFIS

OD REDAKCJI

Szanowni Państwo,

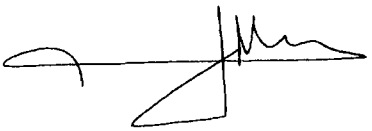
otrzymujecie Suplement nr 14(17) kwartalnika „Żywność. Technologia. Jakość", w którym publikujemy artykuły będące pokłosiem VIII International Starch Convention (VIII Międzynarodowej Konferencji Skrobiowej).

Teksty artykułów drukujemy w języku angielskim, ze streszczeniami w języku polskim, gdyż Konferencja miała charakter międzynarodowy.

Wyrażamy nadzieję, że zamieszczone w tym Suplemencie artykuły zostaną przyjęte z zainteresowaniem nie tylko przez Czytelników zajmujących się problematyką skrobi.

Kraków, grudzień 1998 r.

Redaktor Naczelny



Tadeusz Sikora

This collection of papers is a regular issue of "Żywność. Technologia. Jakość." ("Food. Technology. Quality.") journal and, at the same time, this volume plays a role of the VIII Starch Convention Proceedings held in Cracow on 16–19 June 1998.

This Convention was kindly sponsored by:

- Bank Gospodarki Żywnościowej S.A., Warszawa,
- Komitet Badań Naukowych,
- Ministerstwo Edukacji Narodowej,
- Protein Technologies International, Warszawa,
- GEA Process Technology Sp. Z o.o., Warszawa,
- "ROLIMPEX" S.A., Warszawa,
- "MERCK" S.A., Warszawa,
- "LAJKONIK SNACKS" S.A., Skawina,
- Zakłady Mięsne "MIĘSTAR", Tarnów,
- Okocimskie Zakłady Piwowarskie "Browar Kraków" S.A., Kraków,
- Zakłady Przemysłu Cukierniczego "WAWEL" S.A., Kraków,
- Przedsiębiorstwo Przemysłu Spirytusowego – Destylarnie "POLMOS", Kraków,
- Spółdzielnia Produkcji Piekarskiej i Ciastkarskiej, Kraków,
- Zakład Przetwórstwa Mięsa "BASO", Olkusz,
- Okręgowe Przedsiębiorstwo Przemysłu Zbożowo-Młynarskiego "PZZ Lublin",
- Lubelskie Zakłady Przemysłu Spirytusowego i Drożdżowego "POLMOS", Lublin,
- Zakłady Przemysłu Ziemniaczanego SA, Niechlów,
- Przedsiębiorstwo Przemysłu Ziemniaczanego, Piła.

The Organizing Committee expresses its deep gratitude.

Prof. Dr. Piotr Tomasik, D. Sc.

(Head organizer)

J. GŁADKOWSKI

THE PERSPECTIVES FOR THE PRODUCTION OF THE STARCH AND ITS DERIVATIVES

Ladies and Gentleman

On behalf of the "Polziem" Ltd. Company celebrating this year it's tenth anniversary, I am honored to welcome the participants of the VIII International Starch Convention in Krakow. I would also like to welcome all the guests representing the most prestigious universities and scientific institutes in Europe and Asia.

I would like to thank the hosts of the School for the organization of our meeting.

Poland facing numerous adjustment processes necessary for joining the European Community, production and processing of potatoes has special position in the National Economic development. In the past five years the average crops of potatoes in Poland exceeded over 30% of the total potato crops (excluding Russia). In our adjustment we need to acknowledge the fact, that the European Community outlook assumes an 40-50% increase of potato crops.

Within last twenty years the area taken for plantation was significantly reduced.

From 2 446 thousand ha in 1976 to 1 479 thousand ha in 1996. It resulted mainly from the diminishing role of the potatoes in feeding pigs.

In our opinion this fast falling tendency will take more gentle pattern, within the few years the level of 1.2 million of ha with the crops of 22–25 million of tonns.

In Polish reality we have to accept a continuous leadership of our country as the potato producer. It requires specified directions of development in the area of the starch processing. Central Research Laboratory of the Potato Industry is our scientific- and research basis in the area of the starch processing and also we cooperate with the Agriculture Academies in Poznań, Kraków, Wrocław, Olsztyn, Bydgoszcz and the Łódź Polytechnic. Over 40 research stations work on developing novel potato varieties, Potato Institute in Bonin with it's leading position.

Polish Potato Industry POLZIEM, Zwierzyniecka St. 18, 60-814 Poznań, Poland

We believe, we follow all the conditions of employment policy and the technical factors meet standards of the European Community.

We have 12 companies in Poland processing the potatoes into the starch and it's modified products. Their location is displayed on the enclosed map NR 2. Their daily output reaches approximately 11 thousand ton of potatoes. Consequently, it provides the annually 250 to 260 ton of starch. It satisfies domestic market for starch and surplus can be exported.

In 1990 Poland exported over 50 thousand tons of starch, and in previous years it's export exceeded sometimes 80 thousand ton of starch and its derivatives.

A come back to the world market is one of the goals of our potato industry.

We are still behind European Union in the utilization of starch and its derivatives in the paper and adhesive industries. Hence, the development of these branches our local consumption of starch and its derivatives should annually by few of thousand tons.

We assume the annual output of wheat starch from the Cargill Milling Company in Bielany at Wrocław and PPZ S.A. Niechlów on the level of 52–55 thousand of tons.

The technologies being used as well as the products are similar to the assortment produced in the European Union.

Several companies (most of them privatized) manufacture such dry products for food industry as potato granulated, puree, lumps, fries, chips and many others. In Poland, annually up to 200 tons for chips. of potatoes per year, for fries about 125 thousand tons for fries and for chips 50 thousand tons.

Processing for the food industry consumes annually about 340 thousand tons of potatoes with the increasing tendency.

Potato enterprises in Poland are shown in map No. 2.

In the last few years some changes took place in the starch industry.

The whole, recently state industry has been transformed either into the limited liabilities companies or has been sold to private investors, The National Investment Funds, and Enterprise 'PEPEES' in Łomża which is present at the Stock Market.

I am convinced that this Eighth International Starch Convention will strengthen relationships between science and industry in Poland and European Union.

I would like to thank to the hosts of this Starch Convention, to the Chairman of the Scientific Committee, and to all the organizers of the eighth Convention for such great organization of our meeting.

I would also like to cordially congratulate in my name as well as in the name of the whole starch industry to the Professor dr Adam Sroczyński and to the Professor dr Mieczysław Pałasiński receiving the titles of dr honorees cause.

I would also like to wish Professor dr Wacław Leszczyński chairing the Agricultural Technology Department in Wrocław a lot of success in his further works for the starch industry.

Adresses of seed producers (Basic seed)
(Adresy przedsiębiorstw hodowlano-nasiennych (prowadzących hodowlę zachowawczą ziemniaka))

1. IHAR, Zakład Doświadczalny Ziemniaka, 76-009 Bonin.
2. IHAR, Zakład Naukowo-Hodowlany Zamarte, 89-430 Kamień Krajeński.
3. Szczecińska Hodowla Roślin, Centrala Nasienna Sp. Z o.o. Stacja Hodowli Roślin Barkowo, 72-310 Płoty.
4. Pomorsko-Mazowiecka Hodowla Ziemniaka Sp. Z o.o. z siedzibą w Strzekęcinie, 76-024 Świeszyno.
5. Hodowla Ziemniaka i Nasiennictwo Gdańsk Sp. Z o.o. 80-874 Gdańsk, ul. Na Stoku 50.
6. Hodowla Roślin Jezierzyce Sp. Z o.o., 76-219 Jezierzyce.
7. Hodowla Roślin Szyldak Sp. Z o.o., 14-106 Szyldak 32A.
8. Olsztyńska Hodowla Ziemniaka i Nasiennictwo OLZNAS-CN Sp. Z o.o., 10-448 Olsztyn, ul. Głowackiego 6.
9. Hodowla Roślin Scholastykowo Sp. Z o.o., 77-420 Lipka

Branches of IHAR
(Oddziały Instytutu Hodowli i Aklimatyzacji Roślin)

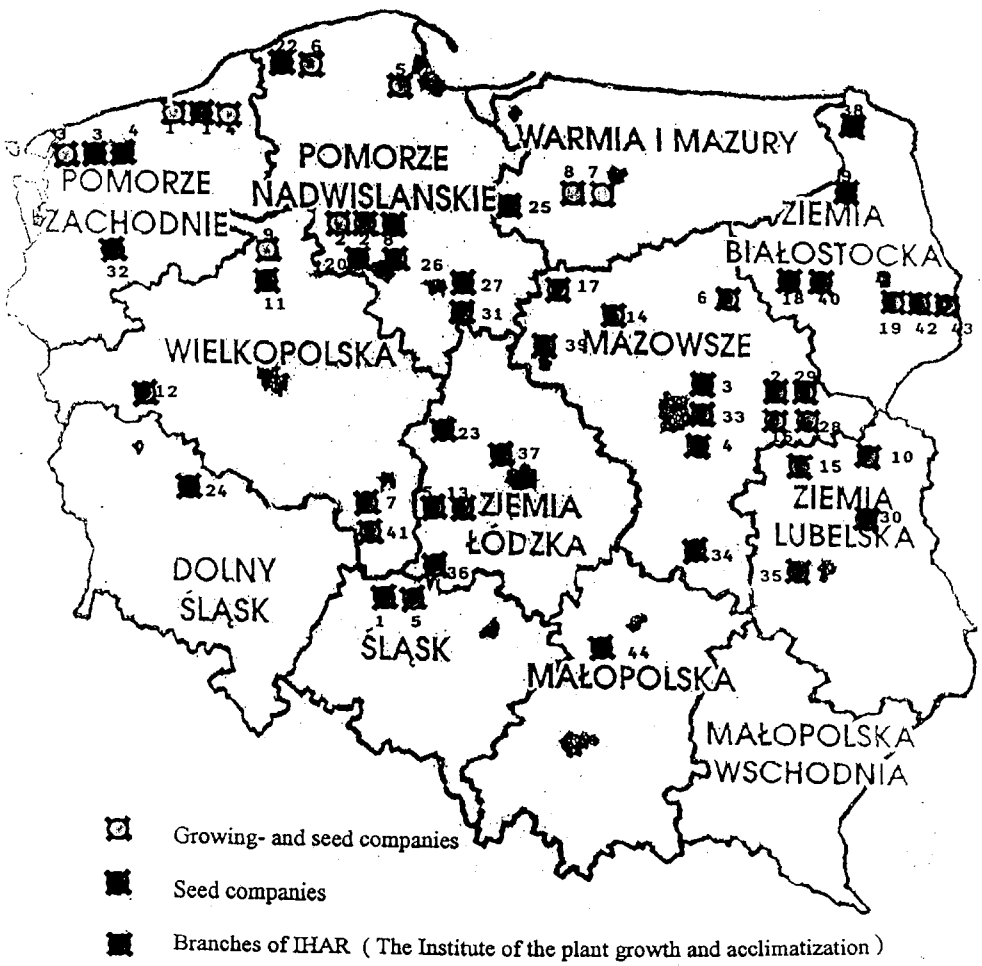
1. Instytut Hodowli i Aklimatyzacji Roślin, Oddział w Boninie.
2. Instytut Hodowli i Aklimatyzacji Roślin, Zakład Naukowo-Hodowlany Zamarte, 89-430 Kamień Krajeński.
3. Instytut Hodowli i Aklimatyzacji Roślin, Oddział w Jadwisinie, 05-140 Serock.
4. Instytut Hodowli i Aklimatyzacji Roślin, Oddział Naukowo-Badawczy Młochów, 05-832 Rozalin.
5. Instytut Hodowli i Aklimatyzacji Roślin, Oddział Stare Olesno.

Adresses of seed producers and seed merchants
(Adresy przedsiębiorstw nasiennych (prowadzących reprodukcję sadzeniaków))

1. IHAR, Oddział w Starym Olesnie, 46-300 Olesno.
2. Przedsiębiorstwo Nasienne "Centrala Nasienna", 08-110 Siedlce, ul. Floriańska 57.
3. Przedsiębiorstwo "Centrala Nasienna" Oddział, 72-200 Nowogard, ul. Nadtorowa 15.
4. Gospodarstwo Nasienne Alfred Jacoby, Zielin, 72-300 Gryfice.
5. Sieradzkie Przedsiębiorstwo Nasienne "Centrala Nasienna", 98-200 Sieradz, ul. POW 30.
6. Przedsiębiorstwo Nasienne "Centrala Nasienna", 07-412 Ostrołęka, ul. Targowa 26.
7. Firma Nasienna "Solanum", Jan Wawrzyniak, Rososzyca, ul. Kościelna 3.
8. Tucholskie Przedsiębiorstwo Nasienne "Centrala Nasienna", 89-500 Tuchola, ul. Świecka 77.
9. Przedsiębiorstwo Handlowo-Nasienne "Cennas" Sp. Z o.o., 19-203 Grajewo, ul. Elewatorska 6.
10. Przedsiębiorstwo "Centrala Nasienna",17-100 Bielsk Podlaski.
11. Kazimierz Grochowski, 77-420 Lipka, ul. Wybudowanie 16 (woj. Piła).
12. Świebodzińskie Przedsiębiorstwo Nasienne "Centrala Nasienna", 66-200 Świebodzin, ul. Jeziorowa 5.

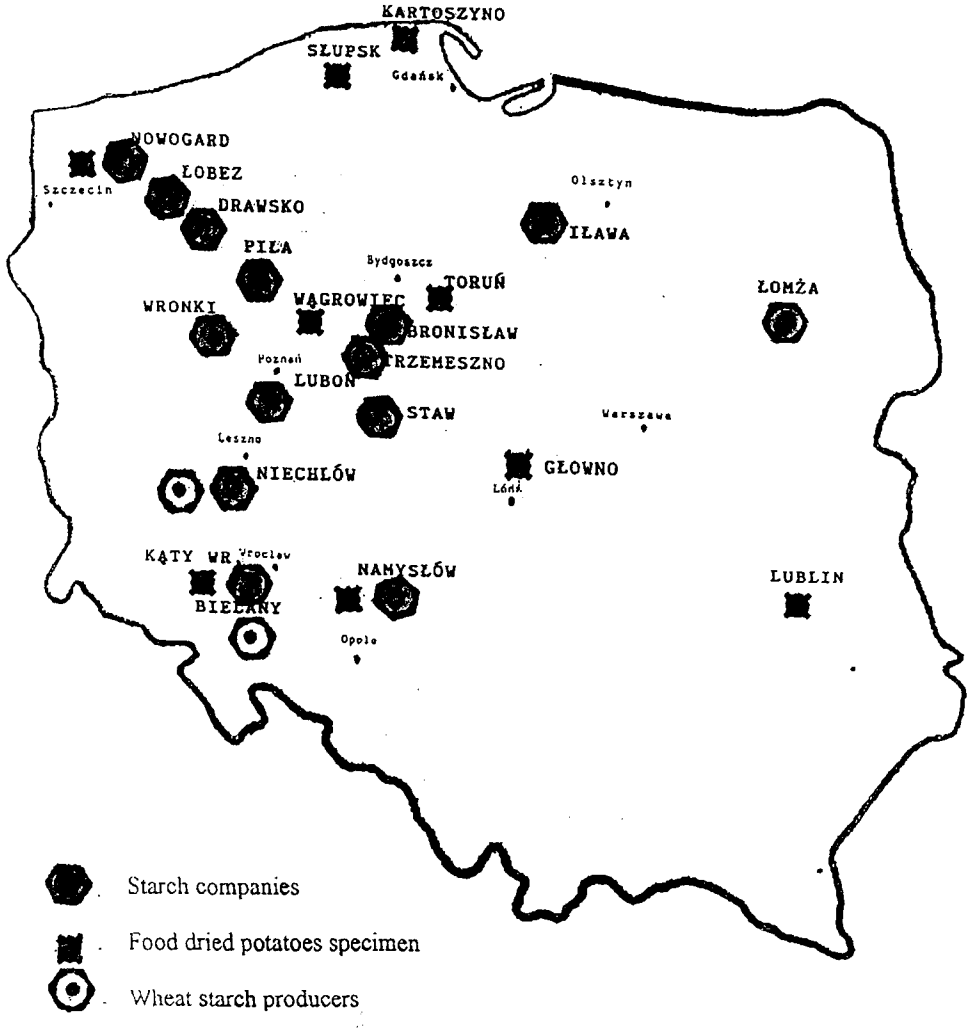
13. Przedsiębiorstwo Nasienne "Agrosad" s.c., ul. Równa 28, 98-235 Błaszki, woj. Sieradzkie.
14. Przedsiębiorstwo Nasienne "Centrala Nasienna", 06-400 Ciechanów, ul. Śmiecińska 14.
15. Przedsiębiorstwo Nasienne Usługowo-Handlowe Sp. Z o.o. "Ziarnopol", 21-400 Łuków, ul. Warszawska 81.
16. Przedsiębiorstwo Usługowo-Handlowe "Solex" Sp. Z o.o., 08-110 Siedlce, ul. Graniczna 31.
17. Nasienne Przedsiębiorstwo Handlowo-Usługowe "Działnas" s.c., 13-200 Działdowo, ul. Norwida 5/31.
18. Łomżyńskie Przedsiębiorstwo Handlowo-Usługowe "Centrala Nasienna", 18-400 Łomża, ul. J. Piłsudskiego 70.
19. Przedsiębiorstwo Produkcyjno-Handlowe "Centrala Nasienna", 15-959 Białystok, ul. Handlowa 1.
20. Gminna Spółdzielnia "Samopomoc Chłopska", 89-410 Więcbork, ul. Mickiewicza 16.
21. Przedsiębiorstwo Produkcyjno-Handlowe "Eksport-Import" "Centrala Nasienna" s.c., 21-200 Parczew, ul. Szkolna 32.
22. Przedsiębiorstwo "Centrala Nasienna", 76-200 Słupsk, ul. Grunwaldzka 11.
23. Przedsiębiorstwo Produkcyjno-Usługowo-Handlowe "Nasiennik" Sp. Z o.o., 62-700 Turek, ul. Milewskiego 14.
24. Gospodarstwo Nasienne Jacek Głodowski, 59-524 Pielgrzymka 149, woj. Legnickie.
25. "Rolimpex" Sp. Z o.o., 14-200 Iława, ul. Kardynała Wyszyńskiego 12.
26. Przedsiębiorstwo Rolno-Handlowe "Awena", 89-600 Chojnice, ul. Drzymały 14.
27. Przedsiębiorstwo Wielobranżowe "Astor" "Centrala Nasienna", 86-200 Chełmno, ul. Osinowska 30.
28. Przedsiębiorstwo "Centrala Nasienna", 21-400 Łuków, ul. Warszawska 84A.
29. Przedsiębiorstwo Nasienno-Handlowe "Agrol", 08-103 Siedlce, ul. Monte Cassino 18.
30. Rolimpex S.A. Oddział, 21-500 Biała Podlaska, ul. Leszczynowa 1.
31. Przedsiębiorstwo Nasienne "CN", 87-400 Golub-Dobrzyń, ul. PTTK 52.
32. Przedsiębiorstwo Usługowo-Handlowo-Produkcyjne "Nasiennik" Sp. Z o.o., 73-200 Choszczno, ul. I Armi WP 2.
33. Przedsiębiorstwo Nasienne "Centrala Nasienna", 02-677 Warszawa, ul. Cybernetyki 19.
34. "Centrala Nasienna" S.A., 26-100 Radom, ul. Małęczyńska 18.
35. Przedsiębiorstwo Handlowo-Nasienne w Lublinie Sp. Z o.o. Oddział "Centrala Nasienna", 20-309 Lublin, ul. Łęczyńska 9.
36. Przedsiębiorstwo "Centrala Nasienna", 98-300 Wieluń, ul. Sieradzka 62.
37. Przedsiębiorstwo "Centrala Nasienna", 91-063 Łódź, ul. Legionów 93.
38. "Rolimpex"-Suwałki Sp. Z o.o., ul. Piaskowa 2, 16-400 Suwałki.
39. Przedsiębiorstwo Nasienne "Centrala Nasienna" Sp. Z o.o., 09-402 Płock, ul. Otolińska 27c.
40. Przedsiębiorstwo Handlowo-Produkcyjne "Zaopatrzenie "Jan Kulesza, Czarnowo, 18-208 Kulesze Kościelne, woj. Łomżyńskie.
41. Przedsiębiorstwo Państwowe "Centrala Nasienna", 63-400 Ostrów Wlkp., ul. Partyzancka 39.
42. Polcoop S.A. Oddział w Białymstoku, ul. Warszawska 43, 15-062 Białystok.
43. Polcoop TIM Sp. Z o.o., ul. Warszawska 43, 15-062 Białystok.
44. Kieleckie Przedsiębiorstwo Nasienne "CN", 32-200 Miechów.

THE LOCALISATION OF THE SEED AND GROWING COMPANIES, SEED AND THE BRANCHES OF IHAR TAKING CARE OF THE GROWING AND SEEDING OF THE POTAOES



Map 1.

THE LOCALISATION OF THE STARCH PRODUCERS AND THE BRANCHES OF THE FOOD DRIED POTATOES SPECIMEN IN POLAND



Map 2.

P. ÅMAN, L. ANDERSSON, R. ANDERSSON, H. FREDRIKSSON,
M. OSCARSSON

BARLEY STARCH

Abstract

Large variations in yield of dry matter and starch were found for different barley cultivars, including barleys with different amylose content. The microstructure, e. g. in granule size distributions, also differed between cultivars. In a waxy barley starch granules with a high content of amylose were found in the subaleurone layer, showing that different types of granules were found in different parts of the endosperm. The amylopectin in starch with different amylose content had similar chain length distributions and gelatinization characteristics.

Introduction

Barley is a very old cultivated crop. Today it is mainly used in animal feed and for malting in developed countries but in certain areas, like Korea and West Asia/North Africa the human consumption of this cereal is still quite high. In Western countries barley may be rediscovered as a food grain since it has many interesting nutritional properties like a high content of mixed-linked β-glucan which may reduce the serum cholesterol levels [1] and relatively resistant endosperm cell walls, which in products with intact structures may reduce the postprandial blood glucose and insulin responses after a meal [2].

Barley is extensively used in genetic studies due to its diploid and self-fertile nature, ease of hybridization and a large number of easily classified characters. The barleys cultivated today have characteristics such as winter/spring, two-rowed/six-rowed, covered/naked, high amylopectin starch (waxy)/low amylopectin starch, high mixed-linked β-glucan/low mixed-linked β-glucan and high protein or lysin. Several of the above mentioned characteristics have been combined in new barley genotypes. For example the starch content in barley varies widely and ranges from 21.2–66.6% [3-4]. This variation may depend on both growing conditions and cultivar.

Department of Food Science, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden

Industrial processing of starch from barley is very limited, and to our knowledge only one full scale plant in Finland is operating [5]. The combination of a relatively low starch content, a high fibre content and that barley contains viscous and gelling materials which need special process steps, including the use of added enzymes, lead to that the products must have an added value compared to starch and byproducts from the traditional plants which generally use corn, wheat or potato as raw materials.

Barley starch with low (0–14%), normal (19–30%) and high (35–45%) amylose content are available today [6-8]. Especially the low amylose starch has rendered great interest during recent time due to its excellent freeze/thaw stability. The high amylose types could be of nutritional interest in products for control of blood glucose and insulin levels due to a significant formation of resistant starch in many processed foods.

In this proceeding we will report some results from our work on new barley types with low, normal or high amylose content. Results from both Swedish field experiments and chemical and physical studies will be presented.

Results and discussion

Effect of cultivar and environment on yield and grain quality

Ten barley cultivars including covered and naked types varying in their content of total starch, amylose, protein and β-glucan, were grown in different years, at various locations and nitrogen fertilization rates [9]. The barleys showed large variations in yield (3250–6690 kg/ha), starch content (51–67 % of DM), starch yield (1870–3840 kg/ha), protein content (8–15% of DM) and β-glucan content (3.5–5.9%). The naked and high amylose barley Hashonucier had the lowest yield and starch yield while the covered feed barley Lina had the highest. As the effect of year and location was generally less pronounced than the effect of N-rate, the yield and analysed variables were calculated as average values for the ten cultivars at the different N-rates. For all barleys the yield and protein content increased with higher nitrogen fertilization rates while the starch content decreased. Most of the barleys also showed higher β-glucan contents.

Composition and microstructure of barley samples

Barley samples with a low (about 8%, waxy, SW 7142-92), normal (about 25%, Golf) and high (about 40%, naked Hashonucier and covered high amylose Glacier) amylose content were used in this investigation on the composition and microstructure (10). Growing location or nitrogen fertilization did not notably influence endosperm thickness or cell size in the different barleys in this limited study. Golf had overall thinner starchy endosperm cell walls and the chemical composition also showed that it contained less β-glucan than the waxy and high amylose samples. In Hashonucier and high amylose Glacier the starch granules were more even in size compared with Golf,

which showed a typical bimodal distribution.In the waxy sample it was shown that medium sized granules which stained black with iodine, indicating a high amylose content, were located in the subaleurone layer while the rest of the granules stained more brownish. Consequently different types of starch granules were present in different parts of the endosperm.

In order to confirm the high amylose content in granules in the subaleurone layer, inner and peripheral parts of the kernels were isolated by pearling [11]. Chemical analyses of amylose content in isolated starch indeed showed that there was a higher concentration of amylose in the outer parts of the starchy endosperm.

Characterization of starch isolated from different types of barley

Starch was isolated from pearled waxy (line 906129) normal (Golf), and high amylose (high amylose Glacier) barleys [12]. Small starch granules tended to associate to the protein fraction and may partly have been lost during the isolation procedure. The starch granules of the barleys with normal and low amylose content had similar size distribution profiles as measured by Coulter Counter and as revealed by scanning electron microscopy. The high amylose barley had smaller A-granules. The content of total amylose, as determined by iodine staining, was 5.6% in the waxy starch, 27% in the normal starch and 37% in the high amylose starch and the content of lipid complexed amylose (LAM) 2.4, 5.5 and 7.5%, respectively. Total amylose content as determined by gel permeation chromatography after debranching was generally 2%-units higher as compared to the results obtained by iodine staining.

The amylopectin unit chain length distribution of the three barleys were examined by high performance size exclusion chromatography, after debranching with isoamylase [12]. All three amylopectins showed a polymodal unit chain length distribution, with local peak maxima or shoulders at DP 12, 18–19 and 46–47. The weight average chain lengths were for the waxy starch 26.7, the normal amylose starch 25.8 and the high amylose starch 24.0. Consequently the high amylose barley seems to have amylopectin with somewhat shorter unit chains than the other two barleys. DSC gelatinization endotherms of the three starches were studied at a starch:water ratio of approximately 1:1. The enthalpy of gelation of the amylopectin were similar in the three samples (range 15.6–16.1 J/g amylopectin). As a general conclusion all three amylopectins had similar structural and gelatinization characteristics.

REFERENCES

[1] Newman R.K., Newman C.W.: Cereal Foods World, **36**, 1991, 800-805.

[2] Granfeldt Y., Liljeberg H., Drews A., Newman R., Björck I.: Am. J. Clin. Nutr., **59**, 1994, 1075-1082.

[3] Åman P., Hesselman K., Tilly A.-C.: J. Cereal. Sci., 3, 1985, 73-77.
[4] Åman P., Newman W.: J. Cereal Sci., 4, 1986, 133-141.
[5] Ahvenainen J., Kuhanen J., Lähdesmäki M., Nevalainen P.: [in] Barley for Food and Malt. ICC/SCF International Symposium. Uppsala, 1992, 100-102.
[6] MacGregor A.W., Fincher G.B.: [in] Barley Chemistry and Technology (MacGregor A.W. and Bhatty, R.S., Eds), 1993, 297-354.
[7] Persson G., Ståhl Å., Johansson L.-Å., Johansson H.: Sveriges Utsädesförenings Tidskrift, **106**, 1996, 79-86 (in Swedish).
[8] Bhatty R.S., Rossnagel B.G.: Cereal. Chem., **74**, 1997, 190-191.
[9] Oscarsson M., Andersson R., Åman P., Olofsson S., Jonsson A.J.: Sci. Food Agric., 1998, in press.
[10] Oscarsson M., Parkkonen T., Autio K., Åman P.: J. Cereal. Sci., **26**, 1997, 259-264.
[11] Andersson L., Fredriksson H., Oscarsson M. Andersson R. Åman P.: Submitted for publication.
[12] Fredriksson H., Silverio J., Andersson R., Eliasson A.-C., Åman P.: Carbohydr. Polym. in press.

SKROBIA JĘCZMIENNA

Streszczenie

Dla różnych odmian jęczmienia, włącznie z odmianami o różnych zawartościach amylozy stwierdzono duże zmiany w zawartości suchej masy oraz skrobi. Mikrostruktura tzn. skład ziarnistości dla różnych odmian również był zróżnicowany. W skrobi jęczmiennej woskowej w warstwie podaleuronowej znaleziono gałeczki o wysokiej zawartości amylozy pokazując, że w różnych częściach endospermy znajdują się różne rodzaje gałęczek. Amylopektyna w skrobi o różnej zawartości amylozy miała podobną długość łańcucha i podobną charakterystykę kleikowania.

A.A.C.M. BEENACKERS, C.J. TIJSEN, J.E. VISSER, E.J. STAMHUIS

NOVEL PROCESSES FOR THE CARBOXYMETHYLATION OF STARCH

Abstract

Two novel processes for the carboxymethylation of starch are presented, and are compared with respect to degree of substitution, substitution patterns, reaction times and yield. The first process is a gel process in a continuous static mixer reactor optimised for handling concentrated aqueous starch pastes. The second process is a slurry process which uses organic liquids as reaction medium. Based on experimental results for both processes it can be concluded that each process has its own characteristic advantages. The static mixer process gives a good selectivity, it has short residence times and superior controllability and safety. Benefits of the non-aqueous slurry process are the possibility to produce granular CMS with a high degree of substitution and a good selectivity.

Keywords: starch, carboxymethylation, Sulzer SMX static mixer, alcohol process, *i*-propanol

Introduction

Native starch is often chemically modified to give it superior properties for dedicated applications. Carboxymethyl starch (CMS) is commonly produced by reacting starch with the sodium salt of monochloracetic acid (SMCA) in an alkaline medium. One of the first papers on the carboxymethylation of starch was published in 1924 by Chowdhury [1].

Carboxymethyl starch has a wide range of applications [2, 3], see Fig. 1. CMS is used for paper making as a binder and as a water resistant coating (insoluble polyvalent metal salts of CMS). In the textile industry CMS is used as a thickener in printing pastes or dyes and in sizing, and in adhesives for water-washable tapes. CMS is also used as glue, as foaming agents, as a coating for seeds, and it is used in oil-well fracturing fluid. Degraded or oxidised CMS is used as detergent components. In the food industry CMS is applied as a fat substitute and as a stabiliser in ice-cream.

Department of Chemical Engineering, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands.

An important characteristic of CMS is the degree of substitution, *DS*, which is defined as the average number of substituents per anhydroglucose unit (AGU), the monomer unit of starch. Each AGU has three hydroxyl groups, so the *DS* lies between zero and three.

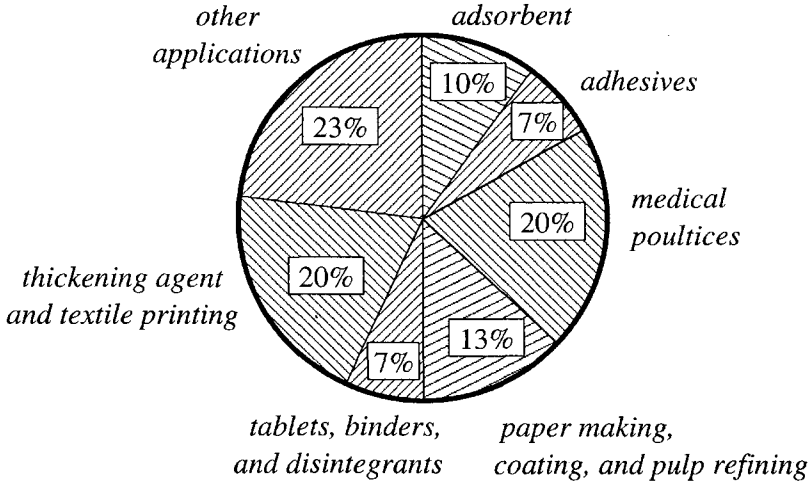


Fig. 1. Pie diagram of the applications of CMS, after [3].

CMS can be manufactured in several ways. In an aqueous slurry the *DS* of CMS is limited to a value of about 0.03 [3]. At higher *DS* values the granules start to swell in water and become sticky which causes agglomeration. For many applications a $DS > 0.03$ is required. However, for a $DS > 0.03$ the starch granules become cold-water soluble which results in a highly viscous paste.

The traditionally used batch reactor with helical ribbon impeller for handling such a viscous starch paste has poor mixing and heat transfer characteristics. A homogeneous product asks for short mixing times relative to the reaction time. Therefore, a novel continuous reactor for the chemical modification of starch paste was developed in our laboratory [4]. This reactor has static mixing elements which provide good mixing, nearly plug flow behaviour and an enhanced heat transfer. The good radial mixing allows for faster reaction rates [5, 6] which results in a smaller reactor for the same production capacity. Other important aspects of a static mixer reactor are the good controllability and safety characteristics. We developed this novel type of reactor for the hydroxypropylation [4] and the carboxymethylation of starch [7]. In the static mixer process the starch loses its granular form and the paste has to be dried on a drum dryer to obtain dry CMS flakes.

To preserve the granular structure of the starch, various processes have been discussed [3, 8]. When salts are added to an aqueous slurry a *DS* of about 0.1 can be reached [8]. A slightly higher *DS* for granular CMS can be obtained after cross-linking of the starch [8]. In a relatively dry process, the maximum *DS* is 0.5 [3]. As a reactor for this dry process a bag at room temperature, a jacketed blender and a fluidized bed have been proposed [8].

Another process for the production of highly substituted granular CMS uses water-miscible organic liquids as a reaction medium. The organic liquids are used to prevent the gelatinisation of the starch granules and to provide a good reaction medium. This gives the possibility to wash and dry the starch easily, which is difficult when the starch is dissolved.

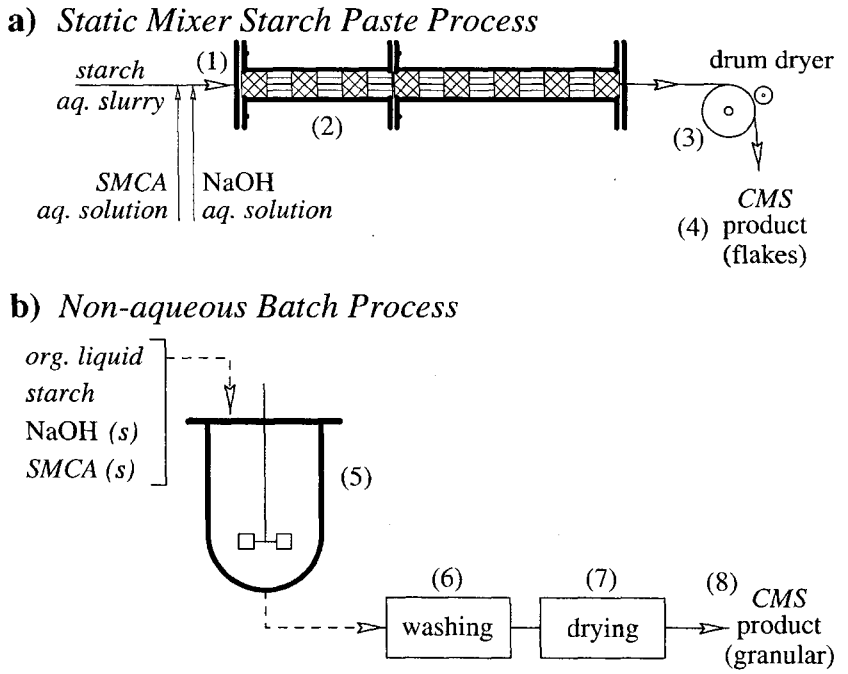


Fig. 2. Schematic diagrams of the static mixer starch paste process (a) and the non-aqueous batch process (b). (1) Gelatinization of starch; (2) Reactor with static mixing elements; (3) Drying of the starch paste; (4) CMS product (flakes); (5) Non-aqueous slurry batch reactor; (6) Washing; (7) Drying of product; (8) Granular CMS product.

In this paper the novel static mixer starch paste process and the novel non-aqueous slurry process are described. Figure 2 shows the schematic flow diagrams of both processes. The advantages and disadvantages of both processes are discussed by comparing the results obtained with these processes.

Theory

Reaction equations

NaOH added to a solution or a slurry of starch gives the following equilibrium reaction with the hydroxyl groups of the starch:

$$ROH + Na^+ OH^- \leftrightarrow RO^- Na^+ + H_2O \quad (1)$$

The activated form of the starch hydroxyl groups, RO^-, is more reactive than the inactivated form ROH [9]. The main reaction is the carboxymethylation of starch:

$$ClCH_2COO^- Na^+ + RO^- Na^+ \rightarrow ROCH_2COO^- Na^+ + Na^+ Cl^- \quad (2)$$

SMCA can also react with OH^-, resulting in a competitive side reaction:

$$ClCH_2COO^- Na^+ + Na^+ OH^- \rightarrow HOCH_2COO^- Na^+ + Na^+ Cl^- \quad (3)$$

SMCA can also react with water, but in an aqueous solution the hydrolysis with OH^- dominates relative to H_2O for $c_{OH^-} > 0.0016$ kmol/m^3 [10].

Because in both the main and the side reaction Cl^- is formed, the conversion of SMCA (A), ζ_A, can be calculated from the chloride production:

$$\zeta_A = 1 - \frac{n_A}{n_{A_0}} = \frac{n_{Cl} - n_{Cl_0}}{n_{A_0}} \quad (4)$$

The selectivity of SMCA towards the product CMS (P) follows from the experimental DS and the number of moles Cl^- formed ($n_{Cl} - n_{Cl_0}$):

$$\sigma_p = \frac{n_P}{n_{A_0} - n_A} = \frac{n_{AGU}}{n_{Cl} - n_{Cl_0}} DS \quad (5)$$

The yield of the product CMS, η_P, is the ratio of the DS and the theoretical DS, DS_t, which is the DS at 100% selectivity and conversion based on the lowest molar amount of the reagents added (SMCA or NaOH):

$$\eta_p = \begin{cases} \text{if } n_{NaOH} \geq n_A & DS_t = n_{A_0}/n_{AGU} \quad \eta_P = \sigma_P \zeta_A \\ \text{if } n_{NaOH} < n_A & DS_t = n_{NaOH_0}/n_{AGU} \quad \eta_P = \sigma_P \zeta_{NaOH} \end{cases} \quad (6)$$

Substitution patterns of CMS

The mole fractions of mono-, di- and tri-substituted AGU were measured with HPLC. However, the technique is not sensitive enough to determine on which C-atom the carboxymethyl groups are situated. Spurlin [11] developed a 'rate of reaction' model which predicts these mole fractions as a function of the DS on the basis of three relative reaction rate constants k_2, k_3 and k_6 for the hydroxyl groups on carbon number C-2 , C-3 and C-6.

Because the position of the carboxymethyl groups is unknown it is impossible to discriminate between k_2, k_3 and k_6. Therefore, the k values are given subscripts a, b and

c. The three *k*-values can be determined separately only if experimental *DS* values are obtained in the full range from zero till three.

Experimental

Materials

Food grade quality potato starch, a slightly oxidised potato starch (Perfectamyl A-4692, AVEBE) and technical grade powder sodium monochloroacetate (SMCA) (Akzo-Nobel, Arnhem, The Netherlands) were a gift of AVEBE (Foxhol, The Netherlands). Elemental analysis of the SMCA showed that the contents of C, Cl, H and Na were 99.4 ± 0.3 wt%, 99.9 ± 0.2 wt%, 100.6 ± 1.7 wt% and 99.9 ± 0.2 wt% of the pure values, respectively (n_r = 2). The molar ratio of chloride and SMCA, was found to be $n_{Cl^-} / n_{SMCA} = 0.68 \times 10^{-3}$. H_2SO_4 used in the eluens of the HPLC analysis was of analytical grade.

Paste process

For the kinetic experiments in a batch reactor the starch was washed three times with demineralised water and dried in a vacuum oven at 55°C to a moisture content between 10–15 wt%. Native starch was used at low concentrations only ($c_{AGU} \leq 0.3$ kmol/m^3). For higher concentrations Perfectamyl was used. For experiments in the static mixers native potato starch from AVEBE was used. The concentration of NaOH in the NaOH-solution (33 wt%, technical grade, Chemproha) was always measured after dilution by titration with 1 N HCl (Titrisol, Merck). The HCl used for hydrolysis of the starch prior to HPLC analysis was of analytical grade.

Non-aqueous batch process

Before use, starch was washed three times with demineralised water and dried in a vacuum oven at 55°C to a moisture content between 10–15 wt%. NaOH pellets were of analytical grade. Dehydrated technical grade *i*-propanol with a purity >99% was used (Acros Chemica, Geel, Belgium). Ethanol, 96% pure, and acetone were both of technical grade. The other organic solvents, *i.e.* methanol, *n*-propanol, *n*-butanol, *s*-butanol and *t*-butanol, were of analytical grade. H_2SO_4 used for hydrolysis of the starch and $BaOH_2$ used for neutralisation of the HPLC samples were of analytical grade.

Experimental set-up for the paste process

The carboxymethylation of starch in aqueous solution was carried out in a batch reactor and in two continuous static mixer reactors of different scale (Static Mixer 1 and 2).

Batch reactor

A jacketed stainless steel batch reactor with a volume $V_r \approx 0.7\times10^{-3}$ m^3 and a stirrer with two turbine impellers ($D_{st}/D_{BR} \approx 0.5$) was used for the batch experiments. Native potato starch was used at concentrations up to $c_{AGU} \leq 0.3$ kmol/m^3. At higher starch concentrations the reaction mixture became too viscous, resulting in poor mixing of the reaction mixture. The use of a slightly oxidised potato starch (Perfectamyl) made it possible to use starch concentrations in the batch reactor of at least $c_{AGU} = 1.9$ kmol/m^3. For $c_{AGU} = 0.3$ kmol/m^3 Perfectamyl proved to have the same reactivity as native potato starch.

The experimental procedure was as follows. A weighed amount of starch with known moisture content together with a weighed amount of milliQ water was stirred in a beaker until no starch lumps could be seen. The beaker was closed with a lid and placed in a waterbath of about 90 °C. The starch slurry was stirred at 5 rps. After 15 minutes the starch started to gelatinise and the starch solution was stirred at 17 rps at 90°C during 45 minutes. The starch solution was weighed to determine the amount of evaporated water before pouring it into the batch reactor. The beaker was weighed again to determine the amount of starch solution in the reactor. Then a specific amount of SMCA powder was added together with a specific amount of water to reach the desired concentrations. The starch solution was brought to the desired reaction temperature with a thermostatic bath (Haake F3-S) which controlled the temperature in the reactor. The reactor was flushed with N_2 to remove O_2 and CO_2. The reaction was started by adding a specific amount of 33 wt% NaOH solution to the reactor with a 50 ml syringe. Sampling took place by suction of 5–20 ml of the reaction mixture into a 50 ml syringe with an extended nozzle and quenching in 100 ml 0.25 M HNO_3.

Small scale static mixer

Static Mixer 1 is a small tubular reactor ($D_t = 27.3$ mm and $L/D_t = 49$) filled with Sulzer SMX static mixing elements (see Fig. 3). Previously, it was used for the hydroxypropylation of starch [4]. For more details on this equipment and procedures, see [4, 7, 12].

Here the operation of the static mixer and some reactor features are discussed briefly. The starch slurry (with ≈12 wt% SMCA) was mixed with a 4.00 M ≈38 wt% SMCA-solution and a 11.2 M ≈33 wt% NaOH-solution in a pre-mixer (1) consisting of 10 Sulzer DN4 static mixing elements. A few seconds after leaving the pre-mixer the starch is gelatinised completely giving a viscous starch paste. Then the starch paste is heated to the desired temperature in a microwave heating section (2). The microwave oven (2.45 GHz) is amplitude controlled and has a tuning facility to maximise the coupling between the microwaves and the starch paste (3a).

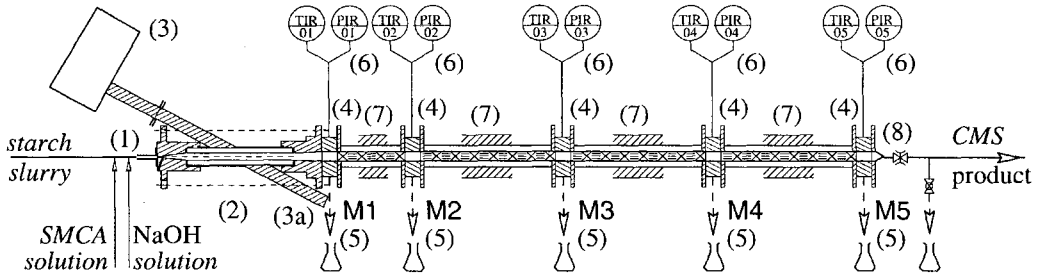


Fig. 3. Experimental setup of Static Mixer 1; (1) Pre-mixer: Sulzer SMX DN4 (D_t = 4.8 mm); (2) Microwave heating section; (3) Microwave generator (microwave tuning (3a)); (4) Measurement sections (M1–M5); (5) Sampling point; (6) Temperature and pressure measurement; (7) Reaction section (Sulzer SMX DN25; D_t = 27.3 mm); (8) Pneumatic valve.

The reactor consists of measurement (4) and reaction sections with Sulzer SMX DN25 static mixing elements (7). The temperature and pressure of the starch paste are measured in the measurement sections (6). A pneumatic valve at the end of the reactor (8) allows for sufficient pressure to take a sample from the last measurement section. Sampling (5) takes place through specially designed valves having a cleaning facility. The reaction was quenched with a stainless steel rod, cooled in liquid nitrogen. Subsequent acidification allowed for further handling of the samples.

Static Mixer 1 was originally designed with four reaction sections of the same length. Because the carboxymethylation of starch is an overall second order reaction the initial reaction rate is relatively high. Therefore, the first reaction section was replaced by a section with 7 instead of 14 SMX static mixing elements.

Larger scale static mixer

To investigate the scale-up of the static mixer reactor for the chemical modification of starch a larger static mixer was built with a 2.5 times larger internal diameter than Static Mixer 1 (Static Mixer 2: D_t = 67.1 mm and L / D_t = 27, see Fig. 4).

This reactor also has the possibility to sample (5) and measure temperature and pressure at several axial locations (6). The temperature of the starch paste can be measured at different radial distances and different angles with respect to the static mixing elements. This way, a radial temperature profile could be measured. A homogeneous temperature is important, because alkaline starch pastes are susceptible for thermal degradation.

Most important differences with Static Mixer 1 are the design of the microwave (3, 3a), the increasing lengths of the reaction sections (7) to obtain a steady increase in conversion at the subsequent measurement sections and the rotatable measurement section (M2) with a movable temperature measuring device inside.

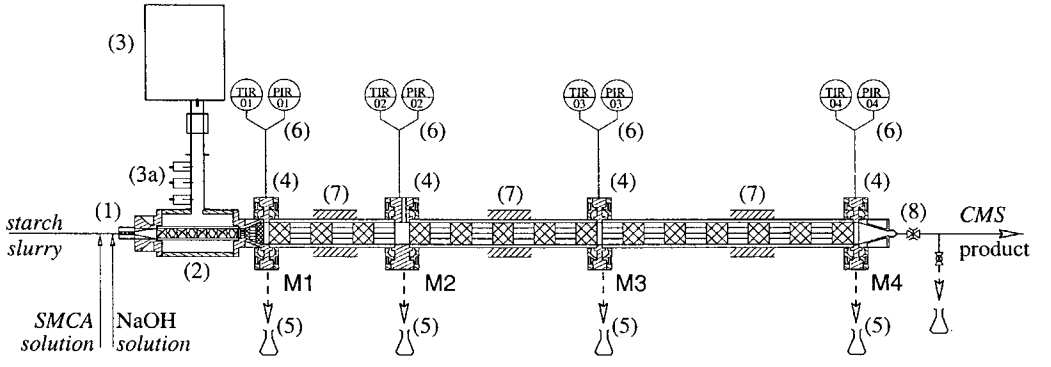


Fig. 4. Experimental setup of Static Mixer 2; (1) Pre-mixer: Sulzer SMX DN4 (D_t = 4.8 mm); (2) Microwave heating section; (3) Microwave generator (microwave tuning (3a)); (4) Measurement sections (M1–M4); (5) Sampling point; (6) Temperature and pressure measurement; (7) Reaction section (Sulzer SMX DN65; D_t = 67.1 mm); (8) Pneumatic valve.

The reactor can be thermostatted with water which circulates at high velocity through the jackets. With air filled jackets and good isolation the reactor can be operated nearly adiabatically.

Experimental set-up for the non-aqueous batch process

The carboxymethylation of granular starch in the non-aqueous batch process was carried out in two different reactors. In the large reactor the *DS* as function of the time was obtained. The conversion, the selectivity and the granular structure was studied in the small reaction vessels.

Large reactor

The organic liquid was first mixed with a specific amount of water. The granular potato starch and the reaction medium were added in a jacketed batch reactor (V_r = 1×10^{-3} m^3), followed by flushing with N_2. Also during the experiment a N_2 flow was used to prevent reaction of NaOH with CO_2 from the air. The reactor was equipped with a reflux cooler to prevent the loss of volatile liquid. Typically, after 15 minutes NaOH was added to the reaction mixture. Subsequently, the vessel was heated to the reaction temperature, and left overnight under stirring to assure equilibrium between the starch and the NaOH. The reaction was started by adding powder SMCA. At given times, a small sample was taken from the reaction suspension for analysis. The reaction was stopped by the addition of H_2SO_4.

Small reaction vessels

Experiments were carried out in magnetically stirred reactors (V_r = 25×10^{-6} m^3) placed in a thermostatic bath. First, NaOH pellets were added to the reactor. Then, the granular potato starch and the reaction medium, with a known composition, were mixed in the reaction vessel. Subsequently, the reaction vessels were heated to the reaction temperature and left overnight under stirring to assure equilibrium between the starch and the NaOH. The reaction was started by adding SMCA and stopped by adding H_2SO_4 to the samples.

Analytical procedures

Degree of substitution

The degree of substitution, *DS*, of the CMS was determined with HPLC [13, 14]. This technique requires hydrolysis of the starch into glucose units while keeping the carboxymethyl groups intact. Hydrolysis of the CMS gives eight possible molecules: glucose, three mono-CMG, three di-CMG and tri-CMG. For the starch paste process a 1 wt% sample was hydrolysed in 1 M HCl at 100°C during 4 hours. For the non-aqueous batch process 0.1 g of CMS was hydrolysed with 18 ml of 0.75 M H_2SO_4 at 100°C for 4 hours, and $BaOH_2$ was used to neutralise and precipitate the sulphate ions as $BaSO_4$.

The hydrolysed samples were injected on two Bio-Rad Aminex HPX-87H ion exclusion columns in series at 65 °C. As eluens 0.75 mM H_2SO_4 was used at a flow rate of 0.5 ml/min. The RI-detection (HP1047A) gave four separate peaks for tri-CMG, di-CMG, mono-CMG and glucose together with peaks for the salt, SMCA, glycolic acid and, if present, organic liquid. The mole fractions x_i of glucose (i = 0), mono-CMG (i = 1), di-CMG (i = 2) and tri-CMG (i = 3) and the *DS* were calculated with the following equations:

$$x_i = \frac{F_i A_i / M_i}{\sum_{j=0}^{3} F_j A_j / M_j} \quad \text{and} \quad DS = \sum_{i=0}^{3} i x_i \tag{7}$$

with the response factors: F_0 = 1, $F_1 = F_2 = F_3$ = 1.03 [15], the molecular masses are M_0 = 180, M_1 = 237, M_2 = 294 and M_3 = 351, and A_i are the peak areas in the chromatogram.

Chloride analysis

The amount of chloride, c_{Cl^-}, was determined by potentiometric titration with 0.1 M $AgNO_3$ and a combined Ag-electrode (Metrohm 6.0404.100) [16].

Results and discussion

Paste process

Reaction kinetics

Some results of a kinetic study in a batch reactor of the carboxymethylation of starch in aqueous solution are presented here [7]. The experimental conditions are given in Table 1 together with the selectivity, σ_P, obtained and the time required for a conversion of 50%, $t_{\zeta_A=0.5}$.

Table 1

Reaction conditions of the carboxymethylation of starch in aqueous solution in a batch reactor

nr	Starch type	T °C	c_{AGU} kmol/m^3	c_{NaOH_0} kmol/m^3	c_{A_0} kmol/m^3	DS_t –	σ_P^e –	$t_{\zeta_A=0.5}$ 10^3 s
a	Native	84.9	0.296	0.654	0.514	1.74	0.210	1.04
b	Native	85.0	0.292	0.653	0.528	1.81	0.205	1.05
c	Native	65.0	0.297	0.665	0.526	1.77	0.235	6.65
d	Native	45.3	0.297	0.664	0.534	1.80	0.290	52.0
e	Native	32.1	0.295	0.672	0.525	1.78	0.320	235
f	Perfect.	65.1	0.300	0.659	0.525	1.75	0.236	6.63
g	Perfect.	64.9	1.87	1.32	1.22	0.652	0.770	1.36
h	Perfect.	45.0	1.89	1.32	1.23	0.651	0.750	10.1
i	Perfect.	30.3	1.85	1.32	1.21	0.654	0.850	53.4

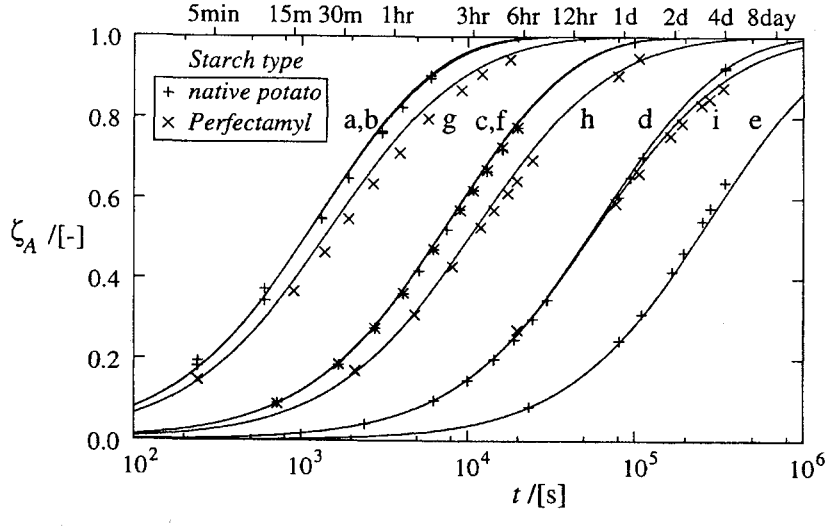


Fig. 5. Conversion of SMCA, ζ_A, as a function of time t at several reaction conditions (see Table 1) for the carboxymethylation of starch in aqueous solution.

Figure 5 gives both the experimental and the modelled conversion of SMCA, ζ_A, as a function of time. The rate equation of Sergeev and Rad'ko [17] was applied for the side-reaction and a similar equation for the main reaction. The duplo measurements (a, b) nearly coincide, which shows that the reproducibility is good. Two experiments (c, f) were carried out under the same reaction conditions to compare the reactivity of Perfectamyl with that of native potato starch. The experimental points nearly coincide which proofs equal reactivity. The experiment with Perfectamyl at high concentration and temperature (g) shows the largest deviation from the modelled curve.

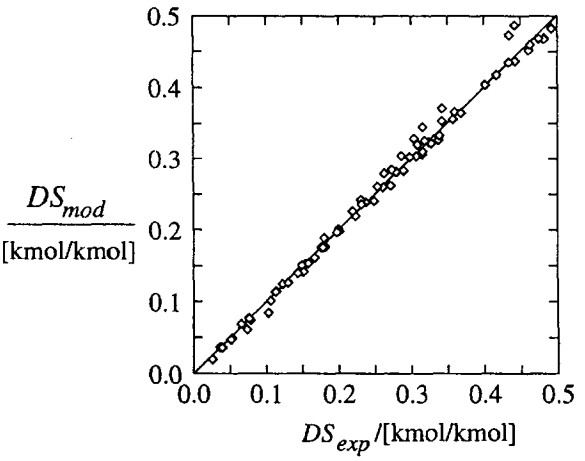


Fig. 6. Parity plot of the *DS* of the carboxymethylation of starch in aqueous solution in a batch reactor.

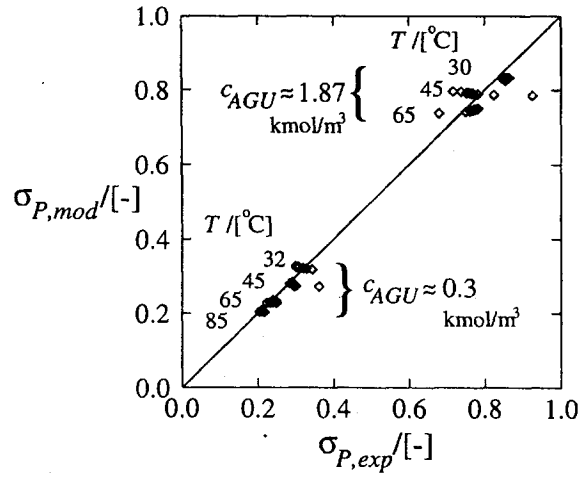


Fig. 7. Parity plot of the selectivity, σ_P, of the carboxymethylation of potato starch in aqueous solution in a batch reactor.

A parity plot for the *DS*, Fig. 6, shows that the modelled *DS* agrees well with the experimental values (MARR = 3.6%). Figure 7 shows a parity plot of the selectivity towards the product CMS, σ_P, for two starch concentrations, c_{AGU}, and several temperatures. It proves that σ_P is much higher for the higher (c_{AGU} = 1.8 kmol/m^3) than for the lower starch concentration (c_{AGU} = 0.3 kmol/m^3). At a high starch concentration, c_{AGU} = 1.85 kmol/m^3, and low temperature, T = 30°C, a selectivity of 0.85 can be reached (exp. i). At a higher temperature and nearly the same starch concentration the selectivity is somewhat lower, 0.77 for experiment g. Also for the low starch concentrations the selectivity decreases with increasing temperature, from 0.32 at 32°C to 0.21 at 85 °C (exp. a–e). Increasing T from 32°C to 65°C gives a 27% decrease in selectivity for the low starch concentration (exp. e → c) while for the high starch concentration the decrease appears to be 9% only (exp. i → g).

Small scale static mixer

The batch experiments showed that the selectivity, σ_P, was better at a higher starch concentration. Therefore the experiments in the static mixer were carried out with a high starch concentration, c_{AGU}. Table 2 gives the experimental conditions of some carboxymethylation experiments in Static Mixer 1.

Table 2

Reaction conditions of the carboxymethylation of starch in aqueous solution in Static Mixer 1. (see Fig. 5 for labels a – d; ζ_{lim} is the conversion of the limiting reactant, SMCA or NaOH)

nr	T_c	c_{AGU}	c_{NaOH_0}	c_{A_0}	DS_{th}	$DS_{\zeta^e_{lim}=1}$	at measurement section M5				
							DS	ζ^e_{lim}	σ^e_P	η^e_P	τ
	°C	kmol/m^3	kmol/m^3	kmol/m^3	kmol/kmol	kmol/kmol	kmol/kmol	–	–	–	s
a	96	1.94	1.41	1.39	0.716	0.56	0.46	0.82	0.78	0.64	1075
b	84	1.95	1.40	1.39	0.713	0.51	0.40	0.78	0.72	0.56	1075
c	76	1.99	1.41	1.46	0.709	0.58	0.35	0.62	0.81	0.50	1069
d	67	1.94	1.37	1.20	0.619	0.45	0.26	0.58	0.73	0.42	1198

T_c is the temperature of the thermostatting water which circulates through the jackets of the reactor. Due to the heat of reaction the temperature of the starch paste is a few degrees higher. The initial starch, NaOH and SMCA concentrations and the mean residence time was approximately the same for all experiments (a – d). The theoretical *DS*, DS_t was calculated with Eq. (6) with the numbers of moles n_i substituted by the concentrations c_i.

The last 5 columns give the experimental values of the *DS* (see also Fig. 8), the conversion of the limiting reactant (either SMCA or NaOH), ζ^e_{lim}, the selectivity, σ^e_P, the yield, η^e_P, and the mean residence time, τ, at measurement section M5. The value $DS(\zeta^e_{\text{lim}} = 1)$ is the *DS* which can be reached with a total conversion of the limiting reactant ($\zeta^e_{\text{lim}} = 1$):

$$DS\left(\zeta^e_{\text{lim}}\right) = \left.\frac{DS}{\zeta^e_{\text{lim}}}\right|_{Mx} \tag{8}$$

with Mx the last measurement section.

Table 2 shows that the conversion at measurement section M5 decreases with decreasing temperature. The selectivity is approximately 0.75 and comparable with the values obtained in the batch experiments with approximately the same starch concentration.

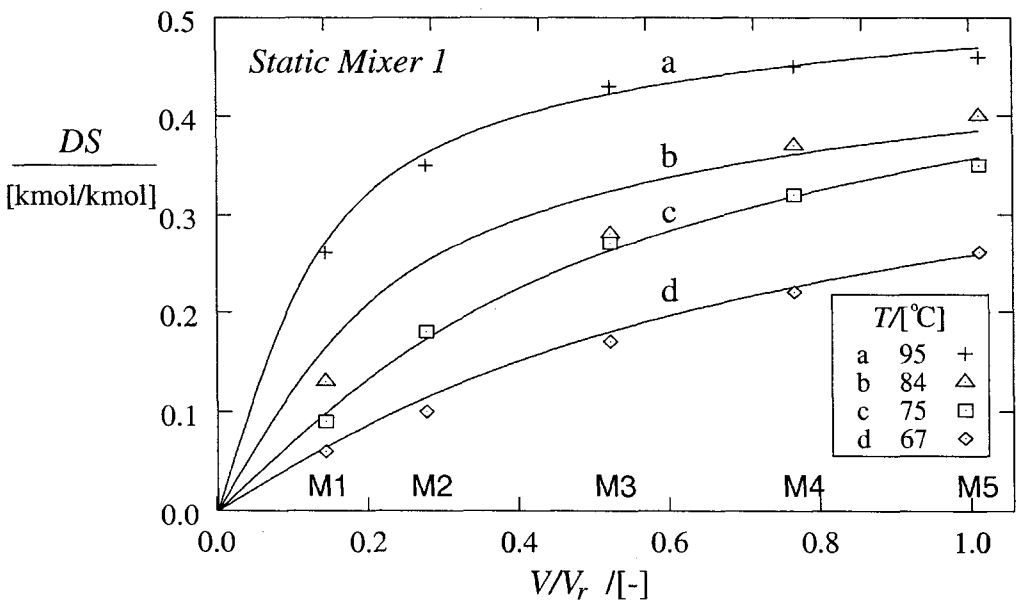


Fig. 8. The *DS* of CMS as a function of the axial position in the static mixer reactor (Static Mixer 1).

Figure 8 shows the *DS* as function of the position in the reactor, given as the ratio of the volume up to that position and the total volume of the reactor, V/V_r. The lines are simulated values of the *DS* on the basis of a model which describes the reactions and the heat transfer in the reactor [7]. The model predicts the experimental *DS* reasonably

good. Occasionally, experimental error, due to difficulties with sampling of the viscous starch paste, caused larger deviations from the predicted value.

Larger scale static mixer

Carboxymethylation of starch was carried out in Static Mixer 2 on a roughly 10 times larger scale than in Static Mixer 1. Two experiments to investigate the scale-up of the carboxymethylation in a static mixer reactor are discussed here. See Table 3 for the experimental conditions. Both experiments have approximately the same initial concentrations and hence the DS_t is almost the same.

Table 3

Reaction conditions of the carboxymethylation of starch in aqueous solution in Static Mixer 2. (V_r = 6.38×10^{-3} m^3; for exp. a: $w_{AGU} = 0.31$, $w_{NaOH_0} = 0.040$, $w_{A_0} = 0.13$ $w_{H_2O_0} = 0.52$)

nr	T_c	c_{AGU}	c_{NaOH_0}	c_{A_0}	DS_{th}	$DS_{\zeta^e_{lim}=1}$	at measurement section M4				
							DS	ζ^e_{lim}	σ^e_P	η^e_P	τ
	°C	kmol/m^3	kmol/m^3	kmol/m^3	kmol/kmol	kmol/kmol	kmol/kmol	–	–	–	s
a	84.5	2.40	1.28	1.43	0.53	0.43	0.37	0.87	0.80	0.70	863
b	–	2.39	1.29	1.33	0.54	0.42	0.40	0.94	0.79	0.74	870

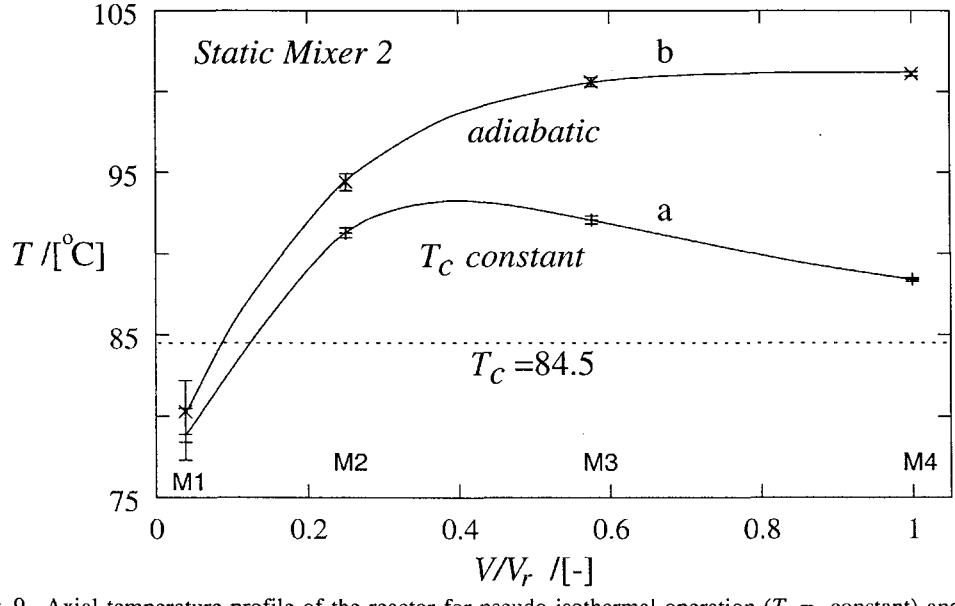


Fig. 9. Axial temperature profile of the reactor for pseudo-isothermal operation (T_c = constant) and for adiabatical operation (Static Mixer 2). The errorbars give the standard deviations of T due to instabilities in time.

In experiment (a) the reactor was thermostatted with water of 84.5 ± 0.1°C. Experiment (b) was carried out adiabatically with approximately the same initial temperature as experiment (a). Figure 9 shows the axial temperature profiles of both experiments. In experiment (a) the temperature difference of the starch paste and the thermostatting water became maximal 8°C between measurement sections M2 and M3. The difference decreases further down in the reactor but is still 3.5°C at the outlet. In experiment (b) the temperature rises continuously until a temperature difference of 21°C is reached between M1 and M4. The slope of the temperature increase at M4 is very small which indicates a high conversion ($\zeta^{e}_{\text{lim}} = 0.94$).

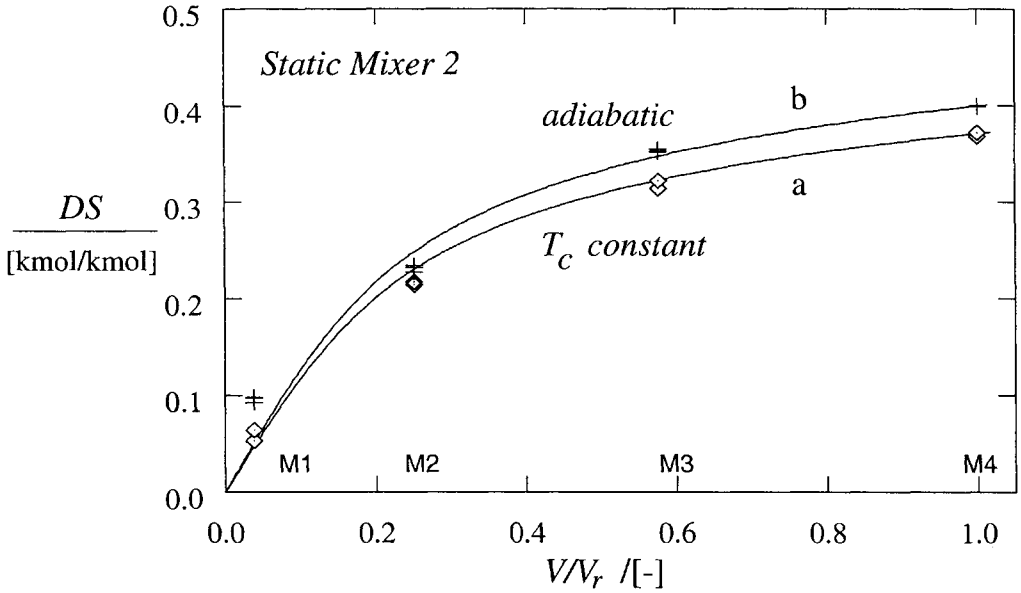


Fig. 10. The *DS* of CMS as function of the axial position in the static mixer reactor (Static Mixer 2). HPLC analysis of the *DS* in duplo.

Figure 10 shows the *DS* as function of the axial position in the reactor for both experiments. In the adiabatic case (exp. b) a higher *DS* is reached due to the higher temperature and corresponding reaction rate. Both experiments gave a white coloured product CMS.

The conversion for experiment (a) is 0.87 after 863 s while it was only 0.78 after 1075 s for experiment (b) in Static Mixer 1 which had approximately the same conditions (see Table 2). Due to the larger scale of Static Mixer 2 the removal of the heat of reaction becomes more difficult. This results in a larger temperature difference with the

thermostatting water. So the starch paste reaches a higher temperature which gives a higher reaction rate.

The selectivities of approximately 0.8 are somewhat higher than in the experiments with the small scale static mixer due to the higher starch concentration applied.

Experiment (a) showed that carboxymethylation in a static mixer with the scale of Static Mixer 2 and the short residence time of 863 s gave a notable temperature increase of 8 °C with respect to the thermostatting water. Experiment (b) proved that also in an adiabatic reactor a good selectivity can be achieved at a high conversion. This is important for the scale-up to commercial size equipment. The adiabatically operated reactor is easier to scale-up than the thermostatted reactor.

Substitution pattern

Figure 11 shows the molar fractions of glucose, mono-CMG and di-CMG as a function of *DS*. The dotted lines are calculated using an equal reactivity for the three hydroxyl groups in the AGU; this means $k_a = k_b = k_c$ in terms of the 'rates of reaction' model of Spurlin [11]. Apparently, the glucose, mono-CMG and di-CMG yields deviate substantially from these curves.

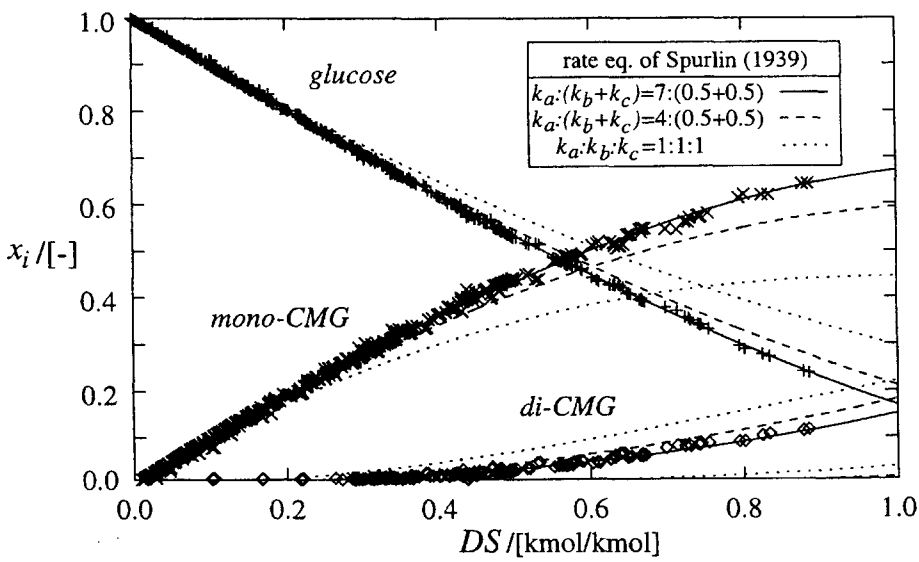


Fig. 11. Mole fractions of glucose, mono-CMG and di-CMG of monomer of carboxymethylated starch in aqueous solution as a function of *DS*, both in batch and in continuous static mixers. Reaction conditions: $30 < T/[°C] < 95$; $0.3 < c_{AGU}/[kmol/m^3] < 2.0$; $0.37 < c_{NaOH_0}/[kmol/m^3] < 2.0$; $0.37 < c_{A_0}/[kmol/m^3] < 1.9$; reaction time $t < 4.3 \times 10^5$ s.

Because the maximally obtained *DS* is in the order of one, k_b and k_c could not be determined separately. For the ratio of the reaction rate constants $k_a : (k_b + k_c)$ equal to 7 : 1 the model curves are in agreement with the experimental values. This means that one of the hydroxyl groups is 7 times more reactive than the sum of the other two. This also implies that under the experimental conditions applied, both in the batch reactor and in the two static mixers, there is no influence of T, c_{AGU}, c_{NaOH_0}, c_{A_0} and reaction time t on the measured substitution patterns. This is a strong indication that the hydroxyl groups of the starch are equally available in both the static mixers and in the batch process.

Temperature homogeneity in a static mixer

In the large static mixer reactor the temperature profile was measured over half of the cross section after six SMX static mixing elements. A temperature difference between the incoming starch paste and the wall of 15°C was applied. Without static mixing elements the temperature profile would have a parabolic shape. Due to the radial mixing by the static mixing elements the temperature profile appears to be flattened (see Fig. 12).

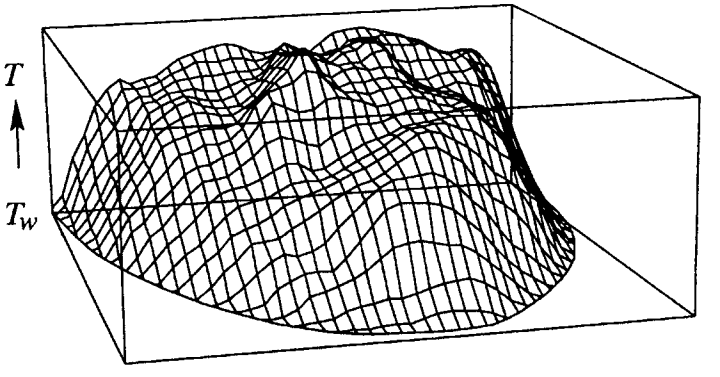


Fig. 12.Measured temperature profile of half the cross section after six SMX elements (Static Mixer 2).

Non-aqueous batch process

For the carboxymethylation of starch in the non-aqueous slurry process first the best media was to be selected, then the optimal amount of water in the system was determined. To obtain highly substituted CMS at a good selectivity, the theoretical *DS*, DS_t, was varied. The optimal reaction temperature, which is a sensitive parameter because of gelatinisation, was selected with experimental design and logistic regression

[18]. Finally, the obtained substitution patterns were compared with models known from the literature.

Best media

The results reported in the literature [19–28] suggest that *i*-propanol is the best organic liquid, but the *DS* and the selectivity, σ_P, values reported are rather low (*DS* < 0.5 and $\sigma_P < 0.3$).

The organic liquids we tested for the carboxymethylation of potato starch were methanol, ethanol, *n*-propanol, *i*-propanol, *n*-butanol, *s*-butanol, *t*-butanol and acetone, all with 10 wt% water. Figure 13 shows the experimental yield, η_P, as a function of time for the different organic liquids. Under the conditions studied, see Table 4, *i*-propanol gave the highest yield and in this case also the highest *DS*. Because the same theoretical *DS* was used for all liquids, DS_t was equal to 1.1.

Table 4

Standard reaction conditions for the carboxymethylation of starch in the non-aqueous batch process

amount of organic liquid	m_l	kg	1.0
water mass fraction	w_{H_2O}	kg/kg	0.1
temperature	T	°C	40
starch mass fraction	w_{AGU}	kg/kg	0.04
NaOH / SMCA molar ratio	–	kmol/kmol	1.0
theoretical DS	DS_t	kmol/kmol	1.1

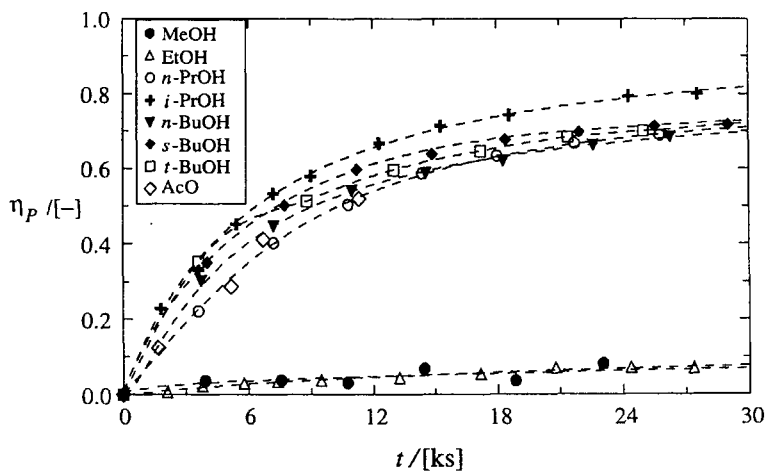


Fig. 13. The yield, η_P, of carboxymethyl potato starch in various organic liquids as a function of reaction time, *t*. Reaction conditions: T = 40 °C, 4 wt% starch, 10 wt% water, n_{NaOH}/n_{SMCA} = 1.0 , and DS_t = 1.1.

After about 30×10^3 s, the yield is equal to 0.80 in *i*-propanol. Acetone, *n*-propanol and the three isomers of butanol gave a slightly lower value of $\eta_P = 0.70$ whereas methanol and ethanol gave $\eta_P = 0.07$, all after a reaction time of 30×10^3 s. A reasonable reproducibility was obtained, triplo results with the same reaction medium differed by 5%, at the most.

It can be concluded that our results confirm the superior behaviour of *i*-propanol known from the literature. However, the *DS* and the yield obtained in this study are much higher than reported in the literature.

Effect of water fraction

The effect of the water fraction on the carboxymethylation was studied in more detail for *i*-propanol. The moisture content of the starch granules was included in the total water fraction in the system. Figure 14 shows the *DS* as a function of time for four *i*-propanol-water mixtures. Because the same DS_t was used in all cases, the *DS* is directly comparable to the yield. The optimal water fraction for *i*-propanol is about 0.1. After 30×10^3 s, the *DS* is equal to 0.91 and the yield is equal to 0.80. After more than 24 hours of reaction the *DS* is 1.0 and the yield, η_P, is 0.88. For the production of granular carboxymethyl potato starch *i*-propanol with 10 wt% is the best reaction medium.

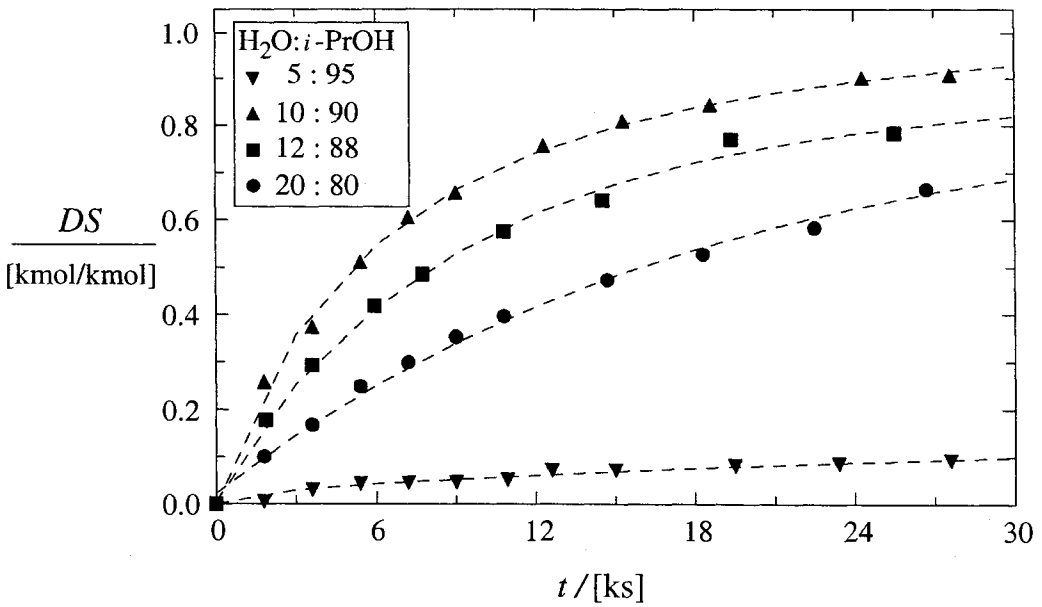


Fig. 14. Effect of the water fraction on the carboxymethylation of potato starch in *i*-propanol; *DS* as function of the reaction time *t*. The reaction conditions are given in Table 4.

Theoretical degree of substitution

From Eqs (1)–(3) it is clear that the DS_t (which is either n_A/n_{AGU} or n_{NaOH}/n_{AGU}, whichever is lower) will influence the final *DS*. Figure 15 shows the final *DS* and the yield as a function of the theoretical degree of substitution. The *DS* increases monotonously with an increase in the DS_t, until a constant level is reached of 1.3. The yield, η_P, decreases monotonously over the whole range.

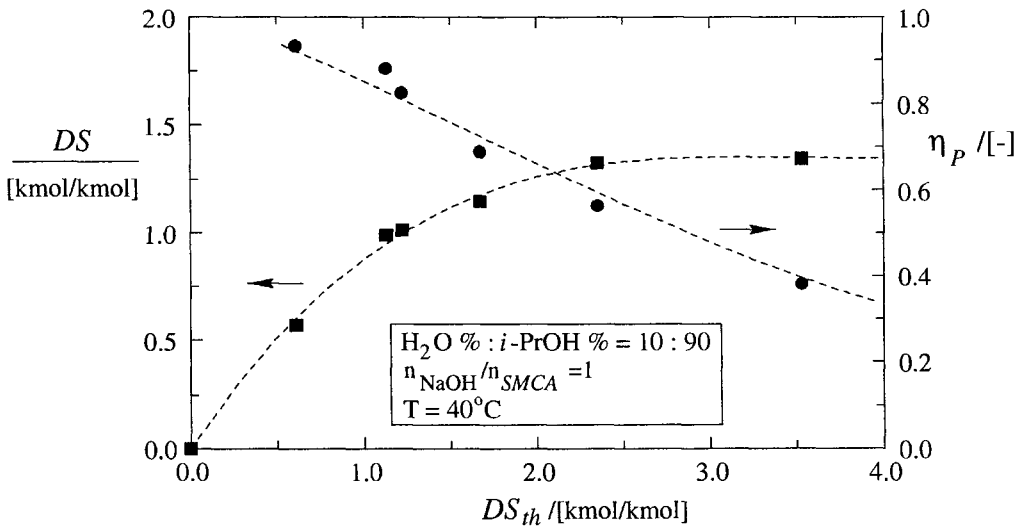


Fig. 15.Final *DS* and yield, η_P, as a function of theoretical DS, DS_t, for *i*-propanol.

To investigate the production of granular carboxymethyl potato starch with a *DS* above 1.3, a novel method was developed. At 40°C granular CMS could be produced with a *DS* as high as 2.2 with a yield of 0.56.

Granular structure

The experimental conditions are restricted because gelatinisation must be avoided. An experimental design study was undertaken to determine the boundaries of the operation conditions. For this study 25 ml vessels were used. Swelling of the starch was checked visually. The study showed that in *i*-propanol the temperature, the water fraction, w_{H2O}, and the fraction of starch, w_{AGU}, were the most sensitive parameters for gelatinisation. In Fig. 16 the operation window is shown where the CMS remains in the granular form. It can be concluded that the operating flexibility with respect to the temperature and the water fraction increases with an increase in the starch fraction.

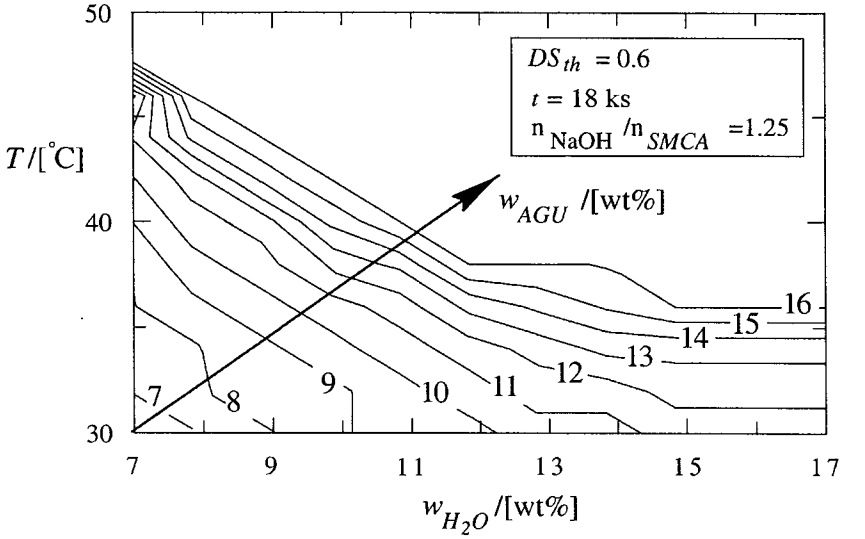


Fig. 16.Contour plot of the starch structure as function of T, w_{H2O}, and w_{AGU}.

Selectivity

The selectivity of SMCA for the main reaction as function of the temperature, the n_{NaOH}/n_{SMCA} ratio, and the DS_t was studied in 25 ml reaction vessels. Figure 17 shows that the selectivity improves with increasing reaction time, leading to $0.7 < \sigma_P < 0.9$ after 6×10^3 s. An important factor for the selectivity is the NaOH concentration. The selectivity decreases with increasing NaOH concentration.

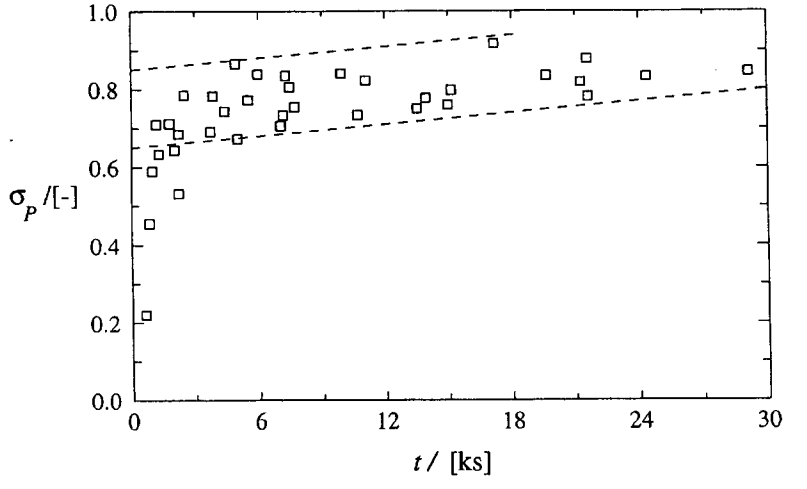


Fig. 17 Selectivity of SMCA towards the product CMS, σ_P , as a function of time. Reaction conditions: *i*-propanol, 10 wt% water, $30 \le T/[°C] \le 50$, 4 wt% starch, $0.5 \le n_{NaOH}/n_{SMCA} \le 1.2$, and $0.6 \le DS_t \le 1.2$.

Substitution pattern

Figure 18 shows the experimental monomer product distribution obtained in the non-aqueous batch process. A comparison is made with distributions calculated from rate equations presented in the literature [11]. The line for $k_a : (k_b + k_c) = 4 : 1$ is in good agreement with the experimental results. This means that one of the hydroxyl groups is 4 times more reactive than the sum of the other two. Because the maximal *DS*, in Fig. 18, is in the order of unity, k_b and k_c could not be determined separately.

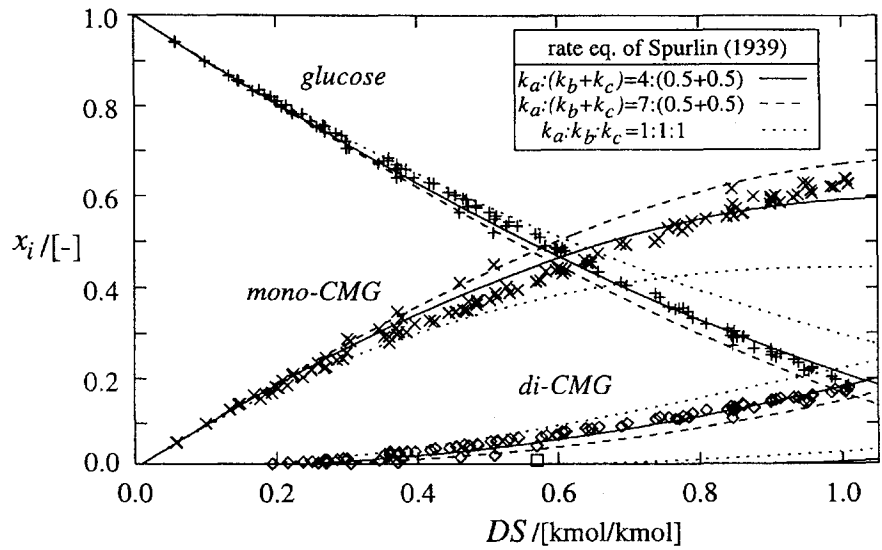


Fig. 18.Mole fractions of glucose, mono-CMG, di-CMG, and tri-CMG of monomer of carboxymethyl starch as function of *DS* for the non-aqueous batch process.

Comparison of both processes

Here, a qualitative comparison is made between the static mixer paste process and the granular non-aqueous batch process for the carboxymethylation of starch. The characteristics of the two processes are compared in Table 5.

Table 5

Comparison between the carboxymethylation of a starch paste in a continuous static mixer reactor and of starch granules in a non-aqueous batch process

	static mixer	non-aqueous batch process
operating mode	continuous	batch
starch form	gelatinised	granular
$t_{\zeta_A=0.5}$ [s] (T = 40°C)	17.3×10^3	4.5×10^3
typical reaction temperature, T_{typ} [°C]	85–90	40
typical reaction time, t [s], at T_{typ}	900	18×10^3
maximal DS [–]	0.9	1.3
maximal selectivity, σ_P [–]	0.80	0.87
$k_a : (k_b + k_c)$ [–]	7 : 1	4 : 1
economics	higher investments	higher variable costs
capacity per m^3 reactor	high	lower
maximal mass fraction starch	0.32	0.10
environmental	water	*i*-propanol

Reaction time

The reaction time of both processes is compared by means of the half-time conversion of SMCA, $t_{\zeta_A=0.5}$. From Fig. 13 $t_{\zeta_A=0.5}$ for the carboxymethylation at 40°C in *i*-propanol is estimated as 4.5×10^3 s. $t_{\zeta_A=0.5}$ in the starch paste process at 40°C is calculated as $t_{\zeta_A=0.5} = 17.3\times10^3$ s (based on reaction times of exp. h and i, see Table 1). The latter value is almost four times higher than for the non-aqueous batch process.

However, raising the temperature to decrease the reaction time is limited in the non-aqueous batch process where gelatinisation has to be avoided. In the starch paste process a higher temperature is even desired to lower the viscosity. But a drawback of a higher temperature is the lower selectivity (see Table 1 and Fig. 7). $t_{\zeta_A=0.5}$ at 85°C at the high starch and reactant concentrations ($c_{AGU} \approx 1.9$, $c_{NaOH_0} \approx 1.3$, $c_{A_0} \approx 1.2$ kmol/m^3, see Table 1) becomes 236 s only. Such short reaction times are possible only if the mixing times are even shorter. Here, the static mixer reactor is superior because of the high intensity mixing characteristics.

Reactor volume

The ratio of the reactor volumes for both processes is calculated on basis of the same production rate of CMS with DS = 0.5 with typical reaction times (τ_{SM} = 900 s; $\tau_{NABP} = 18\times10^3$ s), starch mass fractions ($w_{AGU,SM}$ = 0.32; $w_{AGU,NABP}$ = 0.10) and tem-

peratures (T_{SM} = 85°C; T_{NABP} = 40°C) of each process (see Table 5). The volume ratio becomes:

$$\frac{V_{NABP}}{V_{SM}} \propto \frac{\tau \; w_{AGU,SM}}{\tau \; w_{AGU,NABP}} = \frac{18 \cdot 10^3}{900} \frac{0.32}{0.10} = 64 \qquad (9)$$

Selectivity

High selectivity in the aqueous starch paste process requires a high concentration of starch as shown in Fig. 7. In the starch paste batch reactor a selectivity of 0.77 (T = 65°C) and in the static mixers a selectivity of 0.8 is reached. The concentrations of starch and reactants in the static mixer are limited by the pumps and by the fact that SMCA is a salt. So, for the static mixer a selectivity of almost 0.8 is maximal.

The selectivities reached in *i*-propanol with 10 wt% water are, beside some initial low values, between 65% and 87% (see Fig. 17). This is higher than in the aqueous starch paste process under the conditions applied.

Both process give a good selectivity which results in a relatively low cost of raw material.

Degree of substitution

In the starch paste process a *DS* of about 0.56 at ζ_{lim} = 1 with $\sigma_P \approx 0.78$ can be reached, see Table 2. A *DS* of 0.9 is possible, Fig. 11, but with a lower selectivity. A further increase of the *DS* requires additional feeding of SMCA and NaOH to the starch paste. To maintain a good selectivity the dilution of the starch paste by this addition should be kept minimal.

The non-aqueous batch process has a larger maximal reachable *DS* than the starch paste process.

Substitution pattern

The experimentally obtained mole fractions of glucose, mono-CMG, and di-CMG in both processes were compared as a function of the *DS* with the 'rate of reaction' model of Spurlin [11], see Fig. 11 and 18. The mole fractions of both processes deviate from the model with equal reactivity for all three hydroxylgroups, *i.e.* $k_a = k_b = k_c$. For the starch paste process the ratio $k_a : (k_b + k_c) = 7 : 1$ agreed with experiment while for the non-aqueous batch process this was $k_a : (k_b + k_c) = 4 : 1$. This means that in the starch paste process the most reactive hydroxylgroup appears to be relatively more reactive than in the non-aqueous batch process.

Economical aspects

Besides the technical differences between both processes discussed above, there are substantial economical differences too. The starch paste process in a static mixer

reactor requires relatively higher investments though these will hardly affect process economics. Evaporation of the water in the starch paste with a drum dryer brings substantial variable costs. A benefit is the lower personnel costs because the process is continuous. Further, such a continuous process can be better controlled leading to lower variations in product specifications. The non-aqueous batch process has the advantage of the optional removal of by-products from the CMS, which is hardly possible in the paste process. Also, the batch process has lower drying costs than the paste process. However, the costs of purification of the non-aqueous liquid are substantial. To minimise these costs the non-aqueous liquid must be circulated as much as possible.

Summary

Each process has its own characteristics (see Table 5). The static mixer process is a continuous process and is therefore appropriate for high production rates with a constant product quality. Although the *DS* in one reaction step with this process is limited. The reaction time can be relatively short at relatively high reaction temperatures due to the short mixing times in the Static Mixer. With the non-aqueous process it is possible to produce granular CMS with a *DS* of 1.3. Purification of the granules is possible. An advantage of the non-aqueous batch process is that the starch granules can be easily separated from the reaction medium. For a particular application a quantitative economic analysis will be required. The result will depend on production capacity, the *DS* required and the need to purify the product.

Conclusions

For the production of carboxymethyl starch, a continuous static mixer process and a batch-wise process with an optimal organic reaction medium preventing starch gelatinisation were compared. Both processes have their own characteristics and the optimal choice of a production method of CMS will depend particularly on the plant capacity desired, the *DS* required and on whether product purification is necessary.

The main technical differences are the way of operation and the form of the CMS product. With the static mixer process a paste is produced and in the non-aqueous batch process the granular form of the starch is preserved. With the static mixer process it is possible to have a higher production rate than with the non-aqueous batch process. For the non-aqueous batch process the low production rate is caused by the limitations in the temperature and the fraction of starch in the reactor. In the paste process, purification of CMS is difficult. In the non-aqueous batch process the separation of the granular CMS from the reaction liquid is easy and purification of the granular product is possible. The maximal selectivity, σ_P, and *DS* are higher in the non-aqueous batch process than in the paste process.

Both processes have their own substitution pattern. In the starch paste process the mole fraction of mono substituted CMG is higher than in the non-aqueous batch process for an equal *DS*.

Acknowledgements Financial support of AVEBE (Veendam, The Netherlands) and The Netherlands Foundation for Chemical Research (SON), the support in equipment of Sulzer Chemtech and the stimulating discussions with AVEBE is kindly acknowledged. The authors are grateful to the following undergraduate students: Jaap-Jan Wever, Onno J. Mulder, Jeroen Zanting and Richard Smeding for their work on the starch paste process, and to Henk Kolk and Han Scherpenkate for their work on the non-aqueous batch process. Aho Ilgun is acknowledged for the analytical work and the AOD and Luuk Balt are acknowledged for the technical support.

Notation

c_i Concentration of species i, kmol/m^3.

D_t Internal diameter of the static mixer tube, m.

DS Degree of substitution, ratio of moles substituent to moles AGU, kmol/kmol.

DS_t Theoretical DS at 100% selectivity of the limiting reactant towards the product CMS, kmol/kmol.

$DS\left(\zeta^e_{\text{lim}} = 1\right)$ Final degree of substitution with total conversion of limiting reactant: $DS\left(\zeta^e_{\text{lim}} = 1\right) = DS/\zeta^e_{\text{lim}}\,|_{\text{Mx}}$, with Mx the last measurement section (M5 for SM1 and M4 for SM2).

F_i Response factor of mono- ($i = 1$), di- ($i = 2$) and tri-CMG ($i = 3$) with respect to glucose ($i = 0$).

k_i Reaction rate constant, with $i = a$, b and c for the three hydroxylgroups of AGU.

L Length of the reactor, m.

MARR Mean absolute relative residual, MARR = $\frac{1}{N}\sum_{i=1}^{N}\left|\left(y_i^e - y_i^m\right)/y_i^e\right|$, for all non-zero y_i^e.

M_i Molecular weight of species i, kg/kmol.

M Molarity, kmol/m^3.

m Mass, kg.

n_r Number of repetitions.

n_i Number of moles of species i, kmol.

ROH Hydroxyl group of an AGU.

RO$^-$ Dissociated hydroxyl group of an AGU.

rps Revolutions per second, s^{-1}.

T Temperature, °C.

T_c Temperature of thermostatting water, °C.

t Time, s or h.

V Volume in reactor from entrance up to a certain axial position, m^3.

V_r Volume of the reactor, m^3.

w_i Weigth fraction of species i, $w_i = m_i / m_{tot}$; except for non-aqueous batch process: $w_{H_2O} = mH_2O / (m_{H_2O} + m_{org})$; $w_{AGU} = m_{AGU} / (m_{AGU} + m_{H_2O} + m_{org})$.

x_i Molar fraction of species i.

y_i^e, y_i^m Quantity obtained by experiment or by model, respectively (e.g. c_{Cl^-}, DS) used in MARR.

Greek

η_P Yield of product CMS based on limiting reactant.
ζ_i Conversion of species *i*.
σ_P Selectivity of SMCA towards CMS.
τ Mean residence time, s.

Subscript

A SMCA.
BR Batch reactor.
exp, mod Experimental and modelled, respectively.
i, j Species *i, j*.
l liquid.
lim Limiting reactant, SMCA or NaOH.
NABP Non-aqueous batch process.
org Organic liquid.
P Product (CMS) .
SM Static Mixer process.
st Stirrer.
tot Total.
typ Typical.
0 Initial value ($t = 0$).

Superscript

e, m Experimental and modelled, respectively.

Abbreviations

AGU Anhydroglucose unit.
CMG Carboxymethyl glucose.
CMS Carboxymethyl starch.
M1 – M5 Measurement sections of the static mixer reactor.
PIR Pressure indication and registration.
RI Refraction Index.
SM Static Mixer.
SMCA Sodium monochloroacetate ($ClCH_2COONa$).
TIR Temperature indication and registration.

REFERENCES

[1] Chowdhury J.K.: Biochem. Z., **148**, 1924, 76–97.
[2] Starch: Chemistry and Technology, vol. II (Edited by Whistler, R.L. and Paschall, E.F.), Academic Press, New York, 1967.
[3] Modified Starches: Properties and Uses (Edited by Wurzburg, O.B.), CRC Press, Boca Raton, Florida, 1986.
[4] Lammers G., Stamhuis E.J., Beenackers A.A.C.M.: Starch/Stärke, **45**, 1993, 227–232.
[5] Streiff F.A.: Proc. Third European Conference on Mixing, (Paper C2), 1979, 171–188.
[6] Gavrilescu M., Tudoze R.Z.: Acta Biotechnol., **15**, 1995, 3–26.
[7] Visser J.E.: Continuous Chemical Modification of Starch in a Static Mixer. Ph.D. thesis, University of Groningen, The Netherlands. To be published.

[8] Roberts J.: [in] Starch: Chemistry and Technology, vol. II (Edited by Whistler, R.L. and Paschall, E.F.), Academic Press, New York, 1967, 293–350.
[9] Lammers, G., Stamhuis, E.J. and Beenackers, A.A.C.M., Ind. Eng. Chem. Res., **32**, 1993, 835–842.
[10] Dawson H.M., Pyckock, E.R.: J. Chem. Soc., 1934, 778–783.
[11] Spurlin H.M.: J. Am. Chem. Soc., **61**, 1939, 2222–2227.
[12] Beenackers A.A.C.M., Lammers G., Kuipers N.J.M.: Zywnosc Technologia Jakosc, Krakow, **2**, 1996, 118–137.
[13] Heinze T., Erler U., Nehls I., Dieter: Die Angewandte Makromolekulare Chemie, **215**, 1994, 107–119.
[14] Verraest D.L., Peters J.A., Batelaan J.G., Van Bekkum H.: Carbohydr. Res., **271**, 1995, 101–112.
[15] Respons factors of mono-, di- and tri-CMG. Private communication with R&D section of Avebe, 1996.
[16] Skoog D.A.,West D.M.: Fundamentals of Analytical Chemistry, 2nd edition. Holt, Rinehart and Winston, London, 1971.
[17] Sergeev E.V., Rad'ko S.I.: Kinetika i Kataliz, **12**, 1971, 555–559.
[18] Kleinbaum D.G.: Logistic Regression: A Self-Learning Text. Springer-Verlag, New York, 1994.
[19] Schrodt A., German Patent 717, 275, 1942.
[20] Sloan J.W., Mehltretter C.L., Senti F.R.: J. Chem. Eng. Data, **7**, 1962, 156.
[21] Thewlis B.H.: Starch/Stärke, **21**, 1969, 21–24.
[22] Čeh M.: Starch/Stärke, **26**, 1972, 124–127
[23] Mofti A., Hassan E., Salama J., El-Sabbah M.: Starch/Stärke, **34**, 1982, 406–410.
[24] Hebeish A., Khalil M.I.: Starch/Stärke, **40**, 1988, 147–150.
[25] Suzuki H., Tadokoro Y., Taketomi N.: Denpun Kogyo Gakkaishi, **9**, 1961, 33–42.
[26] Khalil M.I., Hashem A., Hebeish, A.: Starch/Stärke, **42**, 1990, 60–63.
[27] Jingwu Z., Dongli L., Yuguan S., Xiaohong Z., Dahua, W.: J. of Tianjin University, **1**, 1993, 124–131.
[28] Bhattacharyya D., Singhal R.S., Kulkarni, P.R.: Carbohydr. Polym., **27**, 1995, 167–169.

NOWE PROCESY DO KARBOKSYMETYLOWANIA SKROBI

Streszczenie

Przedstawiono dwa nowe procesy służące do karboksymetylowania skrobi. Porównano je pod względem dającego się uzyskać stopnia podstawienia, miejsc podstawiania, niezbędnego czasu reakcji i wydajności. Pierwszy proces wymaga skrobi zżelowanej i odbywa się w statycznym, pracującym w ruchu ciągłym reaktorze z mieszaniem, który zoptymalizowano w celu otrzymywania stężonych wodnych past skrobiowych. Drugi proces zachodzi w zawiesinie w rozpuszczalnikach organicznych.

W oparciu o dane doświadczalne z obu procesów można stwierdzić, że każdy z procesów ma swe specyficzne zalety. Proces w reaktorze statycznym z mieszaniem zapewnia dobrą selektywność, jest krótszy bardzo łatwo jest go kontrolować i jest bezpieczny. Korzyścią wynikającą ze stosowania procesu w zawiesinie w rozpuszczalnikach organicznych jest zachowanie gałeczkowej struktury kaboksymetyloskrobi nawet przy wysokim stopniu podstawienia i dobrej selektywności.

W. BERGTHALLER, M.G. LINDHAUER, H. ZWINGELBERG, M. EHRING

LABORATORY SCALE EVALUATION OF STARCH EXTRACTABILITY OF WHEAT VARIETIES*

Abstract

Since several years the structure of the European starch industry undergoes exceptional changes. Potato starch production stagnates as a result of agro-political decisions of the EU commission resulting in strict quota regulations. With corn starch growth rates are rather marginal. In case of wheat starch production huge capacities have been installed just recently or are in state of construction. As a result, one of the main targets is to improve the competitive situation of wheat starch and to provide appropriate source material. Concerning isolation techniques, recent developments in process technology allowed at the same time a withdrawal from wheat and wheat flour quality standards based predominantly on protein and mineral content. These standards were valid in the minimum for bread wheat. They could maintain their strong position over a relative long period as long as the Martin process was the prevailing technology.

However, modern processes based on centrifugal separation principles and pre-treated wheat flour and water mixtures opened a turn towards an economically more beneficial wheat and wheat flour quality. In fact, it became well known meanwhile that industry is able to process wheat flours having a significantly different property profile. If wheat quality characteristics of the German system of wheat classes are applied, these lots will probably be ascribed to feed wheat.

The described situation that could not be ignored any more initiated investigations on wheat quality characteristics relevant for modern technology in industrial wheat starch extraction. Basis for first studies was the well-known extraction procedure using the Glutomatic 2000 system for recovering starch A and B fractions and gluten from a conventional flour dough. Besides also the so-called „mixer test", a rather time and labour consuming testing system has been applied. Coming from industry its background is practical and achieved experimental results can be applied well in plant construction. At the same time a lately tested rapid method adapted to conditions of modern wheat starch production is used for evaluation of the formation of a workable wheat flour/water mixture. With regard to changes induced in the initial phase of the batter formation by mixing flour and water this method has been indicated as „gluten agglomeration test". Two groups of test samples have been used in this investigation. The first set of wheat samples comprised breeding material produced in a normal agricultural regime while the second set was part of a N_2-fertilisation and phyto-sanitation trial. The trial resulted in wheat samples and flours with well-defined protein concentrations that varied according to fertilisation level. Relationships between results of general

Institute for Cereal, Potato and Starch Technology, Federal Centre for Cereal, Potato and Lipid Research, Schützenberg 12, D-32756 Detmold, Germany

**Publ. No.: 6970 of the Federal Centre for Cereal, Potato and Lipid Research, Detmold and Münster*

bread wheat quality assessment and the described methods have been tested by rank correlation analysis. The lately established gluten agglomeration test allowed the desired differentiation and provided information about rate and extent of gluten agglomeration that differed extremely depending on cultivar and fertilisation regime. Characteristics of the gluten agglomeration test did not show very significant and close connections with grain and flour criteria. However, they all were related to protein quality. The results achieved in the mixer-test allowed evaluation and differentiation for the whole set of samples. It confirmed that only samples with adequate high protein level can be processed satisfactorily.

Introduction

Economic situation of wheat starch production

In Germany and, in particular, Western Europe the role of wheat as substrate in relation to maize and potato is becoming more important. Extraordinary large production capacities for wheat starch are in construction, above all in France. While a somewhat older statistic [1] shows a total starch production of $7.0 \cdot 10^6$ t for the European Union consisting of 15 member states and a 25% portion for wheat starch in 1996, a just recently published study [2] is giving a total starch production of approx. $7.3 \cdot 10^6$ t for 1997 in Europe. The corresponding share of wheat starch is now said to be $2.55 \cdot 10^6$ t which represents 35% of the total production.

Several factors promoted the development towards an increased use of wheat in starch production. In the European Union wheat prices were almost steadily reduced since 1983/84. At that time costs per ton were 220 ECU as compared to 120 ECU in 1994/95. Looking on net costs of wheat or maize as a raw material for starch the costs relevant for wheat were significantly smaller until 1997 [2]. Besides, many wheat starch plants are situated in or near by main areas of wheat production. However, from an economical point of view maize should be the more attracting substrate. An estimation of net starch production costs evaluated on the basis of figures raised in Germany in the period 1986–1992 indicated 740 DM/t for maize starch versus 840 DM/t for wheat starch and 1020 DM/t for potato starch [3]. Nevertheless, wheat seems to be brought in focus as a substrate that can equalise the position of maize. Besides, potatoes will not be increasingly used as substrates since EU quotations limited potato starch production to in total approx. $0.7 \cdot 10^6$ t (including an extra quota for investments made in new federal provinces shortly after reunion).

Recent developments in starch extraction procedures

The principles of modern starch production from wheat have been described just recently by Meuser [4]. Wheat flour remained the unique material, although numerous attempts have been made to replace the ground product by whole grains [5]. The still ongoing economic interest in wheat gluten as an important side-stream product was at most responsible for retaining in wheat flour as starting material. The wet extraction of

a concentrated flour-water system (1 part flour to 0.6 part water) similar in consistency to a bakers bread dough dominated industrial processing until centrifugal separation could be introduced as the more promising principle in separating starch and gluten and at the same time in reducing water consumption to less than 5:1. In contrast to the new hydrocyclone (4-5:1) or decanter (4:1) based processes the Martin process consumed still the 15 fold amount of process water and the batter process the 5-7 fold based on flour weight [6]. This development was a decisive milestone in modernising wheat wet milling processes irrespective of the applied centrifugal technique [4, 6-8]. The well-known density differences between A starch, as the main fraction, and gluten plus B starch, fibres and pentosans allowed an arrangement of these fractions in more or less separated layers in the gravity field [6, 9]. In the following, hydrocyclones or decanters were the central units in separating diluted flour/water mixtures after pre-treatment of their concentrates. The pre-treatment was at most prerequisite for splitting off hydrated wheat protein bodies from starch granules and the subsequent gluten formation. Both principles dominate technology of wheat starch production since that time (10).

Adaptation of wheat varieties to starch production

As result of the described changes in principal process techniques the source material should have been adapted to new processing conditions. In particular, the former requirements in protein quality relied on quality characteristics relevant for baking flour and the preparation of a stiff dough and finally the production of a more or less rubbery, chewing gum like gluten. With new process technologies Barr [10] still mentioned the necessity of wheat having a higher protein content and better processing characteristic without being specific in detail. Details in specification of wheat and wheat flour suitable to wheat starch production have been presented by Witt [7] for the first time and in more recent time by Lindhauer and Zwingelberg [11]. They described wheat quality at much lower protein levels of 12 to 12.5% d.b. (nitrogen conversion factor 5.7) giving a flour protein content of approx. 11 to 11.5% d.b.. Using the nitrogen conversion factor accepted in wheat starch industry (6.25) flours should reach a protein level of 12 to 12.5% d.b. Besides, starch and protein content are inversely correlated, i.e. reduced grain protein concentrations induced by low input-oriented N_2 fertilisation produce an increase in starch content. Further wheat characteristics promising higher starch yields were given as low endosperm hardness and small shares of mechanically damaged starch granules. At last these authors concluded that from an agronomical point of view wheat cultivars should have a soft to medium endosperm structure, low protein and pentosan concentrations and good starch quality (high falling number and amylograph data). Concerning milling wheat cultivars should show good millability that means production of a high flour yield having a mineral content 0.6%

d.b. (in Germany: type 550). A flour standard quality produced from low-protein wheat of soft structure could be enriched by adding high-protein wheat if a protein rich flour is required by the starch factory [11, 12].

New developments in laboratory-scale procedures

Development in testing procedures both of laboratory and small technical scale have been published just recently in summarised form [13]. The ultimate state in laboratory-scale methods was a mixer method used and provided by the relevant machinery industry [14]. There, the fractionation of wheat flour into its components is based on a batter formation by high speed mixing treatments of a concentrated flour/water mixture (ratio: approx. 1:0,9). For stimulation of gluten agglomeration and separation water is added until a ratio of 1:1,8 is reached. Wet gluten is recovered after washing out starch, pentosans, and fibres in a manual procedure. After removing fibres by wet sieving the resulting starch slurry is concentrated by centrifugation and separated carefully by hand into fractions of A and B starch [13].

Material and methods

Grain samples

Two different sets of winter wheat grain samples have been used in this study. The first set comprised 6 samples of winter wheat grain (cv. Kanzler, Ritmo, Contur, Crousty, and Soissons) produced conventionally in northern Germany by breeders. Their history remained in detail unknown. The total weight of each sample was 6 kg.

The second set provided by Prof. Hellriegel Institute e.V. Bernburg, Bernburg, Germany, consisted of 8 grain samples each having a weight of 6 kg. They were produced in a fertilisation trial using 1 German and 1 EU variety (Contra and Soissons) and 1 breed (LP 235194). In order to induce a differing protein composition besides the control variant (no nitrogen fertilisation), the plants have got nitrogen at two and three stages of development, resp. The 50+50 kg/ha variant received its doses at tillering and bolting and the 70+70+50 kg/ha variant at tillering, bolting, and breading. Besides, both fertilised variants have been treated with fungicide.

Prior to analytical characterisation and grinding the samples have been cleaned by aspiration. Then 500 g subsamples have been taken for grain characterisation by dividing. The residual samples have been moistened and stored over night for equilibration. These samples were finally milled in a Bühler mill, type MLU-202. Flour streams passing 180 µm flat sieves and 250 µm rotation cylindrical sieves were combined and taken for analytical characterisation.

Grain characterisation

Grain samples were ground in a Falling number mill and the resulting breaks were stored until analysis in tight polyethylene boxes. The crude protein content was determined by using ICC Standard Method 105/1 (factor 5.7). The sedimentation value was determined by ICC Standard Method 116/118. For evaluation of the gluten content ICC Standard Method 137 was used [15].

Flour characterisation

Flour moisture content as well as protein (factor 5.7) were determined by using ICC standard techniques (ICC Standard Method 110 and 105/1)). For determination of the sedimentation value the method described in ICC Standard Method 116 has been applied [15]. For determination of the starch content the DIN EN ISO 10520 procedure [16] was used. The relevant Farinogram and Extensogram characteristics were derived from respective diagrams as described in standard methods (ICC Standard Method 115 and 116, resp.) [15]. The nitrogen conversion factor for protein evaluation in gluten was 6.25.

Agglomeration test

Referring to a procedure described by Hanneforth, Zwingelberg, and Gebhard [17] for rapid evaluation of protein quality with regard to agglomeration in batters for wafer production a concentrated flour/water suspension is produced in a standard mixer blender using conditions of batter formation according to starch separation processes. After formation of a homogenous suspension the energy consumption during mixing is registered by an ammeter and evaluated by means of specific software.

In detail 120 grams of wheat flour, type 550, are mixed in a blender (type Rotor-Blender GT 1000, neoLab, Heidelberg, Germany) with 120 grams of water heated to +35°C. Two treatments each lasting 20 s are done for batter formation using level 3.5 of the mixer scale. Then, current consumption is measured during the following mixing period of 240 s using level 4.5. For registration a analog/digital multimeter (type Metrahit 15 S, Gossen-Metrawatt GmbH, Nürnberg, Germany) connected with a memory interface (type Metrahit SI 232, Gossen-Metrawatt GmbH, Nürnberg, Germany) allowing direct transmission of data to a PC is used. Data transformation into diagrams is done under Metrawin 10 as software (Gossen-Metrawatt GmbH, Nürnberg, Germany). Available data concerning time until current consumption onset (agglomeration time) and maximum current consumption are derived from diagrams.

Mixer method

The wet separation of flour components yielding fractions of A starch, B starch, wet gluten, fibres, and solubles within process water is investigated by applying the mixer method [13]. In order to allow determination and comparison of dry substance and protein dispersed in water used for the gluten and fibres washing process water volume is limited each to in total 6500 ml.

In detail, 87 grams of tap water heated up to +35°C are mixed with 100 grams of wheat flour substance in a suitable blender (type MX 32, Braun AG, Kronberg, Germany) for 2 x 20 s using level 3 of the mixer scale. Material thrown to the mixer bowl wall is combined with the main part prior to a second treatment. Then, further 100 grams of tap water (+35°C) are added and the blender is switched on four times 1 s each.

The resulting suspension is then transferred quantitatively to a sieve of 200 µm mesh. Residues that remained in the beaker are removed with a small amount of tap water. In order to wash out starch from gluten water is added drop wise (approx. 4.5 L plus approx. 1.5 L for fibre washing). The suspension passing the sieve is recovered in 5 L beakers. In order to combine agglomerated small gluten particles the mixture remaining on the sieve is treated first carefully with a rubber wiper blade. Later on the formed gluten ball can be washed starch-free manually. At least, surplus water is removed from the gluten ball by manual pressing until gluten tends to stick to the hand. The moist gluten is weighed and finally dried in small portions with a gluten dryer (type Glutork 2020, Perten Instruments AB, Huddinge, Sweden). For separating fibre from starch the suspension is transferred to a vibration sieve (type L 2426, Rhewum GmbH, Remscheid, Germany) having 56 µm mesh. As soon as fibres could be washed starch-free they are poured quantitatively in a petri dish for pre-drying in a drying chamber using 40 to 50°C air temperature. The pre-dried fibres are then dried moisture-free by applying 130°C for 90 min. After cooling to room temperature in a desiccator the amount of fibres and insoluble pentosans is determined by weighing.

The starch layer settled from the suspension and containing A and B starch as well is resuspended and poured into centrifuge beakers in adequate portions and centrifuged for 10 min at rotation speed of 4000 min^{-1} (centrifuge type Varifuge S, Haereus Holding GmbH, Osterode). Specifically more dense A starch settles first and will be covered by less dense protein-rich B starch. The top layer of process water containing soluble protein is decanted and collected in a beaker. Finally all starch sediments are put together, re-suspended and again centrifuged. The resulting sediment is then stored in a refrigerator over night to allow stabilisation. The following day A and B starch layer can be successfully separated by hand with a spatula. In general, both layers can be distinguished clearly by its colours. The weight of total process water (washing liquid) is determined and registered and the solution carefully suspended again. A

quantity of 5 ml is immediately filled in a weighing glass for determination of dry matter content. Its drying is done at 130°C during 90 minutes. Besides, the drying procedure in detail is done as described with fibre material.

Finally, A starch and B starch are dried in an agitated drying chamber at an air temperature of 40°C. For moisture equilibration of dried starch samples remain at room climate for at least 2 h. The recovered samples of gluten and starch are broken to small pieces and then finely ground in a laboratory mill equipped with a ring sieve with 0.5 mm perforation (type ZM 100, Retsch GmbH & Co.KG, Haan, Germany).

Statistical analysis

For the analysis of relationships of investigated characteristics the non-parametric rank correlation coefficient due to Spearman has been used since the basis of samples (n = 6) was rather small and not normally distributed [18].

Results and discussion

Composition of wheat flours

The composition of flour samples (type 550) milled from conventionally produced grain samples of the wheat cultivars Kanzler, Ritmo, Contur, Crousty, and Soissons is given in Table 1. Because of protein levels in grains, generally unexpected low for that production area, the flour protein content comprised only a range of 10.0 to 11.6% in dry basis. According to expectations, the flour starch content followed inversely corresponding flour protein contents. The range was given by 80.6 to 82.4% in dry basis.

Table 1

Composition of 6 fours (type 550) from soft winter wheat - breeder samples

Variety	Quality grade*	Moisture content, %	Protein content, % d.b.	Starch content, % d.b.
Kanzler	B	14.2	11.6	80.6
Ritmo	B	14.5	10.6	81.7
Contur**	-	14.4	10.5	82.4
Soissons***	-	14.4	11.6	80.9
Crousty I**	-	14.4	10.8	81.6
Crousty II**	-	13.8	10.0	81.6

* *German testing system for cultivars*

** *German breeds (soft endosperm structure) (comparable to quality grade A to B)*

*** *French cultivar (comparable to quality grade A to B)*

The flour composition of samples produced in N_2 fertilisation trial on the basis of the wheat cultivars Contra, Soissons and the breed LP 235194 was compiled in Table 2. The protein content represented clearly the used levels of N_2 application. Without N_2 the protein content was extremely small (5.7 to 5.8%). A divided fertilisation of two portions of 50 kg per ha each resulted in a level of 7.6 to 8.0% and a fertilisation regime of two 70 kg portions together with a final 50 kg portion which was described as optimal for the production area produced a range of 9.9 to 10.6% d.b. similar to the conventionally produced set of wheat samples. The flour starch content followed clearly, but in inverse direction the effects of fertilisation producing the highest content without N_2 application (84.2 to 85.2% d.b.). Flours from cultivar Soissons had always the highest starch content in respective fertilisation levels

Table 2

Composition of flours (type 550) from soft winter wheat - samples from fertilisation trials

Sample	Moisture content, %	Protein content, % d.b.	Starch content, % d.b.
Contra			
without N-fert.	14.0	5.7	85.2
50+50	13.0	8.0	83.6
70+70+50	13.3	10.2	81.7
LP 235194			
without N-fert.	13.2	5.8	84.2
50+50	12.9	7.6	82.5
70+70+50	13.2	9.9	80.8
Soissons			
50+50	13.6	7.9	85.0
70+70+50	13.9	10.6	83.8

Results of correlation analysis

Based on results of usual wheat quality evaluation and on the other hand on estimates of the agglomeration test (gluten agglomeration time and maximum current consumption) divers relationships have been tested by means of correlation coefficients. Although a rather low probability level (P = 90%) has been accepted only a few connections, with i.e. sedimentation value, moist gluten weight, and different characteristics derived from Farinogram and Extensogram diagrams, could be found being significant (Table 3).

With respect to the assessment of wheat breaks a relevant correlation existed only between maximum current consumption and moist gluten content, but, this connection was rather wide (100 r^2 approx. 50) and not enough consistent. This situation was

similar for the investigations done with flour, too. With regard to gluten agglomeration time only an inversely oriented connection to the sedimentation value could be established. Looking again on the maximum current consumption there existed direct connections to water absorption capacity and dough stability determined in the Farinograph and to extensibility evaluated in the Extensograph. To dough softening, another Farinograph characteristic, an inverse connection could be determined in agreement with expectations. But, because of the rather small proportions associated to the tested characteristics (100 r^2 almost 50) the achieved results, however, have not been accepted as sufficient at all and have therefore not been used in further considerations.

Table 3

Results of a correlation analysis of gluten agglomeration test and wheat quality assessment characteristics

Source	Gluten Agglomeration Test Characteristic	Wheat Quality Assessment Characteristic	Rank Correlation Coefficient
Flour	Agglomeration time	Sedimentation value	-0.786
Breaks	Maximum current consumption	Moist gluten weight (Glutomatic)	0.7714
Flour	Maximum current consumption	Water absorption capacity (Farinogram)	0.829
Flour	Maximum current consumption	Dough stability (Farinogram)	0.7714
Flour	Maximum current consumption	Dough softening (Farinogram)	-0.7714
Flour	Maximum current consumption	Extensibility (Extensogram)	0.7714

Test statistic (P = 90%; n = 6): 0.771

Agglomeration test

The ability of the agglomeration test to discriminate between flours of the above mentioned cultivars is shown well in diagrams of Figure 1. The flour samples could be separated into three groups. In case of cv. Ritmo a certain degree of agglomeration occurred even during batter formation. Further mixing needed high current consumption instantly. For the flour samples of cv. Kanzler, Contur and Crousty II, with 35 to 65 s agglomeration took place significantly later. With 120–130 s, the flours of the third group (cv. Soissons and Crousty I) required much more time for agglomeration. Maximum current consumption was in between 3 and 4 A for all samples showing no specific trend.

A quite different situation was given with flour samples from N_2 fertilisation trials. In case of adequate N_2 supplementation the 70+70+50 variants allowed obviously the formation of a sufficient amount of high molecular gluten proteins. As a result ag-

glomeration, observed by maximum current consumption, took place in the same manner as previously shown with conventionally produced wheat (Figures 2 to 4).

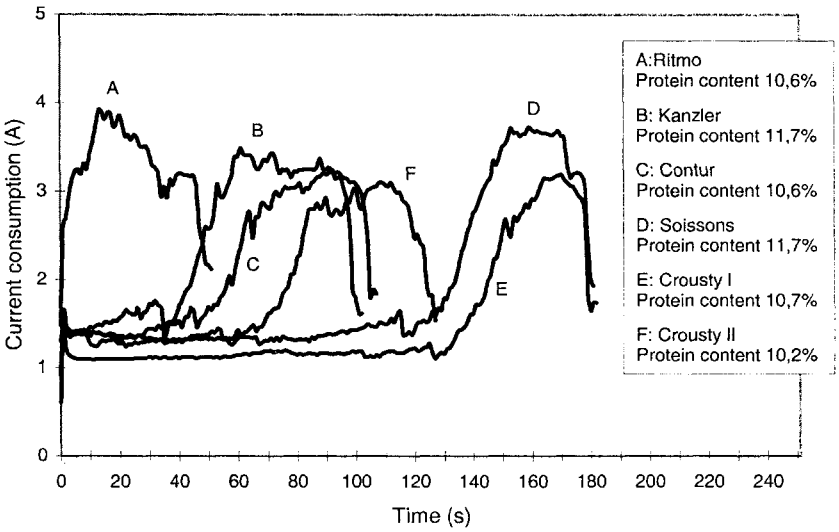


Fig. 1. Gluten agglomeration test applied to conventionally produced wheat varieties - cv. Ritmo, Kanzler, Contur, Soissons and Crousty (I + II)

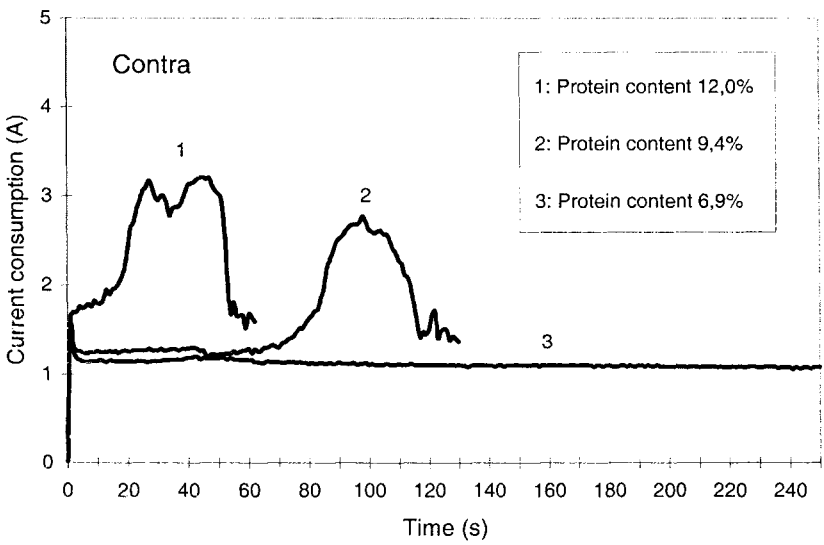


Fig. 2. Gluten agglomeration test applied to wheat samples of cv. contra produced in a N_2 fertilisation test.

Nevertheless, differences due to the tested cultivars were obvious in agglomeration time, in particular. Effects of reduced fertilisation levels became visible, too, in two respects. The 50+50 variant seemed to prevent the formation of a sufficient amount of high molecular gluten proteins which resulted in delayed and retarded gluten agglomeration as measured by agglomeration time and maximum current consumption. This effect was much more pronounced in the variant without fertilisation, where in case of cultivar Contra agglomeration could not be measured, at all (Figure 2).

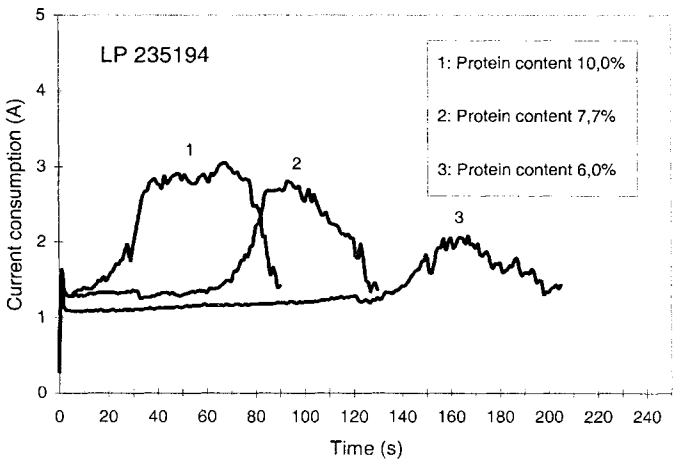


Fig. 3. Gluten agglomeration test applied to wheat samples of breed lp 235194 produced in a N_2 fertilisation test.

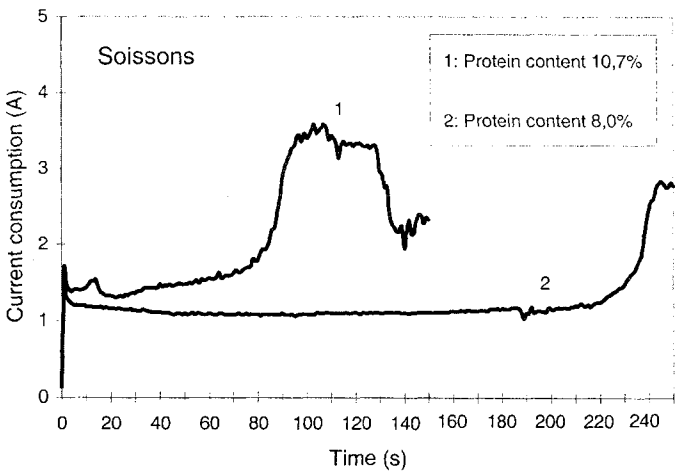


Fig. 4. Gluten agglomeration test applied to wheat samples of cv. soissons produced in a N_2 fertilisation test.

Mixer test

Looking on results of the mixer test of the first set of flours (Table 4) moist gluten weight as well as dry gluten yield followed well the flour protein content. For yields of A starch and B starch fractions, however, remarkable differences could be seen. With one exception represented by cv. Ritmo total starch yield was in good accordance with flour starch content, i.e. total starch yield exceeded the flour starch content by 0.1 to 1.6%. This could be explained as a result of impurities recovered with B starch. For Ritmo, in contrast, a reduction of 0.9% was observed together with serious losses of A starch. The difference could be found as an increase of B starch yield. These results in combination with the absence of measurable agglomeration time were reason to assume a methodological deficiency.

With respect to B starch yield the data allowed the formation of two groups. In the first group consisting of cv. Kanzler, Contur, and Crousty II B starch yield comprised a range of 7.5 to 9.6% which corresponded well with the lower range described for commercially produced wheat flour samples of a previous investigation [8]. But, also B starch yield represented by the second group of Ritmo, Soissons, and Crousty I was covered by once reported results. The range of this group was 11.3 to 14.2%.

Another differentiation of samples could be seen with fibre yield, too. The first group comprised Kanzler, Ritmo, Contur, and Crousty II (range: 1.09-1.19%) and the second group Soissons and Crousty I (range: 1.57–1.76). Besides, these observations concerning the discrimination between the analysed samples could be found partially by comparing yield values of B starch and fibres with agglomeration time. Soissons and Crousty I needed more than two times the mixing time than cv. Kanzler, Contur, and Crousty II as given by the significant difference of 35 to 65 s versus 120 to 130 s.

For the yield of solubles recovered as dry matter of the process water the over all range was 4.0 to 6.7%. A pronounced difference between the investigated samples could not be found.

Finally, the divergence in agglomeration behaviour of two growth samples of variety Crousty, in particular Crousty I and Crousty II, effected clearly extractability and purity of A starch, B starch, and fibres in giving better results at shorter agglomeration time.

The very distinct differences observed in agglomeration tests for effects of fertilisation with growth samples of breed LP 235194 and the varieties Contra and Soissons (Figure 2 to 4) could be found in equivalent order in results of the mixer test, too (Table 5). With cv. Contra agglomeration could not be observed within time of regular measurements and for LP 235194 current consumption was very small. The observed situation indicated that in a deficient situation of N_2 fertilisation plants of tested samples could not store enough protein and obviously could not form the quantity of gluten proteins required for sufficient agglomeration. Looking on results of the mixer test

these samples could not be separated well into components. In particular, dry gluten yield was extremely small while B starch yield and fibre yield were not acceptable in their amount. With level 50+50 effects of fertilisation were in no manner acceptable with regard to extractability of starch and gluten, however negative effects on B starch yield and fibres were less pronounced. Very problematic were the small yields of gluten. Soissons reacted extremely on the applied level of reduced nitrogen fertilisation

Table 4

Results of the mixer test of 6 flours from conventionally produced wheat - breeder samples

Cultivar	Ritmo	Kanzler	Contur	Crousty II	Soissions	Crousty I
Agglomeration time (s)	0	35	45	65	120	130
Flour protein content (% d.b.)	10.6	11.6	10.5	10.0	11.6	10.8
Flour starch content (% c.d.)	81.7	80.6	82.4	81.6	80.9	81.6
Moist gluten (g)	25.9	29.5	25.8	24.2	29.4	26.3
Dry gluten yield (% d.b.)	9.7	11.0	10.0	9.4	10.9	10.1
Total starch yield (% d.b.)	80.8	84.8	83.0	83.2	81.0	82.4
A Starch (% d.b.)	**66,6**	73.8	75.0	73.6	69.7	70.8
B Starch (% d.b.)	**14.2**	7.5	8.0	9.6	**11.3**	**11.6**
Fibre (% d.b.)	1.1	1.1	1.2	1.2	**1.8**	**1.6**
Solubles (% d.b.)	6.3	4.0	5.9	6.1	6.7	5.5

Table 5

Results of the mixer test of flours (Type 550) from soft winter wheat - samples from n-fertilisation trials

N_2-Fertilisation	without		50+50			70+70+50		
	Contra	LP*	Soissons	Contra	LP*	Soissons	Contra	LP*
Agglomeration time (s)	n.b.**	130	235	85	70	65	20	25
Flour protein content (% d.b.)	5.7	5.8	7.9	8.0	7.6	10.2	10.2	9.9
Flour starch content (% d.b.)	82.5	84.2	85.0	83.6	82.5	83.8	81.7	80.8
Moist gluten (g)	**1.3**	**5.0**	**6.6**	**13.3**	**14.2**	25.7	23.0	21.8
Dry gluten yield (% d.b.)	**0.6**	**1.9**	**2.9**	**6.1**	**6.1**	9.7	9.6	9.0
Total starch yield (% d.b.)	88.7	86.1	86.7	84.9	84.6	83.3	83.0	83.2
A Starch (% d.b.)	74.1	75.9	75.1	76.1	76.0	73.5	74.4	74.0
B Starch (% d.b.)	**14.6**	**10.2**	**11.6**	8.8	8.6	9.8	8.6	9.2
Fibre yield (% d.b.)	**3.3**	**3.1**	**4.1**	**1.7**	**1.6**	1.1	1.1	1.2
Solubles (% d.b.)	6.6	9.2	6.7	7.3	7.8	8.9	6.0	6.5

* LP 235 194

** not determined

that might be compared with measures of extensive plant production. The 70+70+50 fertilisation level provided a sufficient nitrogen supplementation. Agglomeration time and maximum current consumption were comparable to the range achieved in variety testing. Corresponding data for moist gluten weight and dry gluten yield reached a somewhat lower, but acceptable level. The amount of A starch and B starch were in the desired range and even the particular fibre yield was in good agreement with data presented for conventionally produced wheat samples of good processing suitability (Table 4).

Conclusion

A comparison of generally used methods for the assessment of protein quality in wheat with results of a batter mixing test system adapted to conditions of gluten agglomeration (= gluten agglomeration test) by means of correlation analysis did not prove any acceptable close relationship between the selected characteristics.

Although further investigations could use only the available set of samples the gluten agglomeration test showed even in a form that needs some improvement a fair potential for beneficial application in the differentiation of wheat flour suitable for starch production. A comparison of agglomeration time of six flour samples of conventionally produced wheat with results of a laboratory test for separation of wheat flour components (yield of moist and dry gluten, A starch, B starch, fibres, and dry matter of solubles) allowed the observation, that slowly occurring gluten agglomeration (120 to 130 s) will result in increased B starch and fibre portions. A more rapid agglomeration (35 to 65 s) reduced the amount of separated B starch significantly and produced yields of approx. 8%. At the same time A starch yield was increased to a generally accepted level. An extreme short agglomeration time signalised flour properties producing methodological difficulties that require further investigations. The described effects of more or less quick and intense gluten agglomeration could be found clearly with flour samples of a nitrogen fertilisation trial, too. An interesting finding was, that in combination with flour protein contents of 6 to 8% high molecular gluten had not been produced in sufficient quantities. As a result serious problems arose in the mixer test, in particular with the separation and purity of B starch and fibres. Wheat flours offering the finally described phenomena will probably not allow acceptable processing in industrial starch production.

REFERENCES

[1] Anonymous. Zahlen und Fakten zur Stärke-Industry. Ausgabe 1997. Bonn: Fachverband der Stärke-Industrie e.V. 1998.

[2] Anonymous: LMC Analysis Starch & Fermentation. Oxford: LMC International Ltd. April 1998.

[3] Wintzer D., Fürniß B., Klein-Vielhauer S., Leible L., Nieke E., Rösch C., Tangen H.: Technikfolgenabschätzung zum Thema Nachwachsende Rohstoffe. Münster: Landwirtschaftsverlag GmbH 1993, V-66 – V-82.

[4] Meuser F.: New developments in the production of wheat starch and gluten. In: van Doren H. and N. van Swaaij: Starch 96. The Book. Perspectives for a Versatile Raw Material on the Threshold of a New Millennium. The Hague: The Carbohydrate Research Foundation & Zestec bv 1997, 65-80.

[5] Tegge G.: Stärke und Stärkederivate. Hamburg: Behr's Verlag 1988.

[6] Zwitserloo, W.R.M.: Production of wheat starch and gluten: Historical review and development into a new approach. In: Pomeranz Y.: Wheat is Unique. St. Paul, Minn.: American Association of Cereal Chemists 1989, 509-519.

[7] Witt W.: Anforderungsprofil an den Rohstoff Weizen. In: Anonymous: Stärke im Nichtnahrungsbereich. Münster-Hiltrup: Landwirtschaftsverlag GmbH 1990, 125-141.

[8] Meuser F., Althoff F., Huster H.: Developments in the extraction of starch and gluten from wheat flour and wheat kernels. In: Pomeranz Y.: Wheat is Unique. St. Paul, Minn.: American Association of Cereal Chemists 1989, 479-499.

[9] Meuer F.: Wheat utilisation for the production of starch, gluten and extruded products. Bushuk, W., and V.F. Rasper: Wheat, Production, Properties and Quality. London, Glasgow, Weinheim, New York, Tokyo, Melbourne, and Madras: Blackie A. & P. 1994, 179-204.

[10] Barr D.J.: The engineering of a modern wheat starch process. In Pomeranz Y.: Wheat is Unique. St. Paul, Minn.: American Association of Cereal Chemists 1989, 501-508.

[11] Lindhauer M.G., Zwingelberg H.: Weizen für besondere Verwendungszwecke - 2. Mitt.: Stärkegewinnung. Getreide Mehl und Brot, **51**, 1997, 67-70.

[12] Zwingelberg H., Lindhauer M.G.: Weizensorten für die Stärkeherstellung. Getreide Mehl und Brot, **50**, 1996, 303-306.

[13] Bergthaller, W., M.G. Lindhauer and H. Zwingelberg: Variety testing of wheat for starch production using a small scale process. In: Pratznik, W., and A. Huber. Carbohydrates as Organic Raw Materials IV. Wien: WUV-Universitätsverlag 1998, 163-175.

[14] Witt W.: Personal communication, 1996.

[15] Arbeitsgemeinschaft Getreideforschung (ed.): Standard-Methoden für Getreide Mehl und Brot. Detmold: Verlag Moritz Schäfer 1994.

[16] Anonymus: Stärke - Bestimmung des Stärkegehaltes - Polarimetrisches Verfahren (DIN EN ISO 10520). Brüssel:CEN 1998.

[17] Hanneforth U., Zwingelberg H., Gebhard M.: Mehle für besondere Verwendungszwecke. 3. Mitt. Untersuchungen zur Charakterisierung von Weizenmehlen zur Herstellung von Flachwaffeln (Flours for special use. 3rd part: Investigations to characterise wheat flours for the production of wafers). Getreide Mehl und Brot, **51**, 1997, 227-231.

[18] Sachs L.: Angewandte Statistik. Berlin, Heidelberg, New York, Tolkyo: Springer Verlag 1984.

EKSTRAHOWALNOŚĆ SKROBI Z RÓŻNYCH SKROBI PSZENNYCH NA PODSTAWIE BADAŃ W SKALI LABORATORYJNEJ

Streszczenie

Od szeregu lat w wyjątkowy sposób zmienia się struktura europejskiego przemysłu skrobiowego. W wyniku polityki agrarnej Unii Europejskiej ograniczającej ilości produkowanej skrobi produkcja skrobi ziemniaczanej została zahamowana. Wzrost produkcji skrobi kukurydzianej jest nieznaczny. W przeciwieństwie do tego szybko wzrasta produkcja skrobi pszennej w związku z czym buduje się nowe krochmalnie i ulepsza technologie wyosobniania tej skrobi i otrzymywania lepszej jakości surowca. W zakresie technologii wydzielania osiągnięto postęp pozwalający wyodrębniać skrobię o wysokiej jakości. Dotychczas, jakość skrobi pszennej określano na podstawie zawartości białka i składników mineralnych.

Nowoczesne procesy stosujące wirowanie oraz stosujące wstępnie przygotowaną mąkę pszenną oraz zawiesiny wodne pozwoliły produkować pszenicę i mąkę z niej w bardziej ekonomiczny sposób i w szerszym asortymencie wysokiej jakości wyrobów.

Podjęto próby określenia jakości pszenicy do przerobu nowymi technologiami określając ekstrahowalność skrobi i glutenu z ciasta.

Y.-Y. CHEN, A. E. MCPHERSON, M. RADOSAVLJEVIC, V. LEE,
K.-S. WONG, J. JANE

EFFECTS OF STARCH CHEMICAL STRUCTURES ON GELATINIZATION AND PASTING PROPERTIES

Abstract

Chemical structures, including amylose contents, amylopectin molecular sizes, branch chain lengths and distributions, and starch phosphate monoester and phospholipid contents, of a wide varieties of starches were analyzed. Thermal properties of starch gelatinization and retrogradation and starch pasting properties were also investigated. Results of the studies have shown that amylopectin branch chain lengths and their distributions govern the starch gelatinization temperature. Short average branch chain lengths and large proportions of short branch chains (DP11-16) relative to chains of DP 18-20 (a shoulder found in many starches) are likely to result in low gelatinization temperatures. Phosphate monoester derivatives also lower gelatinization temperature. With the same amylopectin structure, increasing amylose contents lower the starch gelatinization temperature. Methods of starch isolation also affect starch gelatinization temperature. Amylose contents significantly affect the pasting properties of starch. Normal cereal starches contain lipids and phospholipids, which display higher pasting temperatures, lower peak viscosity, and lower shear thinning than their waxy starch counterparts. Without lipids, normal potato starch displays a higher peak viscosity than waxy potato starch. Branch chain length of amylopectin also affects the pasting properties of starch. The presence of very long branch chains restrict starch swelling and increase the pasting temperature of starch and decrease the shear thinning. Phosphate monoester derivatives decrease the pasting temperature and enhance the viscosity by charge repelling. In contrast, phospholipids, by complexing with amylose and long branch chains of amylopectin, restrict the starch swelling and reduce the viscosity.

Introduction

Starches isolated from different sources are known to have different granule shapes, sizes, [1] chemical structures [2] and physical properties. Great research efforts have been devoted to reveal how the chemical structures affect the functional properties of starch. With advances in genetic engineering of starch structure modifications,

Department of Food Science and Human Nutrition & Center for Crops Utilization Research, Iowa State University, Ames, IA 50011, USA

there are increasing needs of understanding how the structures of starch should be changed to deliver desired properties for market needs.

The advances in analytical techniques have enabled us to elucidate the fine structures of starch and to gain additional knowledge of how chemical structures of starches govern the functional properties. In the past decade, my colleagues and I have focused our efforts on the study of starch structures and functional properties and to develop correlation between the two. In this lecture, I will report some results of our recent findings on amylose contents, amylopectin molecular sizes, branch chain lengths, distributions, and starch phosphate monoesters and phospholipids contents of a wide variety of starches and their effects on the gelatinization, retrogradation, and pasting properties of starch.

How do starch chemical structures affect the gelatinization and retrogradation?

Studies have shown that different starches gelatinize at different temperatures which vary in a wide range. For example, potato starch, wheat starch, and waxy rice starch are known to have relatively low onset gelatinization temperatures, about 57–58°C, whereas high-amylose maize, ae waxy maize, and normal rice starches have high onset gelatinization temperature at about 71°C (Table 1). Onset gelatinization temperatures of normal maize and waxy maize starches are similar (~64°C), and that of normal glacier, waxy, and high-amylose glacier barley starches are also similar (59–60°C). These results confirm that increasing amylose contents do not cause an increase in gelatinization temperature. When the amylopectin structures of two starches are similar, increasing amylose contents is known to slightly decrease the gelatinization temperature. This can be attributed to amylose being interspersed among amylopectin molecules and interrupts the crystalline structure of starch granules.

Branch chain lengths of amylopectin and their distributions play the major role on affecting the gelatinization temperature of starch. Without other structural differences, such as phosphate monoester contents, a starch which contains longer branch chain lengths displays a higher gelatinization temperature. For example, high-amylose maize starches and ae-waxy maize starch, containing longer branch chains, display substantially higher gelatinization temperatures. In contrast, waxy and sweet rice starches have short branch chains and display low gelatinization temperatures.

Branch chain-length distributions of amylopectin (proportions of short and long branch chains) also significantly affect starch gelatinization temperature. Profiles of high-performance anion-exchange chromatography equipped with a post-column enzyme reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD) [3, 4] have shown that most starches display a shoulder at DP 18–20 [5, 6]. On the basis of 3.5 Å for each glucose anhydrous unit, a branch chain of DP 19 has a chain length of about

66.5Å, equivalent to the full length of the crystalline region in amylopectin clusters obtained by neutron scattering studies (7). We have observed that a lower population ratio of branch chains with DP 18–20 compared with the population of peak chain-length (vary from DP 12 to DP 16) results in a lower gelatinization temperature of the starch. HPAEC-ENZ-PAD histograms of debranched amylopectins of high-amylose maize starch and ae-waxy maize starch display a shoulder of DP 18–20 at about the same intensity level as the peak chain length (DP 16), indicating a large proportion of branch chains extending full length of the crystalline region. Consequently, the amylopectin crystallites of the high-amylose maize and ae-waxy maize starch have fewer

Table 1

Thermal properties of starch gelatinization determined by differential scanning calorimetry[a]

Type	T_o[b] (°C)	T_p[c] (°C)	T_c[d] (°C)	ΔH[e] (J/g)
Normal maize	64.4 ± 0.4[sd]	69.4 ± 0.2	80.4 ± 0.4	13.2 ± 0.2
Waxy maize	64.2 ± 0.2	69.4 ± 0.1	81.2 ± 0.2	15.8 ± 0.2
Du waxy maize	65.6 ± 0.5	73.6 ± 0.1	88.3 ± 0.0	16.3 ± 0.3
Ae waxy maize	71.3 ± 0.2	78.8 ± 0.4	95.8 ± 0.7	18.8 ± 0.2
Amylomaize V	71.0 ± 0.4	81.3 ± 0.4	112.6 ± 1.2	19.5 ± 1.5
Amylomaize VII	70.6 ± 0.3	NA	129.4 ± 2.0	16.2 ± 0.8
Normal rice	71.1 ± 0.4	75.9 ± 0.5	87.6 ± 0.4	15.9 ± 0.6
Sweet rice	59.1 ± 0.4	64.7 ± 0.4	77.4 ± 0.6	15.8 ± 0.3
Waxy rice	57.1 ± 0.5	63.2 ± 0.5	80.1 ± 0.6	15.3 ± 0.1
Wheat	57.5 ± 0.4	61.7 ± 0.3	73.9 ± 0.4	11.3 ± 0.1
Barley	57.3 ± 0.2	60.3 ± 0.2	71.1 ± 0.3	10.2 ± 0.6
Waxy amaranth	70.4 ± 0.4	75.1 ± 0.0	83.3 ± 0.2	13.2 ± 0.5
Cattail millet	67.6 ± 0.6	72.0 ± 0.1	83.3 ± 0.2	15.4 ± 0.5
Mung bean	69.7 ± 0.1	72.3 ± 0.2	84.2 ± 0.3	12.5 ± 0.4
Chinese taro	67.9 ± 0.2	72.8 ± 0.6	88.0 ± 0.6	16.4 ± 0.2
Tapioca	62.4 ± 0.2	67.7 ± 0.5	86.2 ± 0.3	15.7 ± 0.5
Potato	58.2 ± 0.1	61.5 ± 0.1	73.8 ± 0.5	17.3 ± 0.1
Lotus root	61.1 ± 0.6	66.3 ± 0.8	77.9 ± 0.6	15.2 ± 0.5
Green leaf canna	59.3 ± 0.3	65.4 ± 0.4	80.3 ± 0.3	15.5 ± 0.4
Green banana	69.3 ± 0.3	72.6 ± 0.3	80.4 ± 0.3	18.4 ± 0.5
Water chestnut	59.3 ± 0.6	70.4 ± 0.8	87.4 ± 0.9	14.9 ± 0.2

[a] The values are averages of at least three starch samples with at least three replicates of each sample.
[b] Onset temperature.
[c] Peak temperature.
[d] Completion temperature.
[e] Enthalpy of starch gelatinization.
[sd] Standard deviation.

defects caused by the presence of short chains. The crystallites are, thus, more difficult to melt and gelatinize at a higher temperature. In contrast, wheat starch and barley starch display a distinguishable shoulder of DP 18–20 at a substantially lower population level than that of peak chain length of DP 11 and 12. The large proportion of short chains present in the crystallites, causing defects to the crystallites, results in substantially lower gelatinization temperatures (57–59°C).

Phosphate monoester derivatives are found in amylopectin of many starches, mostly tuber, root and legume starches. Phosphate monoesters are covalently attached mainly to carbon-6 of glucose which is at least nine glucose units away from the branch linkage (reducing end) [8, 9]. Results obtained from X-ray crystallography [10], DSC thermal properties [11], and Naegeli dextrin [12] have shown that the phosphate monoesters are located in the crystalline region. Starch phosphate monoesters each carries two negative charges; the phosphate groups repel one another and destabilize the starch crystalline structure, thus, reduce the gelatinization temperature. The best known example is potato starch. Potato amylopectin has one of the longest average branch chain length, but because the amylopectin carries a large number of phosphate monoesters (~0.08% phosphorus), it has a very low gelatinization temperature at 57°C.

Methods of starch isolation also affect the gelatinization temperature of starch. We have observed difference between waxy amaranth starch isolated by alkaline method [13] and by enzymatic method [14]. The same starch isolated by alkaline method displayed an onset gelatinization (T_0) of 70.6°C with an enthalpy change of 16.3 J/g, whereas the one by enzymatic method displayed T_0 of 66.7°C and an enthalpy change of 13.2 J/g. The difference can be attributed to alkaline treatment causing damage to the crystalline structure of the starch.

Retrogradation rate of starch is mainly dependent on branch chain length of amylopectin and phosphate monoester derivatives. Starches which have very short average branch chains tend to have slow retrogradation rates. Examples are waxy and sweet rice starches and waxy amaranth starch. Starches with long branch chains and high-amylose contents have high tendency to retrograde, such as high-amylose maize, high-amylose barley and ae-waxy maize starch. du-Waxy maize starch, which has a relatively short average chain length but possesses some very long branch chains, also displays a fast retrogradation rate.

How do starch chemical structures affect the pasting properties?

Amylose is known to be present radially in a starch granule and intertwined with amylopectin [15, 16]. Lipid contents in cereal starches are found to correlate positively with the amylose contents [17]. Up to 50% of amylose has been found complexed with

lipids in a single helical conformation [18]. Therefore, the amylose content of starch significantly affect starch swelling and pasting properties.

For all the cereal starches investigated by using an amylograph, the pasting temperature increase with the increase of amylose content. For example, onset pasting temperatures of waxy maize, normal maize, and high-amylose maize V starches are 69.5°C, 82°C, and 95°C, respectively (Figure 1). Onset pasting temperatures of waxy rice and normal rice starches are 64.1°C and 79.9°C, respectively (Figure 2). The increased onset pasting temperature can be attributed to amylose-amylopectin interaction. This interaction in cereal starch is further enhanced by amylose-lipid complex. As a consequence, the amylose restricts the swelling of starch granule after gelatinization. Viscosity development is delayed to a higher pasting temperature. Peak viscosities of cereal starches are also reduced with the increase of amylose content as demonstrated in maize (Figure 1) and in rice starches (Figure 2) containing different amylose contents.

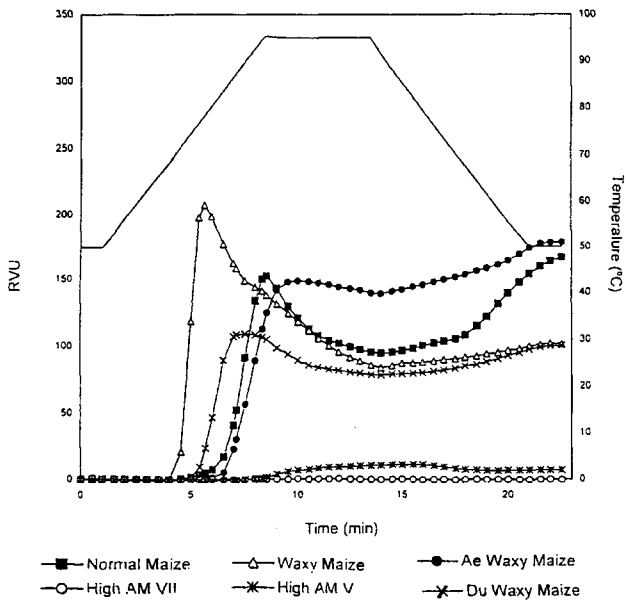


Fig. 1. Rapid Visco-Analyzer pasting profiles of various maize starches.

Increasing amylose contents, however, reduces shear thinning and results in a higher hot-past viscosity. This phenomenon is related to restricted granule swelling. When a starch granule is not fully swollen, it is less fragile and can resist shear force and retains integrity. Increasing amylose also increases set-back viscosity, a difference

in viscosity between hot-past viscosity and final viscosity after cooling. Set back is a phenomenon attributed to amylose in the solution forming a gel network matrix.

Branch chain lengths of amylopectin also affect pasting properties. Examples are shown in waxy maize, du waxy maize starch, and ae waxy maize (Figure 1). All three waxy starches contain no amylose, but branch chain lengths of the three differ. Waxy maize, du waxy maize, and ae waxy maize starch consist of average chain length of DP 23.5, DP 23.1, and DP 29.5, respectively. du Waxy maize starch consists of a small number of very long branch chains, up to DP 80, whereas waxy maize starch consists of no detectable chains longer than DP 73. ae Waxy maize starch has a large number of long chains to DP 84. With the increase of long branch chains, the pasting temperature of du waxy maize and ae waxy maize starch increase. The onset pasting temperatures of waxy maize, du waxy maize, and ae waxy maize are 69.5°C, 75.7°C, and 83.2°C, respectively. Peak viscosity of the three starches are 205 Rapid ViscoAnalyzer unit (rvu), 109 rvu, and 162 rvu, respectively, and the shear-thinning of the starches decrease to 121 rvu, 32 rvu, and 12 rvu, respectively. These results suggest that the presence of very long branch chains in starch also retain the integrity of swollen granules and resist to shear thinning.

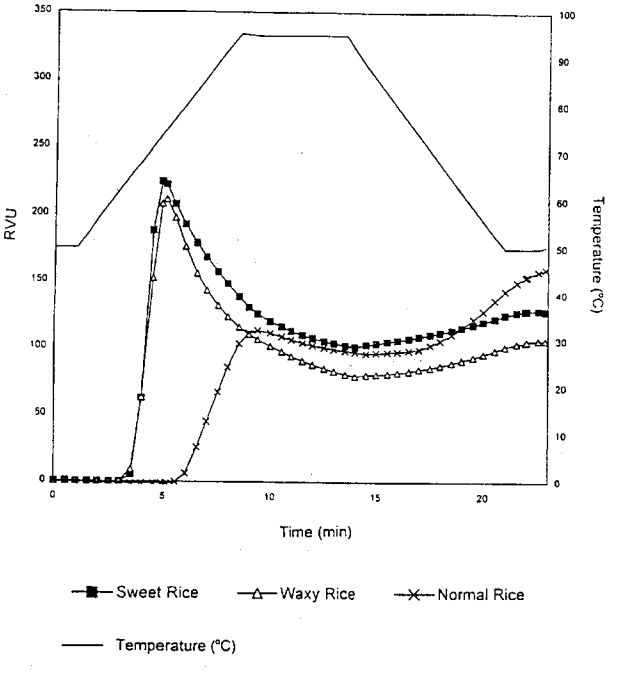


Fig. 2. Rapid Visco-Analyzer pasting profiles of waxy, sweet, and normal rice starches.

Starch phosphate monoesters enhance the peak viscosity and clarity of starch pastes, resulted from their charge repelling. The best example in nature is potato starch. Potato starch displays a very high peak viscosity of 702 rvu, compared with 152 rvu of normal maize starch and 173 rvu for tapioca starch. As a result of highly swelling of starch granules, potato starch paste also suffers a substantial amount of shear thinning of ~537 rvu. Phosphate monoester derivatives also enhance the stability of starch paste, slowing down starch retrogradation.

In contrary to the general observation that waxy starch has a higher peak viscosity than its normal starch counterpart, waxy potato starch has a lower peak viscosity and a slightly higher onset pasting temperature than does normal potato starch (Figure 3). Waxy potato starch has a slightly lower phosphate monoester content (0.066%) and slightly shorter branch chain lengths. The difference in their peak viscosity is mainly attributed to that amylose in the normal potato starch intertwine with amylopectin and hold the integrity of highly swollen granules, which results in the extremely high peak viscosity. Without amylose, waxy potato starch granules disperse promptly and, thus, does not reach to the same level of peak viscosity as does normal potato starch.

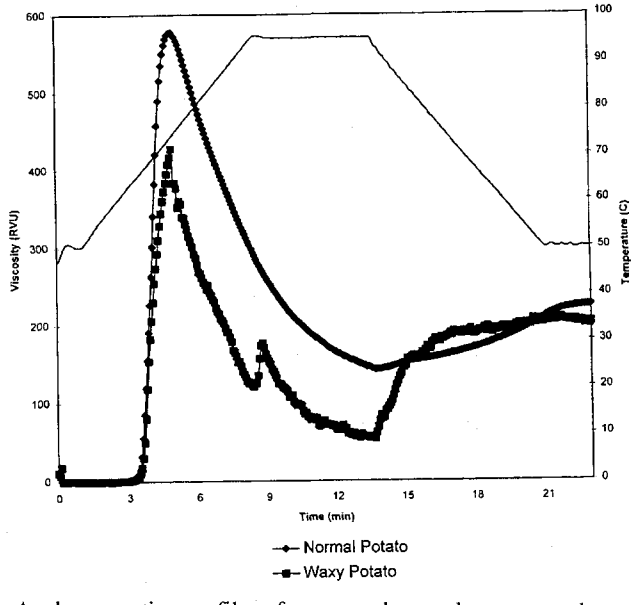


Fig. 3. Rapid Visco-Analyzer pasting profiles of waxy, and normal potato starches.

In contrast to phosphate monoesters, phospholipids have opposite effects on starch paste properties. Phospholipids are powerful complexing agents, which form helical complex with amylose and with long chains of amylopectin. As a result of the helical complex formation with amylose, starch granule swelling is retarded and di-

splays a very low peak viscosity, such as for wheat and barley starch. Wheat and barley starches have very low peak viscosity of 104 rvu and 88 rvu, respectively.

In conclusion, there is a better understanding of how starch structures control the gelatinization and pasting properties. Experimental results have shown that branch chain length and distribution govern the gelatinization temperature, enthalpy changes, rates of retrogradation, and pasting temperature and paste viscosity. Amylose contents predominantly affect pasting properties and rate of retrogradation. Phosphate monoester derivatives significantly affect the gelatinization, retrogradation, and pasting properties, and phospholipids increase the pasting temperature and reduce the peak viscosity.

REFERENCES

[1] Jane J., Kasemsuwan T., Leas S., Zobel H.F., Robyt J.F.: Starch/Stärke, **46**, 1994, 121.
[2] Hizukuri S.: [in] Carbohydrate in Food. Ed. A.-C. Eliasson, Marcel Dekker, New York, NY, 1996, 347.
[3] Wong K.S., Jane J.: J. Liq. Chromatogr., **18**, 1995, 63.
[4] Wong K.S., Jane J.: J. Liq. Chromatog., **20**, 1997, 297.
[5] Hanashiro I., Abe J., Hizukuri S.: Carbohydr. Res., **283**, 1996, 151.
[6] Shi Y.-C., Seib P.A.: Carbohydr. Polym., **26**, 1995, 141.
[7] Jenkins P.J., Cameron R.E., Donald A.M.:Starch/Staerke, **45**, 1993, 417.
[8] Hizukuri S., Shirasaka K., Juliano B.O.: Starch/Staerke, 1983, **35**, 348.
[9] Hizukuri S., Abe J.: [in] Plant Polymeric Carbohydrate, Royal Soc. Chem., 1993, 16.
[10] Muhrbeck P., Svensson E., Eliasson A.-C.: Starch/Staerke, **43**, 1991, 466.
[11] Muhrbeck P., Eliasson A.-C.: J. Sci. Food Agric., **55**, 1991, 13.
[12] McPherson A.E., Jane J.: Unpublished data.
[13] Myers D.J., Fox S.R.: Cereal Chem., **71**, 1994, 96.
[14] Radosavljevic M., Jane J., Johnson L.A.: Cereal Chem. In press.
[15] Jane J., Xu A., Rodosovljivic M., Seib P.A.: Cereal Chem., **69**, 1992, 405.
[16] Kasemsuwan T., Jane J.: Cereal Chem., **71**, 1994, 282.
[17] Morrison W.R., Tester R.F., Snape C.E., Law R., Gidley M.J.: Cereal Chem., **70**, 1993, 385.
[18] Morrison W.R.: [in] Seed Storage compounds: Biosynthesis, interactions and manipulation. P.R. Shewry and A.K. Stobart, Eds. Oxford University Press, Oxford, 1993.

WPŁYW CHEMICZNEJ STRUKTURY SKROBI NA KLEIKOWANIE SKROBI

Streszczenie

Zanalizowano budowę chemiczna wielu odmian skrobi łącznie z zawartością amylozy, rozmiarami cząsteczek amylopektyny, długością łańcuchów w rozgałęzieniach oraz zawartością monofosforanu i fosfolipidów. Zbadano też kleikowanie i retrogradację tych skrobi. Wyniki badań pokazały, że tempperaturą kleikowania rządzi długość łańcuchów stanowiących rozgałęzienia amylopektyny i ich rozmieszczenie. Łańcuchy o przeciętnej długości i wysoki stosunek rozgałęziających łańcuchów krótkich (DP 11-16) do łańcuchów długich (DP 18-20) najprawdopodobniej odpowiadają za niską temperaturę kleikowa-

nia. Skrobie zestryfikowane kwasem fosforowym (monoestry) także mają niższą tempeaturę kleikowania. Przy tej samej strukturze amylopektyny wzrost zawartości amylozy obniża temperaturę kleikowania. Na tę temperaturę ma także wpływ metoda izolowania skrobi. Zawartość amylozy wpływa też na temperaturę tworzenia past. Zwykłe skrobie zbożowe zawierające lipidy i fosfolipidy mają wyższe temperatury tworzenia past, niższe maksymalne lepkości i mniej są rozrzedzane ścinaniem niż ich woskowe odpowiedniki. Zwykła skrobia ziemniaczana bez lipidów ma wyższą maksymalną lepkość niż ziemniaczana skrobia woskowa. Długość odgałęzień w amylopektynie także wpływa na tworzenie past przez skrobię. Bardzo długie łańcuchy ograniczają pęcznienie skrobi , podwyższają temperaturę tworzenia past i obniżają rozcieńczanie ścinaniem. Skrobie estryfikowane kwasem fosforowym mają niższe temperatury tworzenia past i podnoszą lepkość przez odpychanie ładunku. W przeciwieństwie do tego fosfolipidy przez skompleksowanie z amylozą i długie odgałęzienia w amylopektynie ograniczają pęcznienie skrobi i obniżają lepkość kleików i past.

CHENG-YI LII[1,2*], VIVIAN M.-F. LAI[3], SHIN LU[4], MEI-LIN TSAI[1]

CORRELATION BETWEEN THE PHYSICAL PROPERTY, EATING QUALITY AND THE MOLECULAR STRUCTURE OF RICE-STARCHY SYSTEMS

Abstract

Investigations on the physicochemical property and molecular structure of starches in Taiwan are reviewed in relation to the eating quality of cooked rice. In addition to some conventional indices (i.e. Brabender viscoamylographic indices, gel consistency, and sensory properties), dynamic rheological parameters are also involved to clarify the importance of molecular properties of starch on the eating quality. The samples discussed were isolated from 9 indica, 9 japonica and 4 waxy varieties. The average number degree of polymerization (DP_n) of their amylopectin molecules are in the order of japonica ≥ waxy ≥ indica; while the average chain length (CL), and average exterior chain length (ECL) are indica ≥ waxy ≥ japonica. Indica amylopectins, especially from the starches of high amylose contents (AC, > 26%), carry a greater proportion of long chains than the other two varieties. As to amylose, the DP_n and CL values of high-molecular-weight subfractions are somewhat higher for indica amylose than for the japonica. Generally, Brabender viscoamylographic indices of rice flours are well correlated with the apparent AC, gel consistency (GC), and sensory cohesiveness as well as stickiness of cooked milled rice. But the flours with 0-21% AC show similar pasting and soft-gel properties. Dynamic rheological measurements suggest more precisely that different type of starches give their individual rheological patterns during gelatinization and retrogradation, primarily depending on AC and the molecular structure of amylopectin, rather than amylose. Although the AC is commonly regarded as the determinant of eating quality of cooked rice, the molecular and granular structures of starches still give potentially important influences on the physical properties of starchy systems including cooked rice or rice paste.

Introduction

The eating quality of rice (*Oryza sativa* L.) differs remarkably between three categories – indica, japonica and waxy rice [1-2]. Since starch is the principal constitu-

[1] *Institute of Chemistry, Academia Sinica, Taipei 115, Taiwan*
[2] *Graduate Institute of Food Science and Technology, National Taiwan University, Taipei 107, Taiwan*
[3] *Department of Food and Nutrition, Providence University, Shalu, Taichung 433, Taiwan*
[4] *Department of Food Science, National Chung-Hsing University, Taichung 402, Taiwan*
[*] *Corresponding author. E-mail: cylii@chem.sinica.edu.tw*

ent of milled rice (~90%), diverse varieties of rice starches have been extensively investigated to elucidate the physicochemical basis of rice quality [2-7]. In order to rationalize the cooking or processing method of rice, to classify rice on the basis of eating quality, or to develop preferential rice cultivars, some physicochemical indices have been noticed in relation to rice quality. There are the water absorption, volume expansion and alkali spreading value of milled rice, the instrumental and sensory texture of cooked rice, and the final gelatinization temperature (GT), gel consistency (GC), viscoamylographic parameter, swelling number, hot-water solubilization, and the apparent as well as hot-water-insoluble amylose content (AC) of rice flours or starches [8]. Correlations between these traditional indices and the eating quality of cooked rice have been reviewed by Juliano [8, 9]. And, the classification of rice quality is generally made on the basis of total or hot-water-insoluble AC, GC, GT, viscoamylographic indices, etc. [4-9]. However, the role of these indices and the structures of starch molecules in the texture of rice products are still ambiguous [3, 9]. Recently, the fine structure of rice starch molecules, i.e. amylose and amylopectin, have been extensively examined in our laboratory and elsewhere [10-16], and giving important influences on the pasting and rheological properties during gelatinization and retrogradation of rice starch systems [10, 13, 17-18]. Therefore, the effects of the AC, molecular structure, and swelling-solubility property of rice starches on the pasting, gelling and dynamic rheological properties are sequentially summarized in relation to the eating quality of rice products.

Correlations between the physicochemical properties of rice flours

The physicochemical properties of 22 rice flours in the previous studies [5, 13, 15, 18-28] are summarized in Table 1. Nine indica rice studied include Tainan Sen 15 (TNS15), Tainan Sen 19 (TNS19), Taichung Native 1 (TCN1), Taichung Sen 17 (TCS17), Kaohsiung Sen 7 (KSS7), Tainung Sen 19 (TNuS19), Taichung Sen 3 (TCS3), Taichung Sen 10 (TCS10) and Tai Sen 1 (TS1). Nine japonica rice analysed are Kaohsiung 1 (KS1), Tainan 5 (TN5), Tainung 67 (TNu67), Kaohsiung 142 (KS142), Tainan 9 (TN9), Taichung 189 (TC189), Tainung 70 (TNu70), Taigune 9 (TG9) and Taichung 65 (TC65). And, waxy rice examined are two indica waxy rice [Taichung Sen Waxy 1 (TCSW1), Hong Kiao Waxy (HKW)] and two japonica waxy rice [Taichung Waxy 70 (TCW70) and Hsinchu Waxy 4 (HCW4)]. The average apparent AC in starch is in the range of 14–35%, 9.2–20.5% and 0.9–1.4% for the indica, japonica and waxy samples, respectively. The crude protein and fat contents of these flours are 6.9–12.5% and 0.41–1.08%, respectively. And, the gel consistency in 0.2 N KOH is 28–35 mm and 73–100 mm for the flours of 26–35% and 0.9–20.5% amylose, respectively.

Table 1

Some physicochemical properties of rice flours[a]

Type	Variety	App. AC, % [b]	Crude protein, %	Crude fat, %	GC[c] mm	Literature cited
Indica	11. TNS15	34.9	10.9	-[d]	35	[19,20]
	12. TNS19	28.5	8.6	0.75	32	[21]
	13. TCN1	29.0	10.7	0.79	31	[5,19-20,22]
	14. TCS17	28.3	11.0	0.53	28	[15,18,22-23]
	15. KSS7	26.7	12.5	-	33	[5,13,18,19-20]
	16. TNuS19	26.3	8.02	0.41	30	[18,24]
	17. TCS3	15.6	10.6		76	[5,19-20]
	18. TCS10	15.6	8.6	0.46	81-89	[5,13,15,19-20,22,25]
	19. TS1	14.4	-	-	-	[15]
Japonica	21. KS1	20.5	10.7	-	87	[19]
	22. TN5	17.5	8.2	-	93	[5,19-20]
	23. TNu67	16.5	8.6	0.54	73-90	[13,18,19-23,26]
	24. KS142	15.6	-	-	-	[27]
	25. TN9	14.5	-	-	-	[27]
	26. TC189	14.3	6.9	0.75	76	[15,18,25]
	27. TNu70	14.0	-	-	-	[15,18]
	28. TG9	12.5	-	-	-	[15]
	29. TC65	9.2	9.2	-	88	[5,19-20]
Waxy	31. TCSW1	1.35	8.68	1.08	93-100	[18,21,23]
	32. HKW	1.18	-	-	-	[27]
	33. TCW70	0.93	7.5	0.92	97	[18,24,26,28]
	34.HCW4	0.87	-	-	-	[27]

[a] Data presented were means results in literature.
[b] Weight percentage on dry starch basis.
[c] Gel consistency in 0.2 N KOH.
[d] Not determined.

The Brabender viscoamylographic measurements [5, 29-30] have demonstrated that the peak (P) and hot-paste viscosities (H) of indica rice flours (10%) are larger than those of the japonica, and the waxy the least. However, the inverse situation is true for rice starches (7%) [30]. Three indica rice flours (e.g. TNS15, TCN1 and KSS7) have greater setback (SB) and total setback viscosities (SB_t), but lower breakdown ratio (BD_r) than the other samples. The viscoamylographic indices and gel consistency, commonly regarded as the important indicators for eating quality of cooked rice [5,7,8], are closely correlated with AC (Table 2) [5]. Significant correlations between AC, GC, and Amylographic indices of rice flours, and the hardness and stickiness of

cooked rice were also found by Song et al. [2] and Juliano [3, 8]. Nonetheless, most of viasoamylographic indices except for H and SB_t are less correlated with the crude protein content. In the case of gel consistency, it shows significantly negative correlation with the H, SB, SB_t, H/P, and C/P ratios ($p < 0.05$), where C is the cold-paste viscosity [5].

Table 2

Correlation coefficients between physicochemical properties and the Brabender viscoamylographic indices of 10% rice flours [5]

Indices	Apparent AC	Crude protein content	Gel consistency
P	0.32	0.44	-0.63
H	0.82**	0.67*[a]	-0.89**
BD	-0.67	-0.25	0.32
BD_r	-0.90**	-0.62	0.64
SB	0.92**	0.53	-0.70*
SB_t	0.98**	0.67*	-0.88**
H/P	0.87**	0.53	-0.72*
C/P	0.89**	0.50	-0.68*
C/H	0.75*	0.40	-0.45

[a] Significant level: *, $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Table 3

Correlation coefficients between the eating quality of cooked milled rice and the physicochemical properties of rice flours [5]

	Cohesiveness	Stickiness	Overall
Crude protein content	-0.52	-0.70*	-0.66*
Apparent AC	-0.72*[a]	-0.94**	-0.87**
Gel consistency	0.38	0.94**	0.80**
Viscoamylographic indices			
Pasting temperature	-0.29	-0.66*	-0.63
P	0.28	-0.41	-0.42
H	-0.26	0.83**	0.66*
BD	-0.18	0.18	0.65*
BD_r	0.84**	0.84**	0.86**
SB	-0.72*	0.86**	-0.87**
SB_t	-0.61	0.93**	-0.91**
H/P	-0.57	0.84**	0.92**
C/P	-0.68*	0.80**	0.82**
C/H	-0.89**	0.69*	0.50

[a] Significant level: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

The above physicochemical properties and the eating quality of cooked milled rice such as cohesiveness, stickiness and overall texture are negatively correlated with ($p<0.01$) the crude protein and apparent amylose contents, but positively with GC (Table 3) [5]. The significant factors are BD_r, SB, C/P and C/H values for cohesiveness, H, BD_r, SB, SB_t, H/P, C/P and C/H for stickiness, and H, BD, BD_r, SB, SB_t, H/P, and C/P for overall scores.

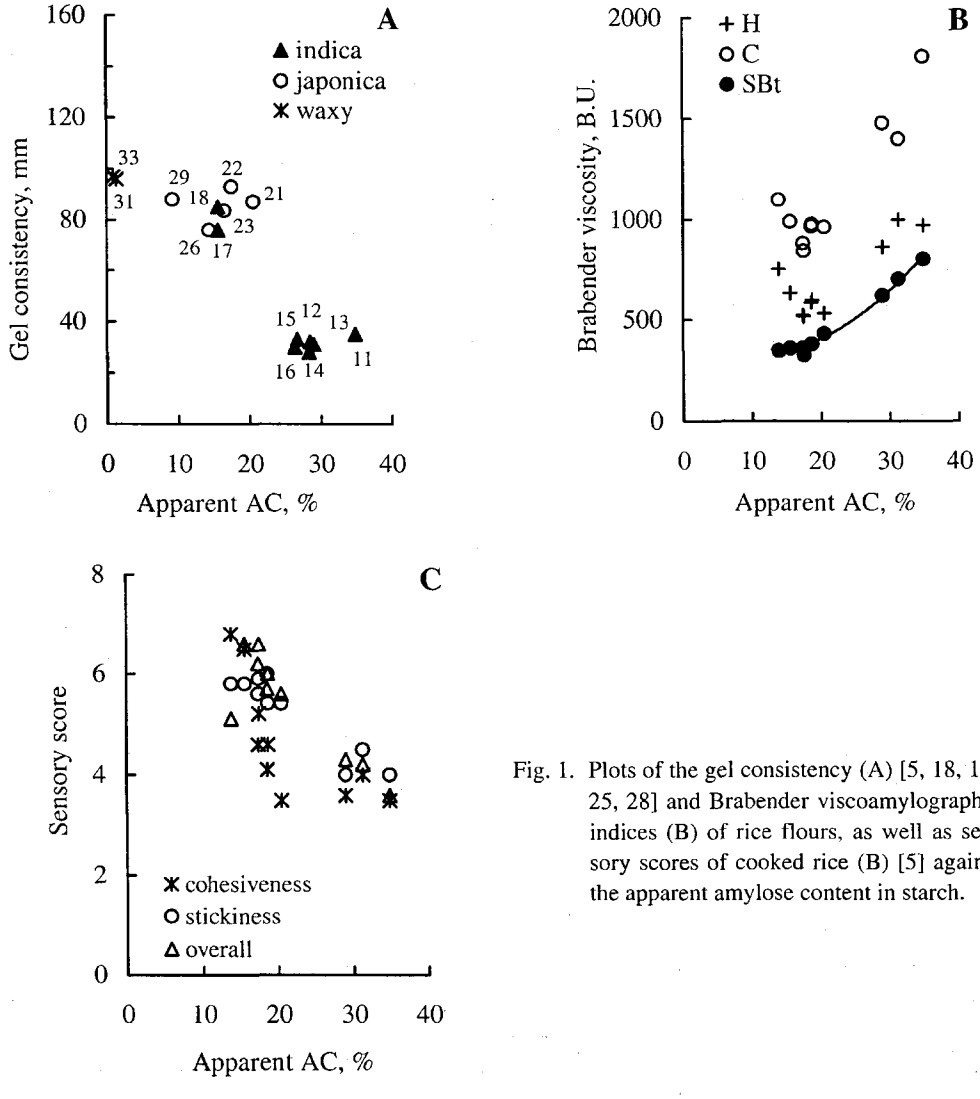


Fig. 1. Plots of the gel consistency (A) [5, 18, 19-25, 28] and Brabender viscoamylographic indices (B) of rice flours, as well as sensory scores of cooked rice (B) [5] against the apparent amylose content in starch.

The Ac-dependencies of the eating quality indices are further depicted in Figure 1. The gel consistency measurements (Figure 1A) [5, 19-25, 28] suggest that the rice flour systems can be divided into hard and soft gels as the AC is ≥ 26% (high-AC) and ≤ 21% (waxy to low-AC) respectively, according to the classification of Juliano [3]. Since the gelling time for GC measurements is short (30 or 60 min) [8], and solely reflecting the properties of short-term retrogradation, it is likely incapable of further identifying the influence of AC and molecular structure. The viscoamylographic indices H, C and SB_t show positive AC-dependence for the high-AC indica flours (Figure 1B) [5]. While, the AC-dependence of paste viscosity can vary with the flour concentration used [31]. In addition, the SB_t value increased with increasing AC ($SB_t = 0.75AC^2 - 13.50AC + 374.85$, $R^2 = 0.99$) due to the effect of water-soluble amylose leaching. This tendency is different from those of BD_r, which decreasing with increasing AC ($BD_r = 0.001AC^2 - 0.110AC + 2.997$, $R^2 = 0.82$). The latter phenomenon could be attributed to the fact that the higher-AC starch granule is more rigid and resistant to swelling and disintegration [6, 7, 17, 26]. As to the sensory properties (Figure 1C) [5], those of low-AC (10-21%) have high scores in cohesiveness, stickiness and overall texture due to the consumer preference [9]. Similarly, Sandhya Rani et al [7] reported that the paste breakdown (BD) of 21 nonwaxy rice flours is inversely correlated to the sensory and viscoelastograph hardness of cooked rice, and the BD at 95°C correlated excellently with total and insoluble AE, sensory and instrumental measures of cooked-rice texture.

Generally, the gelatinized high-AC rice, rice flours or starches show greater firmness, higher cold-pasting viscosity [5, 29], Amylographic consistency, total setback [3, 5], and gel rigidity [3, 17, 32] due to notable retrogradation within granule or to granular rigidity [6-7], as comparing with the low-AC. Accordingly, starch granule rigidity or fragility may be the basic element in rice quality [7]. The texture of cooked rice is primarily governed by the apparent or water-insoluble AC, rather than by crude protein [5, 7-9], in agreement with the findings of Bhattacharya et al. [4]. However, some disagreements are also found as compared with the data of Juliano, who discovering that protein has significant influences on the GC, P, and SB values of rice flours, and the hardness, rather than stickiness, of cooked rice [8].

Correlations between the fine structure and retrogradation property of amylopectin

Fine structure of rice amylopectin

Two typical profiles of size exclusion chromatography (SEC) were found for the chain distribution of 14 rice amylopectins (Figure 2) [14]. The type I profile has three peaks including the extralong chain fraction (a) of chain length (CL) > 100 g.u.), long

chain fraction (b) of CL = 25–100 g.u. and the short chain fraction (c) of CL < 25 g.u.. While the second type (II) only contains the b and c fractions.

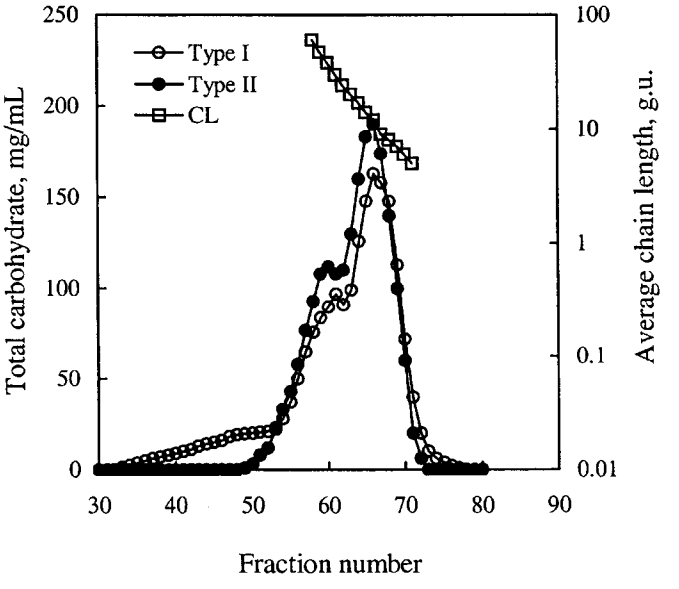


Fig. 2. Molecular chain distributions of rice amylopectins measured by size exclusion chromatography [14].

The molecular properties of 14 amylopectins (APs) from indica, japonica and waxy rice can be obtained from the SEC results, including the molar fractions of a, b, and c, the (a+b)/c ratio, the number-average degree of polymerization (DP_n), average chain length (CL), average exterior chain length (ECL), and average interior chain length (ICL). As indicated in Table 4, three indica amylopectins (TCN1, TCS17 and KSS7) show type I profile carrying 4~11% extralong chains, 29~36% long chains, and 58~65% short chains with (a+b)/c ratios of 0.54–0.71 [14]. The DP_n, CL, ECL and ICL of these three APs are 1743~2885, 20.2~22.1, 14.5~15.8 and 4.7~5.3 g.u., respectively. The other APs give type II profiles with negligible amount of extralong chains, 34-36% long chains and 64–66% short chains with (a+b)/c ratios 0.51-0.56. And, the DP_n, CL, ECL and ICL are 6481~11931, 15.4~19.8, 11.3~13.4, and 3.2~5.7 g.u., respectively. Generally, the DP_n value is indica ≤ waxy ≤ japonica; the CL and ECL, indica ≥ waxy ≥ japonica; and the ICL similar for three varieties of APs. The above results are consistent with those of Reddy et al. [11] that the APs from the highest-amylose-equivalent (AE) variety have the largest proportion of long B chains in the exterior region and the lowest proportion of short chains, while the reverse was true for waxy rice. By studying on 8 varieties of rice AP fractions, Huzukuri and his coworkers [33]

also diaplayed that the CL values of indica rice APs (20–22 g.u.) are slightly greater than those of the japonica (19–20 g.u.), and indica APs having greater molecular sizes. Nonetheless, Juliano found that the nonwaxy and waxy rice APs have similar ratios of A to B-chains [3].

Table 4

Chain distributions and molecular properties of amylopectins from various rice starches [a] [14]

Variety	Extralong, a % [b]	Long b % [b]	short c % [b]	Chain ratio, (a+b)/c	DP_n g.u.	CL g.u.	ECL g.u.	ICL g.u.
13. TCN1	10.1	31.4	58.5	0.71	2827	22.1	15.8	5.3
14. TCS17	10.9	29.2	59.9	0.67	2885	21.7	15.4	5.3
15. KSS7	3.7	35.6	60.7	0.65	1743	20.2	14.5	4.7
18. TCS10	nd[c]	36.2	63.9	0.57	6481	18.8	13.4	4.3
19. TS1	nd	34.9	65.1	0.54	7850	18.5	13.2	4.2
23. TNu67	nd	34.5	65.5	0.53	8812	17.5	12.6	3.9
24. KS142	nd	34.3	65.7	0.52	7327	17.3	12.2	4.1
25. TN9	nd	34.5	65.5	0.53	9540	16.3	11.7	3.6
26. TC189	nd	35.0	65.1	0.54	10470	15.4	11.3	3.2
27. TNu70	nd	35.4	64.6	0.55	11931	15.7	11.5	3.2
31. TCSW1	nd	35.7	64.3	0.56	7721	19.8	13.1	5.7
32. HKW	nd	35.0	65.0	0.54	8270	19.1	13.2	4.8
33. TCW70	nd	34.2	65.8	0.52	9101	17.6	12.2	4.4
34. HCW4	nd	33.9	66.1	0.51	9844	17.4	11.8	4.6

[a] Means with different letters in the same column are significantly different ($p < 0.05$)
[b] Molar percentage determined by SEC.
[c] Not detectable

Influence of amylopectin structure on the retrogradation property itself

It is known that the hardness or gel strength of starch gels or cooked rice is proportional to the retrogradation enthalpy. And, the changes in the retrogradation enthalpy of AP gels can be described according to the Avrami equation [34]:

$$\log\{-\ln[(\Delta H_\infty-\Delta H_t)/(\Delta H_\infty-\Delta H_o)]\} = \log k + n \log t$$

where k is the rate constant, and n is the Avrami exponent implying the geometry of crystallites [35]. The double logarithmic Avrami plots of TCS17 (indica), KS142 (japonica) and TCSW1 (waxy) AP gels (60%) are examplified in Figure 3 [36]. Obviously, a two-stage retrogradation process was suggested with a slope deviating at the first 7th day of storage at 5°C. Such kind of two-stage retrogradation behavior was also found in potato starch gels [37]. The slope (n_1) of short-term retrogradation (I) is

TCS17 < TCSW1 < KS142, and those (n_{II}) of the long-term (II) TCS17 ≈ TCSW1 < KS142.

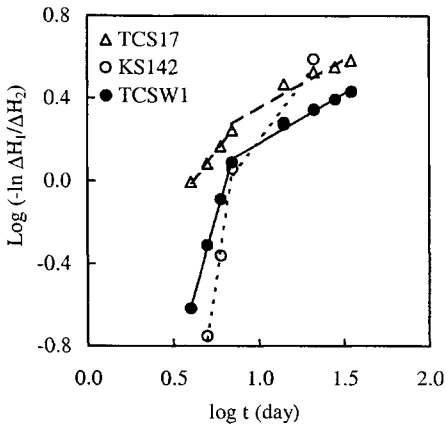


Fig. 3. Changes in the retrogradation enthalpy of 60% rice amylopectin with aging time (at 5°C) according to the double logarithmic form of Avrami equation. ($\Delta H_1 = \Delta H_\infty - \Delta H_t$, $\Delta H_2 = \Delta H_\infty - \Delta H_0$, and ΔH_0, ΔH_t and ΔH_∞ are the retrogradation enthalpy changes at 0, t and infinite ($t \rightarrow \infty$) days of storage, respectively) [36].

Correlation coefficients between the n_I, k_I, n_{II} and k_{II} values and the molecular properties of AP are tabulated in Table 5 [36]. For the short-term retrogradation stage the n_I value significantly increased with increasing the molar fraction of short chain (c) and DP_n, and with decreasing the molar fraction of extralong chain (a), and (a+b)/c ratio, CL, ECL, and ICL. The k_I value increases with increasing the molar fraction of extralong chain (a), (a+b)/c ratio, CL, and ECL, and with decreasing the molar fraction of short chain (c) as well as DP_n. For the long-term retrogradation stage (II), the above molecular properties give insignificant influences on the n_{II}; but increasing the a value or decreasing b value results in the greatening k_{II} significantly.

Table 5

Correlation coefficients between the two-stage retrogradation characteristics and molecular properties of rice amylopectins [a] [36]

Properties	Short-term (stage I)		Long-term (stage II)	
	n_I	k_I	n_{II}	k_{II}
Extralong chain (a), %	-0.64*	0.70*	-0.40	0.59*
Long chain (b), %	0.41	-0.58	0.46	-0.65*
Short chain (c), %	0.72*	-0.68*	0.29	-0.44
(a+b)/c ratio	-0.71*	0.68*	-0.29	0.44
DP_n, g.u.	0.75**[b]	-0.72*	0.16	-0.36
CL, g.u.	-0.79**	0.61*	-0.32	0.42
ECL, g.u.	-0.82**	0.66*	-0.24	0.40
ICL, g.u.	-0.64*	0.44	-0.45	0.40

[a] Retrogradation levels determined by DSC on 60% amylopectin systems.
[b] Significant level: *, $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Obviously, the molecular structure and chain distribution of APs significantly governs the geometry of crystallites and the retrogradation rate during short-term storage. While, the molecular chain distribution still influences the retrogradation rate of long-term storage. As comparing the results of Table 4 with the Table 1 and Figure 1, it is found that the high-AC rice (e.g. TCN1, TCS17 and KSS7), which having hard-gel property, high viscosity, and low stickiness, possesses the AP of a lower DP_n, higher CL and ECL, and higher (a+b)/c ratio, as compared with the low-AC. And, the proportion of extralong and long chains of AP would contribute greatly to the hardness caused by short-term retrogradation of the parent starch, flour or cooked rice. Based on the results of 8 varieties rice starch, Juliano and his coworkers [3, 38] also showed that the APs of waxy rices with high GT (≥75°C) have larger molecular weights than those of rices with low GT. Hence, the harder texture of cooked rice products from high-GT waxy rices may be due to the higher molecular weight of their APs. Similarly, Shi and Seib [39] observed that AP retrogradation is directly proportional to the fraction of DP 16–30, and inversely to the fraction of DP 6-11. They also attributed a higher onset melting temperature and retrogradation enthalpy to a greater proportion of long chains (DP > 16) in the AP [39]. The positive correlation between the n_l and c value is consistent with the findings of Mua and Jackson [40, 41] that the corn AP with an intermediate to low M_w shows a marked retrogradation. Since the retrogradation enthalpy of starch is proportional to the amylopectin content and hardness [42], the above results further confirm the importance of the AP structure in the eating quality of cooked rice or rice products.

Correlations between the fine structure of amylose and the physical property of parent starches

Fine structure of rice amylose

Generally, rice amylose (AM) consists of 40–67 wt% linear and 33–60 wt% branched fractions [9]. The molecular properties of AM molecules fractionated from Taiwan indica and japonica rice were examined by size-exclusion chromatography and are tabulated in Table 6 [13, 15-16], where the F1 and F2 represent high and low-molecular-weight subfractions respectively. It is shown that the DP_n and CL are in the range of 987~1225 and 276~430 g.u., respectively, for both indica and japonica AMs. These results are somewhat different from those of Hizukuri and his coworkers [12, 43, 44] on 7 varieties of AMs from other rice cultivars, of which the DP_n is 532–910 g.u.(linear chains) or 1130-1660 g.u. (branched chains) and the CL 101–250 g.u, depending on the recrystallization condition [12]. In addition, the DP_n and CL of subfraction F1 (Table 6) appear to be fairly higher for the indica AM (1486~2011 and

324~385 g.u.) than for the japonica (1472~1696 and 247~317 g.u. respectively). While, the DP_n and CL of subfraction F2 (353–441 g.u. and 152–309 g.u. respectively) are comparable for both varieties. These results are also somewhat different from the data of Hizukuri [50] that the DP_n and CL are 2230 and 330 g.u. for the F1, 1670 and 520 g.u. for the F2, and 410 and 295 g.u for the F3 subfraction, respectively. Among the AM molecules examined, the CL of indica AMs is not certainly greater than the japonica, also disagreeing with the reports of Juliano [9].

Table 6

Molecular properties of rice amylose fractions and subfractions [a]

Variety	Whole AM		F1 subfraction		F2 subfraction	
	DP_n	CL	DP_n	CL	DP_n	CL
13. TCN1	1135	335	2011	347	378	252
14. TCS17	1225	276	1623	324	353	168
15. KSS7	1075	430	1486	364	436	291
18. TCS10	1160	393	1776	385	437	309
19. TS1	1157	402	1665	340	409	241
23. TNu67	1004	287	1472	294	441	152
26. TC189	1114	333	1696	298	373	266
27. TNu70	1204	365	1533	247	447	248
28. TG9	987	332	1578	317	401	267

[a] Data in glucose unit were obtained from [13, 15-16].

Influence of amylose structure and swelling-solubility property on the rheological property of starch

Since the tan δ and G′ values are highly correlated with the stickiness and hardness of cooked rice [45], correlating the dynamic rheological and swelling-solubility properties of starch systems (30%) [18] to the molecular properties of amylose fractions [13, 15-16] were interested. Table 7 exhibits that the swelling power (SP), storage modulus and loss tangent at 95°C (G'_{95} and tan δ_{95}) during gelatinization, and the G′ and tan δ at 25°C (G'_{25} and tan δ_{25}) as well as the exponent ($n_{G'25}$) of starch concentration ($G'_{25} \propto C^n$) on retrogradation are significantly correlated with AC. Increasing AC tends to reduce the SP, tan δ_{95} and tan δ_{25} ($p < 0.05$), and to increase the G'_{95}, G'_{25} and $n_{G'25}$ ($p < 0.01$). Interestingly, these rheological parameters are insignificantly correlated with the DP_n and CL of amylose and its subfractions. And, the water soluble index (WSI) on gelatinization is mainly influenced by the DP_n of F2 subfraction (low-DP fraction), instead of AC. The lower the DP_n of F2, the higher is the WSI.

Table 7

Correlation coefficients between the physical properties of rice starches and the molecular properties of their amylose fractions [a]

Property	Gelatinization					Retrogradation		
	SP	WSI	G'_{95}	tan δ_{95}		G'_{25}	tan δ_{25}	$n_{G'25}$ [b]
AM content	-0.82**[c]	0.57	0.90***	-0.71*		0.90***	-0.74*	0.88**
AM-DP_n	-0.11	-0.11	0.06	-0.46		-0.08	0.09	-0.12
AM-CL	0.04	-0.66	-0.38	-0.14		-0.27	0.12	-0.67
AMF1-DP_n	-0.41	0.48	0.38	0.23		-0.28	0.20	-0.36
AMF1-CL	-0.56	-0.07	0.32	-0.24		0.32	-0.45	-0.09
AMF2-DP_n	0.19	-0.83*	-0.57	0.19		-0.37	0.35	-0.54
AMF2-CL	0.13	-0.53	-0.38	-0.14		-0.41	0.20	-0.75

[a] Correlation coefficients obtained by correlating the data of [15, 16] to the [18].
[b] Exponents of starch concentration (G'_{25} - C^n) relationships, where C is starch concentration.
[c] Significant level: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Sharp [44] has present close relationships between the rapid viscosity analyzer (RVA) and standard Bradender viscoamylographic indices. Hence, the correlation between RVA parameters of starch systems (8%) [18] and the molecular property of amyloses [15-16] as well as the swelling-solubility property of starch itself [18] were analyzed. The RVA parameters examined (Table 8) include T_o (onset temperature of viscosity increase), T_p (temperature of peak viscosity), P (paste viscosity at 95°C), H (holding viscosity at 95°C for 4 min), P-H (difference between P and H), C (cold viscosity at 35°C), C-H (difference between C and H) and F (final viscosity at 35°C for 5 min). And, the swelling-solubility properties of rice starch systems (1%) at 95°C include SP (swelling power), WSI (water soluble index), BV (blue value) and λ_{max} (maximum wavelength). The apparent AC, ins. AC (hot-water-insoluble AC) and lipid-AC (the amount of amylose binding with lipid) [18] were also involved. The correlation coefficients (Table 8) suggest that the AC, ins. AC, lipid-AC and swelling-solubility properties gives significant effects on most of the RVA parameters. But, the molecular structure of amylose and its subfractions give a negligible effect on the RVA properties, in agreement with the dynamic rheological parameters. This implies that the swelling property of starches, which principally caused by AP molecules, is more important for the rheological characteristics and consequently texture of rice starch systems.

Table 8

Correlation coefficients between rapid viscosity analyzer indices and physicochemical properties of rice starches [a]

Property	T_o	T_p	P	H	P-H	C	C-H	F
App. AC	0.69*[b]	0.71*	-0.56	0.61	-0.88**	0.88**	0.89**	0.89**
Ins. AC	0.60	0.63	-0.53	0.52	-0.81**	0.90***	0.93***	0.91***
Lipid-AC	0.76*	0.96***	-0.75*	0.20	-0.86**	0.68*	0.75*	0.68*
AM-DP_n	0.19	0.85*	0.56	0.59	0.24	0.53	0.46	0.48
AM-CL	0.49	-0.12	-0.12	0.03	-0.24	0.06	0.05	0.02
AMF1-DP_n	-0.06	0.05	0.67	0.41	0.65	-0.02	-0.15	-0.07
AMF1-CL	0.71	-0.42	0.13	0.45	-0.32	0.47	0.44	0.47
AMF2-DP_n	-0.15	-0.30	-0.59	-0.58	-0.32	-0.36	-0.27	-0.35
AMF2-CL	0.41	-0.34	0.46	0.29	0.42	-0.13	-0.27	-0.23
SP	-0.69*	-0.97***	0.72*	-0.30	0.89**	-0.73*	-0.78*	-0.73*
WSI	0.34	0.53	-0.38	0.26	-0.52	0.74*	0.81**	0.75*
BV	0.66	0.94***	-0.77*	0.17	-0.87**	0.67*	0.75*	0.68*
λ_{max}	0.67*	0.98***	-0.73*	0.17	-0.83**	0.63	0.70*	0.63

[a] Correlation coefficients obtained by correlating the data of [15-16] to [18].
[b] Significant level: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Conclusion

Generally, the apparent AC or hot-water-insoluble AC has notable effects on the rheolgical properties and texture of rice starches, flours and rice products. However, the granular rigidity, which resulting from the arrangement of AP and AM molecules, appears to be another important factor responsible for the change in the rheological and eating characteristics. Between these two molecules, the molecular structure of AP shows a more close correlation to the rheological properties interested, possibly due to that AP is the principal component of starch. However, for the pasting property of rice starches and flours, AM and AP molecules would be of the same importance, since the combination of longer-chain AP and the intermediate-molecular-weight AM may synergistically increase the pasting viscosity as in the case of corn starch [47]. Nonetheless, since the gelatinization and retrogradation mechanisms of starches are quite complicated, further studies on the relationships between the granular structure of starch composite and the dynamic rheological indices as well as the eating quality of rice products are required.

REFERENCE

[1] Juliano B.O.: Starch: Chemistry and Technology, 2nd ed., Academic Press, New York, 1984, 507-528.

[2] Song S., Hsu A.-N., Hong M.-C.: Rice Grain Quality. Proc. Symp. TDAIS, S. Song and M.-C. Hong, eds. Taichung District Agricultural Improvement Station, Changhua, Taiwan, 1988.

[3] Juliano B.O.: J. Jap. Soc. Starch Sci., **29**, 1982, 305-317.

[4] Bhattacharya K.R., Sowbhagya C.M., Indudhara Swamy Y.M.: J. Food Sci., **47**, 1982, 564-569.

[5] Lii C.-Y., Chang Y.-H.: Symposium of rice quality in Taiwan, 1988, 31-43.

[6] Radhika Reddy K., Subramanian R., Zakiuddin Ali S., Bhattacharya K.R.: Cereal Chem., **71**, 1994, 548-552.

[7] Sandhya Rani M.R., Bhattacharya K.R.: J. Text. Stud., **26**, 1995, 587-598.

[8] Juliano B.O.: Rice: Chemistry and Technology, 1985, 443-524.

[9] Juliano B.O.: Cereal Foods World, **43**, 1998, 207-221.

[10] Chinnaswamy R., Bhattacharya K.R.: Starch/Stärke, **38**, 1986, 51-57.

[11] Radhika Reddy K., Zakiuddin Ali S., Bhattacharya K.R.: Carbohydr. Polym., **22**, 1993, 267-275.

[12] Hizukuri S., Shirasaka K., Juliano B.O.: Starch/Stärke, **35**, 1983, 348-350.

[13] Tseng Y.-H.: MS thesis. National Taiwan University, Taiwan, 1996.

[14] Lu S., Chen L.-N., Lii C.-Y.: Cereal Chem., **74**, 1997, 34-39.

[15] Huang R.-M., Tseng J.-Y., Lii, C.-Y.: Food Sci. (Chinese), **25**, 1998a, 210-221.

[16] Huang R.-M., Tseng J.-Y., Lii C.-Y.: Food Sci. (Chinese), **25**, 1998b (accepted).

[17] Lii C.-Y., Lai M.-F., Tsai M.-L.: Food. Technology. Quality. (Poland), **2** (7), 1996, 27-53.

[18] Tsai M.-L.: Ph.D. dissertation. National Taiwan University, Taiwan, 1997.

[19] Lii C.-Y. Chang S.M., Yang H.L.: Bull. Inst. Chem., Academia Sinica, **33**, 1986, 55-62.

[20] Chang S.-M., Lii, C.-Y.: Bull. Inst. Chem., Academia Sinica, **32**, 1985, 57-61.

[21] Wu et al.: J. Chinese Agric. Chem. Soc., **30**, 1992, 337-348.

[22] Huang R.-M., Lii C.-Y.: J. Chinese Agric. Chem. Soc., **28**, 1990, 267-275.

[23] Lu S., Wu S.-J., Lii C.-Y.: Food Sci. (Chinese), **15**, 1988, 280-286.

[24] Huang R.-M., Lii, C.-Y.: Cereal Chem., **71**, 1994, 202-207.

[25] Lin S.-Y., Lii, C.-Y.: J. Chinese Agric. Chem. Soc., **33**, 1995, 482-493.

[26] Lii C.-Y., Shao Y.-Y., Tseng K.-H.: Cereal Chem., **72**, 393-400.

[27] Council of Agriculture, Executive Yuan: Rice Varieties in Taiwan, Taiwan, 1987.

[28] Lin S.-Y., Lii, C.-Y.: Food Sci. (Chinese), **22**, 1995, 407-418.

[29] Yang C.-C., Lai H.-M., Lii C.-Y.: Food Sci. (Chinese), **14**, 1987, 212-221.

[30] Chang Y.-H., Chu L.-W., Su N.-C.: Food Sci. (Chinese), **23**, 1996, 739-751.

[31] Sandhya Rani M.R., Bhattacharya K.R.: J. Texture Stud., **20**, 1989, 127-137.

[32] Lii C.-Y., Tsai M.-L., Tseng K.-H.: Cereal Chem., **73**, 415-420.

[33] Hizukuri S.: Carbohydrates in Food, New York., 1996, 347-429.

[34] Avrami M.: J. Chem. Phys., **8**, 1940, 212-224.

[35] Sperling L.H.: Introduction to Physical Polymer Science, New York, 1993, 232-235.

[36] Lii C.-Y., Lu S., Lai V. M.-F., Chen L.-N.: J. Cereal Sci. (submitted).

[37] Mita T.: Carbohydr. Polym., **17**, 1992, 269-276.

[38] Juliano B.O., Villareal, R.M.: Starch/Stärke, **39**, 1987, 298-301.

[39] Shi Y.-C., Seib, P.A.: Carbohydr. Polym., **26**, 1995, 141-147.

[40] Mua J.P., Jackson D.S: J. Agric. Food Chem., **45**, 1997, 3848-3854.

[41] Mua J.P., Jackson D.S.: J. Cereal Sci., **27**, 1998, 157-166.

[42] Wootton M., Mahdar D.: Starch/Stärke, **45**, 1993, 295-299.

[43] Takeda Y., Hizukuri S., Juliano B.O.: Carbohydr. Res., **148**, 1986, 299-308.
[44] Hizukuri S., Takeda Y., Maruta N., Juliano B.O.: Carbohydr. Res., **189**, 1989, 227-235.
[45] Otobe K., Naito S., Sugiyama J., Kikuchi Y.: Nippon Shokuhin Kagaku Kaishi, **42**, 1995, 748-755.
[46] Sharp R.N.: Cereal Chem., **63**, 1986, 325-326.
[47] Jane J., Chen J.F.: Cereal Chem., **69**, 1992, 60-65.

KORELACJA MIĘDZY WŁAŚCIWOŚCIAMI FIZYCZNYMI, SMAKOWITOŚCIĄ I STRUKTURĄ CZĄSTECZKOWĄ SKROBI RYŻOWEJ

Streszczenie

Dokonano przeglądu badań nad fizykochemicznymi właściwościami i strukturą cząsteczkową skrobi tajwańskich i odniesiono wyniki tych badań do smakowitości gotowanego ryżu. Aby wyjaśnić związek pomiędzy strukturą cząsteczkową skrobi a smakowitością poza takimi zwykłymi wskaźnikami jak parametry charakterystyki kleikowania Brabendera, konsystencją żelu i właściwościami sensorycznymi wzięto pod uwagę parametry z dynamicznych pomiarów reologicznych. Próbki skrobi wyodrębniono z 9 odmian ryżu indica, 9 odmian japonica i 4 skrobi woskowych. Średni stopień polimeryzacji (DP_n) ich cząsteczek amylopektyny układał się w szeregu japonica_> woskowa > indica, podczas gdy średnia długość łańcucha (CL) i średnia zewnętrzna długość łańcucha (ECL) malały w szeregu indica > woskowa > japonica. Amyloektyna z odmian indica, szczeg/ólnie ze skrobi wysokoamylozowych (AC > 26%) maiły większy udział łańcuchów długich niż amylopektyna z pozostałych odmian. Natomiast dla amylozy, DP_n i CL wysokocząsteczkowych podfrakcji są nieco wyższe w przypadku odmian indica niż odmian japonica. Ogólnie, parametry charakterystyki kleikowania dla mąk ryżowych dobrze korelują z pozorną zawartością amylozy, AC, GC i sensoryczną kohezyjnością oraz skleikowatością gotowanego zmielonego ryżu. Jednak mąki o AC 0–21% podobnie kleikowały i a ich żele miały podobną miękkośći. Dynamiczne pomiary lepkości lepiej pokazują, że różne rodzaje skrobi mają swe indywidualne charakterystyki reologiczne w czasie kleikowania i retrogradacji. Zależą one przede wszystkim od AC i struktury cząsteczkowej amylopektyny, a mniej od struktury amylozy. Chociaż AC powszechnie uważa się za wyznacznik smakowitości gotowanego ryżu struktury cząsteczkowa i gałeczkowa wciąż w istotny sposób wpływają na fizyczne i właściwości układów skrobiowych z gotowanego ryżu i pasty ryżowej.

Z. CZUCHAJOWSKA[1], A. KLAMCZYNSKI

STRUCTURE AND FUNCTIONALITY OF BARLEY STARCHES

Abstract

Amylose contents of prime starches from non-waxy (regular) and high-amylose barley determined by colorimetric method were 24.6% and 48.7% respectively, while the waxy starch showed only a trace (0.04%) of amylose. The isoamylase debranched amylopectin showed little difference between non-waxy and high-amylose barleys, while amylopectin from waxy barley had significantly higher percentage of fraction with degree of polymerization (DP) < 15 (45%).

The x-ray diffraction pattern of waxy starch differed from non-waxy and high-amylose. Waxy starch had sharper peaks at 0.58 nm, 0.51 nm, 0.49 nm and 0.38 nm than non-waxy and high-amylose starches. The *d*-spacing at 0.44 nm characterizing the amylose-lipids complex was the most evident for high-amylose starch but was not observed in waxy starch.

DSC thermograms of prime starch of non-waxy and high-amylose barleys exhibited two prominent transition peaks: one above 60°C corresponded to starch gelatinization, and the second above 100°C corresponded to the amylose-lipid complex. The starch from waxy barley had only one endothermic peak of gelatinization of amylopectin with an enthalpy value of 16.0 J/g.

The retrogradation of gelatinized starch of three types of barley stored at 4°C showed that amylopectin recrystallization rates of non-waxy and high-amylose barley were comparable when recrystallization enthalpy was calculated based on the percentage of amylopectin. No recrystallization peak of amylopectin was observed in waxy barley.

Storage time showed a strong influence on the recrystallization of amylopectin. The enthalpy value for non-waxy barley increased from 1.93 J/g after 24 hr of storage to 3.74 J/g after 120 hr. When gel was rescanned every 24 hr a significant decrease in enthalpy was recorded.

A highly statistically significant correlation (r = 0.991) between DSC values of retrograded starch of non-waxy barley and gel hardness was obtained. The correlation between starch enthalpy value and gel hardness of starch concentrate indicates that texture of gel was mainly due to its starch structure and functionality. The relationship between properties of starch and starch concentrate might favor the application of barley starch concentrate without the necessity of using the wet fractionation process.

[1] *Associate Professor (Correspondent Author) and Graduate Research Assistant*
Department of Food Science and Human Nutrition, Washington State University, Pullman, WA 99164-6376

Introduction

Starch in barley is the largest single component, representing up to 65% of kernel dry weight and providing a valuable source of energy. Despite such availability of barley starch, relatively little research has been carried out on its functional properties, when compared to wheat or corn starches. Part of the reason for such neglect is the fact that a high proportion of barley grain is used in animal feed without any processing. Another reason could be the difficulty of isolating the starch from barley as a pure product by the wet process, which is complex, lengthy and requires a large amount of water. The high water-holding capacity of barley meal is primarily due to the presence of β-glucans, which absorb a lot of water and make isolation of starch by the wet process difficult.

Previous studies on barley starch have been carried out primarily on non-waxy types of barley cultivars in which starch contains about 25% amylose, while starches of two other types of barley, waxy and high-amylose, have attracted interest only recently (Czuchajowska et al 1992). During any food preparation, starch undergoes partial or complete gelatinization, as well as interacting with other food components (Czuchajowska and Smolinski 1997). Therefore, in order to promote greater utilization of barley in human consumption, research on thermal behavior of isolated starches and on flours of different types of barley must be conducted.

Well-documented results of this research will be of great value to the food industry in selecting the right type of barley for a specific product or process.

Therefore, the objectives of this study were:

- to evaluate the thermal behavior of starches isolated from non-waxy, waxy and high-amylose barleys;
- to examine the thermal behavior of flours differing in composition as the result of abrading; and,
- to study the retrogradation rate of amylopectin and the gel strength of three types of starch as compared to the strength of gel from starch concentrate.

Materials and methods

Samples

Three types of hull-less barley, non-waxy (cv. Glacier), high-amylose (cv. Glacier) and waxy (cv. Wanubet) were provided by Dr. C.W. Newman from Montana State University and Dr. S. Ullrich from Washington State University. The barley starch was isolated from three types of barley by wet process according to the methodology by Szczodrak and Pomeranz (1991).

Abrasion of barley samples

Barley was abraded using a Tangential Abrasive Dehulling Device (TADD, Venables Machine Works, Ltd., Saskatoon, Canada) to 10%, 20% and 40% of kernel weight.

Chemical analyses

Protein contents (N x 6.25) of samples were determined by a Leco nitrogen analyzer (Leco Corporation, St. Joseph, MI) equipped with a thermal conductivity detector. Moisture content was determined by oven drying for 1 hr at 130°C (AACC Method 44-15A, 1995), ash content by dry combustion for 16 hr at 580°C (AACC Method 08-01, 1995) and free lipids content by petroleum ether extraction (AACC Method 30-25, 1995). Starch content was determined according to Prosky et al 1988. Beta-glucans content was measured enzymatically, as described by Ahluwalia and Ellis (1984).

Characteristics of starch

Amylose content of prime starch was determined by colorimetric method (Knutson and Grove 1994) and by high-performance size-exclusion chromatography (HPSEC). The starch solubilization procedures for HPSEC for estimation of amylose content was carried out according to Bradbury and Bello (1993). The amylose content of starch was determined by HPSEC as the ratio between total peak area and the peak area corresponding to amylose (Kobayashi et al 1985). Isoamylase-debranched amylopectin was prepared according to the method described by Yuan et al (1993) and modified by Lin and Czuchajowska (1997). DP of the linear fractions in debranched amylopectin was calculated as MW/162 (Bradbury and Bello 1993). Solubilized starch (100 μL) or isoamylase-debranched starch solution was injected into a two-column HPSEC system (Bio-Sil SEC 125, 300 x 7.8 mm, Bio-Rad, Richmond, CA) using 30% DMSO as the mobile phase at a flow rate of 0.5 mL/min. The two-column system was preceded by a guard column (80 x 7.8 mm) and a 2 μm precolumn filter (A315, Upchurch Scientific, Oak Harbor, WA). The HPSEC system consisted of an autosampler (model 1050, Hewlett Packard, Wilmington, DE), a solvent-delivering system (model 2350 HPLC pump and model 2360 gradient programmer, ISCO, Inc., Lincoln, NE), a differential refractometer (R401, Waters Associates, Inc., Milford, MA) and a computer equipped with HPLC 3D ChemStation software (Hewlett Packard). The columns and detector (sensitivity at 32Tx) were maintained at a constant temperature (35°C). The HPSEC system was calibrated using four pullulan standards (Polymer Laboratories, Amherst, MA) with MW 112,000, 22,800, 5,900 and 738, respectively. A linear relationship ($r^2 = 0.992$) between log molecular weight and retention time was obtained.

Wide-angle x-ray diffraction of barley starch

X-ray diffraction patterns of the starches were recorded on a Siemens D 500 diffractometer (Madison, WI) operating at 35 kV, 30 mA. Diffractograms were obtained from 4° 2θ to 30° 2θ with a step of 0.05° 2θ, counting 4 sec on each step. Integrated normalized intensities were calculated on the basis of the number of counts recorded by the scintillation counter (Sievert et al 1991, Czuchajowska et al 1991).

Differential scanning calorimetry (DSC)

Thermal behavior of three types of barley starches and flours was followed by DSC, as described by Czuchajowska and Pomeranz (1989), on a Perkin-Elmer DSC-2 instrument (Perkin-Elmer Corporation, Norwalk, CT).

Experiment 1

In the first experiment 30 sub-samples of dry starch (10 mg) isolated from non-waxy and waxy barley were weighed into large pans, water was added (20 μL), and then the pans were sealed and scanned from 20°C to 180°C at a heating rate of 10°C/min. Next, the 30 pans were divided into six sets of five pans each and stored at 4°C. Each set was rescanned once after 24 hr, 48 hr, 72 hr, 96 hr and 120 hr intervals.

Experiment 2

In the second experiment, contrary to the first, six replications of non-waxy and waxy prime starch were scanned at every 24 hr period up to 120 hr. After each scanning the pans were stored at 4°C to evaluate the effect of repeated heating on the intensity of retrogradation of non-waxy and waxy barley starches.

Pasting properties of abraded barley

The pasting properties of the material were measured by Brabender Visco-Amylograph according to Shuey and Tipples (1980).

Hardness of gel prepared from prime starch and starch concentrate

The texture of gel representing 60% of the inner part of the barley kernel (starch concentrate) was measured using a TX-XT2 Texture Analyzer (Stable Micro System, Haslemeres, England). The gel was prepared in a Brabender Visco-Amylograph. The slurry of 10% starch or 10% flour was heated to 90°C and held for 20 min at that temperature. The hot paste was poured into molds (30 mm in diameter and 35 mm in height), covered to avoid evaporation of water and stored at 4°C for up to 120 hr. Texture of the gel was measured by using two attachments: a plexiglass plunger (12 mm diameter) or a disc (60 mm diameter).

Gel prepared from waxy barley was too weak to stand by itself. Therefore, to have comparable data, texture of gels prepared from non-waxy, high-amylose and waxy barleys was measured using the plexiglass plunger. The gels were penetrated once by the plunger to 30% of their height and the force of penetration recorded. Non-waxy and high-amylose barley gels were compressed by disc to 30% of the gel height. Hardness of gel was recorded. The texture of gels was measured during a 120 hr period in 24 hr intervals.

Statistical analysis

All tests were run at least in duplicate. Least significant difference (LSD), analysis of variance (ANOVA) and correlation analysis were performed using the Statistical Analysis System (SAS Institute, Cary, NC 1986).

Results and discussion

Effect of abrasion on composition of barley

The changes in barley composition due to abrasion at 10%, 20% and 40% are summarized in Table 1. Compared to whole kernel, protein content significantly decreased when the percentage of abrading was increased. A similar pattern was observed for all three types of barley. The largest change in protein content, a decrease of 4.2%,

Table 1

Composition of abraded barley

Type of Barley	Abrasion Level	Protein (%)	Ash (%)	Free Lipids (%)	Starch (%)	Total β-glucans
Regular	Whole kernel	13.6 a	2.11 a	2.60 a	67.6 d	6.2 ab
	10%	12.3 b	1.68 b	1.88 b	73.7 c	6.6 a
	20%	11.6 c	1.45 c	1.47 c	77.2 b	6.8 a
	40%	10.2 d	1.00 d	0.95 d	82.9 a	6.8 a
High-amylose	Whole kernel	12.5 a	2.05 a	2.16 a	65.2 d	5.6 c
	10%	10.3 b	1.62 b	1.62 b	71.0 c	6.4 b
	20%	9.9 c	1.36 c	1.18 c	77.8 b	6.5 b
	40%	8.3 d	0.92 d	0.80 d	81.0 a	7.1 a
Waxy	Whole kernel	15.5 a	2.61 a	2.65 a	65.6 d	6.60 cd
	10%	14.6 b	1.90 b	1.76 b	70.5 c	6.87 c
	20%	13.5 c	1.46 c	1.23 c	74.6 b	7.31 b
	40%	11.9 d	0.96 d	0.86 d	78.0 a	8.00 a

[a] Values with different letters in a column within each type of barley are significantly different at the 5% level.

took place in high-amylose barley at 40% abrading, compared to whole grain, while non-waxy and waxy barleys decreased in protein content by 3.4% and 3.6%, respectively, compared to whole grain. The ash and free lipids content decreased more than two times, due to the removal of 40% of the outer layer of the kernels. Contrary to protein, ash and free lipids, starch content increased by abrading. In the 40% abraded kernel starch content increased by 12.4% in waxy barley, and by more than 15% in non-waxy and high-amylose barleys. Similar changes in barley composition due to abrading were reported by Baik and Czuchajowska (1997). In all three types of barley β-glucans content was highest in the inner part when abraded at the 40% level.

Composition of isolated barley starches

The composition of purified prime starch is presented in Table 2. As indicated by average protein and ash contents of 0.5% and 0.2%, respectively, all three types of barley starches were of high purity. Beta-glucans were not detected in these starches. Independent of applied methodology, the highest amylose content was found in starch from high-amylose barley, followed by starches from non-waxy and waxy barleys. The amylose content determined by the colorimetric method in these three types of starches agreed with previous work by Czuchajowska et al (1992). The waxy starch had 0.04% amylose, while amylose content in high-amylose barley reached almost 50%. The prime starch from non-waxy barley had an amylose content of around 25%, which is close to most starch of wheat.

Table 2

Composition of prime starch from three types of barley

Type of Barley	Protein (%)	Ash (%)	Starch (%)	Amylose in Starch	
				HPSEC (%)	Iodometric (%)
Regular	0.56 b	0.21 b	97.4 b	32. 7 b	24.6 b
High-amylose	0.61 a	0.18 c	98.4 a	39.7 a	48.7 a
Waxy	0.35 c	0.23 a	97.8 b	7.4 c	0.04 c

[a] Values with different letters in a column are significantly different at the 5% level.

Fine structure of amylopectin

The distribution of average molecular weight of the branch chains of all three evaluated starches is summarized in Table 3. The average branch chain distribution of amylopectin showed little difference between non-waxy and high-amylose barleys. Waxy barley contained significantly more HMW and LMW, but less IMW fractions of debranched amylopectin than non-waxy and high-amylose barleys. Actual data were in agreement with those of MacGregor and Morgan (1984), in that HMW fraction of de-

branched amylopectin from waxy barley represented about 20% of the total relative peak area.

Table 3

Branch chain distribution of amylopectin debranched by isoamylase

Type of Barley	HMW[a] (%)	IMW (%)	LMW (%)
Regular	15.5 b[b]	47.5 a	37.0 b
High-amylose	16.3 b	46.4 a	37.4 b
Waxy	19.7 a	35.4 b	45.0 a

[a] HMW - Degree of Polymerization > 35: IMW - 35 < Degree of Polymerization < 15; LMW - Degree of Polymerization < 15.

[b] Values with different letters in a column are significantly different at the 5% level.

X-ray diffraction pattern of barley starches

All three barley starches exhibited the A-type x-ray diffraction pattern, as shown in Figure 1. A higher percent relative intensity (PRI) indicates a higher degree of crystallinity of starch. Major peaks of barley starches were observed around *d*-spacings 0.58 nm, 0.51 nm, 0.49 nm, 0.44 nm and 0.38 nm. Waxy barley starch differed from non-waxy and high-amylose barley by having sharper peaks at 0.58 nm, 0.51 nm, 0.49 nm and 0.38 nm. The *d*-spacing at 0.44 nm is characteristic of the amylose-lipid complex. No peak was observed in waxy starch at 0.44 nm, but high-amylose barley had the most evident peak of 0.44 nm of all three starches.

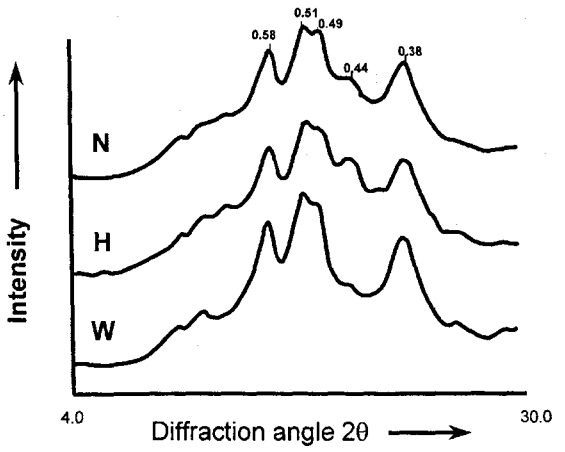


Fig. 1. X-ray diffraction patterns of barley starches. N, H and W indicate non-waxy, high-amylose and waxy starches. Numbers indicate the starch crystal *d*-spacings (2θ) of the major diffraction peaks.

Viscosity of abraded barley

The large changes in composition of barley kernels due to abrading (see Table 1) caused significant changes in thermal behavior of abraded barley. The increase in starch content due to abrading resulted in increased viscosity of all three types of barley. In non-waxy barley the viscosity increased from 680 BU of whole meal to 970 BU of flour from 40% abraded kernels (Table 4). This increase in viscosity was mainly due to an increase in starch content from 67.6% to 82.9%, since total β-glucans showed no significant changes due to abrading.

Table 4

Amylograph parameters of abraded barley

Type of Barley	Abrasion Level	Peak Temperature (°C)	Peak Viscosity (B.U.)
Regular	Whole kernels	85	680
	10%	85	860
	20%	85	930
	40%	85	970
High-amylose	Whole kernels	83	-
	10%	83	-
	20%	83	930
	40%	83	940
Waxy	Whole kernels	64	510
	10%	64	980
	20%	64	1380
	40%	65	1580

For high-amylose barley, viscosities were comparable to the non-waxy type. The largest changes in viscosity due to abrading took place in waxy barley. The viscosity of 40% abraded barley was three times higher than that of whole meal. It is interesting that this large increase in peak viscosity of all three types of barley was not accompanied by increases in peak temperature.

DSC thermograms of starch

DSC thermograms of starch gelatinization and recrystallization of the three types of barley during storage are presented in Figure 2A and 2B. Both non-waxy and high-amylose barleys exhibited two prominent transitions over similar temperature ranges. The first transition temperature above 60°C corresponded to endotherms of starch gelatinization. The second transition above 100°C corresponded to the amylose-lipid complex. The retrogradation of gelatinized starch of three types of barley stored at 4°C for 2 weeks is graphically presented in Figure 2B. The enthalpy values of retrogradated

amylopectin of non-waxy and high-amylose barley were comparable when the recrystallization was calculated based on the percentage of amylopectin. No recrystallization peak of amylopectin was found in waxy barley. This result may be explained by the presence of a high percentage of low degree polymerization of branches of waxy amylopectin determined by HPSEC of isoamylase debranched amylopectin (Table 3).

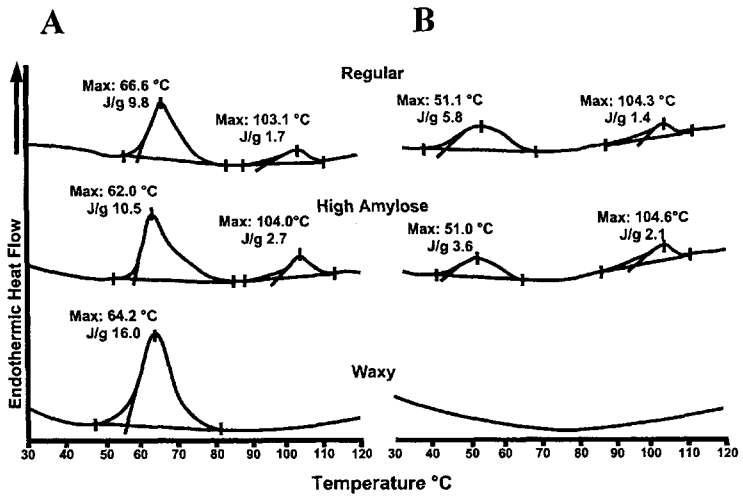


Fig. 2. DSC thermograms of prime starch from (non-waxy) regular, high-amylose and waxy barleys. A - first scan without storage; B - rescanned after two weeks of storage at 4°C.

Retrogradation of amylopectin – DSC study

The retrogradation rate of prime starch from non-waxy barley is graphically presented in Figure 3A. As indicated by the size of the enthalpy peak, it is clear that the retrogradation of amylopectin increases with storage time. After 120 hr, enthalpy of 3.74 J/g was recorded. It is interesting that the onset temperature of recrystallized amylopectin after 120 hr of storage was 38°C, almost 10°C lower than the onset of gelatinized starch. The lower temperature and lower enthalpy peak of recrystallized amylopectin indicates its less perfectly ordered structure. In waxy barley, again, the retrogradation of amylopectin did not occur during storage of the gel under the same conditions (Figure 3B). Since waxy starch did not show recrystallization enthalpy during storage in the first or second experiment, the changes in recrystallization due to rescanning of each gel 5 times are shown graphically only for starch of non-waxy barley (Figure 4A). Storage time showed a strong influence on the recrystallization of amylopectin (Figure 4A). The enthalpy value increased from 1.93 J/g after 24 hr to 3.74 J/g after 120 hr. However, a significant decrease in enthalpy was recorded when

gels were rescanned 5 times in 24 hr intervals (Figure 4B). These results indicate that not only is a certain amount of time needed to recrystallize amylopectin, but also that frequent melting can change the inner structure of amylopectin and delay retrogradation. This observation could be important to the food industry, because it may give processors an option to affect the texture of products by delaying recrystallization.

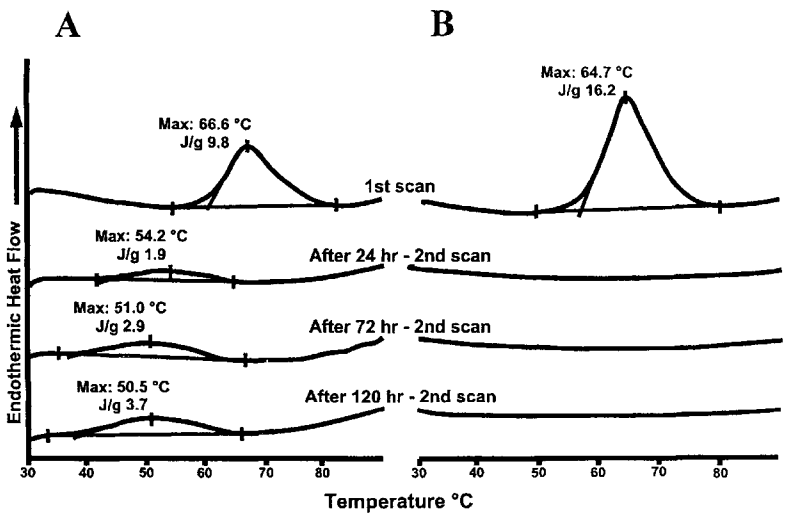


Fig. 3. DSC thermograms of starches from non-waxy (A) and waxy (B) barleys. Starches were scanned and rescanned a second time up to 120 hr at 4°C.

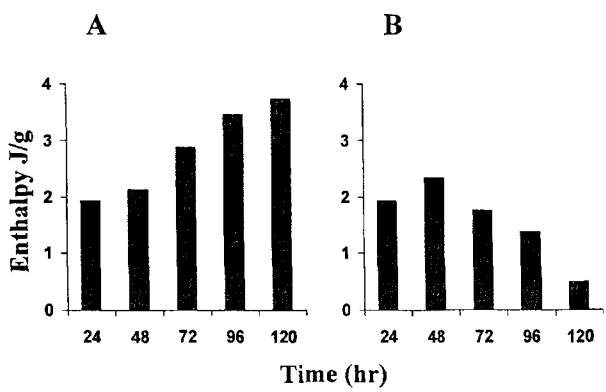


Fig. 4. Enthalpy values of retrograded starch from non-waxy barley. A - rescanned a second time after storage up to 120 hr at 4°C at 24 hr intervals. B - rescanned repeatedly at 24 hr intervals up to 120 hr.

Texture of gel

The effect of storage on texture of gels from prime starch and starch concentrate of three types of barley measured by plunger is graphically presented in Figure 5. The hardness of waxy starch gel was below 0.7 N and did not change during storage. Gels from non-waxy and high-amylose starch showed distinctly higher hardness than waxy starch, ranging from 5.8 N to 11.1 N (Figure 5A). The texture of gel from starch concentrate, also measured by plunger, showed a similar pattern, but slightly lower values (Figure 5B). Hardness of gel from waxy starch concentrate was below 0.6 N, while hardness of gels from non-waxy and high-amylose starch concentrate ranged from 5.2 N to 9.5 N. The hardness of gel from starch of non-waxy barley increased during storage from 4.1 N to 7.2 N, while hardness of gel from high-amylose starch increased from 6.6 N to 9.2 N. The higher relative value of gel hardness from high-amylose starch could be due to an almost 2 times higher amylose content in high-amylose starch than in non-waxy starch, as determined by colorimetric method. Hardness of gel prepared from prime starch and starch concentrate of non-waxy barley measured by disc are graphically presented in Figure 6. When the storage time was increased, the texture of gel increased significantly for both starch and starch concentrates. However, starch concentrate produced a softer gel due to the presence of other components. A statistically significant positive correlation was obtained between hardness storage time of gels prepared from starch and starch concentrate for non-waxy ($r = 0.997$) and for high-amylose barley ($r = 0.964$). The gradual increase in gel hardness (starch and starch concentrate) could be mainly due to retrogradation of amylopectin, especially since changes of gel hardness were greater for non-waxy than for high-amylose gel. Therefore, a relationship should exist between starch gel hardness and DSC enthalpy values during storage.

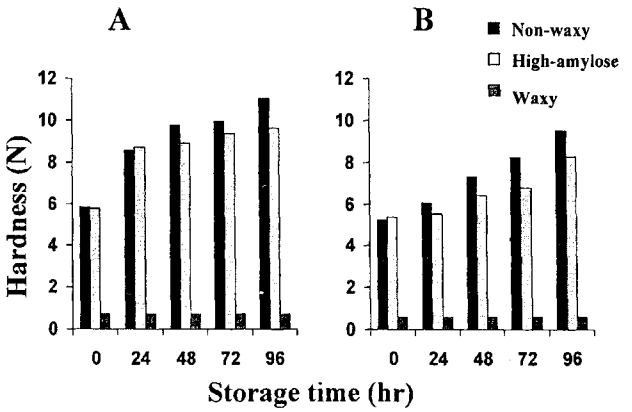


Fig. 5. Hardness of gels prepared from prime starch (A) and starch concentrate (B) measured using a TX-XT2 texture analyzer equipped with a plunger.

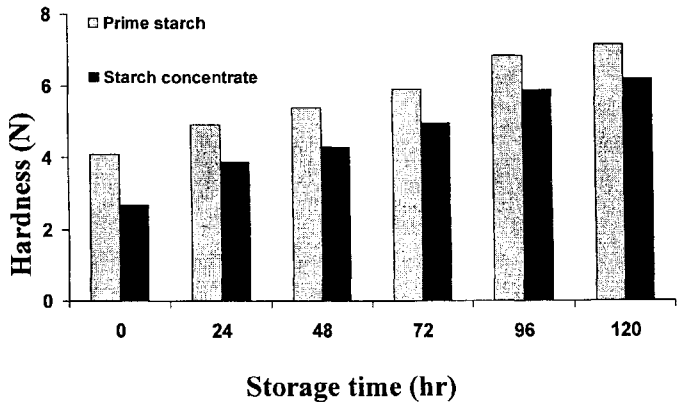


Fig. 6. Hardness of gels prepared from prime starch and starch concentrate of non-waxy barley measured using a TX-XT2 texture analyzer equipped with a disc.

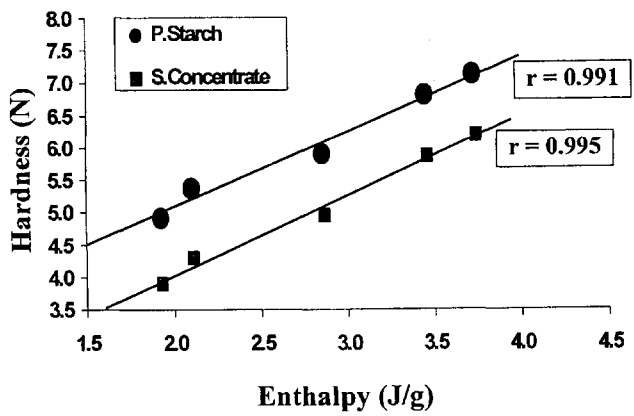


Fig. 7. Correlations between enthalpy value of prime starch and hardness of gels from prime starch and starch concentrate for non-waxy barley.

The correlations between DSC values (J/g) and gel hardness (N) for prime starch (r = 0.991) and starch concentrate (r = 0.995) of non-waxy barley are shown in Figure 7. The strong correlation between starch enthalpy values and hardness of starch concentrates indicates that the texture of starch gel concentrate is mainly due to its starch content, structure and functionality (Table I). The fact that this relationship appears for starch concentrate might be of particular importance for the food industry, because it would favor the application of barley starch concentrate without the necessity of using the wet fractionation process to isolate starch.

REFERENCES

[1] Ahluwalia B., Ellis E.E.: J. Inst. Brew., **90**, 1984, 254-260.
[2] American Association of Cereal Chemists, The Association, St. Paul, MN, 1995. Approved Methods of the AACC, 8th ed. Methods: 08-01, 30-25 and 44-15A, approved October 1975, revised October 1981.
[3] Baik B.-K., Czuchajowska Z.: Food Sci. and Technol. International., **3**, 1997, 423-435.
[4] Bradbury A.G.W., Bello A.B.: Cereal Chem., **70**, 1993, 543-547.
[5] Czuchajowska Z., Pomeranz Y.: Cereal Chem., **66**, 1989, 305-309.
[6] Czuchajowska Z., Sievert D., Pomeranz Y.: Cereal Chem., **68**, 1991, 537-542.
[7] Czuchajowska Z., Smolinski S.: Cereal Foods World, **42**, 1997, 526-532.
[8] Czuchajowska Z., Szczodrak J., Pomeranz Y.: Cereal Chem., **69**, 1992, 413-418.
[9] Knutson C.A., Grove M.J.: Cereal Chem., **71**, 1994, 469-471.
[10] Kobayashi S., Schwartz S.J., Lineback D.R.: J. Chromatogr., **319**, 1985, 205-214.
[11] Lin P.-Y., Czuchajowska Z.: Cereal Chem., **74**, 1997, 639-646.
[12] MacGregor A.W., Morgan J.E.: Cereal Chem., **61**, 1984, 222-228.
[13] Prosky L., Asp N.G., Schweizer T.F., Devries J.W., Forda I.: J. Assoc. of Anal. Chem., **71**, 1988, 1017-1023.
[14] SAS. 1986. SAS User's Guide: Statistics, 5th ed. The Institute: Cary, NC.
[15] Shuey W.C., Tipples K.H.: Am. Assoc. Cereal Chem. St. Paul, MN., 1980.
[16] Sievert D., Czuchajowska Z., Pomeranz Y.: Cereal Chem., **68**, 1991, 86-91.
[17] Szczodrak J., Pomeranz Y.: Cereal Chem., **68**, 1991, 589-596.
[18] Yuan R.C., Thompson D.B., Boyer C.D.: Cereal Chem., **70**, 1993, 81-89.

SKŁAD, WŁAŚCIWOŚCI TERMICZNE I TEKSTURA ŻELU SKROBI SUPERIOR I SECUNDA Z FASOLI GARBANZO I GROCHU

Streszczenie

Otrzymano frakcje skrobi superior i secunda z fasoli garbanzo i grochu i określono ich właściwości fizyczne, termiczne oraz właściwości żelu z nich. W skrobi z fasoli garbanzo gałeczki o rozmiarach poniżej 35 μm stanowiły 85%, w skrobi grochowej gładkich odmian Latah 66,8% i SS Alaska tylko 18,4% ogólnej liczby gałeczek. W skrobi superior z garbanzo było 35,9% amylozy, w skrobiach grochowych powyżej wymienionych gładkich odmian amylozy było odpowiednio 44,5 i 48,8% a w skrobi z grochu pomarszczonej odmiany Scout amylozy było aż 86,0%. Skrobie secunda miały o co najmniej 8% więcej amylozy niż poprzednie. Endotermiczne entalpie dla skrobi z garbanzo i grochowych superior z odmian Latah i SS Alaska mieściły się w zakresie 12,1 do 14,2 J/g podczas gdy dla takiej frakcji ze skrobi Scout entalpia wynosiła zaledwie 1,1 J/g. Endotermiczna entalpia ze skanningowej kalorymetrii różnicowej i amylograficzne właściwości kleikujące skrobi superior wyraźnie zależały od zawartości amylozy ($P < 0,05$). Skrobie superior garbanzo i grochu odmian Latah i SS Alaska dawały bardzo zwarte, elastyczne żele. Skrobia superior z grochu odmiany Scout dawała mocne, lecz kruche żele. Ich twardość była wysoka wynosiła 21,8 N, a zwartość i sprężystość była niska (odpowiednio 0,29 i 0,82). Twardość żelu przechowywanego w 22 i 4°C wzrastała z zawartością amylozy w skrobi.

Z. CZUCHAJOWSKA, T. OTTO, B. PASZCZYNSKA, B.-K. BAIK

COMPOSITION, THERMAL BEHAVIOR AND GEL TEXTURE OF PRIME AND TAILINGS STARCHES FROM GARBANZO BEANS AND PEAS

Abstract

Prime and tailings starches of garbanzo beans and peas were separated and their chemical composition, physical properties, thermal behavior and gel properties were determined. Starch granules smaller than 35μm was 85% in garbanzo beans, 66.8% in a smooth pea cv. Latah and only 18.4% in a smooth pea cv. SS Alaska. Amylose content of prime starch was 35.9% in garbanzo beans, 44.5% to 48.8% in smooth peas and 86.0% in wrinkled pea cv. Scout. Tailings starch had a higher amylose content by at least 8% than its corresponding prime starch. The endothermic enthalpy value of garbanzo bean and two smooth pea prime starches ranged from 12.1 to 14.2 J/g, while prime starch from wrinkled peas gave a distinctly lower enthalpy value of 1.1 J/g. Differential scanning calorimetry endothermic enthalpy and amylograph pasting properties of prime starch were significantly related to its amylose content ($P<0.05$). Prime starches of garbanzo beans and smooth peas produced highly cohesive elastic gels. Wrinkled pea prime starch formed the strongest, though brittle, gel, as indicated by high hardness (21.8 N), low cohesiveness (0.29) and low springiness (0.82). Hardness of gel stored at 22°C and at 4°C was positively correlated with amylose content of starch.

Introduction

Legumes have been consumed traditionally as whole seeds or as a ground flour after dehulling. The rapidly growing food industry constantly demands new ingredients, which has drawn the attention of researchers to legume components obtained by the wet fractionation process (Schoch and Maywald 1968, Czuchajowska and Pomeranz 1994). At present there is a strongly visible public interest in natural, unmodified sources of food ingredients. For example, unmodified starch is gaining attention because labeling a food product as "natural" is attractive to consumers. The fractionation of legumes into their main components (starch, protein and fiber) is one way to increase the value of legumes by

Associate Professor, Graduate Research Assistant, Research Associate and Research Associate respectively, Department of Food Science and Human Nutrition, Washington State University, Pullman, WA 99164-6376.

broadening their application. These legume fractions could be used to supplement non-legume food products to improve their textural and nutritional value.

The purposes of this study were: to evaluate the chemical composition and thermal behavior of prime and tailings starches obtained from garbanzo beans and smooth and wrinkled peas; to determine size distribution of prime starch granules by image analysis; to examine the physical properties of prime and tailings starch gels during storage at different temperatures; and, to relate chemical composition of starches to textural properties of gels.

Materials and methods

Materials

Garbanzo bean flour, stone milled from split or broken seeds, was provided by Blue Mountain Seeds, Inc. (Walla Walla, WA). One smooth pea, cv. Latah, was obtained from Dr. F. Muehlbaur, ARS-USDA, Pullman, WA. A second smooth pea, cv. SS Alaska, and a wrinkled pea, cv. Scout, were purchased from Crites Co. (Moscow, ID).

Preparation of starch

Garbanzo bean flour, with particle size smaller than 86 μm, and pea flours, a blend of three breaks and first reduction, were fractionated into prime starch, tailings starch and water solubles according to the method of Otto et al (1997a).

Chemical characteristics of isolated legume starches

Protein content (N x 6.25) of both prime and tailings starches was determined by a Leco instrument (Leco Corp., St. Joseph, MI) equipped with a thermoconductivity detector. Moisture content was determined by oven-drying for 1 hr at 130 °C (AACC Method 44-15A 1995), ash by dry combustion for 16 hr at 580°C (AACC Method 08-01, 1995) and free lipids by petroleum ether extraction, followed by evaporation to constant weight under vacuum (AACC Method 30-25 1995). Insoluble and soluble dietary fiber were determined by the procedure of Prosky et al (1988). Starch content was determined after its enzymatic conversion to glucose by successive treatment with α-amylase, protease and amyloglucosidase (Prosky et al 1988). The released glucose was measured with the glucose oxidase-peroxidase reagent (Lloyd and Whelan 1969). Amylose content of starch was determined by the procedure of Knutson and Grove (1994).

Physical properties of starch

The size distribution of the garbanzo bean and smooth pea starches was determined by image analysis. The granule image was transferred from an Olympus BH-2 photo microscope to a Quadra 950 Macintosh computer via a Pulnix BW CCD camera (Pulnix, Sunnyvale, CA). Each image was analyzed by NIH Image Analysis version 1.52 (Be-

thesda, MD) and converted to micrometers. Five hundred granules of each starch were measured by free-hand circling on the computer screen.

Differential Scanning Calorimetry (DSC)

DSC characteristics of starches were determined with a DSC-4 instrument (Perkins-Elmer Corp., Norwalk, CT). An indium standard was used for temperature and enthalpy calibration. A 10 mg sample and 20 µl of distilled water were placed in a stainless steel capsule, sealed and allowed to equilibrate for 30 min at room temperature. The sample was then heated from 20°C to 180°C at a rate of 10°C per min. A capsule with inert material ($Al_2 O_3$) and water served as the reference sample. For each endothermic curve, temperature of transition onset, peak and completion were determined by data processing software. The transition enthalpy was calculated from the peak area by software and expressed as joules per gram (J/g) of dry matter.

Amylograph

Pasting properties of starch were determined with a Brabender amylograph by a modification of the method of Shuey and Tipples (1980).

Starch gel texture

Starch slurry in distilled water (8%) was prepared and heated at a rate of 1.5°C/min to 93.5°C using the Brabender viscoamylograph and held at that temperature for 10 min. The paste was then poured into eight individual cylindrical stainless steel molds (3.5 cm height and 3.5 cm inside diameter). Gels for wrinkled pea starch were prepared by autoclaving starch slurry (8%) at 126°C for 1 hr. Duplicate gels were stored for 24 hr and 72 hr at 22°C and 4°C. The gels to be stored for 72 hr at 22°C were vacuum packaged in gas impermeable plastic bags to minimize microbial growth.

The gel texture was then evaluated at least in duplicate by texture profile analysis (TPA) using the TA-XT2 Texture Analyzer (Stable Micro Systems, Haslemeres, England). Each cylindrical gel was placed upright on a metal plate and compressed at a rate of 1.0 mm per sec to 30% of its original height using a 5 cm diameter metal disk. The compression was repeated twice to generate a force-time curve from which hardness (height of the first peak) and springiness (ratio between recovered height after the first compression and the original gel height) were determined. Cohesiveness was calculated as the ratio between the area under the second peak and the area under the first peak (Bourne 1968, Friedman et al 1968).

Statistical analyses

All physical and chemical measurements of samples at each treatment were performed at least in duplicate. Analysis of variance (ANOVA) and least significant differ-

ences (LSD) were calculated by the Statistical Analysis System (SAS, 1986). Significance was defined at the 5% level.

Results and discussion

Characteristics of starches

Chemical compositions of both prime and tailings starches separated from garbanzo beans and peas are summarized in Table 1. Starch content of prime starch determined by enzymatic assay ranged from 97.5% in the wrinkled pea cv. Scout to 99.4% in the smooth pea cv. SS Alaska. Protein and ash contents of prime starches from garbanzo beans and smooth pea cvs. Latah and SS Alaska were lower than 0.35% and 0.19%, respectively, indicating that isolated starches were very pure. The prime starch of wrinkled pea cv. Scout had significantly higher protein (0.96%) and ash (0.31%) content than did the prime starch from garbanzo beans and smooth peas. This result could be explained by the difficulty in the fractionation process of wrinkled pea cv. Scout due to its high fiber content and composite starch granules (Otto et al 1997a).

Table 1

Characteristics of prime and tailings starches from garbanzo beans and peas[a]

Starch	Starch (%)	Protein[b] (%)	Ash (%)	Total Fiber (%)	Amylose (%)
Prime Starch					
Garbanzo Bean	99.1	0.17	0.17	trace	35.9
Smooth Pea					
cv. Latah	99.0	0.33	0.01	trace	44.5
cv. SS Alaska	99.4	0.35	0.08	trace	48.8
Wrinkled Pea					
cv. Scout	97.5	0.96	0.31	trace	86.0
LSD	0.66	0.28	0.01	...	0.54
Tailings Starch					
Garbanzo Bean	38.5	7.22	2.08	51.9	49.1
Smooth Pea					
cv. Latah	64.8	7.61	1.29	26.2	52.6
cv. SS Alaska	63.2	4.55	1.37	30.6	57.0
Wrinkled Pea					
cv. Scout	45.1	10.20	1.21	43.1	94.0
LSD	0.42	1.19	0.11	0.69	0.75

[a]Results expressed on a dry weight basis.

[b]N x 6.25.

Contrary to the high purity of prime starches, tailings starches from garbanzo beans and peas contained considerable amounts of protein, ash and fiber. The starch content of isolated tailings starches was over 63.2% in the two smooth peas, 45.1% in wrinkled pea

cv. Scout and 38.5% in garbanzo beans. The protein content of tailings starches ranged from 4.6% to 10.2%, ash from 1.21% to 2.08% and fiber from 26.2% to 51.9%. The high concentration of these components indicates that tailings starches are a mixture of starch granules, cell wall materials and insoluble proteins.

Amylose content was 35.9% for garbanzo bean prime starch, 44.5% for cv. Latah and 48.8% for SS Alaska (Table I). Wrinkled pea cv. Scout had the highest amylose content (86.0%) in prime starch. Amylose content of tailings starch was always higher by at least 8% than that of prime starch in both garbanzo beans and pea cultivars. This can be explained by the large population of small-sized granules within the tailings starch. The smaller starch granules are typically higher in amylose than are larger granules (Szczodrak and Pomeranz 1991, MacGregor and Fincher 1993).

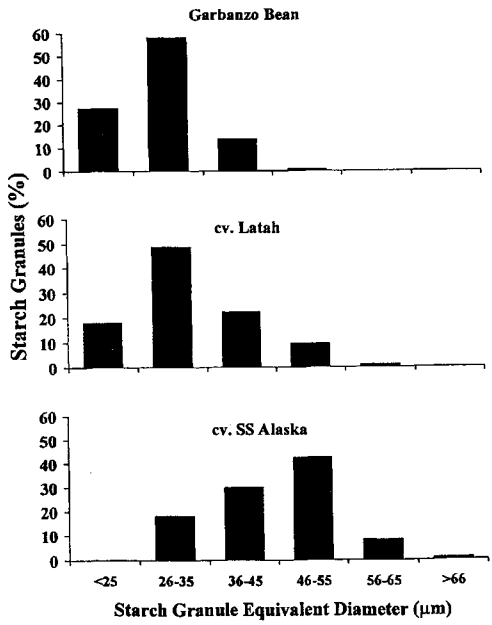


Fig. 1. Size distribution of prime starch granules from garbanzo beans and smooth pea cvs. Latah and SS Alaska determined by image analysis.

Size distributions of prime starch granules from garbanzo beans and the two smooth pea cultivars, determined by image analysis, are graphically presented in Figure 1. Garbanzo bean prime starch granules were smaller than those of smooth peas and ranged from 25 μm to 55 μm in diameter. Granules smaller than 35 μm represented 85% of total garbanzo bean starch. While granules smaller than 25 μm made up 27.2% in garbanzo bean starch, granules larger than 45μm were only 1%. Prime starch from cv. Latah represented a broader range of granule size than that from garbanzo beans, ranging from 25 μm to

65 μm, with a dominant granule size of 26–35 μm. Starch from cv. SS Alaska covered the broadest range of granule size, from 26 μm to greater than 66 μm, with 40% of the granules ranging from 46 to 55 μm. Distribution of starch granules from the wrinkled pea cv. Scout was not determined, as many of the compound granules were broken, leaving small granular pieces that were difficult to distinguish from the intact granules.

Thermal behavior of starches

The thermal behavior of prime and tailings starches was evaluated by DSC and amylograph tests. The DSC transition temperatures and enthalpy values of prime and tailings starches are given in Table 2. The onset temperature of prime starch of garbanzo beans did not differ significantly from that of smooth or wrinkled peas. However, the transition temperature of wrinkled pea starch was about 6°C higher than for garbanzo beans and the two smooth peas. Garbanzo beans had the highest endothermic enthalpy value of starch (14.2 J/g), followed by smooth pea cvs. SS Alaska (12.8 J/g) and Latah (12.1 J/g).

Table 2

Differential scanning calorimetry characteristics of prime and tailings starches from garbanzo beans and peas[a]

Starch	T_o[b] (°C)	T_p[c] (°C)	ΔH[d] (J/g)
Prime Starch			
Garbanzo Bean	62.8	68.1	14.2
Smooth Pea			
cv. Latah	62.1	68.6	12.1
cv. SS Alaska	61.4	67.7	12.8
Wrinkled Pea			
cv. Scout	62.4	74.7	1.1
LSD[e]	0.87	1.33	1.83
Tailings Starch			
Garbanzo Bean	60.5	73.2	1.5
Smooth Pea			
cv. Latah	58.0	69.5	5.4
cv. SS Alaska	56.6	67.2	4.6
Wrinkled Pea			
cv. Scout	70.9	86.8	0.8
LSD	2.26	3.45	1.22

[a]Values are averages of two replications.
[b]T_o = onset temperature.
[c]T_p = peak temperature.
[d]_H = transition enthalpy.
[e]Least significant difference (P = 0.05). Differences between two means exceeding this value are significant.

Prime starch of wrinkled pea cv. Scout gave the lowest enthalpy value of 1.1 J/g due to its high amylose content (86%). The highest enthalpy value of garbanzo bean starch was due to its high amylopectin content. Prime starches from the two smooth peas, which had a lower enthalpy value by at least 1.4 J/g than that of garbanzo beans, also had approximately 10% more amylose content (Table 1).

Transition enthalpies of tailings starches were significantly lower than those of corresponding prime starches. Several factors can explain the differences in enthalpy values between prime and tailings starches: total starch content (Table 1), size and mechanical damage of starch granules (Otto et al 1997b), amylose content (Table 1) and interactions between other tailings starch components. These differences in transition enthalpy between prime and tailings starches from garbanzo beans and peas are visualized by the size and shape of DSC enthalpy curves (Figure 2). Prime starches from garbanzo beans and smooth peas, consisting of pure and undamaged granules with at least 52% amylopectin, showed narrow and well defined endothermic peaks. Tailings starches contained small and damaged granules admixed with insoluble protein and fiber, and showed rather flat and broad DSC endothermic curves.

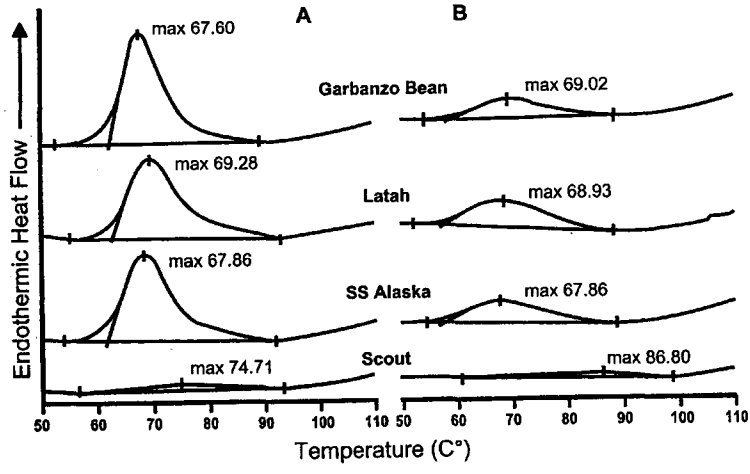


Fig. 2. Differential scanning calorimetry thermograms of prime starch (A) and tailings starch (B) from garbanzo beans, smooth pea cvs. Latah and SS Alaska and wrinkled pea cv. Scout.

Pasting characteristics of the legume starches, as determined by amylograph, are shown in Table 3. Prime starches of garbanzo beans and both varieties of smooth peas had similar patterns of pasting curves. Pasting viscosity at 93.5°C was 395 BU in garbanzo beans and 305 BU and 490 BU in the smooth pea cvs. Latah and SS Alaska, respectively. During 30 min holding at 93.5°C with constant shear, viscosity increased by over 70 BU. During the cooling period from 93.5°C to 50°C, set-back was extensive, as shown by the

large increases in viscosity. Pasting viscosity further increased during the holding period at 50°C with constant shear. Wrinkled pea cv. Scout showed no viscosity development, as temperatures were not high enough for the starch granules to fully swell and gelatinize the granules.

Table 3

Amylograph pasting properties of prime and tailings starches from garbanzo beans and peas

Starch	Viscosity (BU)			
	at 93.5°C 0 min	at 93.5°C 30 min	at 50°C 0 min	at 50°C 30 min
Prime Starch				
Garbanzo Bean	395	495	740	800
Smooth Pea				
cv. Latah	305	377	655	750
cv. SS Alaska	470	540	910	1050
Wrinkled Pea				
cv. Scout	0	0	5	10
Tailings Starch				
Garbanzo Bean	80	170	218	200
Smooth Pea				
cv. Latah	370	455	595	575
cv. SS Alaska	255	337	417	405
Wrinkled Pea				
cv. Scout	5	5	18	18

Pasting properties of tailings starches from garbanzo beans and smooth peas were generally lower than corresponding prime starches and showed lower stability during the holding period at 50°C (Table 3). The higher viscosities of tailings starches from smooth pea cvs. SS Alaska and Latah than that of garbanzo beans could be due to their higher starch content. The viscosity development in the tailings starch of cv. Scout was due to protein and cell wall components, since starch did not contribute to any viscosity as shown in prime starch (Table 3).

Characteristics of starch gels

The TPA parameters, hardness, cohesiveness and springiness of starch gels varied broadly among starches within each storage temperature (Table 4). Hardness of prime starch gels, an important textural parameter reflecting the strength of gel, was 7.9 N in garbanzo beans, 11.4 N in cv. Latah and 11.1 N in cv. SS Alaska, when stored at 22°C. The prime starch gel of wrinkled pea cv. Scout showed a significantly lower hardness value of only 0.98 N because of incomplete gelatinization of starch at 93.5°C. However,

when the prime starch slurry of cv. Scout was autoclaved at 126°C for 1 hr, a strong gel with a hardness value of 21.8 N was formed. The hardness of gels stored at 4°C followed the same pattern as those stored at 22°C. However, due to facilitated retrogradation at 4°C, the hardness of gels increased by 3.8 N for garbanzo beans, 5.3 N for smooth pea cv. Latah, 8.4 N for cv. SS Alaska and 4.7 N for autoclaved starch of wrinkled pea cv. Scout. A strong positive correlation was obtained between amylose contents and hardness of gels. Correlation coefficients were 0.965 for gels stored at 22°C and 0.967 for gels stored at 4°C. Cohesiveness, which reflects gel structure, was highest in garbanzo beans (0.95), followed by cv. SS Alaska (0.91) and cv. Latah (0.88). Storing gels at 4°C did not significantly affect cohesiveness. The lowest gel cohesiveness value (0.29) for cv. Scout indicated that the gel was very strong but brittle. Springiness of gels from garbanzo beans and smooth peas was over 0.96, indicating high recovery of gel height after first compression. Gels from cv. Scout showed a much lower springiness value than others, as could be expected from its low cohesiveness value.

Table 4

Texture profile analysis parameters of prime starch gels stored at 22°C and 4°C for 24 hrs[a]

Starch sample	Hardness (N)		Cohesiveness (ratio)		Springiness (ratio)	
	22°C	4°C	22°C	4°C	22°C	4°C
Garbanzo Bean	7.90	11.7	0.95	0.94	0.99	0.98
Smooth Pea						
cv. Latah	11.4	17.7	0.88	0.84	0.96	0.95
cv. SS Alaska	11.1	19.5	0.91	0.85	0.98	0.97
Wrinkled Pea						
cv. Scout	0.98	1.10	0.39	0.43	0.66	0.64
cv. Scout (AC[b])	21.8	26.5	0.29	0.29	0.82	0.76
LSD[c]	0.96	1.35	0.05	0.03	0.02	0.04

[a]Gels contain 8% starch on a dry weight basis.

[b]Autoclaved at 126°C for 1 hr.

[c]Least significant difference (P=0.05). Differences between two means exceeding this value are significant.

The increase in hardness due to accelerating retrogradation during storage at 4°C for all legume prime starch gels is shown graphically in Figure 3. Statistically significant increases in gel hardness were obtained for all gels by extending storage from 24 hr to 72 hr.

Gel hardness of tailings starches from smooth and wrinkled peas stored at 22°C and 4°C for 24 and 72 hr are shown in Figure 4. Garbanzo bean tailings starch, containing less than 40% starch, did not form a gel. The large differences in composition of tailings starches originating from different legumes (Table 1) make it difficult to compare gel texture. The hardness of tailings starch gels stored at 4°C was higher than for those stored at

22°C. Storage time also had a significant effect on gel hardness (except cv. Latah). These changes in hardness due to storage temperature and time indicate that starch plays an important role in the textural properties of tailings starch gel.

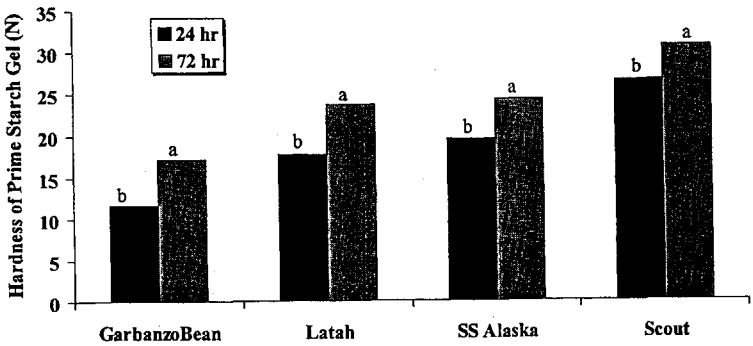


Fig. 3. Hardness of prime starch gels from garbanzo beans, smooth pea cvs. Latah and SS Alaska and wrinkled pea cv. Scout stored at 4°C for 24 and 72 hr. Gels for each legume starch were prepared by heating to 93.5°C for 10 min, except cv. Scout, which was prepared by autoclaving at 126°C for 1 hr. The letters at the top of each bar indicate significant differences at the 5% level.

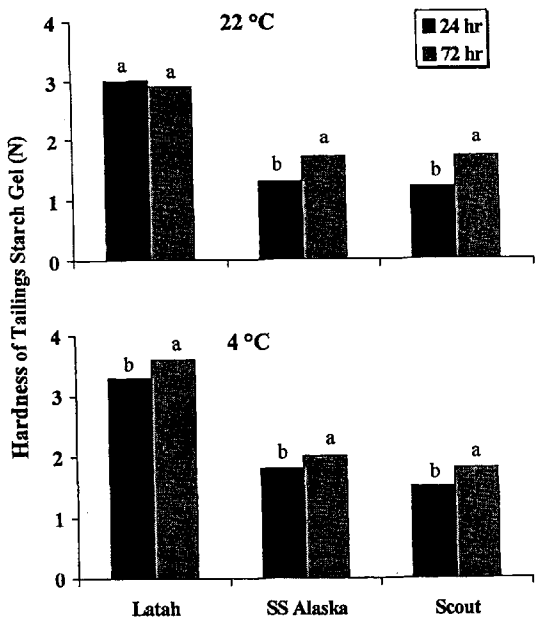


Fig. 4. Hardness of tailings starch gels from smooth pea cvs. Latah and SS Alaska and wrinkled pea cv. Scout stored at 22°C and 4°C for 24 and 72 hr. Gels for each legume tailings starch were prepared by heating to 93.5°C 10 min, except cv. Scout, which was autoclaved at 126°C for 1 hr. The letters at the top of each bar indicate significant differences between storage times at the 5% level.

REFERENCES

[1] American Association of Cereal Chemists, Approved Methods of the AACC, 8th ed., Method 44-15A, approved October 1975, revised October 1981; Method 08-01, approved April 1961, revised October 1981; Method 30-25, approved April 1961, revised October 1976, October 1981; Method 76-31, approved September 1992, revised October 1994. The Association: St. Paul, MN, 1995.
[2] Bourne M.C.: J. Food Sci., **33**, 1968, 223-226.
[3] Czuchajowska Z., Pomeranz Y.: U.S. Pat. 5,364,471, 1994.
[4] Friedman H.H., Whitney, J.E., Szczesniak A.S.: J. Food Sci., **28**, 1968, 390-396.
[5] Knutson C.A., Grove M.J., Cereal Chem., **71**, 5, 1994, 469-471.
[6] Lloyd J.B., Whelan, W.J.: Anal. Biochem., **30**, 1969, 467-470.
[7] MacGregor A.W., Fincher G.B.: Barley Chemistry and Technology. A.W. MacGregor and R.S. Bhatty (Eds.), Am Assoc. Cereal Chem., St. Paul, MN, 1993, 73-130.
[8] Otto T., Baik B.-K., Czuchajowska Z.: Cereal Chem., **74**, 1997a., 141-146.
[9] Otto T., Baik B.-K., Czuchajowska Z.: Cereal Chem., **74**, 1997b., 445-451.
[10] Prosky L., Asp N.G., Schweizer T.F., Devries J.W., Furda J.: J. Assoc. Off. Anal. Chem., **71**, 1988, 1017-1023.
[11] SAS User's Guide: Statistics, SAS Institute, Inc., Statistical Analysis Systems Institute, Cary, NC, 1985.
[12] Schoch T.J., Maywald E.C., Cereal Chem., **45**, 1968, 564-573.
[13] Shuey W.C., Tipples K.H., eds.: The Amylograph Handbook, Am. Assoc. Cereal Chem., St. Paul, MN, 1980.
[14] Szczodrak J., Pomeranz, Y.: Cereal Chem., **68**, 6, 1991, 589-596.

STRUKTURA I FUNKCJONALNOŚĆ SKROBI JĘCZMIENNYCH

Streszczenie

Zawartość amylozy w zwykłych i wysoko amylozowych skrobiach jęczmiennych oznaczono metodą kolorymetryczną. Ustalono, że wynosi ona odpowiednio 24,6 i 48,7%, natomiast w skrobi woskowej znaleziono zaledwie ślad (0,04%) amylozy. Amylopektyna pozbawiona rozgałęzień izoamylazą mało różniła się od zwykłych i wysoko amylozowych skrobi jęczmiennych, podczas gdy amylopektyna z woskowej skrobi jęczmiennej miała wyraźnie mniej, bo 45%, frakcji o wysokim stopniu spolimeryzowania (DP < 15).

Skrobia woskowa, normalna i wysoko amylozowa miały różne dyfraktogramy proszkowe. Skrobia woskowa wykazywała ostrzejsze piki przy 0,58; 0,51; 0,49 i 0,38 nm niż skrobia zwykła i i wysoko amylozowa. Odstęp *d* przy 0,44 nm charakteryzujący kompleksy amylozowo-lipidowe był najwyraźniejszy w przypadku skrobi wysoko amylozowej i nie pojawiał się w widmach skrobi woskowej.

Termogramy DSC skrobi zwykłej i wysoko amylozowej miały dwa wyraźne piki przy 60°C odpowiadający kleikowaniu skrobi i powyżej 100°C dotyczący kompleksu amylozowo-lipidowego. Skrobia z jęczmienia woskowego dawała tylko jeden endotermiczny pik kleikowania amylopektyny o entalpii 16,0 J/g.

Retrogradacja skrobi skleikowanej trzech typów jęczmienia przechowywanej w 4°C pokazała, że szybkość rekrystalizacji amylpektyny ze skrobi zwykłej i wysoko amylozowej była porównywalna gdy entalpię rekrystalizacji obliczano na procentową zawartość amylopektyny. W skrobi woskowej nie zaobserwowano piku rekrystalizacji amylopektyny.

Czas przechowywania miał duży wpływ na rekrystalizację amylopektyny. Entalpia dla zwykłej skrobi wzrosła z 1,93 J/g po 24 h przechowywania do 3,74 J/g po 120 h. Gdy żel mieszano co 24 h entalpia wyraźnie spadała.

Stwierdzono wysoce statystycznie istotną korelację (r = 0,991) między parametrami DSC retrogradowanej skrobi zwykłej i twardościa żeli z niej. Korelacja między entalpiami i twardością żeli wskazuje, że struktura żelu zależała przede wszystkim od struktury i funkcjonalności skrobi. Zależność między właściwościami skrobi i stężeniem skrobi może powodować, że przy produkcji pożądanych kleików korzystniejsze będzie operowanie stężeniem skrobi bez konieczności stosowania frakcjonowania skrobi na mokro.

S.R. ERLANDER

BIOSYNTHESIS OF STARCH

Abstract:

Evidence is presented which supports the proposal (Erlander, S.R., Enzymologia, 1958, 19, 273-283) that plant glycogen is a required intermediate in the synthesis of starch. Its synthesis temporarily ceases in a specific cell after a period of about three days, and at that point debranching enzymes are then activated which remove its exterior A-chains. The partially debranched glycogen is amylopectin and the removed branches are degraded by soluble starch synthases (SSS I and II) to produce ADPglucose (ADPGlu) which is the sole source of glucose for the production of amylose by the granular bound starch synthase (GBSS). Two independent cytosol/plastid transport systems activate either phosphorylase (from transported ADPGlu) or its back-up system SSS II (from transported Glu-6-P). Both use ADP glucose pyrophosphorylase in the synthesis of glycogen. The linear chains of debranched amylopectin have the narrow *Poisson* size distribution, whereas those of linear amylose have the broad size distribution of an A-B condensation polymer. Thus amylose can not be a precursor to amylopectin. Inner, short A-chains, located particularly on the 3rd and 5th tiers of the precursor glycogen, account for changes in the A/B chain ratio and for clusters.

Introduction

About forty years ago it was proposed [1] that starch is synthesized in the plant, not by first synthesizing amylose-type chains, but rather by first synthesizing a highly branched plant glycogen from which both amylose and amylopectin are produced. Thus it was proposed [1] in 1958 that there must exist in the plant a debranching enzyme which removes exterior A-chains from this precursor glycogen and then allows these removed chains to be converted into amylose. The partially debranched glycogen then becomes, with its elongated exterior chains, amylopectin. And the newly created and elongated exterior chains, which now have become about twice the length of the average distance between branch points, allows the amylopectin to retrograde (form double helices) and thus to form starch granules. As pointed out by Wursch and Gumy [2], any exterior amylopectin chain which is equal to or less than eleven glucose units long will not participate in a retrogradation process. Thus starch can not form unless this partial debranching occurs first.

Erlander's Natural Products, P.O. Box 106, Altadena, CA 91003 USA

About eleven years after the proposal of this glycogen precursor mechanism, the predicted, essential debranching enzymes were discovered [3-5] in sweet corn. Thus as stated in 1984 by Pan and Nelson [5]: "We believe that Erlander's hypothesis that sugary maize is defective in debranching enzyme activity is correct, but not his conjecture that phytoglycogen is a normal intermediate in starch synthesis." However, if all of the debranching enzymes are removed by genetic manipulation, then according to the proposed mechanism, the only product which should be formed is the precursor glycogen *if* the glycogen is indeed a "normal intermediate." And this is exactly what Mouille, et.al., [6] discovered in 1996 when they genetically eliminated the debranching enzyme and consequently found only a 9% branched polysaccharide and no starch (no amylopectin *and* no amylose). Further support of the proposed glycogen precursor mechanism was given when it was shown [7] that plant glycogen exists, not only in sweet corn, but also exists in dent, waxy and *ae* corn. In addition, other experimental data, as given in reviews both recently [8-10] and previously [11], also support this mechanism.

Furthermore, it is now known that the linear chains of amylose are synthesized by the granular bound starch synthase (GBSS). As pointed out previously [10, 11], there exists a quantitative relationship between the amount of branches removed from the glycogen and the amount of amylose found in starch granules. In order to retain this relationship and prevent glucose obtained from these debranched chains from entering the general pool for further glycogen synthesis, it was proposed [11] in 1970 that these undegraded chains must be connected together by some unknown mechanism to form amylose. In the extended glycogen precursor mechanism [10], it is shown that this previous explanation is not necessary since this quantitative relationship can be maintained even though the removed branches are degraded to glucose if it is assumed that the synthesis of the glycogen precursor is halted after three or four days of synthesis in a *specific* cell and before this glycogen is converted into starch.

The role of phosphorylase in starch synthesis

Initially, phosphorylase plus branching enzymes were proposed [1] as those enzymes which produced the precursor glycogen. However, it has now been generally concluded by others that the soluble starch synthases (SSS I and SSS II) produce the linear chains from ADPglucose (ADPGlu) using ADPglucose pyrophosphorylase (ADPGlu pp) and glucose-1-phosphate (Glu-1-P). In contrast, mechanisms [10] presented here illustrate that phosphorylase is still valid as the primary enzyme for the production of linear chains in the precursor glycogen and incorporate ADPGlu pp, which, as pointed out by Preiss [12], is essential for the synthesis of starch.

It was discovered by Akazawa and his group [13] that a translocator exists for the transfer of ADPGlu from the cytosol into the plastid. Afterwards Mohlmann, et al. [14] and Chen, et al. [15] observed that ADPGlu pp exists in both the cytosol and the plas-

tid and thus Glu-1-P could be converted into ADPGlu in the cytosol. In addition, Mohlmann, et al. [14], showed that two independent transport systems are used for starch synthesis: one for ADPGlu and another for glucose-6-phosphate (Glu-6-P). Based on this and other information, it was concluded [10] that these two independent transport systems lead to two mechanisms for the synthesis of starch: 1) the transport of ADPGlu involves phosphorylase, considered here as the primary mechanism, and 2) the transport of Glu-6-P involves SSS II, considered here as the back-up system.

The validity of using phosphorylase for starch synthesis has been supported by the recent results of Duwenig, et.al. [16] as well as by other results [10]. In addition, the SSS II enzyme can produce a polymodal debranched amylopectin, whereas the SSS I enzyme does not [8-10]. Consequently, since native starch contains a polymodal-type amylopectin, then this observation rules out the use of SSS I in starch synthesis and thus allows the SSS II system to be the potential back-up for starch synthesis if the phosphorylase system fails.

The glycogen precursor mechanism

The production of either ADPGlu or Glu-6-P in the cytosol is initiated by sucrose synthase which converts sucrose into UDPGlu plus fructose. These two components can then be converted into either ADPGlu or Glu-6-P. Using cytosolic ADPGlu pp and the enzymatic systems of Schaffer and Petreikov [17], then UDPGlu and fructose are changed into ADPGlu in the cytosol as follows: UDPGlu→Glu-1-P (using UDPGlu pp) →ADPGlu (using ADPGlu pp); and fructose→fructose-6-P (using fructokinase) →Glu-6-P (using phosphoglucoisomerase) →Glu-1-P (using phosphoglucomutase) →ADPGlu (using ADPGlu pp). Also for the back-up system, the sucrose could be converted into Glu-6-P in the cytosol by use of the same enzyme-type systems. Either ADPGlu or Glu-6-P is then transported into the plastid.

Using the phosphorylase as the primary mechanism, it is proposed [10] that the transported ADPGlu is converted into glycogen in the plastid by the following mechanism:

$$\text{ADPGlu} + \text{PPi} \rightarrow \text{ATP} + \text{Glu-1-P} \quad \text{(using ADPGlu pp)} \qquad (1)$$

$$\text{Glu-1-P} + G_{n-1} \rightarrow P_i + G_n \text{ (glycogen) (using phosphorylase and branching enzymes)} \qquad (2)$$

In the back-up system, the Glu-1-P (from transported Glu-6-P) is used by reversing Eq.1:

$$\text{Glu-1-P} + \text{ATP} \rightarrow \text{PPi} + \text{ADPGlu} \quad \text{(using ADPGlu pp)} \qquad (3)$$

$$\text{ADPGlu} + G_{n-1} \rightarrow \text{ADP} + G_n \text{ (glycogen) (using SSS II and branching enzymes)} \qquad (4)$$

Thus both systems rely on the critical ADPGlu pp enzyme to produce the glycogen.

It should be noted that in order to transfer the ADPGlu from the cytosol to the

plastid, there is involved an exchange with AMP [14]. In addition, the transfer of Glu-6-P from the cytosol to the plastid involves a counter exchange with Pi. In the case of the ADPGlu transport mechanism the production of the AMP can occur from fat metabolism in the plastid:

$$ATP + RCO_2H + HS\text{-}CoA \rightarrow AMP + PPi + RCO\text{-}S\text{-}CoA + H_2O$$

The summary of the reaction would be: ATP → AMP + PPi. Thus this reaction could supply the needed AMP for the translocation of the ADPGlu and in addition the needed PPi for Eq. 1. Plus the production of protein in the cytosol with the use of transfer RNA produces the same result: ATP → AMP + PPi. Extra PPi is also generated in the cytosol in the conversion of fructose to ADPGlu. The PPi could thus be supplied by a counter transfer with Pi. That is, it is known that Pi is involved in a homo transfer with itself (Pi) or in a hetero transfer with Glu-6-P [14]. Hence, it is possible that this same translocator could also transfer PPi with a counter transfer of Pi. In addition, AMP could be produced with the use of plastid myokinase: ADP → ATP + AMP [14]. Thus the necessary ingredients for Eq. 1 can be produced.

Each transfer mechanism is independent and it appears that each suppresses the other. Hence, when ADPGlu transport is used to produce starch, it suppresses the use of the Glu-6-P transport mechanism and vice-versa. With respect to the Glu-6-P transport mechanism, Eq. 3 shows that ADPGlu is produced which in turn can suppress phosphorylase (Eq. 2) which is used by the ADPGlu transport system. In addition, the production of Pi in Eq. 2 for the ADPGlu could compete with the transfer of Glu-6-P from the cytosol from the homo transfer of Pi/Pi or the hetero transfer of PPi/Pi.

Mohlmann, et al. [14] were puzzled as to why ATP inhibits starch synthesis through the ADPGlu transport mechanism, whereas ATP enhances and is required for the Glu-6-P transport mechanism. This apparent contradiction can be explained by the use of the phosphorylase system (Eqs. 1, 2) since now ATP becomes a product (Eq. 1) rather than a substrate (Eq. 3). If excess ATP is added to the ADPGlu transport mechanism, then that excess ATP will suppress starch synthesis by partially reversing Eq. 1. The result adds further proof for the proposed mechanism.

It was concluded previously [18] from a study of radioactive data on starch synthesis, particularly from the data given by Whistler and Young [19], that the synthesis of the plant glycogen occurs over a period of three or four days. After the glycogen synthesis reaches a critical point (perhaps at a maximum concentration of glycogen for the plastid), then it was proposed [10] that the enzyme system ADPGlu pp, and consequently the synthesis of glycogen, is stopped. After the branching enzymes have further branched the external chains of the newly synthesized glycogen without the addition of more glucose units (the production of short, external "stub" branches), then – and only then – are the debranching enzymes released, which in turn removes the stub

branches plus the newly formed A-chains to produce amylopectin.

Thus it is proposed that the resulting glycogen, from either the phosphorylase or the SSS II system, is converted into amylopectin plus (in non-waxy plants) amylose as follows:

G_n (glycogen) $\rightarrow$ G_{n-x} (amylopectin) + G_x (removed branch) (using debranching enzymes) (5)

G_x (removed branch) + ADP $\rightarrow$ G_{x-1} + ADPGlu (using SSS I and SSS II) (6)

ADPGlu (from removed branches only) + G_a (amylose) $\rightarrow$ ADP+ G_{a+1} (using GBSS) (7)

Hence, the partially debranched precursor glycogen becomes amylopectin (Eq. 5) and the removed branches become amylose (Eq. 6 and 7). The proposal that the synthesis of the precursor glycogen is stopped before the release of the debranching enzymes ensures that the only glucose units used in the synthesis of amylose are those glucose units which come from the removed branches. In this manner, as proposed initially [1] in 1958, there is established a quantitative relationship between the yield of amylose and the extent of debranching of the precursor glycogen.

The three or four day period for glycogen synthesis based on radioactive studies

The proposed mechanism [10] can explain why the maximum in yield of glycogen and the midpoint in the increase in the yield of starch both occurred at 7 p.m. (1900) for the 26th day sweet corn [20]. In addition, the mechanism explains why the yield of plant glycogen from dent and waxy corn for the 7 p.m.(1900) sample was about twice the yield of the 9 a.m. (0900) morning sample [7]. That is, if all of the glycogen were converted into starch in a single day rather than the proposed three or four day period, then essentially all of the glycogen would have disappeared by the following morning and consequently the yield of glycogen for the 9 a.m. (0900) sample should have been near zero rather than about one-half of the evening sample. Indeed, the existence itself of plant glycogen in dent, waxy and even *ae* corn [7], as well as its maximum yield at the midpoint of starch synthesis [20], backs the validity of the glycogen precursor mechanism.

In addition, the initially high radioactivity of the amylose, as observed by Whistler and Young [19], also supports the three or four day synthesis since this length of time allows a mixture of mature and immature glycogen to exist together in the plant, but each in separate cells. It is concluded that when the radioactive sucrose was inserted into the plant by Whistler and Young [19], then the more randomly labeled, immature glycogen was retained, whereas the mature glycogen, with radioactivity in only its exterior chains, was converted into starch. And since the debranching enzymes are proposed to be inactive until the glycogen is mature, then it is the exterior radioactive A-chains of these mature glycogens which are the first to be removed by the debranching enzymes to produce in this case an initially high radioactivity in amylose.

But this high radioactivity in amylose then tapers off as uniformly labeled precursor glycogens begin to be converted into starch after three or four days.

The use of the SSS II back-up system

The questions arises: Are there any examples of a shift from the proposed primary phosphorylase system to the SSS II system? As pointed out by Mohlmann, et. al. [14], corn (maize) and normal barley use the ADPGlu transport system, whereas wheat uses the G-6-P transport system. Moreover, such a transition from the phosphorylase system to the SSS II system could have occurred in the antisense experiments of Sonnewald, et. al. [21], where antisense repression of the major leaf plastidic type L phosphorylase took place. The transition would explain the continual production of starch despite the destruction of this phosphorylase. Another possible example is the conversion of Bomi to *shx* barley. It was concluded [8-10] from an analysis of the data of Schulman, et. al. [22] on the genetic change from Bomi barley to *shx* barley, that this genetic change involves a transition from phosphorylase to the SSS II enzyme system. In other words, the removal of one of the three SSS I enzymes (but none of the SSS II enzymes) in this conversion could not possibly account for the dramatic 69% loss of starch. That is, the proposed back-up system using SSS II produces a polymodal debranched amylopectin, but SSS I enzyme does not [8-10] and thus the SSS I can not be a major contributor (or even contribute at all) to the synthesis of the precursor glycogen.

In connection with this possible conversion of the phosphorylase system in Bomi to the SSS II system in *shx* barley, the phosphorylase system produces ATP (Eq. 1) which is in essence a direct transportation of ATP to the plastid. The ATP also inactivates the Glu-6-P transport system (the SSS II system) [14]. This extra energy could in part account for the greater yield of starch in the Bomi barley. Moreover, Mohlmann, et. al [14] observed (their Table 2) that the rate of starch synthesis using transported ADPGlu is about six times faster (99.5/16.8) than that rate produced by using the transported Glu-6-P. According to the proposed equations, this dramatic difference in rates for the two transport systems could establish phosphorylase as the primary system and, in addition, could account for the observed [22] loss of about two-thirds of the starch yield in going from Bomi to *shx* barley.

The polymer size distributions of debranched amylopectin chains and amylose

It was concluded in 1988 [23] and more recently [8] that the polymodal size distribution of the completely debranched amylopectin can be correlated with individual polymers. It was then proposed that these individual polymers have their origin in the tier structures of the precursor glycogen and its resulting amylopectin. For example, if there are no branches attached in its original structure (an A-chain), then its degree of polymerization would be approximately $x_n = 10$. However, the external A-chains of the

amylopectin would have a value of $x_n = 20$ or so, since in the precursor glycogen this external A-chain was a B-chain with one branch attached to it (before stub branching had occurred). Thus theoretically, there should be no chains in the amylopectin structure which produce a polymer of the size $x_n = 10$ or so. Examination of the polymodal behavior of debranched amylopectin chains shows, however, that there exists two and possibly three such polymers, with perhaps degrees of polymerization of about $x_n = 10$ and 12. These two short polymers are considered to be hidden in the interior structure and are produced because of steric hindrance in the growing precursor glycogen. Shorter polymers, of perhaps $x_n = 3$ or 4, are shown to exist in polymodal patterns of Schulman, et.al. [22] for debranched amylopectins from Bomi and *shx* barley starches, and these shorter polymers can be ascribed to remnants of the proposed stub branching. A method was developed in 1988 [23] and presently [8] which allows the calculation of the fraction of the assumed polymers in the mixture of the polymodal debranched amylopectin chains. In the calculations, it is assumed that each polymer is distinct and has the *Poisson* size distribution, which agrees with the observation by Bailey and Whelan [24] in 1961 that phosphorylase produces a synthetic amylose which has a *Poisson* size distribution.

Later, in 1994, Ong, et al. [25] made similar calculations using the Gaussian curve for size distribution calculations and similar results were obtained. However, the Gaussian curve becomes much broader at the higher molecular weights and thus when applied to the structure of amylopectin would produce less branching in the amylopectin interior which contradicts the properties of the branching enzyme and the greater steric hindrance in the more exterior chains.

In contrast to the *Poisson* size distribution for the various distinct polymers of debranched amylopectin, it was observed [8, 23] that linear amylose has an extremely broad size distribution and behaves as an A-B type condensation polymer. This contrast in size distributions shows that amylose can not be the precursor to amylopectin since different enzyme systems must be involved in their production. In other words, the granular bound starch synthase (GBSS) and SSS I do not produce a polymodal-type amylopectin [10], and thus do not synthesize linear chains in the same manner as the polymodal-producing phosphorylase or SSS II. Hence, amylopectin can not be produced by first synthesizing a short, precursor amylose polymer. Consequently, based on these size distributions, the amylose precursor mechanism is erroneous.

The branched polymers of amylose can also be examined by correlating the properties of these polymers with size distributions for various theoretical polymers using Flory's equations [26] for linear A-B condensation polymers and our mathematical extension [27] to represent multiple branched A-R-B_{f-1} condensation polymers. These equations were applied [8] to the results of Everett and Foster [28] and Takeda, et al.

[29]. Takeda, et al. [29] separated individual polymers of amylose at 40°C (two branched structures and one linear structure). A comparison [8] of the theoretical model with the experimental results [29] showed that one of the branched polymers of amylose behaved as an A-R-B_2 type condensation polymer, the other branched polymer as a non-statistical A-R-B_2 type condensation polymer (a transition polymer), and the third polymer as a completely linear A-B type condensation polymer.

The location of "hidden" a-chains in the interior structure of amylopectin

Baba, et al. [30] showed that debranching of their resulting radioactive amylopectin produced short linear chains which were not radioactive. These short chains, which appeared to have an approximate degree of polymerization of $x_n = 10$, most likely were the above "hidden" chains [10]. Further proof of this is given by the fact that *beta* – amylolysis of the amylopectin showed that the radioactivity was in the longer exterior chains ($x_n = 20$) of the amylopectin [30]. Thus the shorter, non-radioactive chains were located in the interior part of the amylopectin. Furthermore, the narrow size distributions of their resulting chains (both the non-radioactive short and radioactive long chains) illustrated that these polymers had the *Poisson* size distribution.

The position of these hidden, inner A-chains was also determined by comparing one variety of debranched amylopectin with another. That is, if shorter A-chains replace much longer B-chains to a greater extent in one variety than in another, then the difference between the two varieties should show a decrease in the longer B-chains when there is an increase in the shorter, inner A-chains. Theoretical *Poisson* polymers were used to produce theoretical curves for debranched amylopectins from wheat, tapioca and barley. In these theoretical curves only one A-chain was used ($x_n = 10$). Because of the very narrow width of the *Poisson* size distribution curves, the theoretical curve had a deep "dip" between ten and twenty glucose unit chain lengths. Similar dips in the debranched amylopectin curves also occurred as well. The amount of the $x_n = 30$ polymer had the following sequence: wheat (4.2%) < tapioca (6.2%) < barley (13.8%), which correlated with the sequence for the amount of the intermediate polymer (as measured by the percentage increase in the experimental dip over that of the theoretical dip): wheat (21%) > tapioca (17%) > barley (10%). Thus the decrease in the $x_n = 30$ polymer is followed by an increase in the $x_n = 12$ polymer. Consequently, the longer inner chain ($x_n = 12$ or so) must be located primarily in the third tier from the exterior of the precursor glycogen, whereas the shorter ($x_n = 10$) inner chain must be located primarily in the more interior fifth tier.

A similar comparison was made between Bomi and *shx* barley, where as discussed above, it is considered that the Bomi barley uses phosphorylase and the *shx* barley uses SSS II in the synthesis of the linear chains of glycogen. A comparison

showed that the $x_n = 12$ was greater for the Bomi barley and the $x_n = 10$ polymer was greater for the *shx* barley. But the greater amount of the $x_n = 12$ polymer in the Bomi barley was associated with lesser values for the $x_n = 30$, 40 and 70 polymers. Likewise, the greater amount of the $x_n = 10$ polymer was associated with lesser amounts of the $x_n = 50$, 60 and 80 polymers. In other words, just as the $x_n = 12$ polymer displaced the $x_n = 30$ polymer in wheat, tapioca and barley, so also the increase in the $x_n = 12$ polymer can be associated with a partial replacement(a decrease) in the $x_n = 30$ polymer, as well as a partial replacement, that is, decrease, in the $x_n = 40$ and 70 polymers. In other words, the location of the $x_n = 12$ polymer is in the third, fourth and seventh tiers of the precursor glycogen and that of the $x_n = 10$ polymer is in its fifth, sixth and eighth tiers. The larger, inner or "hidden" polymer therefore appears in general to be in the exterior tiers of the glycogen and amylopectin structures.

The statistical model for amylopectin and its precursor glycogen

A comparison was made between amylopectins and a model based on all possible structures (a statistical model) which have three branch points (a total of 5 structures) which is comparable to those structures which have six branches (132 structures) and seven branches (429 structures) as studied previously [31]. It is seen that the change in the ratio of A/B chains in going from glycogen to amylopectin can be explained by using hidden A-chains. The Meyer model for the precursor glycogen, modified by placing short, inner A-chains in its structure, gave similar results.

These hidden A-chains can also explain the presence of clusters. The size of these clusters, as described by Zhu and Bertoft [32], is approximately that of the three branched structures, or possibly slightly larger. Furthermore, these structures are produced from random branching, as in the formation of a statistical structure, since as pointed out by Zhu and Bertoft [32] at least three different structures exist in these clusters. Hence, the statistical model, with inner, short A-chains and with a *Poisson* size distribution for the linear chains, represents the amylopectin and its clusters.

Conclusions

The proposed glycogen precursor mechanism [1, 10] for starch synthesis is supported by experimental evidence: the predicted debranching enzymes have been found and the predicted glycogen intermediate has also been found. That is, a 9% branched glycogen was observed by Mouille, et.al. [6], when the debranching enzymes were inactivated and 8% branched glycogens were isolated not only from sweet corn, but also from dent and waxy corn solubles, as well as the 6% branched glycogen from *ae* corn solubles [7]. The postulated use of phosphorylase as the primary enzyme for one of two predicted independent pathways for starch synthesis [10] is also supported by

experimental evidence and is based on the observed [14] existance of two transport systems. The continuation of starch synthesis when the phosphorylase is inactivated, possibly by destroying the translocator for ADPGlu, can be explained by a switch to a back-up system, that is, the transport of the Glu-6-P into the plastid. This in turn must instigate the production of the precursor glycogen with SSS II and branching enzymes. Both mechanisms (phosphorylase and SSS II) are dependent upon the use of the critical enzyme ADPGlu pp.

The statistical model and the proposed glycogen precursor mechanism illustrate that the amount of removed branches correlates with the amount of amylose found in starches, that "stub" branching is needed to produce the 8.0% branching observed [7] in dent, waxy and sweet corn glycogens, that the $x_n = 30$ polymer exists in amylopectins but is reduced in amount because of a partial, but variable, replacement of these chains with short A-chains ($x_n = 12$), and that slightly shorter A-chains ($x_n = 10$) have also replaced many of the $x_n = 50$ polymers in wheat and barley amylopectins. Moreover, amylose can not be a precursor to amylopectin since the linear amylose behaves as a broad A-B condensation polymer, whereas the debranched amylopectin chains behave as *Poisson*-type polymers with a very narrow size distribution.

Aggregation (retrogradation) of amylopectin occurs by the debranching of the exterior chains after glycogen synthesis stops. Further aggregation must occur with proteins to produce reversible aggregates in waxy (disaggregation in 0.05M sodium phosphate at pH = 7 [33] and irreversible aggregates in dent corn amylopectins [8].

Emes and Neuhaus [34] consider the possibility that the ADPGlu transport system allows the use of ADPGlu pp in the cytosol, the consequent transfer of the ADPGlu, and then the production of linear glucose chains by the direct use of the SSS enzymes without the use of ADPGlu in the plastid. However, even with activity of ADPGlu pp in the cytosol, there is still activity of this enzyme in the amyloplast. Consequently, it is concluded that the above proposed mechanism using phosphorylase explains the use of the ADPGlu pp in *both* the cytosol and the plastid.

One of the arguments against the proposed phosphorylase mechanism is that as seen in Eq. 2 there is a production of phosphate which it is argued would inhibit the ADPGlu pp enzyme. However, as pointed out by Tetlow, et.al. [35], phosphate does not accumulate in the plastid during starch synthesis even though it could, and that its removal can not at present be accounted for. Thus they consider that some unknown transport mechanism transfers the phosphate into the cytosol: "Presumably, mechanisms exist in the amyloplasts for the removal of the additional phosphate" [35]. Consequently, these data indicate that at present unknown transport mechanisms could also remove the phosphate generated by Eq. 2 (or PPi in Eq.3), such as a counter exchange of Pi and PPi.

Genetic alterations of the genes controlling the ADPGlu pp show that the activity of the ADPGlu pp can be increased tremendously [36]. With respect to this increase in ADPGlu pp activity, in unpublished work by M.J. Emes, as reported by J. Preiss [37], a mutation increased by four fold the activity of this enzyme (from 110 to 339 activity units) and at the same time substantially increased the yield of starch. Interestingly, with this increase in both the ADPGlu pp activity and the yield of starch, there was also a dramatic *decrease* in the activity of the soluble starch synthases (from 3527 to 2231 activity units) plus a slight increase in the activity of phosphorylase (from 355 to 376 activity units). The corresponding one-third drop in SSS activity indicates that the SSS is not used in this case in the production of the precursor glycogen, but most likely only for the conversion of the removed branches into amylose, as proposed in Eq. 6 above. On the other hand, the presence of phosphorylase plus simultaneous increases in *both* the activity of phosphorylase and that of ADPGlu pp illustrate that in this case phosphorylase in involved in starch synthesis. Furthermore, the use of phosphorylase in the ADPGlu transport system explains the mystery [14] of why added ATP suppresses the ADPGlu transport system (Eqs. 1 and 2) but in contrast enhances the Glu-6-P transport system (Eqs. 3 and 4). Moreover, the mutation results [37] may have involved a shift from the Glu-6-P transport system to the ADPGlu transport system and this shift could also have increased both the activity of ADPGlu pp (since *both* cytosolic and plastidic ADPGlu pp would be used) and the yield of starch (since the rate of starch synthesis is six times faster for the ADPGlu transport system [14].

REFERENCES

[1] Erlander S.R.: Enzymologia, **19**, 1958, 273-283.
[2] Wursch P., Gumy D.: Carbohydr. Res., **256**, 1994, 129-137.
[3] Manners D.J., Rowe K.I.: Carbodhydr. Res., **9**, 1969, 107-121.
[4] Lee E.Y.C., Marshall J.J., Whelan W.J.: Arch. Biochem. Biophys., **143**, 1971, 365-374.
[5] Pan D., Nelson O.E.: Plant Physiol., **74**, 1984, 324-328.
[6] Mouille G., Maddelein M.-L., Libessart N., Talaga P., Decq A., Delrue B., Ball S., Plant Cell, **8**, 1996, 1353-1366.
[7] Erlander S.R., Griffin J.L.: Starch/Starke, **19**, 1967, 34-41.
[8] Erlander S.R.: Starch/Stärke, 1998, Starch Biosynthesis. I. submitted.
[9] Erlander S.R.: Starch/Stärke, 1998, Starch Biosynthesis. II. submitted.
[10] Erlander S.R.: Starch/Stärke, 1998, Starch Biosynthesis. III. submitted.
[11] Erlander S.R.: Starch/Stärke, **22**, 1970, 352-362 and 393-401.
[12] Preiss J.: [in] Oxford Surveys of Plant Molecular and Cell Biology, **7**, 1992, 59-114.
[13] Pozueta-Romero J., Frehner M., Viale A.M., Akazawa T.: Proc. Nat. Acad. Sci. USA, **88**, 1991, 5769-5773.
[14] Mohlmann T., Tjaden J., Henrichs G., Quick W.P., Hausler R., Neuhaus H.E.: Biochem. J., **324**, 1997, 503-509.

[15] Chen B.-Y., Wang Y., Janes H.W.: Plant Physiol., **116**, 1998, 101-106.
[16] Duwenig E., Steup M., Kossman J.: Planta, **203**, 1997, 111-120.
[17] Schaffer A.A., Petreikov M.: Plant Physiol., **113**, 1997, 739-746.
[18] Erlander S.R.: Starch/Stärke, **19**, 1967, 42-48, 99-110.
[19] Whistler R.L., Young J.R.: Cereal Chem., **37**, 1960, 204-211.
[20] Erlander S.R., McGuire J.P.: Starch/Stärke, **19**, 1967, 402-410.
[21] Sonnewald U., Basner A., Greve B., Steup M.: Plant Mol. Biol., **27**, 1995, 567-576.
[22] Schulman A.H., Tomooka S., Suzuki A., Myllarinen P., Hizukuri S.: Carbohydr. Res., **275**, 1995, 361-369.
[23] Erlander S.R.: 14th Intern. Carbohydr. Symp., Stockholm, Sweden, 1988, Aug. 14-19, Abstract C77.
[24] Bailey S.J., Whelan W.J.: J. Biol. Chem., **236**, 1961, 969-973.
[25] Ong M.J., Jumel K., Tokarczuk P.F., Blanshar J.M.V., Harding S.E.: Carbohydr. Res., **260**, 1994, 99-117.
[26] Flory P.J.: Principles of Polymer chemistry, Cornell U.P., Ithaca, N.Y., 1953, 336-427.
[27] Erlander S.R., French D.: J. Polymer Sci., **20**, 1956, 7-28.
[28] Everett W.W., Foster J.F.: J. Am. Chem. Soc., **81**, 1959, 3459-3469.
[29] Takeda Y., Shitaozono T., Hizukuri S.: Carbohydr. Res., **199**, 1990, 207-214.
[30] Baba T., Yoshii M., Kainuma K.: Starch/Stärke, **39**, 1987, 2-56.
[31] Erlander S.R.: J. Polymer Sci., **37**, 1959, 91-101.
[32] Zhu Q., Bertoft E.: Carbohydr. Res., **288**, 1996, 155-174.
[33] Erlander S.R., Tobin R.: Starch/Stärke, **19**, 1967, 73-78, 94-99.
[34] Emes M.J., Neuhase H.E.: J. Experimental Botany, **48**, 1997, 1995-2005.
[35] Tetlow I.J, Blissett K..J., Emes M.J.: Planta, **204**, 1997, 100-108.
[36] Meyer C.R., Yirsa J., Gott B., Preiss J.: Arch. Biochem. Biophys., **352**, 1998, 247-254.
[37] Preiss J.: Plant Polysaccharide Symposium '98, Davis, California, U.S.A., August 4-7, 1998.

BIOSYNTEZA SKROBI

Streszczenie

Przedstawiono dowód podtrzymujący sugestię (Erlander Enzymologia 1958, 19, 273-283), że glikogen jest niezbędnym związkiem pośrednim w syntezie skrobi. Jego synteza w specyficznych komórkach czasowo ustaje po około trzech dniach i uaktywniają się enzymy odcinające rozgałęzienia usuwając jego zewnętrzne łańcuchy A. Glikogen z częściowo usuniętymi odgałęzieniami stanowi amylopektynę, a odcięte łańcuchy ulegają degradacji przez syntazę rozpuszczalnej skrobi (SSS I i II) dając ADP-glukozę (ADPGlu), która jest jedynym źródłem glukozy przekształcanej w amylozę przez syntazę gałeczkowej skrobi związanej (GBSS). Dwa niezależne układy transportu cytosol/plastyd albo aktywują fosforylazę (z transportowanej ADPGlu) lub wspierającego układu SSS (z transportowanej Glu-6-P). W syntezie glikogenu oba te systemy korzystają z fosforylazy ADPGlu. Liniowe łańcuchy amylopektyny pozbawione odgałęzień mają niewielki zakres rozrzutu rozmiarów wg *Poissona*, podczas gdy łańcuchy amylozy, polimeru z kondensacji A z B, mają bardzo zróżnicowane rozmiary. Zatem amyloza nie może być prekrsorem amylopektyny. Wewnętrzne, krótkie łańcuchy A znajdujące się przede wszystkim na 3 i 5 jednostkach glukozowych glikogenu odpowiadają za zamiany stosunku liczby łańcuchów A i B (A/B) i za powstawanie klasterów.

T. FORTUNA[1], L. JUSZCZAK[1], M. PAŁASIŃSKI[2]

CHANGE IN THE GRANULE POROSITY ON MODIFICATION OF STARCH

Abstract

Potato, wheat, maize and oat starches were modified by physical, chemical and enzymatic treatment. The obtained preparations were characterized in terms of specific surface area, volume of mesopores and their average diameter with ASAP 2000 apparatus, by method of low-temperature adsorption of nitrogen. Changes in the properties of starch granules due to the applied modifications were observed. It was proved, that enzymatic modification had the highest influence on porosity. Among the α-amylase modified preparations, the highest increase of porosity occurred in oat and wheat starch, lower in maize and the lowest in potato starch. The above results were confirmed by granules morphology analysis performed by means of scanning microscopy.

Introduction

Native starches have limited application in technological processes. To obtain required properties starch is being modified. The most frequently used are physical, chemical and enzymatic methods of starch modification [Fornal, 1985; Wurzburg, 1988; Muzimbaranda, Tomasik, 1994; Słomińska, 1997]. Starch susceptibility to modifying agents depends on botanical origin of parent plant, size and structure of starch granules, amylose/amylopectin ratio and phosphoric acid content in starch granule [Swinkels, 1985; Lewandowicz, 1990; Soral-Śmietana, 1995]. Apart from carbohydrate substance an integral part of starch is water and the interaction starch granule – water is decisive for starch modification process [Bączkowicz, Tomasik, 1989]

Cereal starches differ from potato starch, among other things, in water binding capacity and rheological properties. They also contain more lipid-protein substances, which partly occur on the granules' surface and partly form stable complexes with amylose helices [Morrison, 1981; Soral-Śmietana, 1995]. Starch reactivity is also influenced by starch granule structure, thus one of the important factors determining

[1] *Department of Analysis and Food Quality Evaluation, University of Agricultural, Cracow, Poland*
[2] *Department of Carbohydrate Technology, University of Agricultural, Cracow, Poland*

starch susceptibility to modification is the specific surface area of granules and their porosity.

Cereal small granules differ from potato starch in larger specific surface area and volume of mesopores, while their average diameters of mesopores are similar [Fannon et al., 1992; Baldwin et al., 1994; Achremowicz et al., 1997]. Starch granules porosity has an impact on physico-chemical properties of starch and is widely investigated [Hellman and Melvin, 1950; Marousis and Saravacos, 1990; Fannon et al., 1992, 1993; Karathanos and Saravacos, 1993]. Various methods are used to measure starch granules porosity, among them: scanning microscopy [Fannon et al., 1992], confocal microscopy and atomic force microscope [Baldwin et al., 1994, 1997], mercuric porosimetry [Karathanos and Saravacos, 1993], helium stereopicnometry [Marousis and Saravacos, 1990], water sorption measurements [Aguerre et al., 1989], and methods based on physical adsorption from gas phase [Xano and Nogai, 1989, Achremowicz et al., 1997] or liquid phase [Fortuna et al., 1996]. The obtained porosity values vary due to starch source but also depend on the applied measurement method.

The aim of this paper was to find what is the influence of physical, chemical and enzymatic modification on the porosity of starch granules.

Material and methods

The following starches were used:
- potato starch 'Superior' produced by Potato Processing Company Pila,
- commercial wheat starch of German origin,
- commercial maize starch of German origin,
- commercial oat starch of Finnish origin

All the above starches were modified by physical, chemical and enzymatic treatment.

I. Physical modification was performed in 3 different ways:
   1. by convectional heating in 130°C for 2 hours
   2. by convectional heating in 200°C for 2 hours
   3. by irradiating with microwaves for 14 min., in microwave oven Moulinex FM A945GS (850 W, 2450 MHz)

II. Chemical modification was performed in water dispersion (pH = 9), using sodium trimetaphosphate as a modifying agent [Lim, Seib, 1993].

III. Enzymatic modification (mild hydrolysis with α-amylase) was performed using bacterial α-amylase preparation Maxamyl (20 μl/1g d.s. starch) after incubation in 40°C

In native as well as in modified starches specific surface area S_{BET} [Branauer et al., 1938], volume of mesopores and their medium diameter were determined. Specific surface area of the preparations and porosity were measured with multifunctional

automatic apparatus ASAP 2000 (Micrometrics, Noxcross, Georgia USA), by means of absorption of highly purified nitrogen at liquid nitrogen temperature. Before the determination the probes were dried in vacuum at 35°C to remove excessive moisture. Then the preparations were additionally desorbed in degassing station working in automatic mode, utilizing washing with pure helium and vacuum treatment. The state of surface degassing was checked and for further experiments completely desorbed probes were used.

Granules' morphology was characterized with scanning electron microscope (SEM) Jeal JSM 5200. The probes were prepared according to Fornal [1985].

Results and discussion

Particular native starches and products obtained by their modification had different specific surface area of granules (Tab. 1). Oat starch was characterized by the largest surface area and potato starch by the smallest. Also the volume of mesopores in oat starch was the biggest. Average mesopores diameters are close for all native starches. Results of Fannon et al. [1992, 1993] confirm differences in porosity between starches from different sources. Also the origin of the pores is different. Part of them appears during starch granules formation in plant tissue [Fornal, 1984], some when thermal or hydrothermal processes take place, while amylose migrates from the inside of granule to its surface [Baldwin et al., 1994], other are mechanical cracks and damages caused by grain processing [Niemann, Whistler, 1992]. Due to the performed modifications the properties changed. Preparations modified by physical treatment obtained from potato starch were distinguished by larger surface area and volume of mesopores as well as their smaller average diameter. In maize starch preparations there such a change was not observed. This proves that maize starch is more resistant towards the modifying agents (convectional heating, microwave irradiation). Stability of corn starch is probably due to the occurrence of lipids in surface and helical amylose complexes [Tomasik et al., 1996]. Hellman and Melwin [1950] in their research determined specific surface area of starch, by means of nitrogen absorption, to be 0.70 m^2/g for maize, 0.28 m^2/g for tapioca and 0.11 m^2/g for potato. According to Karathanos and Saravacos [1993] specific surface area of waxy corn was 0.39 when determined by low pressure mercuric porosimeter.

Estrification of the starches with ortophosphoric (V) acid also resulted in changes of granules porosity increasing surface area and volume of mesopores (except maize starch). Oat starch after chemical modification showed a little increase of surface area while volume and mean diameter of mesopores were highly enlarged.

Table 1

Porosity (pore characteristics) of initial and modified starch granules

Starch source	Specific surface area [m^2/g]	Volume of mesopores [cm^3/g] x 10^{-3}	Average diameter of mesopores [m] x 10^{-10}
POTATO: initial	0.24	0.35	57.2
convectional heating at 130°C	0.34	0.40	47.4
convectional heating at 200°C	0.35	0.37	42.7
microwave heating	0.51	0.57	44.7
chemical modification	0.40	0.55	54.0
enzymatic modification	0.62	1.36	87.7
WHEAT: initial	0.53	0.76	57.0
chemical modification	0.70	1.26	71.8
enzymatic modification	3.74	10.65	113.9
MAIZE: initial	0.69	1.10	64.2
convectional heating at 130°C	0.71	1.13	64.0
convectional heating at 200°C	0.77	1.24	64.4
microwave heating	0.68	1.11	65.3
chemical modification	0.65	1.07	66.1
enzymatic modification	3.12	7.67	98.3
OAT: initial	1.22	1.80	58.8
chemical modification	1.26	2.45	77.6
enzymatic modification	8.41	22.50	107.1

The greatest changes in porosity of starch granules were observed in enzymatically modified preparations. The highest surface area after α-amylase treatment was found for oat starch: 8.41 m^2/g, much lower for wheat and maize and the lowest for potato. A similar trend concerned volume and mean diameter of mesopores. When amylolytic enzymes act on native starches, porosity of its granules plays an important role. Starches with larger surface area could be easier attacked by amylolytic enzymes. Starch granules from roots and tubers are more resistant towards amylases then those from grains [Sugimoto et al., 1980; Słomińska, 1997]. Sugimoto [1980] showed that potato and banana starch granules are more resistant towards amylases then maize starch granules.

Yamada et. al. [1995] in the scanning microscopy experiments on porosity of maize starch after amylase action, found that the enzyme mainly attacks amorphous regions.

Figs. 1-4 present photos from scanning electron microscopy of modified starches preparations. Granules morphology confirms the greatest changes occurring in probes treated with α-amylase.

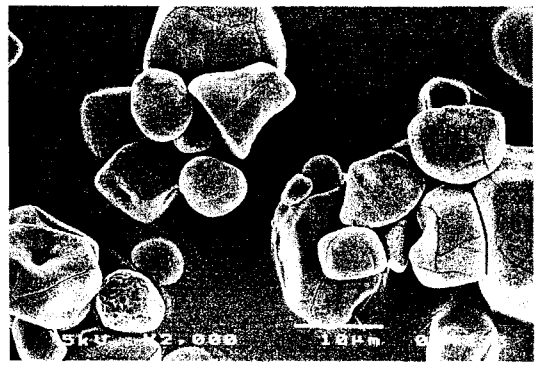


Fig. 1. Maize starch after convectional heating at 200°C.

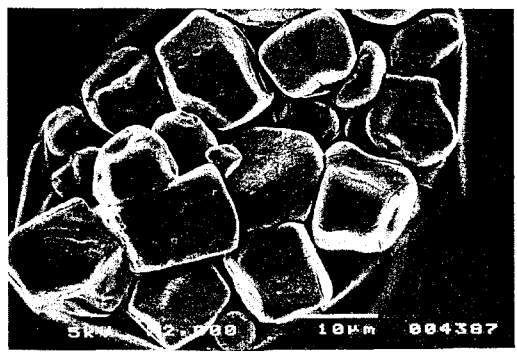


Fig. 2. Maize starch after microwave heating.

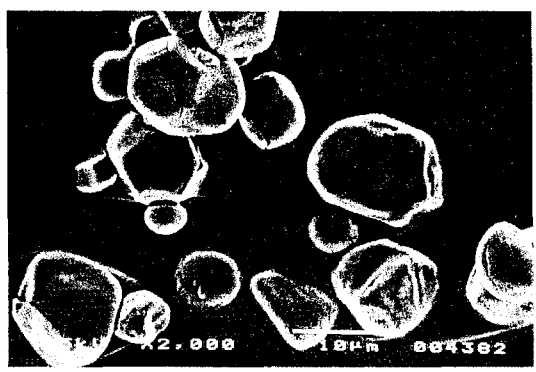


Fig. 3. Maize starch after chemical modification.

Fig. 4. Maize starch after enzymatic modification.

Conclusions

1. Modification of starch granules with the applied physical treatments causes different changes of starch properties, depending on its origin (enlargement of surface area and volume of mesopores and decrease of their average diameter in potato starch granules).
2. Chemical modification also caused porosity changes, increasing surface area of granules and volume of mesopores (except maize starch).
3. The greatest changes in porosity were obtained after enzymatic modification. The highest increase of specific surface area among the enzymatically modified preparations was found for oat starch, much lower for wheat and corn starches, and the lowest for potato starch.
4. Morphology analysis of starch granules confirmed the above results.

REFERENCES

[1] Achremowicz B., Fortuna T., Januszewska R., Juszczak L., Kielski A., Pałasiński M.: Żywność, Technologia, Jakość, **3**, 12, 1997, 28-35.
[2] Aguerre R.J., Suarez, C., Viollaz, P.E.: Journal of Food Engineering, **9**, 1, 1989, 71-80.
[3] Baldwin P.M., Adler J., Davies M.C., Melia C.D.: Starch/Stärke, **46**, 9, 1994, 341-346.
[4] Baldwin P.M., Davies M.C., Melia C.D.: International Journal of Biological Macromolecules, **21**, 1997, 103-107.
[5] Branauer S., Emmett P.H., Teller E.: Journal of the Amarican Chemical Society, 60, 1938, 309-319.
[6] Bączkowicz M., Tomasik P.: Starch/Stärke, **41**, 1989, 449-453.
[7] Fannon J.E., Hauber R.J., BeMiller J.N.: Cereal Chemistry, **69**, 3, 1992, 284-288.
[8] Fannon J.E., Shull J.M., BeMiller J.N.: Cereal Chemistry, **70**, 3, 1993, 611-613.
[9] Fornal J.: Zeszyty Naukowe ART Olsztyn, **20**, 1984, 1-45.
[10] Fornal J.: Acta Alimentaria Polonica, **11**, 1985, 141-151.

[11] Fortuna T., Januszewska R., Wąchalewski T.: Zeszyty Naukowe AR w Krakowie, Technologia Żywności, **3**, 1996, 29-37.
[12] Hellman N.N., Melvin E.H.: Journal of the American Chemical Society, **72**, 1950, 5186-5188.
[13] Karathanos, V.T., Saravacos G.D.: Journal of Food Engineering, **18**, 3, 1993, 259-280.
[14] Lewandowicz G., Mączyński M.: Chemik, **3**, 1990, 69-71.
[15] Lim S., Seib P.A.: Cereal Chemistry, **70**, 1993, 137-144.
[16] Marousis S.N., Saravacos G.D.: Journal of Food Sciences, **55**, 5, 1990, 1367-1372.
[17] Morrison W.R.: Starch lipids: a reappraisal. Starch/Stärke, **33**, 12, 1981, 405-410.
[18] Muzimbaranda C , Tomasik P.: Starch/Stärke, **46**, 1994, 469-474.
[19] Niemann C., Whistler R.L.: Starch/Stärke, **44**, 1992, 409-414.
[20] Słomińska L.: Przemysł Spożywczy, **3**, 1997, 9-12.
[21] Soral-Śmietana M.: Proced European Conference on grain legumes; „Improving, production and utilisation" (ed AEP). Copenhagen, Denmark, 1995, 364-365.
[22] Sugimoto Y., Fujita S., Takaya T., Fuwa H.: Starch/Stärke, **32**, 1980, 290-294.
[23] Swinkels J.J.M.: Starch/Stärke, **37**, 1985, 1-5.
[24] Tomasik P., Ciesielski W, Sikora M., Sychowska B.: VII Międzynarodowa Konferencja Skrobiowa, Kraków, 1996, 83-99.
[25] Wurzburg O.B.: Modified starches properties and uses, Boca Raton, Florida, 1988, CRS Press Inc.
[26] Xano T., Nogai T.: Journal of Food Engineering, **18**, 1989, 259-280.
[27] Yamada T., Hisamatsu M., Teranishi K., Katsuro K., Hesegawa N., Hayashi M.: Starch/Stärke, 9, 46, 1995, 358-361.

WPŁYW MODYFIKACJI SKROBI NA POROWATOŚĆ JEJ ZIARN

Streszczenie

Skrobie ziemniaczaną, pszenną, kukurydzianą i owsianą poddano modyfikacji fizycznej, chemicznej i enzymatycznej. Uzyskane preparaty przebadano odnośnie powierzchni właściwej, objętości mezoporów i średniej ich średnicy za pomocą aparatu ASAP 2000, przy zastosowaniu metody niskotemperaturowej adsorpcji azotu. W wyniku zastosowanych modyfikacji stwierdzono zmiany badanych właściwości ziarn skrobiowych. Wykazano, że największe zmiany w porowatości spowodowała modyfikacja α-amylazą. Wśród preparatów modyfikowanych enzymatycznie największym wzrostem porowatości odznaczała się skrobia owsiana i pszenna, mniejszym kukurydziana, a najmniejszym ziemniaczana. Powyższe wyniki potwierdziła analiza morfologii ziarn wykonana za pomocą mikroskopii skaningowej.

N. INOUCHI, K. NISHI, S. TANAKA, T. ASAI, Y. KAWASE, Y. HATA, Y. KONISHI*, S. YUE**, H. FUWA

CHARACTERIZATION OF SMALL GRANULAR SIZED STARCHES – AMARANTHUS AND QUINOA STARCHES

Abstract

Starch granules were prepared from mature grains of 9 samples of *Amaranthus* and 4 samples of *Chenopodium quinoa*. By the ordinary GPC of *Pseudomonas* isoamylase-debranched starch materials the amylose content of amaranth starches was in a range of 0-28 %. Thus we confirmed that there were normal, low-amylose, and waxy-types of amaranth starches. The amylose content of quinoa starches was 25-27 %. The ratio of short chains to long chains of amylopectin of these starches was in a range of 2.2-3.3 and somewhat lower than or similar to that of the normal maize starch. Isoamylase-debranched materials were separated by HPLC with differential refractometer (RI) and low-angle laser light scattering photometer (LALLS) as detectors in one hand, and by high performance anion exchange chromatography with pulsed amperometric detector (HPAEC-PAD) in other hand. We found that amylopectins of amaranth and quinoa had increased amounts of long B chains and decreased amounts of short chains as compared with the waxy maize amylopectin, however, they had increased amounts of short chains with degree of polymerization (DP) from 6 to 12. Amaranth starches had slightly higher temperatures of gelatinization (To, Tp, and To) and smaller heats of gelatinization (ΔH) by diferential scaning calorimetry (DSC) comparing with the normal maize starch. Quinoa starch showed lower To, Tp, and Tc and smaller ΔH. Amaranth and quinoa starch granules were digested by amylases faster than those of the noraml maize.

Introduction

The granular shape and size of starches depend upon their original plant species. As some representatives of starch granules with small sizes (mean particle size, around 1–1.5 μm), we have been studied structure and properties of starches obtained from

Department of Food Science and Technology, Fukuyama University, Higashimura-cho, Fukuyama 729-0251, Japan,

* *Department of Food and Nutrition, Osaka City University, Sugimoto, Osaka 558-0022, Japan*

** *Department of Genetics, Institute of Crop Breeding and Cultivation, Chinese Academy of Agricultural Sciences (CAAS), Baishiqiao Rd., Beijin 100081, China*

grain amaranth [1-3], konjac [4], and taro [5, 6]. Ranhotra et al. [7] reported that quinoa (*Chenopodium quinoa*) has starch granules with small size.

Amaranth and quinoa have potential agronomic importance, because their seeds are generally higher in protein, fat, ash, and fiber in comparison to common cereals [8, 9] Moreover, the amino acid balance of these seeds are better than that of wheat and maize, because the first limiting amino acid, lysine, is present in relatively higher amounts in these seeds. Nevertheless, the main components of the seeds are starches. Accordingly the objective of this study is to know the structural characteristics and functional properties of starches of *Amaranthus* and *Chenopodium quinoa*.

Sample starch or sample seeds	Original place	Obtained through
Normal maize starch	USA	Sanwa Denpun Kogyo, Co., Ltd.
Waxy maize starch	USA	Sanwa Denpun Kogyo, Co., Ltd.
Amaranthus hypochondriacus K343	USA	Shinkyo Sangyo, Co., Ltd.
Amaranthus cruentus R104	China mainland	Dr. S. Yue (1996)
Amaranthus cruentus R104	China mainland	Dr. S. Yue (1997)
Amaranthus cruentus K112	China mainland	Dr. S. Yue (1996)
Amaranthus cruentus K112	China mainland	Dr. S. Yue (1997)
Amaranthus cruentus K350	China mainland	Dr. S. Yue (1997)
Amaranthus cruentus K459	China mainland	Dr. S. Yue (1997)
Amaranthus cruentus K472	China mainland	Dr. S. Yue (1997)
Amaranthus hybridus D88-1	China mainland	Dr. S. Yue (1997)
Chenopodium quinoa Quinua Real	Bolivia	Dainihon Meiji Seito, Co., Ltd.
Chenopodium quinoa	Peru	Dainihon Meiji Seito, Co., Ltd
Chenopodium quinoa Quinua ①-B	Bolivia	Dr. Takashi Akazawa
Chenopodium quinoa Quinua ②-B	Bolivia	Dr. Takashi Akazawa

Materials and methods

Sample seeds and preparation of starches

Starch granules were prepared from mature grains of 9 samples of *Amaranthus* and 4 samples of *Chenopodium quinoa* by a modification of Schoch's method [10]. Sample seeds were obtained as shown above. Commercial normal and waxy maize starches were used as references.

General methods

High performance gel permeation chromatography (HPLC) with differential refractometer (RI) and low-angle laser light scattering photometer (LALLS) as detectors and high performance anion exchange chromatography with pulsed amperometric detector (HPAEC-PAD) of isoamylase-debranched materials of amylopectin

The procedure for HPLC-RI-LALLS and Dionex chromatography (HPAEC-PAD) were reported earlier [11] except for the following minor change in the procedure for HPAEC-PAD. Namely, PAD-SC cell was used instead of PAD-standard cell and 0.1 M $NaNO_3$ was used in the elution solution instead of 0.5 M CH_3COONa.

Other methods

Contents of amylose and chain length distributions of amylopectin were determined by gel permeation chromatography (GPC) of *Pseudomonas* isoamylase-debranched starches. The methods for debranching of starch, GPC of debranched starch, analytical methods for fractionated materials have been reported previously [12, 13]. Some chemical and physical properties of starches were also determined. The method for recording absorption spectra of starch iodine complexes [14], the method for determination of starch-granule digestibility to amylase [15], and the procedure for differential scanning calorimetry (DSC) has been described elsewhere [16].

Results and discussion

The amylose content and amylopectin chain length distribution of starches by GPC

We have shown that there were normal, waxy, and low-amylose types of amaranths [1-3] From the data shown in Tables 1 and 2 we confiremed our previous results. Namely, two amaranth starches belong to normal, three to waxy, and two to low-amylose (amylose contents; 6.6 and 12.6 %, respectively) types among 7 different kinds of amaranth starches tested (Table 2). Possibility of cross contamination of normal pollen to waxy plants was cleared by microscopic observation of iodine stained starch granules obtained from *A. cruentus* K350 which stained purple instead of blue for normal starch and red for waxy one.

The ratio of short chains to long chains of amylopectins of amaranth starches (Fr. III/Fr. II) were in a range of 2.2 to 2.6 and slightly lower than those (around 3) of maize amylopectins. These results suggest that amaranth amylopectins have increased amounts of long chains and/or decreased amounts of short chains comparing with the normal and waxy maize amylopectins.

Table 1

Some characteristics of absrption curves of iodine complexes of starches obtained from maize, amaranth, and quinoa

Starch sample	Blue value*	λmax (nm)
Normal maize	0.36 0.02	587 0.8
A. hypocondriacus K343	0.08 0.00	530 0.8
A. cruentus R104 ('96)	0.12 0.00	538 0.1
A. cruentus R104 ('97)	0.10 0.00	530 0.8
A. cruentus K112 ('96)	0.31 0.01	585 0.6
A. cruentus K112 ('97)	0.40 0.01	588 0.5
A. cruentus K350 ('97)	0.15 0.00	541 0.9
A. cruentus K459 ('97)	0.25 0.01	567 1.4
A. cruentus K472 ('97)	0.43 0.03	591 0.6
A. hybridus D88-1 ('97)	0.09 0.00	529 1.0
C. quinoa Qinua Real	0.32 0.02	591 2.7
C. quinoa	0.32 0.01	587 0.7
C. quinoa 1-B	0.40 0.06	594 3.4
C. quinoa 2-B	0.41 0.03	596 1.9

*Optical density (OD) at 680nm **Wave length at the absorption maximum

Table 2

Characteristics of isoamylase-debranched materials by GPC of starches obtained from maize, amaranth, and quinoa

Starch sample	Fr. I (%)	Int. Fr. (%)	Fr. II (%)	Fr. III (%)	Fr. III/Fr. II
Normal maize	30.4	4.9	15.8	48.9	3.1
Waxy maize	0.0	4.4	24.5	71.1	2.9
A. hypocondriacus K343	0.0	5.7	26.7	67.6	2.6
A. cruentus R104 ('96)	0.0	6.8	26.3	66.8	2.6
A. cruentus R104 ('97)	0.0	5.2	27.4	67.4	2.5
A. cruentus K112 ('96)	19.4	7.4	21.9	51.4	2.4
A. cruentus K112 ('97)	27.8	5.6	19.1	47.5	2.5
A. cruentus K350 ('97)	6.6	2.3	28.4	62.7	2.2
A. cruentus K459 ('97)	12.6	5.8	22.7	58.9	2.6
A. cruentus K472 ('97)	24.1	6.0	21.1	48.8	2.3
A. hybridus D88-1 ('97)	0.0	3.4	27.5	69.2	2.5
C. quinoa Qinua Real	27.0	5.5	20.4	47.2	2.3
C. quinoa	24.7	7.9	21.2	46.3	2.2
C. quinoa 1-B	26.7	6.6	20.2	47.5	2.4
C. quinoa 2-B	26.4	3.9	16.7	52.9	3.2

*Each fraction (Fr.) was devided according to λmax of carbohydrate-iodine complexes as follows; Fr. I, λmax 620nm, Intermediate Fr., 620nm λmax 600nm, Fr. II, 600nm λmax 540nm, Fr. III, 540nm λmax.

The amylose content of quinoa starches was in a range of 24.7 to 27.0 %, however, 7 and 15 % amylose contents were recently reported by other investigators for different quinoa samples [7, 17]. Quinoa starch has received relatively little attention. Information regarding quinoa starch has been incomplete and contradictory. The variations in the results, probably due to in part to environmental, agronomic and genetic factors, but also due to the analytical procedures employed. Fr. III/Fr. II for quinoa amylopectins tended to be lower than those of maize amylopectins.

The amylopectin chain length distribution by HPLC-RI-LALLS

We showed amaranth amylopectins have increased amounts of long B chains and decreased amounts of short chains by HPLC-RI-LALLS (Fig. 1 and Table 3). Interestingly, short chains (F.3 in Fig. 1) of amaranth amylopectins have two peaks instead of one peak for the waxy maize amylopectin. These types of F.3 curves with two peaks were reported for amylopectins of the dull (du) maize mutants [18].

Table 3

Characteristics of isoamylase-debranched materials of maize and amaranth amylopectin by HPLC-LALLS

					ACL			ACLp	
Sample starch	F.2 %	F.3 %	F.3/ F.2	MW/MN	TCL	F.2	F.3	F.2	F.3
waxy maize	27.1	72.9	2.7	1.37	29.3	54.6	20.2	32.1	15.2
*A.C.R.*104	29.6	70.4	2.4	1.46	29.4	55.2	18.5	48.5	21.4/14.7
*A.C.R.*350	27.1	72.9	2.7	1.40	28.2	53.7	18.7	48.3	15.2/14.5
*A.hyb.*D88-1	29.2	70.8	2.4	1.37	28.7	54.4	18.2	47.6	19.2/14.4

*F.2 and F.3 are long and short chains of amylopectin, respectively. MW and MN are weight average and number average molecular weights, respectivly. TCL, ACL, and ACLp are total chain length, average chain length, and ACL at the apieces of the curve, respectively .

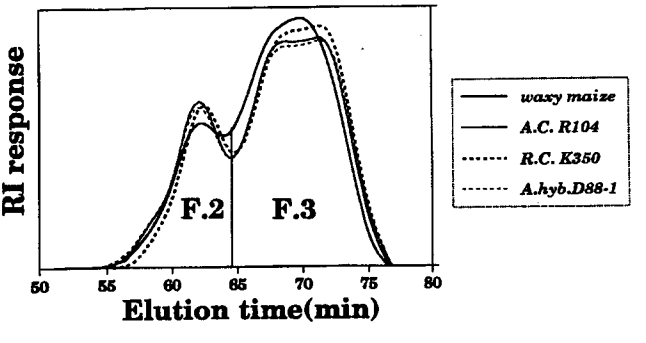


Fig. I. HPAEC-PAD traces for isoamylase-debranched materials of starches obtained from rice plants grown under different temperature conditions after anthesis. (a) group I-5; and (b) group II-5.

The amylopectin short chain-length distribution by HPAEC-PAD

Figure 2 shows the short chain-length distrubutions of isoamylase-debranched materials of amaranth and quinoa amylopectins with comparison to waxy maize amylopectin by Dionex chromatography. The amaranth and quinoa amylopectins have increased amounts of chains with degree of polymerization (DP) from 6 to 12 and some decreased amounts of chains with DP from 13 to 20 in comparision to the waxy maize amylopectin.

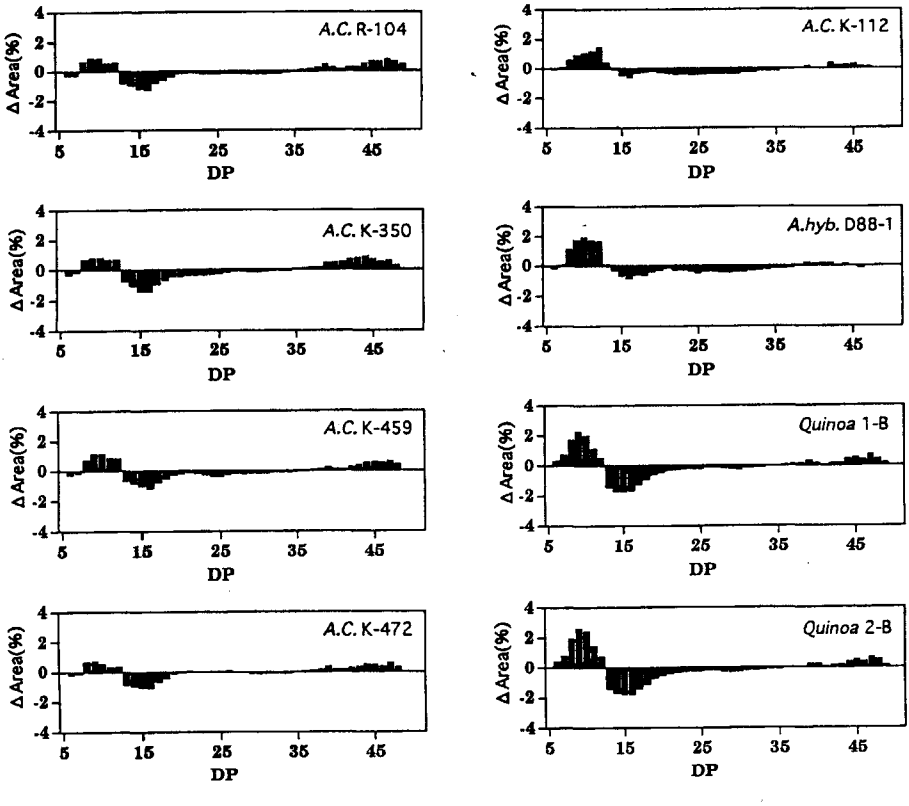


Fig. 2. Chain-length distributions of debranched amylopectins of rice plants grown under different temperature conditions after antesis. (a) group I-5; and (b) group II-5.

DSC characteristics of amaranth and quinoa starches

Amaranth starches had slightly higher temperatures for gelatinization (To, Tp, and Tc) and smaller heats of gelatinization (ΔH) comparing with the normal maize starches (Table 4). Quinoa starches showed lower To, Tp, and Tc and smaller ΔH comparing with the normal maize starches (Table 4).

Table 4

DSC characteristics of starches obtained from maize, amaranth, and quinoa

Starch sample	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)
Normal maize	65.3 ± 0.3	69.9 ± 0.2	75.2 ± 0.4	14.8 ± 0.1
A. hypocondriacus K343	63.2 ± 0.5	72.4 ± 0.1	80. ±1 0.5	10.0 ± 0.0
A. cruentus R104 ('96)	70.0 ± 0.2	76.1 ± 0.1	82.2 ± 0.3	11.1 ± 0.2
A. cruentus R104 ('97)	65.9 ± 0.6	74.6 ± 0.7	80.7 ± 0.8	10.9 ± 0.4
A. cruentus K112 ('96)	65.8 ± 0.5	71.2 ± 0.3	77.9 ± 0.9	9.5 ± 0.0
A. cruentus K112 ('97)	67.7 ± 0.5	74.8 ± 0.2	82.8 ± 0.2	13.5 ± 0.3
A. cruentus K350 ('97)	71.6 ± 0.4	76.9 ± 0.5	83.9 ± 0.7	12.4 ± 0.8
A. cruentus K459 ('97)	67.4 ± 0.1	75.2 ± 0.0	81.7 ± 0.0	12.3 ± 1.1
A. cruentus K472 ('97)	66.3 ± 0.7	73.6 ± 0.1	80.7 ± 0.1	11.9 ± 0.9
A. hybridus D88-1 ('97)	65.3 ± 0.1	72.8 ± 0.1	79.4 ± 0.2	12.6 ± 0.7
C. quinoa Qinua Real	52.8 ± 0.7	60.2 ± 0.1	67.8 ± 0.3	8.1 ± 0.8
C. quinoa	57.4 ± 0.4	61.9 ± 0.3	67.6 ± 0.5	7.3 ± 0.1
C. quinoa 1-B	52.2 ± 0.2	58.9 ± 0.2	68.5 ± 0.5	10.3 ± 0.1
C. quinoa 2-B	46.1 ± 0.4	54.2 ± 0.1	66.2 ± 0.5	10.5 ± 0.3

*To, Tp, and Tc are onset, peak, and conclusion temperatures for gelatinization and ΔH is heat of gelatinization, respctively.

Digestibility of amaranth and quinoa starch granules

Starch granules of amaranth and quinoa were digested by a mixture of glucoamylase and α-amylase faster than those of the normal maize (Tables 5 and 6). The main reason may be the smaller sizes of these two kinds of starch granules than those of the normal maize.

Table 5

Degradation of starch granules (% degradation) obtained from maize, amaranth, and quinoa by amylase* – 1

Starch sample	Duration of enzyme reaction (hr)			
	1	3	6	24
Normal maize	21.7	54.9	83.6	99.1
A. hypocondriacus K343	57.5	90.5	97.7	99.3
A. cruentus R104 ('96)	59.2	89.7	93.9	94.5
A. cruentus K112 ('96)	65.8	90.3	89.4	100.6
C. quinoa Qinua Real	77.7	100.4	100.4	101.3
C. quinoa	70.7	96.8	99.5	102.2

*Commercial preparation composed of a mixture of α-amylase and glucoamylase obtained from Aspergillus sp. K- 27.

Table 6

Degradation of starch granules (% degradation) obtained from maize, amaranth, and quinoa by amylase* – 2

Starch sample	Duration of enzyme reaction (hr)			
	1	3	6	24
Normal maize	17.0 2.3	50.9 2.6	79.6 4.8	92.2 5.5
A. cruentus R104 ('97)	59.8 0.5	87.2 0.3	99.7 0.1	96.7 7.5
A. cruentus K112 ('97)	55.7 2.4	90.5 0.2	92.8 2.3	91.1 0.0
A. cruentus K350 ('97)	49.4 4.5	91.1 1.2	94.4 3.7	100 0.0
A. cruentus K459 ('97)	57.2 0.1	82.9 3.5	97.7 4.9	97.3 2.4
A. cruentus K472 ('97)	53.1 4.4	93.3 2.1	86.8 5.6	89.1 4.0
A. hybridus D88-1 ('97)	57.3 3.1	90.7 3.8	91.8 2.9	91.2 1.2
C. quinoa 1-B	46.6 3.5	90.2 1.7	85.1 7.5	86.1 2.4
C. quinoa 2-B	55.4 1.2	94.5 1.6	94.1 1.5	89.8 4.5

*Commercial preparation composed of a mixture of α-amylase and glucoamylase obtained from Aspergillus sp. K- 27.

Conclusions

We found that amaranth and quinoa amylopectins had unique short chain length distributions. Namely they had increased amounts of long chains and decreased amounts of short chains, however, they had increased amounts of chains with DP from 6 to 12 comparing with the waxy maize amylopectin. Moreover there were wide variations in the amylose content of amaranth starch.

The textural contribution of starch to food and non-food industrial products varies with size, proportion, and degree of branching of the starch molecules present in them, in addition to their granular size and structure. There have been several investigations for application of amaranth and quinoa starches [18-22] These investigations are, however, immature. I hope our studies offer useful information for food and other industrial uses of these starches.

REFERENCES

[1] Sugimoto Y., Yamada Y., Sakamoto S., Fuwa H.: Starch/Stärke, **33**, 1981, 112-116.
[2] Tomita Y., Sugimoto Y., Sakamoto S., Fuwa H.: J. Nutr. Sci. Vitaminol., **27**, 1981, 471-484.
[3] Konishi Y., Nojima H., Okuno K., Asaoka M., Fuwa H.: Agric. Biol. Chem., **49**, 1985, 1965-1971.
[4] Sugimoto Y., Yamashita Y., Kunishige N., Fuwa H.: Oyo Toshitsu Kagaku (in Japanese), **42**, 1995, 223-229.
[5] Sugimoto Y., Nishihara K., Fuwa H.: Denpun Kagaku (in Japanese), **33**, 1986, 169-176.

[6] Sugimoto Y., Yamamoto Y., Abe K., Fujita S., Fuwa H.: Denpun Kagaku (in Japanese), **34**, 1987, 1-10.
[7] Ranhotra G.S., Gelroth J.A., Glasser B.K., Lorenz K.J., John D.L.: Cereal Chem., **70**, 1993, 303-308.
[8] Lopez M.G., Bello-Perez L.A., Paredes-Lopez O.: [in] "Amaranth: Biology, Chemistry, and Technology", ed. by Paredes-Lopez O., CRC Press, Boca Raton, 1994, 110-140.
[9] Coulter L., Lorenz K.: Lebensm.-Wiss. u. -Technol., **23**, 1990, 203-207.
[10] Schoch T.J.: [in] "Method in Enzymol:', III, ed. by Colowick S.P., Kaplan N.O., Academic Press, New York, 1954, 5.
[11] Ando H., Inouchi N., Asaoka M., Okuno K., Fuwa H.: Żywność. Technologia. Jakość., **2**, 1996, 9-15.
[12] Inouchi N., Glover D.V., Fuwa H.: Starch/Stärke, **39**, 1987, 259-266.
[13] Inouchi N., Glover D.V., Sugimoto Y., Fuwa H.: Starch/Stärke, **36**, 1987, 8-12.
[14] Inouchi N., Glover D.V., Fuwa H.: Starch/Stärke, **47**, 1994, 421-426.
[15] Fuwa H., Nakajima M., Hamada A., Glover D.V.: Cereal Chem., **53**, 1977, 355-364.
[16] Inouchi N., Glover D.V., Sugimoto Y., Fuwa H.: Starch/Stärke, **43**, 1991, 468-472.
[17] To K.-G., Shimizu M., Toshida T., Watanabe K., Mitsunaga T.: 36th Meeting for Kinki Branch of Japanese Society for Food and Nutrition, Abstracts, 1997, 37.
[18] Zhao J., Whistler R.L.: Food Technol., **48**, 1994, 104-109.
[19] Ahamed N.T., Singhal R.S., Kulkarni P.R., Kale D.D., Pal M.: Carbohydr. Polymers, **31**, 1996, 157-160.
[20] Teli M.D., Shanbag V., Kulkarni P.R., Singhal R.S.: Carbohydr. Polymers, **31**, 1996, 119-122.
[21] Ahamed N.T., Singhal R.S., Kulkarni P.R., Pal M.: Carbohydr. Polymers, **31**, 1996, 99-103.
[22] Walkowski A., Fornal J., Lewandowiez G., Sadowska J.: Polish J. Food Nutr. Sci., **6/47**, 1997, 11-22.

CHARAKTERYSTYKA SKROBI O MAŁYCH GAŁECZKACH – SKROBIA Z AMARANTUSA I QUINOA

Streszczenie

Gałeczki skrobiowe wydzielono z dojrzałych ziaren 9 próbek amarantusa i 4 próbek *Chenopodium quinoa.* Za pomocą zwykłej chromatografii żelowej (GPC) skrobi pozbawionej odgałęzień za pomocą izoamylazy z *Pseudomonas* stwierdzono, że skrobia amarantusowa zawiera od 0 do 28% amylozy. W skrobi z quinoa znaleziono 25 do 27% amylozy. Stosunek liczby łańcuchów krótkich do łańcuchów długich w amylopektynie wynosił dla tych skrobi od 2,2 do 3,3 i był nieco niższy niż dla zwykłej skrobi kukurydzianej. Materiał pozbawiony odgałęzień za pomocą izoamylazy rozdzielono za pomocą wysokosprawnej chromatografii cieczowej (HPLC) z refraktometrem różnicowym (RI) i niskokątowym fotometrem laserowym światła rozproszonego (LALLS) jako detektorami oraz za pomocą wysokorozdzielczej chromatografii jonowymiennej z amperometrycznym detektorem pulsacyjnym (HPAEC-PAD). Stwierdziliśmy, że amylopektyny z amarantusa i z quinoa miały więcej długich łańcuchów B i mniej krótkich łańcuchów aniżeli amylopektyna ze skrobi kukurydzianej woskowej. Jednakże, miały one więcej łańcuchów krótkich o stopniu polimeryzacji (DP) od 6 do 12. W porównaniu ze zwykłą skrobią kukurydzianą skrobie amarantusowe miały nieco wyższą temperaturę kleikowania (T_o, T_p i T_c) i mniejsze ciepła kleikowania (ΔH) zmierzone różnicowym kalorymetrem skanningowym (DSC). Skrobia z quinoa miała niższe T_o, T_p i T_c i mniejsze ΔH. Gałeczki skrobi z amarantusa i z quinoa były trawione przez amylazę szybciej niż gałeczki zwykłej skrobi kukurydzianej.

V.N. KISLENKO, A. A. BERLIN, N.I. LITOVCHENKO

KINETICS OF GRAFT POLYMERIZATION OF METHYL ACRYLATE ONTO HYDROXYETHYL- AND CARBOXYMETHYL CELLULOSE

Abstract

An influence of the process condition on the grafting efficiency and molecular mass of the acrylic chains of graft copolymers of poly(methyl acrylate) with hydroxyethyl and carboxymethyl cellulose (HEC and CMC) has been studied.

The kinetics of graft polymerization of methyl acrylate onto hydroxyethyl and carboxymethyl cellulose initiated by peroxydisulfate ion in the wide region of reagent concentrations has been investigated. The reaction order with respect to the monomer is about 1, to peroxydisulfate ion and initial polymer is about zero for polymerization and grafting of methyl acrylate onto CMC and HEC. Effective activation energies are 102 and 110 kJ/mol for HEC and 78 and 80 kJ/mol for CMC in these reactions respectively.

The kinetics of the peroxydisulfate ion decomposition and the change of molecular mass of HEC and CMC in their water solutions has been investigated. The reaction order with respect to peroxydisulfate is about 1 and with respect to cellulose derivatives changes from 0.3 to zero when the cellulose ether concentration increases in the reaction of peroxydisulfate decomposition. It has been showed that the process of polymer destruction and formation of cross-linked cellulose derivatives proceeds in their water solutions in the presence of peroxydisulfate.

Graft polymerization of monomers is one of the universal and effective methods of chemical modification of polymers, natural ones in particular.

Graft copolymers of vinyl monomers and water soluble derivatives of cellulose can be obtained in a form of stable water dispersions or solutions suitable for direct applications. That gives a possibility to use them as stabilizers of emulsions and dispersions. For example, grafting of methyl acrylate onto methyl or hydroxyethyl cellulose with following hydrolysis of grafted chains gives stable products.Their emulsifying power is greater by several folds than one for bacbone cellulose ethers (Table 1).

Amphiphilio graft copolymers can reduce the adsorption strength of metals due to the Rebinder effect. Therefore they can be used as metal-cutting fluids at metal machining (Table 2). The wear of a cutting tool is significantly reduced and the surface

Lviv Polytechnic State University 12 S.Band

quality rises at application of metal-working fluid on a base of graft copolymer of methyl methacrylate with HEC.

Amphiphilic nature of graft copolymers causes the fact that their water dispersions create films insoluble neither in water nor in organic solvents. It allows us to use them as film former. For instaance, dispersions of graft copolymers of fluor-containing monomers and carboxymethyl cellulose are good for fabrio finishing. In paarticular, graft copolymer of perfluoro-heptyl acrylate and carboxymethyl cellulose gives to a fabrio oil- and dirt-repulsive properties. Herewith the fabrios feep them even after 10 washing (Table 3).

Table 1

Emulsifying ability of cellulose ethers and their graaft copolymers

Specimen	T, °C	Time of half – separation, s
Methyl cellulose	20	9,8
	50	emulsion is not formed
Graft copolymer of PMA with MC	20	7200
	50	5400
Hydroxyethyl cellulose	20	25
	70	emulsion is not formed
Graft copolymer of PMA with HEC	20	> 10 000
	70	< 10 000

Table 2

Effect of metal-working fluid on wear of drills (steel P6M5), diameter 9.2 mm) at drilling of steel X12M. Angle rate of drilling is 59 rad/s, feed is 0.022 mm/rad (regime 1) and 0,03 mm/rad (regime 2)

Metal-working fluid	Durability of instrument under wear, s		Wear on back surface, m		Surface quality, class	
	1	2	1	2	1	2
Emulsol	720	240	0.8	1.1	3	3
Graft copolymer of poly (methyl Methacrylate) with						
CMC	5160	2280	0.7	0.7	4	4
HEC	4980	2160	0.7	0.7	4	4

Table 3

Change of properties of a fabrio treated with graft copolymer of PFHA with CMC

Property	Change of properties after washing		
	0	5	10
Oil resistance	130	110	90
Quality of washing, %	94	89	87
Water absorption, %	119	–	–

Experimental part

Methyl acrylate was purified by a method presented in Ref. [1] and rectified. Ammonium peroxydisulfate was recrystallized twice from water. HEC with the polymerization degree of 812 and ethylene oxide content of 32%, Na-CMC with molecular mass $3.64 \cdot 10^4$ and the substitution degree of 0.74 was used. The Process was carried out in a glass vessel under argon atmosphere at 55–75°C. Concentration of monomer was 0.12–0.98 mol/l, ammonium peroxydisulfate was 2.2–8.8 mmol/l, and cellulose ether was 5–20 g/l. The monomer conversion, S, in the samples determined by a gravimetrio method. The grafting efficiency, δ, was determined by complete extraction of methyl acrylate homopolymer with acetone in Soxhlet extraction apparatus.

Formation of graft copolymers of poly(methyl acrylate) with cellulose ethers was confirmed by IR-spectroscopy investigation of products of graft polymerization (after complete extraction of methyl acrylate homopolymer by acetone and unmodified cellulose ether by water). The absorption bands of cellulose ethers (1068, 1026, 885 cm^{-1}) and of poly (methyl acrylate) (1732, 1160, 1198, 1256, 826 cm^{-1}) were bound in the products after extraction.

Results and discussion

The investigation of an influence of the process condition on the properties of graft copolymers of poly(methyl acrylate) with hydroxyethyl- and carboxymethyl cellulose showed (Table 4), that the monomer conversion increased, grafting efficiency as well as molecular mass of polyacrylic chains decreased when the monomer/cellulose derivative ratio, initiator concentration and temperature increased. The grafting degree (GD) for all samples is high enough.

Kinetics and mechanism of monomer graft polymerization onto cellulose ethers were investigated to find optimum conditions for copolymer preparation.

The monomer conversion, determining the general polymerization rate of methyl acrylate in the presence of HEC or CMC, and its product by the grafting efficiency, Sδ

(characterising the strict grafting) change proportionally to time at the initial stage of kinetic curves (Fig. 1, 2). An increase in concentrations of a backbone polymer, potassium peroxydisulfate does not depend on the process rate. An increase of temperature carries out to the rise reaction rate. It is worth noting that the rates of the general polymerization process and of strict grafting of MA onto CMC and HEC are close to each other. It is evidence on the faint influence of the nature of a backbone polymer onto the reaction kinetics.

Table 4

Chemical properties of graft copolymers of poly (methyl acrylate) with hydroxyethyl cellulose

[MA]/[HEC] by mass	$[S_2O_3^{2-}]$, % of mass HEC	T, °C	S, %	GD, %	δ, %	$M.10^{-5}$
2.0	10	30	94	160	85	2.43
2.0	10	40	94	151	81	2.28
2.0	10	50	95	124	65	2.00
2.0	10	60	95	118	62	1.66
2.0	10	70	96	114	60	1.39
2.0	2.5	60	151	151	99	–
2.0	5	60	137	137	76	1.66
2.0	10	60	118	118	62	1.47
2.0	20	60	89	89	47	1.33
1.0	10	60	92	92	99	-
2.0	10	60	118	118	62	1.66
3.0	10	60	112	112	39	2.86
4.0	10	60	124	124	32	3.94
6.0	10	60	136	136	23	4.75

The reaction order, calculated from the initial rates, is close to 1 with respect to monomer and to zero with respect to initiator and backbone polymer for the general polymerization process and strict grafting of MA onto HEC and CMC. Effective activation energies are 102 ±12 and 110±14 kJ/mol for the MA – HEC system, as well as 78±10 and 80±10 kJ/mol for the MA – CMC system (for the general polymerization process and strict grafting respectively). One should notice that the values of activation energy are close to each other for the general polymerization process and strict grafting.

Interaction of peroxydisulfate with polysaccharides proceeds by radical chain mechanism, to which a reaction rate decrease testifies when radical inhibitors are introduced. Initiation of this processs takes place only trough peroxydisulfate ion homolysis. The reaction rate increases when the hydroxyethyl cellulose concentration grows to

10 g/l then it reachess constaant value (Fig. 3). The rate of peroxydisulfate decomposition is less in CMC solution. The presence of carboxyle groups in the polymer macromolecule considerably decreases peroxydisulfate decomposition rate.

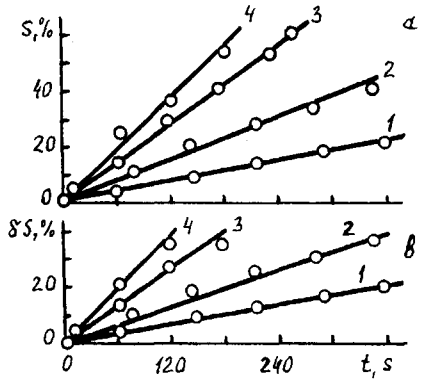


Fig. 1. Change of monomer conversion (a) and of Sδ (b) in time for methyl acrylate grafting onto HEC. The initial concentration of monomer is 0.23 mol/l, HEC 20 g/l, ammonium peroxydisulfate 4.4. mmol/l, and temperature 55 (1), 60 (2) 65 (3) and 70° C (4).

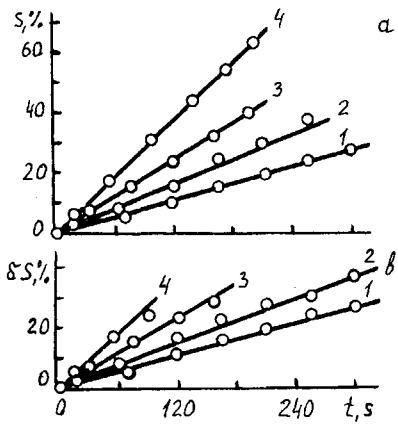


Fig. 2. Change of monomer conversion (a) and of Sδ (b) in time for methyl acrylate grafting onto CMC. The initial concentration of monomer is 0.23 mol/l, CMC 20 g/l, ammonium peroxydisulfate 4.4 mmol/l, and temperature 55 (1), 60 (2), 65 (3) and 70°C (4).

The reaction order, calculated from the initial rates, is close to 1 with respect to peroxydisulfate and changes from 0.3 (when hydroxyethyl cellulose concentration is less than 10 g/l) to zero (at the higher concentrations) with respect to hydroxyethyl cellulose. The reaction order with respect to carboxymethyl cellulose is about zero in

the whole range of concentrations. Effective activation energies are 87±4 and 129±8 kJ/mol for peroxydisulfate decomposition in the presence of hydroxyethyl and carboxymethyl cellulose respectively.

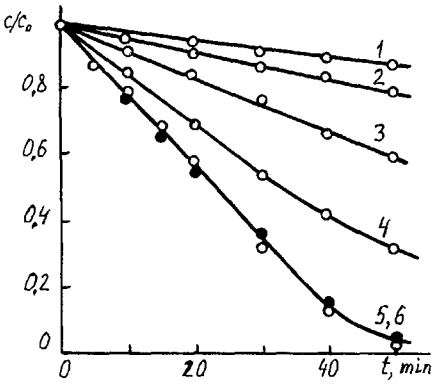


Fig. 3. Kinetic curves of ammonium peroxydisulfate decompossition in water (1) and in the presense of hydroxyethyl cellulose at initial concentration of peroxydisulfate 4.4 mmol/l, temperature 70°C and hydroxyethyl cellulose concentration 0.001 (1), 0.01 (2), 0.1 (3), 1 (4) and 3% (5).

It is necessary to note, that under peroxydisulfate action, ecomposition of polysaccharide takes place. Investigation of hydroxyethyl cellulose molecular mas dependence on reaction conversion and peroxydisulfate ion concentration showed, that the polymer decomposition process proceeds (Fig. 4). In addition to this process, the formation of cross-linked polysaccharide has been observed. At the low enough concentration of peroxydisulfate only the anomalous increase in polymer molecular mass proceeds (Fig. 4). This phenomenon may be connect to a polymer radical recombination with the formation of longer chains.

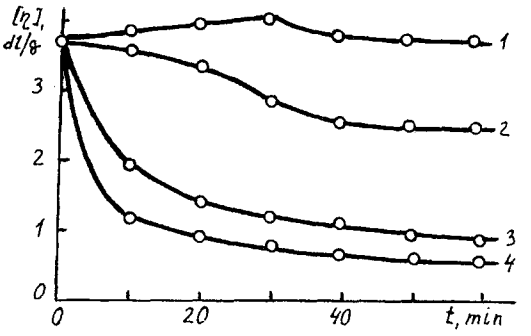


Fig. 4. Changes of intrinsic viscosity of hydroxyethyl cellulose with time at 70°C and peroxidisulfate concentration 0.044 (1), 0.22 (2), 2.2 (3) and 8.8 mol/l (4).

Therefore the process of graft polymerization of acrylic monomers onto polysaccharides includes two stages. In the first of them the homolysis of peroxydisulfate and monomer polymerization proceeds. The graft copolymer formation has been observed in this stage. Then the radical chin decomposition of peroxydisulfate and destruction of water soluble cellulose derivatives takes place.

REFERENCES

[1] Grigoriev A.P., Laboratory Manual for Technology of Polymerizing Plastics, Vysshaya Shkola, Moscow, 1964.

KINETYKA SZCZEPIONEJ POLIMERYZACJI AKRYLANU METYLU Z HYDROKSYETYLO- I KARBOKSYETYLOCELULOZĄ

Streszczenie

Badano wpływ warunków procesu na efektywność szczepiania i masę cząsteczkową łańcuchów akrylowych szczepionych polimerów poli(metyloakrylanu) z hydroksyetylo- (HEC) i karboksymetylocelulozy (CMC).

Prześledzono w szerokim zakresie stężeń reagentów kinetykę szczepionej polimeryzacji akrylanu metylu z hydroksyetylo- i karboksymetylocelulozą inicjowaną jonem peroksydisiarczanowym. Zarówno w przypadku CMC jak i HEC reakcja względem monomeru jest ok. pierwszego rzędu, a względem inicjatora dla polimeryzacji jak i szczepiania jest rzędu zerowego. Efektywna energia aktywacji dla HEC wynosi 78 kJ/mol a dla CMC 80kJ/mol.

Badano kinetykę rozkładu jonu peroksydisiarczanowego i zmiany masy cząsteczkowej HEC i CMC w ich roztworach wodnych. Reakcja jest ok. pierwszego rzędu względem tego jonu a względem pochodnych celulozy zmienia się od 0,3 do zera wraz ze wzrostem stężenia pochodnej celulozy. Pokazano, że w roztworze wodnym w obecności peroksydisiarczanu zachodzi rozkład polimeru i że pochodne celulozy ulegają sieciowaniu.

J. KOROLCZUK, V. BRETON-DOLLET, J.P. TISSIER, J.F. MAINGONNAT

EFFECT OF HEAT TREATMENT ON THE RHEOLOGY AND MICROSTRUCTURE OF MAIZE STARCH GELS

Abstract

The normal and waxy maize starch pastes presented at 60°C a shear thinning, power law type behaviour, with the consistency index decreasing with the rise of the pasting temperature from 100 to 130°C. The structure index, for both types of pastes was on average about 0.4 and it was only slightly increasing when the pasting temperature rose. The maize starch pastes presented much greater thixotropic properties than did the waxy starch pastes. The gelation et 25°C followed the kinetic of the first order reaction. The reaction rate constant was about 10 times lower for the waxy then for the normal maize starch gels. The maize starch gels have the filamentous structure, while the waxy starch gels are composed of grapes of small (0.1 μm), roughly spherical particles.

Introduction

The main reason for using starch and its derivatives as additives in food preparation is to retain water and to increase the product viscosity. Native starch granules have semicrystalline structure and contain about 10-20% water [7, 17, 20-23, 31-32, 39-41]. At low water content (<1.5%) the crystalline structure remains unchanged even after high temperature (232°C) treatment [7]. While heated in presence of excess water, water, above so called gelatinization temperature the starch granules loose their crystalline structure. They are swelling and retain up to 80 g of water per gram of dry matter depending mainly on the starch species [2-4, 5-13, 26, 29, 32]. A part of starch (mainly amylose) is solubilized during the gelatinization process [14-15, 19, 24-26, 33-34]. During cooling and storage the solubilized and hydrated amylose and amylopectin either precipitate (at low concentrations) or form a gel [1-2, 9-10, 12, 16-18, 27-30, 33-38] and eventually partly recrystallize [32, 35].

INRA-LGPTA, 369 rue Jules Guesde, B.P. 39, 59651 Villeneuve-d'Ascq, France.

The aim of this work was to study the effect of heating at temperatures over 100°C on the rheological properties and the microstructure of the normal and waxy maize starch gels.

Materials and methods

The following raw materials were used: maize starch and waxy starch (Sigma, Saint Quentin Fallavier, France). A controlled stress rhrometer type Carri-Med CS100 (Rheo, UK) with a cone (4°, 6 cm) and plate geometry was used for rheological measurements.

Two hundred millilitres of water suspensions containing 4% or 5% starch were heated during 30 minutes at 100 to 135°C in a small (250 ml) stainless steel reactor vessel with magnetic stirring. Then the pastes were cooled to 90°C and transferred on tothe plate of the rheometer for rheological measurement. For microscopic studies the gel samples were prepared from the starch suspensions containing 10% of normal or waxy starch. They were heated at 110°C during 30 minutes then cooled to 25°C. The heating and cooling rate was 1°C/min. After cooling the samples were left overnight at room temperature. Then they were dehydrated by the Critical Point Drying with CO_2, carried out in an Emscope CPD 75, coated with Polaron E5 100 and then observed in a JEOL 35 CF Scanning Electron Microscope at 5 to 15 kV.

Results and discussion

All analysed samples (Fig. 1) show shear thinning behaviour, with the logarithm of the apparent viscosity (η_a in Pa·s) being proportional to the logarithm of the shear rate (γ in s^{-1}):

$$\mathrm{Log}(\eta_a) = K + (n - 1)\,\mathrm{Log}\,(\gamma) \qquad (1)$$

where: K = consistency index or the logarithm of the apparent viscosity for the shear rate $\gamma = 1\ s^{-1}$, n – structure or behaviour index.

The consistency index (K) decreases with the increase in pasting temperature, following the Arrhenius type relation:

$$K = A + E/RT \qquad (2)$$

where: A = hypethetical apparent viscosity level for T = infinity and $\gamma = 1\ s^{-1}$, E = activation energy of flow in J/mol, R – gas constant = 8.314 $J/mol^{-1}\ .\ K^{-1}$, T = absolute temperature (K).

With the increase of the pasting temperature, the apparent viscosity at 60°C, for a given shear rate was decreasing, probably due to the progressing solubilisation of amylose and amylopectin and the description of starch granules. The amplitude of the

apparent viscosity changes in relation with the pasting temperature was higher for 5% maize starch paste (M5%) than for the 4% waxy (M4%) starch paste (Fig. 1). The coefficient (A) from the equation (2) was –10.14 (±0.112) for the M5% against –4.84 (±0.103) for the W4%. The activation energy of flow (E) from the equation (2) was respectively 34 (±1.65) and 17 (±2.0) kJ/mol for normal and waxy starch pastes (Fig. 2). The apparent viscosity of the pastes, measured at 60°C, was divided by a factor of 1.9 and 1.4 respectively for normal and waxy starches when the pasting temperature rose by 10°C. But if taking into account a quite important dispersion of the experimental results the differences in the paste viscosity can only be observed for low (<100°C) and high (.125°C) pasting temperatures.

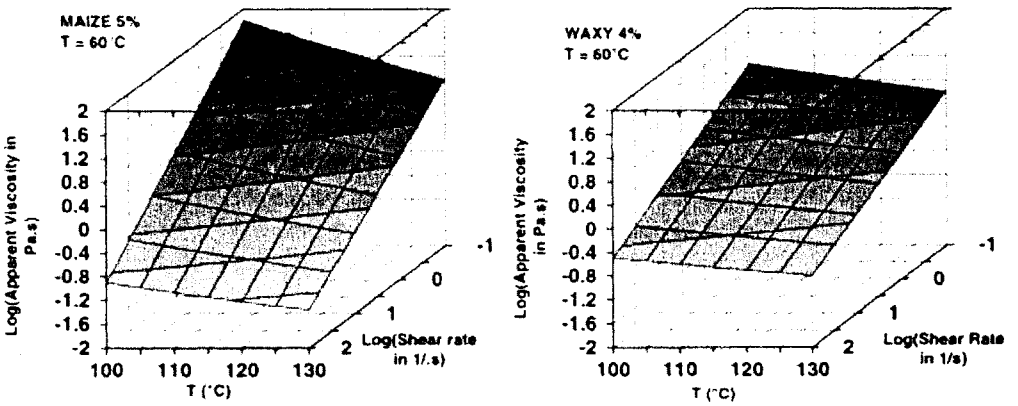


Fig. 1. Logarithm of the apparent viscosity at 60°C as a function of the pasting temperature and the logarithm of the shear rate for the 5% maize starch and 4% waxy starch pastes.

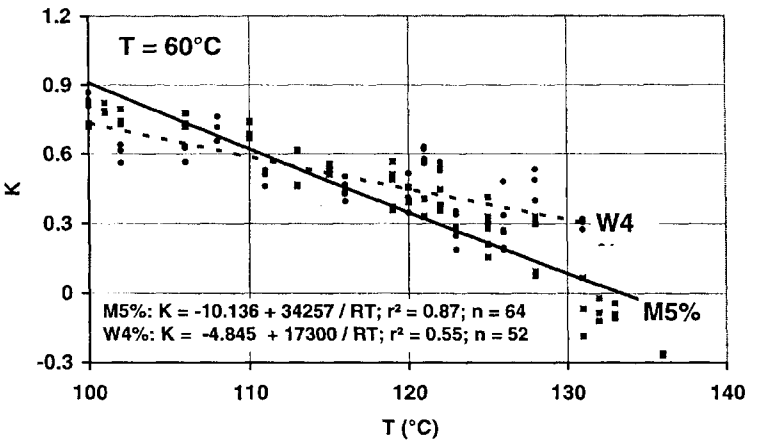


Fig. 2. Consistency index (K) at 60°C as a function of the pasting temperature (T in °C) for the 5% maize (M5%) starch and 4% waxy (W4%) starch pastes.

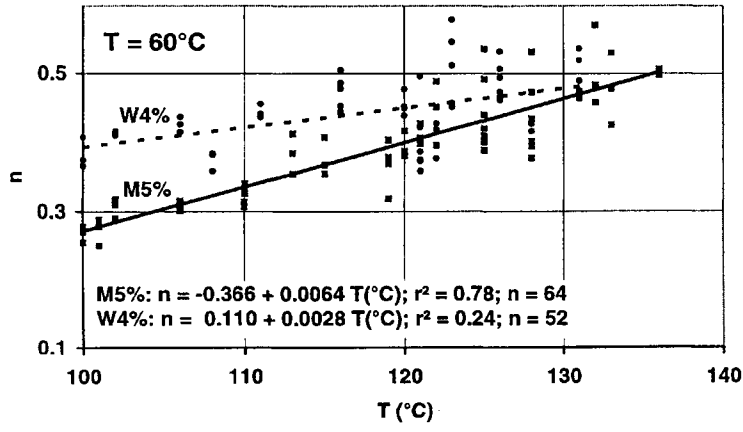


Fig. 3. Structure or behaviour index (n) at 60°C as a function of the pasting temperature (T in °C) for the 5% maize (M5%) starch and 4% waxy (M4%) starch pastes.

The structure of behaviour index (n) from the equation (1) was on average about 0.4 and it was slightly rising with the increase of the pasting temperature (Fig. 3). For both types of starch this increase was relatively small if compared with the scattering of the experimental results. The standard deviation was about 0.04, while the average amplitude of the increase of the coefficient (n) was 0.19 for the normal and 0.09 for the waxy starch pastes for the pasting temperature rise from 100°C to 130°C.

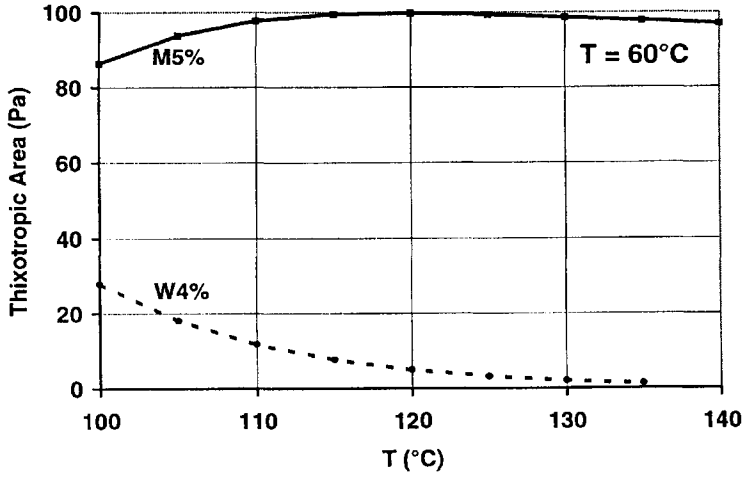


Fig. 4. Thixotropic area at 60°C as a function of the pasting temperature (T in °C) for the 5% maize (M5%) starch and 4% waxy (W4%) starch pastes.

Basing on the experimentally found evolution of (K) and (n) coefficients from the equation (1) as a function of the gelatinization temperature, we calculated the thixo-

tropic areas enclosed withim the hysteresis loops for the plots of the apparent viscosity against the shear rate (Fig. 4). For the normal maize starch pastes, for the first shearing cycle, the thixotropic area at 60°C is almost independent on the gelatinization temperature. For the first cycle the thixotropic area represents 50–80% of the total area of the apparent viscosity versus shear rate plots for the increasing shear rate. It decreases to only about 20-30% for the successive shearing cycles. It means that already at 60°C the maize starch paste is quite well structured. For the waxy starch pastes the thixotropic ares decreases with the rising of the gelatinization temperature (Fig. 4). At the same time the relative importance of the thixotropic area is much smaller. It represents only between 10 and 25% of the apparent viscosity versus shear rate plots area for the increasing shear rate. This confirm the low structuring capacity of the waxy starch, composed in 99% of the amylopectin.

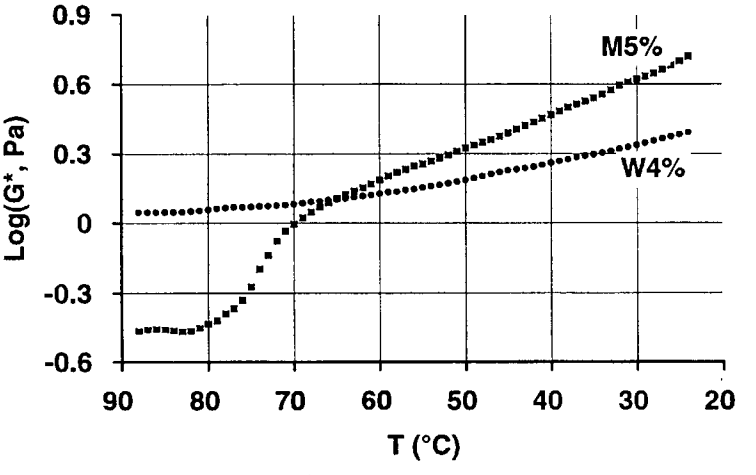


Fig. 5. Logarithm of the complex modulus (G^*) evolution during cooling the maize 5% (M5%) and waxy 4% (W4%) pastes from 90°C to 25°C.

During the cooling from 90 to 25°C, the semi-liquid starch pastes are transformed in semi-solid gels. The complex modulus (G^*) was gradually increasing for both types of starch, but the amplitude of the modulus rise was much higher for the M5% than for the W4% paste (Fig. 5). Also the kinetic of the modulus changes was different for both types of pastes. Between 80 add 70°C the modulus increase was very high for the M5% paste, white for the W4% paste the rate of modulus increase was low and almost uniform for the whole temperature range (90–25°C).

The shift angle was very high (~80°C) for the W4% paste at 90°C and it decreased only slightly, to ~50° at 25°C (Fig. 6). For the M5% paste already at 90°C the shift angle was ~55°C and it decreased rapidly between 80 and 70°C to ~35°C and then

slowly to ~10°C at 25°C. The shift angle is 90° for purely viscous bodies and 0° for purely elastic bodies. It is in between 90 and 0° for viscoelastic materials. From this point view the W4% paste is more viscous than elastic and the M5% paste is more elastic than viscous. This is due to the presence of amylose in the M5% pastes. Similar type of the viscosity, modulus and the shift angle evolution during cooling the potato and barley, wheat and maize starch pastes [1, 33].

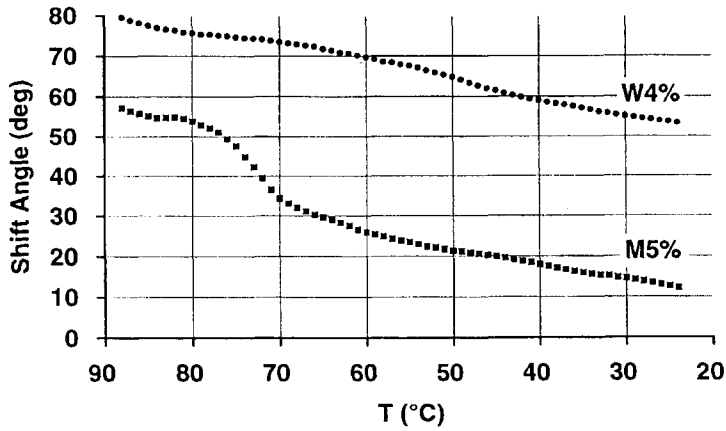


Fig. 6. Shift angle evolution during cooling the maize 5% (M5%) and waxy 4% (W4%) pastes from 90°C to 25°C.

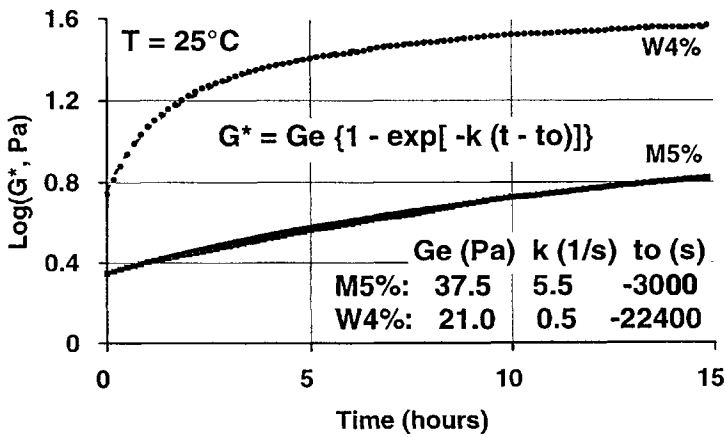


Fig. 7. Logarithm of the complex modulus (G^*) evolution during 15 hours storage aat 25 °C of the maize 5% (M5%) and waxy 4% (W4%) pastes.

During the storage at 25°C, the modulus continued to grow (Fig. 7), following the kinetic of the order reaction:

$$G_t = G_e \{1 - \exp[-k(t - t_o)]\} \quad (3)$$

where: G_t = complex modulus level (Pa) after time t (s), G_e = equilibrium level of the complex modulus, t_o = latency time (s) and k = reaction rate constant (s^{-1}).

All three parameters of the equation (3) were very much different for both analysed types (M5% and W4%) of starch pastes (Fig. 7). The equilibrium modulus (G_e) was about twice higher and the reaction rate constant (k) was ~10 times higher for the M5% than for the W4% gels. As the gelation process was already well advanced when the product temperature was decreased to 25°C, so the latency time (t_o) here is the hypothetical time period between the beginning of the storage period at 25°C and the moment when the modulus versus time curve, calculated by the equation (3), crosses $G^* = 0$ Pa level.

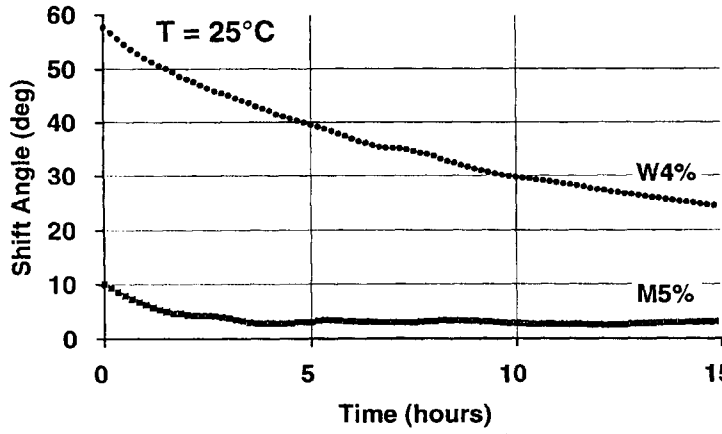


Fig. 8. Shift angle evolution during 15 hours storage at 25 °C of the maize 5% (M5%) and waxy 4% (W4%) pastes.

The shift angle continued to decrease during the storage at 25°C (Fig. 8). The equilibrium level of about 2–3° was reached after about 3 hours of storage for the M5% gel. With the shift angle close to 0°, the M5% gel is almost purely elastic. On the other hand the evolution of the shift angle for the W4% gel was much slower. It passed through 45° level, considered sometimes ass the sometimes as the gel point, after about 3 hours of storage at 25°C and it continued to decrease, reaching 24° after 15 hours of storage.

The starch gels, prepared from 10% normal and waxy starch suspensions have quite different microstructure (Fig. 9). In the native maize starch gel (M10%) the swollen starch granules are still visible. They have a filamentous structure and they are interconnected by a network of filaments 0.1–0.2 μm thick and several μm long. Similar filamentous structure was observed outside the starch granules in the wheat starch gels [29]. In the waxy starch gels the starch granules in observed. The gel is composed

of the small (0.1–0.2 μm) roughly spherical particles quite densely aggregated in grapes of variable dimensions, interconnected by the filaments, composed of more or less linearly aggregated small particles.

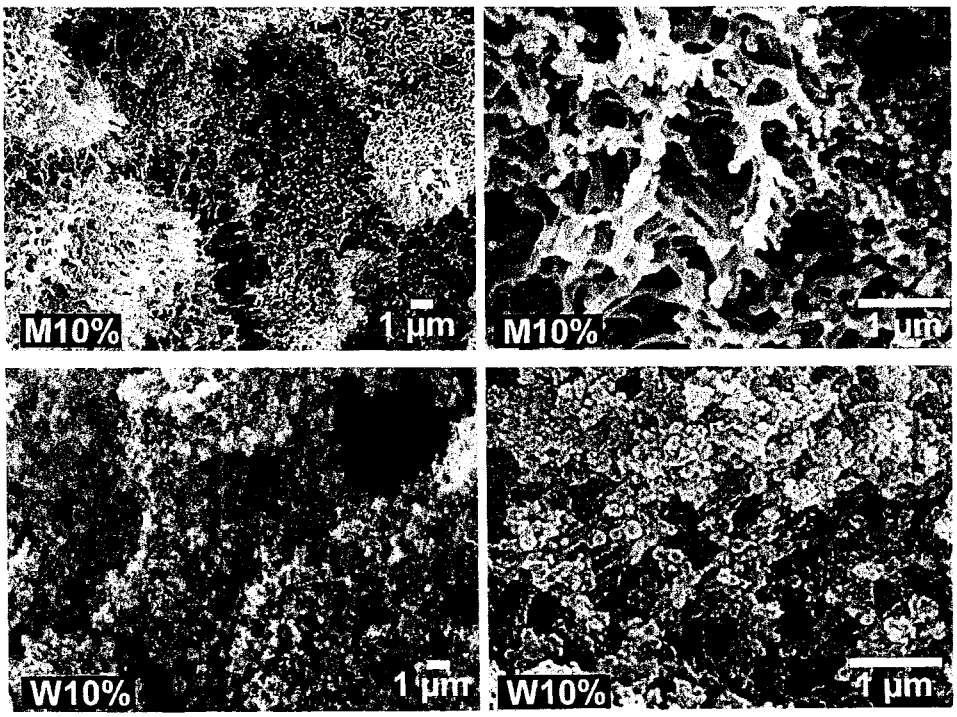


Fig. 9. Scanning electron micrographs of 10% waxy (W10%) and normal maize (M10%) starch gels.

REFERENCES

[1] Autio K.: Rheological and microstructural changes of oat and barley starches during heating and cooling. Food Struct. **9**, 1990, 297-304.

[2] Autio K., Poutanen K., Suortti T., Pessa E.: Heat-induced structural changes in acid -modified barley starch dispersions. Food Strukt., **11**, 1992, 315-322.

[3] Bagley E.B., Christianson D.D.: Swelling capacity of starch and its relationship to suspension viscosity – Effect of cooking time, temperature and concentration. J. Text. Stud., **13**, 1982, 115-126.

[4] Bagley E.B., Christianson D.D.: Yield stresses in cooked wheat starch dispersions. Starch, **35**, 1983, 81-86.

[5] Bloksma A.M.: Effect of heating rate on viscosity of wheat flour doughs. J.Text. Stud., **10**, 1980, 261-269.

[6] Breton V., Korolczuk J., Doublier J.L., Maingonnat J.F.: Rheological properties of maize starch pastes and gels. Rheology 5, 1995, 24-28.

[7] Burt D.J., Russell P.L.: Gelatinization of low water content wheat starch – water mixtures. Starch, **35**, 1983, 354-360.

[8] Cheng-Yi Lii, Min-Feng Lai, Mei-Lin Tsai: Studies on starch gelatinization and retrogradation with dynamic rheometry. The influence of starch granular structure and composition. Żywność Technologia Jakośc. Proceding of VII International Starch Convention, Kraków, 12-14 June 1996, 27-53.

[9] Christianson D.D., Baker F.L., Loffredo A.R., Bagley E.B.: Correlation of microscopic structure of corn starch with rheological properties of cooked pastes. Food Microstruc., **1**, 1982, 13-24.

[10] Christianson D.D., Baglwey e.B.: Yield stresses in dispersion of swollen, deformable cornstarch granules. Cereal Chem., **61**, 1984, 500-503.

[11] Doublier J.L.: Rheological studies on starch – Flow behaviour of wheat starch pastes Starch,**33** 1981, 415-420.

[12] Doublier J.L.: A rheological comparison of wheat, maize, faba bean and smooth pea starches. J. Cereal Sci., **5**, 1987, 247-262.

[13] Doublier J.L., Choplin L.,: A rheological description of amylose gelation. Carbohydr. Res., **193**, 1989, 215-226.

[14] Doublier J.L., Llamas G., Le Meur M.: A Rheological investigation of cereal starch pastes and gels. Effect of pasting procedures. Carbohydr. Polym., **7, 1987, 257-275.**

[15] Doublier J.L., Paton D., Llamas G.: A rheological investigation of oat starch pastes Cereal Chem., **64**, 1987, 21-26.

[16] Eliasson A.C. Viscoelastic behavior during the gelatinization of starch. I. Comparison of wheat, maaize, potato and waxy-barley starches. J. Text. Stud., **17,** 1986, 253-265.

[17] Eliasson A.C., Finstad H., Ljunger G. – 1988 – A study of starch-lipid interactions for some naative and modified maize starches. Starch, **40**, 1988, 95-100.

[18] Evans I.D., Haisman D.R.: Rheology of gelatinization starch suspension. J. Text. Stud. **10**, 1979, 347-370.

[19] Fujita S., Morita T., Fujiyama G.: The study of melting temperature and entalpy of starch from rice, barley, wheat, foxtail and proso millets. Starch, **45**, 1993, 436-441.

[20] Gallant D.J., Guilbot A.: Etude de l`ultrastructure du grain d`amidon `a l` aide de nouvelles methodes de preparation en microscope electronique. Staarch, **21**, 1969, 156-163.

[21] Gallant D.J., Guilbot A.: Developpement des connaissances sur l` ultrastructure du grain d` amidon. I. L`amidon de ble. Starch, **25**, 1973, 335-342.

[22] Ghiasi K., Hosney R.C., Varriano-Marston E.: Gelatinization of wheat-starch. I. Excess-water systems. Cereal Chem., **59**, 1982, 81-85.

[23] Guilbot A., Charbonniere R., Drapon R.: Sur la contribution de l` eau a l` organisation des chaines macromoleculaires de l` amidon. Die Starke, **13**, 1961, 204-207.

[24] Jane J.L., Chen J.F.,: Effect of amylose molecular size and amylopectin branch chain lenght on paste properties of starch. Cereal Chem., **69**, 1992, 60-65.

[25] Korolczuk J., Breton-Dollet J.P., Tissier J.F., Maingonnat J.F.: Rheological properties and microstructure of maize starch / milk proteins gelss. Żywność Technologia Jakość. Procesing of VII Internationaal Starch Convention, Kraków, 12-14 June 1966, 67-74.

[26] Leach W., McCoven L.D., Schoch T.J.: Structure of the starch granule. I. Swelling and solubilitie patterns of various starches. Cereal Chem., **36**, 1959, 534-544.

[27] Miles M.J., Morris V.J., Ring S.G.: Gelation of amylose. Carbohydr. Res. **135**, 1985, 257- 269.

[28] Miles M.J., Morris V.J., Orford P.D., Ring S.G.: The roles of amylose and amylopectin in the gelation and retrogradation of starch. Carbohydr. Res., **135**, 1985, 271-281.

[29] Miller B.S., Derby R.I., Trimbo H.B.: -1973- A pictorial explanation for the increase in viscosity of a heated wheat starch – water suspension. Cereal Chem., **50**, 1973, 271-280.

[30] Muhrbeck P., Eliasson A.C.: Influence of pH and ionic strength on the viscoelastic properties of starch gels – A comparison of potato and cassava starches. Carbohydr. Polym., **7**, 1987 291-300.

[31] Robin J.P., Mercier C., Charbonniere R., Guilbot A.: Lintnerized starches. Gel filtration and enzymatic studies of insoluble residues from prolonged acid treatment of potato starch. Cereal Chem., 51, 1974, 389-406.
[32] Sterling C.: Reviev paper. Textural qualities and molecular structure of starch products. J. Text. Stud., **9**, 1974, 225-255.
[33] Svegmark K., Hermanssson A.M.: Shear induced changes in the viscoelastic behaviour of heat treated potato starch dispersion. Carbohydrate Polymers, **13**, 1990, 29-45.
[34] Svegmark K., Hermansson A.M.: Disstribution of amylose and amylopectin paptato starch pastes: Effects of heating and shearing. Food Structure, **10**, 1991, 117-129.
[35] Varriano-Marston E., Zelaznak K., Nowotna A.: Structural characteristics of gelatinized starch. Starch, **37**, 1985, 326-329.
[36] Wong R.B.K., Lelievre J.: Viscoelastic behaviour of wheaat starch pastes. Rheol. Acta, **20**, 1981, 299-307.
[37] Wong R.B.K., Lelievre J.: Rheological characteristics of wheat starch pastes measured under steady shear conditions. J.Appl. Polym. Sci., **27**, 1982, 1433-1440.
[38] Wong R.B.K., Lelievre J.: Effects of storage on dynamic rheological properties of wheat starch pastes. Starch, **34**, 1982, 231-233.
[39] Żelaznak K.J. Hoseney R.C.: The glass transition in starch. Cereal Chem., **64**, 1987, 121-124.
[40] Zobel H.F.: Molecules to granules: a comprehensive starch review. Starch, **40**, 1988, 44-50.
[41] Zobel H.F., Young S.N., Rocca L.A.: Starch gelatinization: an X-ray diffusion study. Cereal Chem., **65**, 1988, 443-446.

WPŁYW OGRZEWANIA NA REOLOGIĘ I MIKROSTRUKTURĘ ŻELI ZE SKROBI KUKURYDZIANEJ

Streszczenie

W temperaturze 60°C lepkość kleików, z normalnej i woskowej skrobi kukurydzianej, obniżyła się wraz ze wzrostem prędkości ścinania. Dla zakresu temperatury kleikowania od 100 do 130°C, indeks konsystencji był odwrotnie proporcjonalny do temperatury kleikowania. Indeks struktury był średnio na poziomie 0,4 i bardzo nieznacznie wzrastał z temperaturą kleikowania. Kleiki z normalnej skrobi kukurydzianej wykazywały znacznie wyższe własności tiksotropowe niż ze skrobi woskowej. Proces żelowania w temperaturze 25°C przebiegał zgodnie z kinetyką reakcji pierwszego rzędu. Stała prędkości reakcji była około 10 razy niższa dla skrobi woskowej. Żele ze skrobi normalnej miały strukturę włóknistą, natomiast ze skrobi woskowej charakteryzowały się strukturą ziarnistą.

H. KOSTYRA, M. SORAL-ŚMIETANA, M. WRONKOWSKA,
H. KMITA-GŁAŻEWSKA

COMPLEXES OF RESISTANT STARCH WITH NUTRIENTS

Abstract

Starch is a very important component of nutritional ecosystem. In virtualy all human populations, starch is the major component of the diet. The aim of this experiment was to explain the possibility of forming complexes between physically modified starches and nutrients, i.e. tyrosine, folic acid, cholic acid and cholesterol in pH corresponding to the intestine environment. The studies were carried out on physically modiefied starches: retrograded tapioca, maize and wheat; termamyl-digested potato and wheat; autoclaved and spray-dried potato and wheat. The obtained results prove that only cholesterol can form the complexes with processed starches. The investigated substances are characterized by different hydrophilic-hydrophobic properties. Formation of the complex only between cholesterol and processed starches suggests that the initial organisation of the complex within a starch granule is specifically changed during the physical modification of starch. Considering our results, we can propose the following model of these changes. V-amylose helix, sugar-lipid complex and free amylose chain form the specific complex with the hydrophobic tunnel domains. This complex we regard as a resistant starch component of starch granule. The hydrophobic character of this complex is the cause of its resistance to the action of enzymes. It means that only hydrophobic substances can interact with this complex. In conclusion, our investigations enable us to suggest an initial hypothesis for the biological role of resistant starch in the intestinal tract. In the intestinal tract, resistant starch could first of all play the role of a thickening agent. Therefore, resistant starch is the complexing agent of nutrients to a lesser degree.

Introduction

Starch is a very important component of nutritional ecosystem. In virtually all human populations, starch is the major component of the diet [1]. Because uncooked starch is poorly digested in the human alimentary tract, the main function of the various methods of cooking starch materials is so convert starch granules into a form that can be attacked readily by the amylolytic enzymes of the digestive system. In nutritional terms it is common practice to describe two groups of polysaccharides; the latter group being major components "dietary fibre" and, in general representing polysaccha-

Institute of Animal Reproduction and Food Research of Polish Academy of Science, Olsztyn, Poland

rides that are resistant to digestion in upper alimentary system of non-ruminants. At present modified starch containing fraction of resistant starch is classified to the second group. This problem concerns two very interesting questions. What is the biological role of resistant starch? To what degree resistant starch is similar to dietary fibre?

It is worth noting that the native components of starch can form the complexes with lipids, proteins, non-protein nitrogen substances and metals [2].

The aim of this experiment was to explain the possibility of forming complexes between physically modified starches and the nutrients, i.e. tyrosine, folic acid, cholic acid and cholesterol in pH corresponding to the intestine.

Materials and methods

The studies were carried out on physically modiefied starches: retrograded tapioca, maize and wheat; Termamyl – digested potato and wheat; autoclaved and spray – dried potato and wheat. Analysis of resistant starch was carried out using the method by Champ [3]

The solutions of the investigated substances and the incubations with nutrients were made as follows:

A. Cholesterol, samples of the processed starches (100mg) were incubated with emulsion consisted of phosphate buffer, pH 6.8, cholesterol (9%), phosphatidyl choline (40%) and cholic acid (51%) at 37°C for 1h. The content of cholesterol was determined by the BIOCHEMTEST cholesterol colorimetric kit (POCH – Gliwice – Poland).
B. Tyrosine, (50 mg) was dissolved in 50ml of phosphate buffer, pH 7.6, at 50°C. The working solutions were prepared as follows: 3.00, 1.50 and 0.25 ml of the starting solution were diluted to 10 ml with phosphate buffer.
C. Cholic acid (6 mg) was dissolved in 1 ml of methanol and diluted to 10 ml with of phosphate buffer.
D. Folic acid (6 mg) was dissolved in 10 ml of phosphate buffer.

The working solutions of the cholic acid and folic acid were prepared as follows: 0.7, 0.5 and 0.3 ml of the starting solutions were diluted to 10 ml with phosphate buffer. The samples of the processed starches (50 mg) were incubated with the working solutions (10ml) of investigated nutrients at 37°C for 2 h and then centrifuged at 3500 x g for 10 min. The supernatants (tyrosine – 275 nm, folic acid – 280 nm, cholic acid – 222 nm) and absorption spectra were measured by using Beckman's spectrophotometer DU 7500.

Results and discussion

It seems logical and necessary before starting of the presentation and discussion of the results to present the chemical nature of starch complexes with lipids, protein and metals and also the hydrophobic – hydrophilic properties of the investigated nutrients.

It is know that only starch granules from cereal grains, including maize and wheat, contain significant amounts of internal lipid [4], potato starch, in common with starch granules from other tubers and from legume seeds does not contain this type of internal lipid. From point of view the forming of the complexes between starch and lipids was the proving that the significance of the monoacyl character of starch internal lipids lies in the fact that these can form helical inclusion complexes with amylose, whereas di-or tri-acyl lipids do not form such complexes [5].

Starch also forms complexes with proteins. Average values for protein content of commercial starches have been quoted as maize (0.35%), wheat (0.40%) and potato (0.06%).

Careful washing can reduce protein values of cereal starches. In precise Lowy's et al. [6] investigations of wheat starch, the true protein content (by amino acid analysis) of well-washed, pure A starch was found to 0.1%, dry weight basis. Approximately 10% of the starch protein appeared to be associated with the granule surface, in that it could be removed with strong salt solutions of sodium dodecylsulphate (SDS) failed to remove any more protein. However, extraction with hot solutions of SDS, in which the starch was gelatinized, liberated the remaining protein.

The phosphorus belongs to the minor components of starch. The phosphorus content of potato starch (0.06 – 0.10 % as P, dry weight), unlike the lipid phosphorus of cereal starch is due to direct esterification of glucose residues in amylopectin and accounts for about 1 phosphate group per 300 glucose nuits [7].

On the basis of these data and from theoretical point of view, we can conclude that the different components of starch can from clathrates and inclusion and adsorption complexes. Stabitity of these complexes depends on the stereochemistry of the components, the kind of bonds and the type of the interactions. The main role in the forming of these complexes is played by the hydrogen bonds, and to a lesser degree ionic bonds and hydrophobic-hydrophilic interactions. As the results of the adsorption of the all investigated nutrients by the processed starches were very similar, the processed wheat starches were selected to exemplity. The results presented in Fig. 1–6 prove that only cholesterol can form the complex with processed starch. The investigated substances are characterized by different hydrophilic-hydrophobic properties. The chemical structure of these substances shows the they can be ranked according to the hydrophilic-hydrophobic properties as follows: folic acid-tyrosine-cholic acid-

cholesterol. Formation of the complex only between cholesterol and processed starches suggests that the initial organisation of the complexes within a starch granule was specifically changed during the physical modification of starch. Considering our results, we can propose the following model of these changes, witch is a transformation of the Blanshard's model, Fig. 7, [8]. V-amylose helix, sugar – lipid complex and free amylose chain form the specific complex with the hydrophobic tunnel domains. This complex we regard as a resistant starch component of starch granule. The hydrophobic character of this complex is the cause of its resistance for the action of enzymes. It means that only low molecular hydrophobic substances can interact with this complex. In the face of this fact it is interesting to ask, why the investigated hydrophobic-hydrophilic molecules do not form the complexes with other components present in a starch molecule? The reason of the phenomenon could be the inverse nature of the hydrophobic-hydrophilic residues in the molecule. The assumption has been made that only part of the hybrid amylose/amylopectin helix can be hydrated. It means that only this area is available for the functional molecules. In this context only hydrophilic part of molecule can form hydrogen bonds with the external hydroxyl glucose groups of the amylose/amylopectin helix. However, these interactions are blocked by the hydrophobic groups because these groups show the tendency to scape from a water medium.

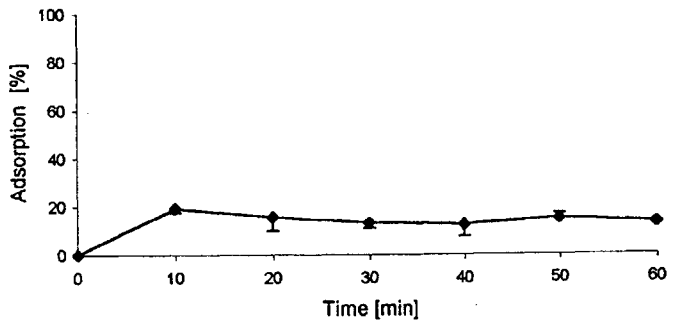


Fig. 1. Adsorption of cholesterol by termamyl-digested wheat starch.

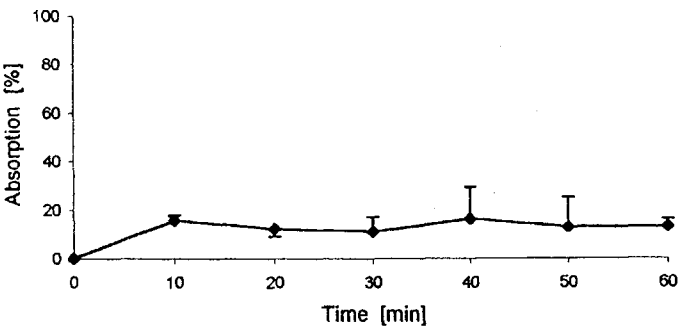


Fig. 2. Adsorption of cholesterol by autoclaved and spray-dried wheat starch.

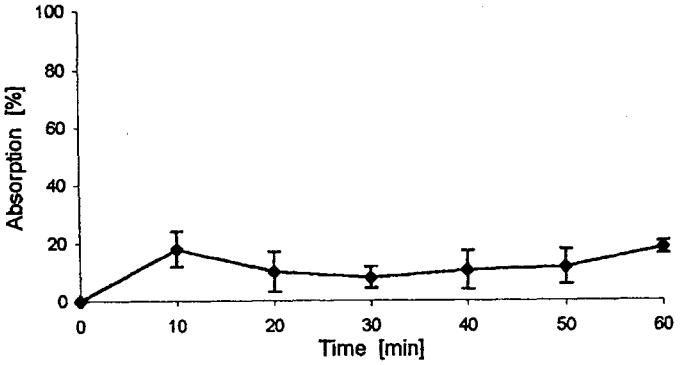


Fig. 3. Adsorption of cholesterol by retrograded wheat starch.

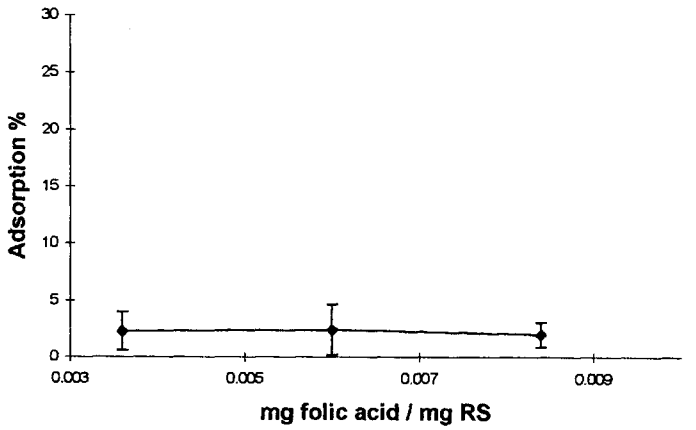


Fig. 4. Adsorption of folic acid by termamyl-digested wheat starch.

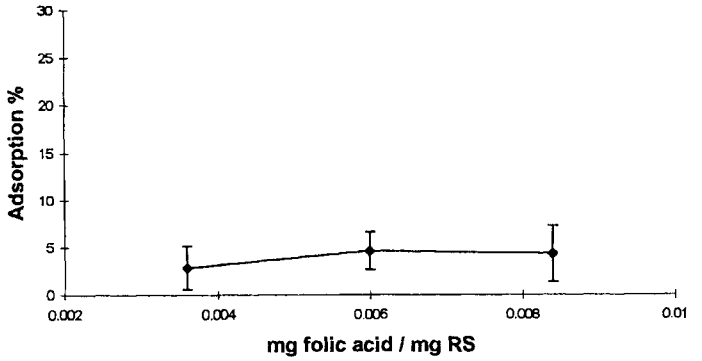


Fig. 5. Adsorption of folic acid by autoclaved and spray-dried wheat starch.

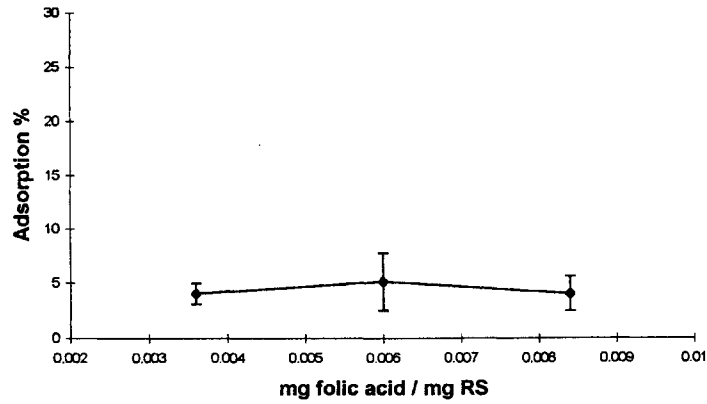


Fig. 6. Adsorption of folic acid by retrograded wheat starch.

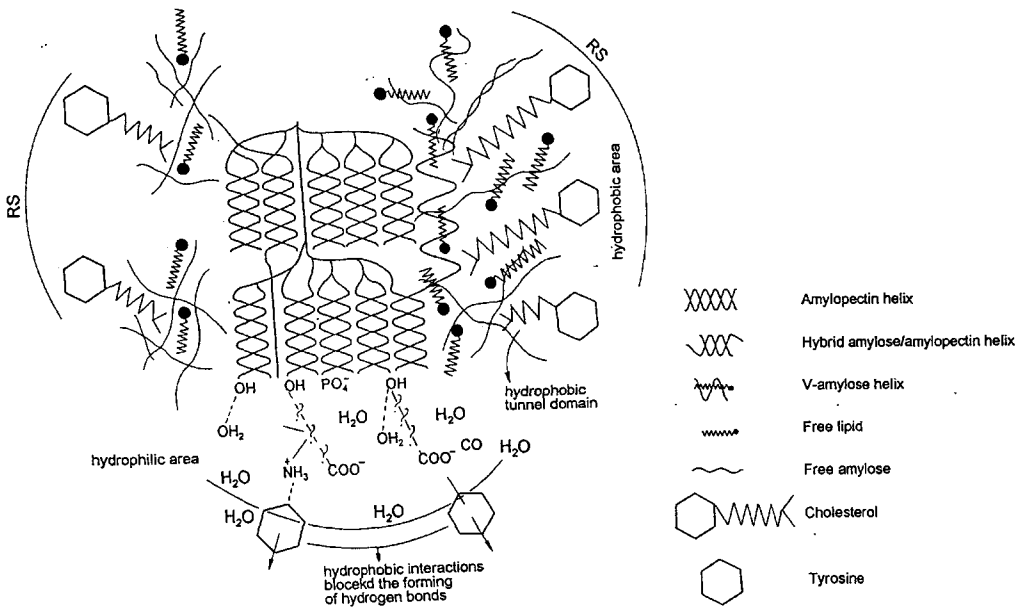


Fig. 7. Model of processed starch showing the possible positivning and interactions of various compounents.

In conclusion our investigations enable us to suggest an initial hypothesis for the biological role of resistant starch in the intestinal tract. In our opinion, resistant starch could play in the intestinal tract first of all the role of a thickening agent. Therefore, resistant starch is a lesser degree the complexing agent of nutrients.

The research was supported by grant no. 5 P06G 008 10 from the State Committee for Scientific Research (KBN).

REFERENCES

[1] Galliard T.: Critical Reports on Applied Chemistry, **13**, 1987, 1-14.
[2] Galliart T., Bowler P.: Critical Reports on Applied Chemistry, **13**, 1987, 55-76.
[3] Champ M.: Eur J. Clin Nutr., **46**, (Suppl. 2), 1992, S109-S111.
[4] Morrison W.R., Laingnelet B.: J. Cereal Sci., 1983, **1**, 3-8.
[5] Banks W., Muir D.D., The Biochemistry of Plants, Academic Press, New York, 1980, 321-369.
[6] Lowy G.D.A., Sargeant J.G., Schofield J.D.: J. Sci. Food Agric., **32**, 1981, 371-377.
[7] Swinkells J.J.M.: Stärke, **35**, 1985, 1-5.
[8] Blanshard J.M.V.: Critical Reports on Applied Chemistry, **13**, 1987, 17-53.

KOMPLEKSY SKROBI ODPORNEJ ZE SKŁADNIKAMI POKARMOWYMI

Streszczenie

Skrobia jest ważnym składnikiem ekosystemu żywieniowego. W rzeczywistości jest ona dla całej ludzkiej populacji głównym składnikiem diety. Celem pracy było wyjaśnienie możliwości tworzenia kompleksów pomiędzy modyfikowanymi skrobiami i składnikami żywności, tj.: tyrozyna, kwas foliowy i cholowy oraz cholesterol w warunkach pH odpowiadających środowisku jelita. Badania przeprowadzono używając fizycznie modyfikowane-retrogradowane, autoklawowane oraz enzymatycznie-po działaniu termamylu skrobie: tapiokową, kukurydzianą, ziemniaczaną i pszenną. Uzyskane wyniki dowiodły, że tylko cholesterol tworzy kompleksy z modyfikowanymi skrobiami.

Uzyskane wyniki sugerują, że podczas modyfikacji skrobi zachodzą przemiany konformacyjne jej składników i tworzą się nowe układy kompleksowe. Helikalna V-amyloza, kompleksy lipidowo-amylozowe i amyloza łańcuchowa tworzą specyficzny kompleks z hydrofobowymi tunelowymi domenami. Ten kompleks uważamy za składnik skrobi odporny na amylolizę. Hydrofobowy charakter tego kompleksu powoduje jego odporność na działanie enzymów. Wydaje się, że tylko hydrofobowe związki mogą wchodzić z tym kompleksem w interakcje. Uzyskane wyniki pozwoliły nam na wysunięcie hipotezy, że rola biologiczna skrobi odpornej na amylolizę w przewodzie pokarmowym polega głównie na pełnieniu funkcji zagęstnika treści pokarmowej, a w mniejszym stopniu jako czynnik kompleksujący.

G. LEWANDOWICZ[1], M. SORAL-SMIETANA[2], J. FORNAL[2]

NEW RS PREPARATIONS – PHYSICOCHEMICAL PROPERTIES AND STRUCTURE

Abstract

New RS preparations were obtained by physical modification of potato, tapioca, maize and wheat starches and they contained respectively 30.84%; 18.34%; 25.48% and 26.34% RS determined according to the Champ method. Starch samples were investigated using the Brabender rheological method, X-ray diffractometry and light and scanning electron microscopy.

It was found that all RS preparations showed lower gelatinisation temperature as compared to native ones. The light microscopy study however proved that RS preparations are more difficult to disperse in water than native starches. It was observed that the dispersion process depends on the amylose content in starch. The scanning electron microscopy study showed that the forms of preparations were similar to those of pregelatinised starches (drum dried or extruded). The X-ray diffraction studies showed that all preparations obtained from both tuber and cereal starches showed a B-type X-ray diffraction pattern characteristic of native tuber starches.

Introduction

Resistant starch (RS) is a physiologically important indigestible starch fraction. It is not digested in the small intensine, but may be fermented by micro-organisms in the large intensine. RS cannot be properly defined chemically due to the fact that the resistance of starch to digestive enzymes is related to hydrolysis conditions (nature of the enzymes, ratio starch/enzyme, characteristic of the hydrolysis, etc.) [1]. There are four types of resistant starch: physically inaccessible, locked in plant cell walls; native resistant starch (represented by native potato, banana and high amylose starch); retrograded or crystalline non-granular starch; and specific, chemically modified or repolymerised starches. Retrograded starch is the most common RS in the diet and, from the technological point of view, is the most important type resistant starch because it forms as a result of food processing [2]. It is generally believed that this resistant starch

[1] *Starch and Potato Products Research Laboratory, Poznań, Poland*
[2] *Institute of Animal Reproduction and Food Research of Polish Academy of Science, Olsztyn, Poland*

fraction consists mainly of retrograded amylose and shows a B-type crystalline structure [3-9]. On the other hand, it is found that RS formed at the temperature of 100°C shows an A-type X-ray diffraction pattern. B-type starch is formed at a significantly lower temperature (0–68°C) [10]. It is also possible to obtain enzyme-resistant starch by retrogradation of amylopectin, but the crystallisation of amylopectin is a slow process continuing over a period of several days or weeks [11]. The melting endotherm of amylopectin is observed at about 60°C, while for amylose crystallites at about 150°C. One of the most important issues in starch chemistry and technology is the correlation between starch structure and its functionality. The aim of our work was to determine the structure and physicochemical properties of new RS preparation invented by a research team from Starch and Potato Products Research Laboratory and the Institute of Animal Reproduction and Food Research of Polish Academy of Sciences.

Materials and methods

Resistant starch preparations were obtained by solubilisation of native starches and water and their isolation from the solution without any non-solvents or complexing agents. The procedure is described in detail in the Polish patent specification [12].

The course of gelatinisation was followed with a Brabender viscograph procedure under the following conditions: measuring cartridge 700 cmg; heating/cooling rate 1.5°C/min; thermostating 30 min.

The starch samples to be examined by light microscopy were prepared by the smear method according to Kaczyńska et al., [13]. Starch suspensions were heated at the initial gelatinization temperature (as measured according to Brabender), and at 90°C. A drop of starch paste was smeared over a microscope glass, cooled, stained with iodine and observed in a Olympus BX60 light microscope.

The starch samples to be examined by scanning electron microscopy were prepared according to Fornal [14] and observed with a Jeol JSM 5200 microscope.

X-ray diffractometry was performed with a TUR 62 (Carl Zeiss, Germany) X-ray diffractometer under the following conditions: X-ray tube CuKα (Ni filter); voltage 30 kV; current 15 mA; scanning from $\Theta = 2°$ to 18°.

Results and discussion

Our resistant starch preparations like many others presented in patent descriptions were not pure indigestible starch fractions, but a mixture of digestible and indigestible ones [15-20]. The variation in the indigestible fraction content (table 1) depended on the nature of starch, mainly on amylose and lipids content. It is generally accepted that the resistant starch formation is based on amylose retrogradation, therefore amylose content could be the most important factor influencing this process [3-9]. It is also

reported that endogenous lipids substantially decrease the yield of RS – due to the amylose-lipids complexes formation [23]. The highest value of indigestible fraction (table 1) was found in the RS preparation obtained from potato starch – a variety characterised by a high amylose content but a low lipids content.

Table 1

Indigestible fraction content in RS preparations

Origin of starch	Resistant starch content in RS preparation, %	Amylose content in native starch [21-23], %	Lipids content in native starch [22], %
Potato	30.84	20-24	0.05
Tapioca	18.34	17	0.1
Maize	25.48	24-28	0.7
Wheat	26.34	22-28	0.8

X-ray diffraction studies (fig. 1 and 2) showed that all preparations obtained from both tuber and cereal starches showed a B-type X-ray diffraction pattern characteristic of native tuber starches which is generally compatible with data reported by other authors [3-9]. Brabender viscosity curves (fig. 3-6) showed that potato and tapioca RS preparations kept high swelling characteristics, whereas maize and wheat changed the type of swelling characteristics to higher than the native ones. High type of swelling characteristics and B-type crystallinity is typical of tuber starches. These data suggest that the presence of B-type of crystallinity is a necessary condition of starch indigestibility.

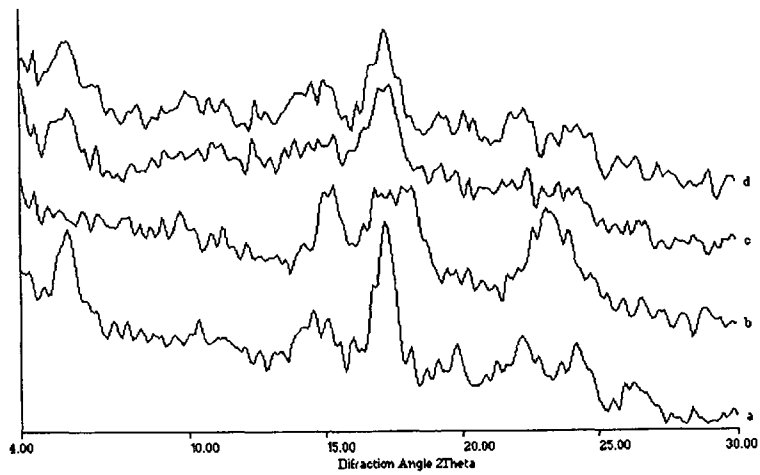


Fig. 1. X-ray diffraction patterns of: a – native potato starch; b – native tapioca starch; c – RS preparation obtained from potato starch; d – RS preparation obtained from tapioca starch.

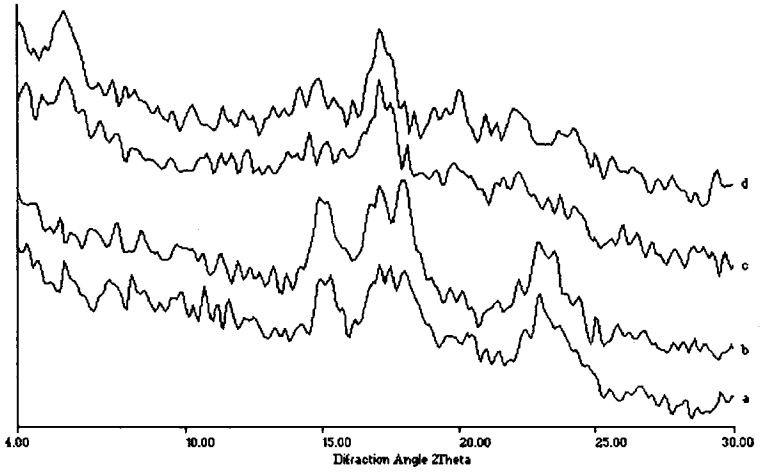


Fig. 2. X-ray diffraction patterns of: a – native wheat starch; b – native maize starch; c – RS preparation obtained from wheat starch; d – RS preparation obtained from maize starch;

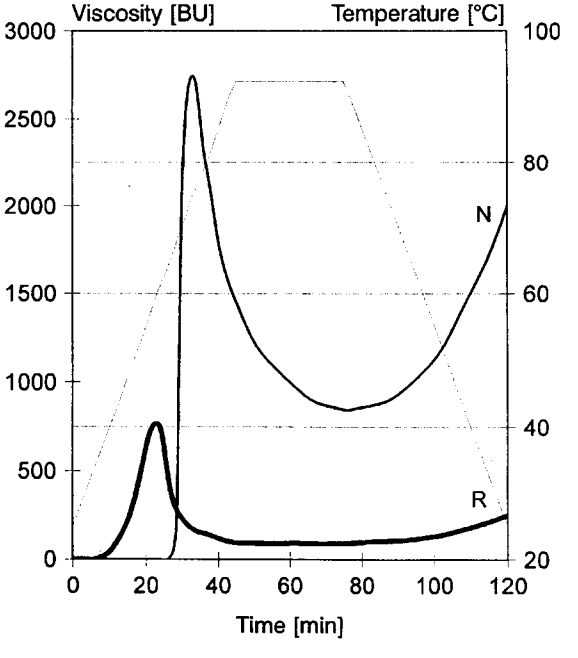


Fig. 3. Brabender viscosity curves for 8% solutions of RS preparation obtained from potato starch as compared to native one: R – resistant starch preparation; N – native starch.

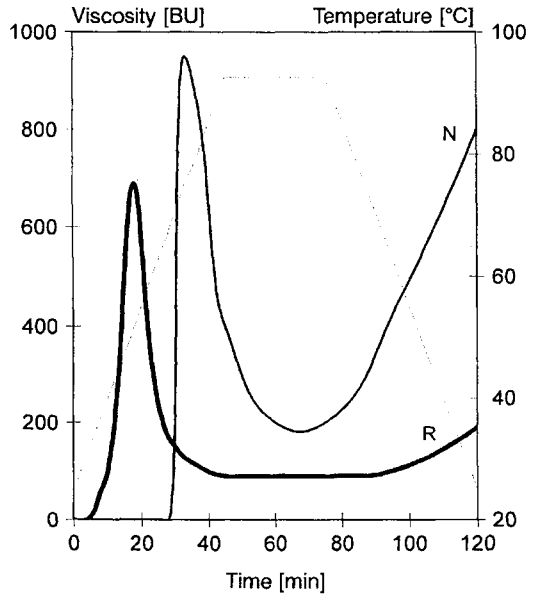


Fig. 4. Brabender viscosity curves for 8% solutions of RS preparation obtained from tapioca starch as compared to native one: R – resistant starch preparation; N – native starch.

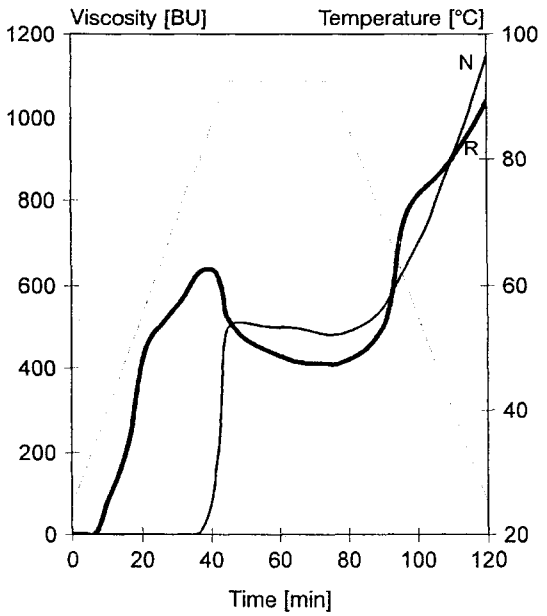


Fig. 5. Brabender viscosity curves for 8% solutions of RS preparation obtained from wheat starch as compared to native one: R – resistant starch preparation; N – native starch.

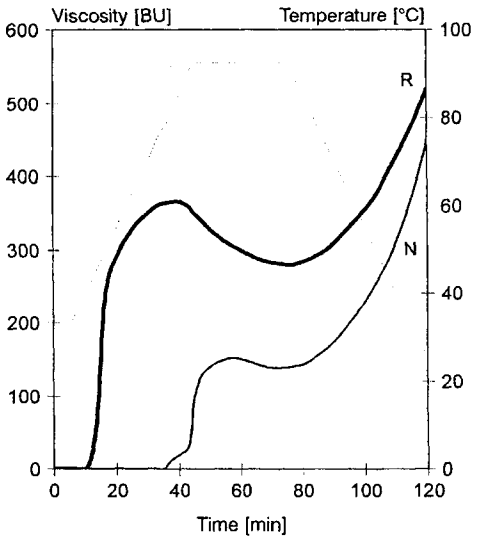


Fig. 6. Brabender viscosity curves for 8% solutions of RS preparation obtained from maize starch as compared to native one: R – resistant starch preparation; N – native starch.

Brabender viscosity curves (fig. 3-6) also showed that starch preparations revealed a lower gelatinisation temperature as compared to native ones. This was unexpected, because starch indigestibility is thought to be connected with their insolubility. However a verification of Brabender's data by light microscopy studies (photographs not presented) proved that RS preparations were more difficult to disperse in water than the native starches. At the temperature of gelatinisation it was impossible to observe any symptoms of solubilisation (for example amylose leakage from the starch granules) and the RS preparation remained unchanged. At a temperature of 90°C the majority of RS preparations showed brown-black coloured images, which indicated some hindrance in starch solubilisation (mainly amylose), and amylose iodine complexation processes. The only exception was the RS preparation obtained from tapioca starch which revealed a similar image as the native starch, which was correlated with low resistant starch and amylose contents. These observations proved that the gelatinisation of RS preparations was based on amylopectin hydration, whereas amylose fraction remained insoluble. Amylose formed very strong inter- and intramolecular starch-starch bonds, nonsusceptible to association with water molecules or to complexation with iodine atoms. These observations still did not provide an answer to the question about the correlation between starch structure and digestibility. It is believed that during gelatinisation the crystal regions of starch are disrupted. On the other hand, it is believed that the resistant starch fraction consists mainly of retrograded amylose and shows a B-type crystalline structure [3-9]. Our RS preparations underwent gelatinisa-

tion, thus their viscosity increased during heating, but up to 90°C they were only partially soluble. These phenomena can be explain in two ways:

- during heating amorphous amylopectin underwent association with water molecules, but amylose of B-type crystallinity remained insoluble, or
- during heating amylopectin of B-type crystallinity underwent association with water molecules, but amorphous amylose remained insoluble

The latter hypothesis is probably more difficult to accept, but probably it is true. It should be taken into consideration that authors reporting about B-type crystallinity of resistant starch do not claim that even pure RS fraction (isolated by enzyme procedure) reveals 100% crystallinity degree. Moreover, native potato starch granules of RS_2 type containing 66.5% RS [25] reveals average crystallinity degree of 28% [26], so the correlation is rather poor. Furthermore, there is also a type of RS_3 which reveals an A-type of X-ray diffraction pattern [11]. So, it is possible that the B-type of crystallinity is not a necessary condition of starch indigestibility. Probably the following two connected processes exist:

- formation of an indigestible starch fraction, and
- formation of a starch fraction of B-type crystallinity.

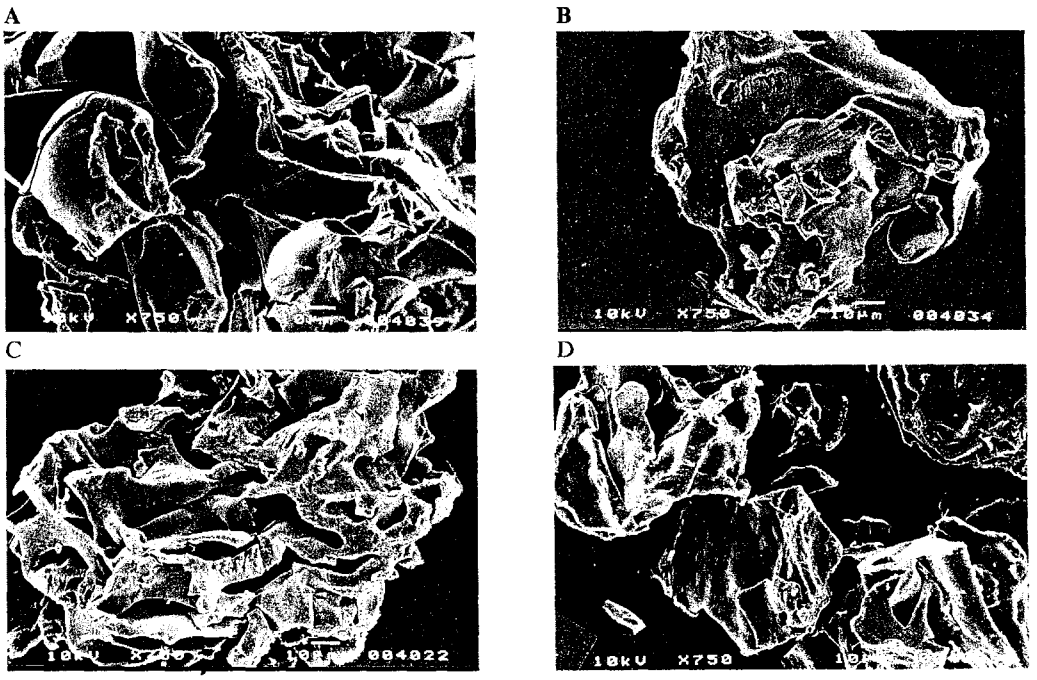


Fig.7. Scanning electron microphotographs of: A – RS preparation obtained from potato starch; B – RS preparation obtained from tapioca starch; C – RS preparation obtained from wheat starch; D – RS preparation obtained from maize starch;

Pictures of RS preparations obtained using scanning electron microscopy did not reveal any significant differences in their structure (fig. 7). Starch granules changed their granular shape into shapeless corrugated particles which final shape and size depended on final grinding procedure. All RS preparations revealed similarity in form to pregelatinised or extruded starches. The particles of RS preparations were thicker than those of extruded starch however [27].

The research was supported by grant no. 5 P06 G 008 10 from the State Committee for Scientific Research (KBN)

REFERENCES

[1] Champ M.: Proceedings of the concluding plenary meeting of EURESTA, ed. Asp N-G. et al p. 1-14.
[2] Escarpa A., Gonzales M.C., Manas E., Garcia-Diz L., Saura-Calixto F.: J. Agric. Food Chem., **44**, 1996, 924-928.
[3] Berry C.S.: J.Cereal Sci., **4**, 1986, 301-314.
[4] Berry C.S., I'Anson I., Miles M., Morris V.J., Russel P.L.: J. Cereal Sci., **8**, 1988, 203-206.
[5] Russel P.L., Berry C.S., Greenwell P.: J. Cereal Sci., **9**, 1989, 1-15.
[6] Siljestrom M., Eliasson A.C., Björck I.: Starch/Stärke **41**, 1989, 147-151.
[7] Sievert C., Pomeranz Y.: Cereal Chem. **67**, 1990, 217-221.
[8] Czujachowska Z., Sievert D., Pomeranz Y.: Cereal Chem. **68**, 1991, 537-542.
[9] Sievert D., Czujachowska Z., Pomeranz Y.: Cereal Chem. **68**, 1991, 86-91.
[10] Eerlinger R.C., Crombez M., Delcour J.A.: Cereal Chem. **70**, 1993, 339-344.
[11] Eerlinger R.C., Jacobs H., Delcour J.A.: Cereal Chem. **71**, 1994, 351-355.
[12] Lewandowicz G., Soral-Śmietana M., Foral J.: Polish Pat. Spec. No, 1998.
[13] Kaczyńska B., Autio K., Fornal J.: Food Structure, **12**, 1993, 217-224.
[14] Fornal J.: Acta Alim. Polonica, **XI**, 1985, 141-151.
[15] Iyengar R., Zaks A., Gross A.: U.S. Pat. 5051271, 1991.
[16] Chiu Ch., Henley M., Altieri P.: U.S. Pat. 5281276, 1994.
[17] Henley M., Chiu Ch.: U.S. Pat. 5409542, 1995.
[18] Shi Y., Trzasko P.T.: U.S. Pat. 5593503, 1997.
[19] Pomeranz Y., Sievert D.: Pat. WO 90/15147, 1990.
[20] Chiu Ch., Henley M., Altieri P.:Pat. Eur. 0564893, 1993.
[21] Howling D.: Food Chemistry, **6**, 1980, 51-61.
[22] Swinkles J.J.M.: Starch/Stärke **37**, 1985, 1-5.
[23] Blenford D.: International Food Ingredients, issue 6, 1994.
[24] Eerlinger R.C., Cillen G., Delcour J.A.: Cereal Chem., **71**, 1994, 170-177.
[25] Würsch P., Delcour J., Proceedings of the concluding plenary meeting of EURESTA, ed. Asp N-G. et al p. 15-19.
[26] Steeneken P.A.M.: Starch/Stärke, **36**, 1984, 13-18.
[27] Śmietana Z., Szpendowski J., Soral-Śmietana M., Świgoń.: Acta Acad. Agricult. Tech. Olst., **6**, 1996, 3-13.

NOWE SKROBIE ODPORNE – WŁAŚCIWOŚCI FIZYKOCHEMICZNE I STRUKTURA

Streszczenie

Nowe skrobie odporne otrzymano przez fizykochemiczną modyfikację skrobi ziemniaczanej, tapiokowej, kukurydzianej i pszennej. Nowe preparaty zawierały odpowiednio 30,84%; 18,34%; 25,48% i 26,34% skrobi odpornej według oznaczeń metodą Champa. Zbadano reologiczne właściwości tych próbek metodą Brabendera, i ich budowę metodą rentgenografii proszkowej i elektronowym mikroskopem skanningowym.

Stwierdzono, że wszystkie preparaty skrobi odpornej miały niższą temperaturę kleikowania w porównaniu ze skrobiami natywnymi. Mikroskopia wykazała, że preparaty skrobi odpornej dyspergowały w wodzie trudniej od skrobi natywnej, dyspersja zależała od zawartości amylosy w danym preparacie. Mikroskopia elektronowa pokazała, że skrobie odporne przypominały swą budową skrobie wstępnie skleikowaną (suszoną w bębnach lub ekstrudowaną). Badania rentgenowskie wykazały, że wszystkie preparaty uzyskane ze skrobi roślin bulwiastych i zbożowych należały do krystalograficznego typu B charakterystycznego dla skrobi z roślin bulwiastych.

D.M. NAPIERAŁA

OBSERVATIONS ON THE AGEING OF POTATO STARCH PASTES MODIFIED WITH COMPLEXING AGENT

Abstract

The effects of gelatinisation and complexing conditions on stability of the potato starch solution were observed in a period of two weeks. Different methods of preparing 2% (w/v) starch aqueous solution with 0.25 μM Rose Bengal were tested. The differences in behaviour of all samples during storage were significant, which proves that the way in which starch pastes with dye have been prepared is of crucial importance. The most effective retrogradation delay and the least significant structural changes during storage were observed in starch – Rose Bengal solution prepared by immediate high temperature treatment above the gelatinisation temperature with preliminary swelling the starch with dye. This treatment facilities more effective intermolecular association with dye molecules resulting in polymer ordering and spatial network stability.

Introduction

Starch solution obtained as a result of boiling an aqueous polymer suspension for a long time under normal pressure conditions exhibits a tendency to aggregate its components during cooling down and long time storing. In an appropriate high concentration such a polymer solution reveals the ability to form a spatial gel network [16]. All these time-varying association – aggregation processes, known as starch retrogradation, have been the subject of many investigations [1-8, 12, 13]. Retrogradation of starch solutions results in many physical changes such as an increase in viscosity, development of opacity and turbidity, precipitation of insoluble crystalline starch and syneresis of water. These physical changes are related to qualitative parameters of starch convention products. Therefore it is very important to design all the factors which may control starch changes during long time storing. One of them are starch paste processing conditions and complexing with low-molecular compound. The ability of amylose to form inclusion complexes with different compounds can be used as

Department of Physics, Agricultural University of Poznań, Wojska Polskiego 38/42, 60-637 Poznań

considerable in influencing the structural changes during storage [14]. Hizukuri *et al.* [4] observed that retrogradation in sufficiently gelatinised starch can be inhibited. The relationship between method of starch gelatinisation and the crystallinity of storaged starch and degree of retrogradation during storage was studied by Mizuno [8].

The purpose of this work was to study the influence of hydrothermal processing conditions and complexing on the stability and physical properties of potato starch aqueous solutions during long time storing in the presence of Rose Bengal, used as complexing agent required for stabilisation of starch solution.

Materials and methods

A soluble potato starch obtained from Polish Chemical Reagent – Gliwice was used in the experiment without further purification. Rose Bengal (RB) sodium salt was obtained from Sigma Co. The starch concentration was 0.02 g/cm^3, whereas dye concentration was 0.25 μM. The methods of hydrothermal processing of starch with Rose Bengal are need to be indicated in detail, because they were modified. All aqueous starch suspensions were heated at boiling temperature in the same time period of 30 minutes. The first modification depended upon heat treatment: for some probes (from no 1 to 5) the contact of starch with water occurred at room temperature and for the others (probes no 6 and 7) – the contact with water occurred immediately at boiling temperature. The next modification concerned the moment of Rose Bengal addition: it was added to starch before heat treatment for probes no 1, 5 and 6; at the end of starch solution boiling – in probes 3 and 7; during starch paste cooling down, at 50°C – in probe no 2 and in probe no 4 – after 1 h of starch paste storing at room temperature. The third processing modification was related with preliminary 1 hour starch swelling in water, without a complexing agent – in probe no 4, and in the presence of Rose Bengal – in probe no 5. All starch solutions, prepared in such a way, were exposed for long time storage at a stable temperature of 27°C.

Measurements of proton spin-lattice relaxation rate, R_1 were carried out using a 30-MHz pulse NMR spectrometer. Signal of free induction decay was recorded after a two-pulse sequence $\pi - \tau - \pi/2$. The data set obtained were analysed using the two-dimensional NMR signal – analysing program CracSpin [15].

Optical rotation (OR) measurements were performed using POLAMAT A produced by Carl Zeiss Jena, in 1dm cell, at three wavelengths: λ = 366 nm, 406 nm and 436 nm.

Results and discussion

Potato starch pastes of 2% (w/v), prepared according to the above description, were observed during long-time storing at a constant temperature. Some main changes observed in starch paste probes during ageing are presented in Table 1.

Table 1

The effect of ageing on some properties of starch pastes (0.02 g/cm^3) with Rose Bengal (25 μM), stored at 27°C

No of probe	Ageing time, days	Turbidity	Precipitation	Microbialogical spoilage	Discolouration
1	1	–	–	–	–
	4	–	–	+	–
	7	–	+	+	–
	10	–	+	++	–
	14	–	++	++	–
2	1	–	–	–	–
	4	–	+	+	–
	7	–	++	+	–
	10	++	+++	++	++
	14	+	+++	++	+++
3	1	–	–	–	–
	4	–	+	+	–
	7	+	+	+	–
	10	++	++	++	+
	14	++	+++	+++	++
4	1	–	–	–	–
	4	+	++	+	–
	7	+	++	++	+
	10	+	++	++	++
	14	+	+++	++	+++
5	1	–	–	–	–
	4	–	–	–	–
	7	–	+	–	–
	10	–	+	–	–
	14	+	+	–	–
6	1	–	–	–	–
	4	–	+	–	–
	7	–	+	+	–
	10	–	+	++	+
	14	–	++	++	++
7	1	–	–	–	–
	4	–	–	–	–
	7	–	–	–	–
	10	–	–	–	–
	14	+	–	–	–

– no effect; + low effect; ++ medium effect; +++ high effect

The comparison of ageing changes in starch pastes with the same ratio of polymer amount and amount of complexing agent, modified during hydrothermal processing, suggests that the molecular structure of polymer with complexing dye in the studied systems is very different. The stability of starch polymer network in water solution is conditioned by efficiency of intermolecular polymer – dye interaction, which can stable amylose helical form and supermolecular ordering.

All starch dispersions were tested after 20 days of storing by optical rotation measurements in spectral range beyond the dye absorption bands. The values of $[\alpha]_\lambda$ for three wavelengths, 366 nm, 406 nm and 436 nm are presented in Table 2.

Table 2

The optical rotation (OR) data at three wavelengths: λ = 366 nm, 406 nm and 436 nm, obtained in potato starch pastes after 20 days of storage at 27°C

Number of probe	$[\alpha]_{366\ nm}$, deg	$[\alpha]_{366\ nm}$, deg	$[\alpha]_{436\ nm}$, deg
Freshly prepared 2% starch paste	20.33		13.50
Freshly prepared 2% starch paste with 0.25μM RB	19.47		13.00
1	19.57	15.32	12.98
2	9.90	7.75	6.55
3	19.24	14.98	12.74
4	16.80	10.90	11.64
5	19.66	14.46	13.30
6	14.44	11.30	9.55
7	18.80	14.20	12.96

Although the changes in starch pastes storing for 20 days at 27°C are considerable, especially the amount of precipitated amylose, discolouration, the differences in optical rotation between freshly prepared starch paste and after long time storing are surprisingly small (Table 2). In probe no 3, for example, in which high amount of precipitate was observed, the optical rotation is comparable with that for probe no 7, the most stable of the starch paste studied. Optical rotation offers one of the simplest and most reliable methods for determining the concentration of polysaccharide in solution [9]. Due to precipitation, starch concentration in long- time storage solution is not the same as before ageing, in freshly prepared starch solution. Nevertheless, the values of OR were not changed in starch pastes with Rose Bengal after long time storing for some probes (Table 2).

It was observed, after 15 days of storing starch pastes with no preservative, two of all starch probes were untouched by micro-organisms. These were probes 5 and 7. The

other solutions were significantly destroyed and discoloured, especially starch pastes no 2, 4 and 6. High turbidity has occurred in starch solution samples 2 and 3. It could be noticed, amylose precipitation, coloured by presence of Rose Bengal molecules, formed radically arranged pattern at the bottom of vessel in starch probes no 2 and 3. No comparable effect was observed in other solutions. In all these, much or less changed starch pastes after 20 days of storing, the measurements of proton relaxation rate were performed from upper layer. Spin-lattice proton relaxation rate, R_1, like to spin-spin relaxation rate analysed in [10], was characterised by multiexponential free induction decay, revealing under measurement conditions at least two water proton subsystems with different molecular mobility. The data obtained by fitting procedure with Crac-Spin program for all probes are presented in Table 3.

Table 3

Spin-lattice proton relaxation rate, R_1 and percent contribution of two proton fractions in potato starch pastes after 15 days of ageing at 27°C

No of probe	R_{11}, s^{-1}	A_{11} ,%	R_{12}, s^{-1}	A_{12},%	R_{13},s^{-1}	A_{13} ,%
1	0.454	97.0	18.5	3.0		
2	0.431	96.6	15.6	3.4		
3	0.445	98.2	22.3	1.8		
4	0.441	94.0	39.1	1.9	1.3	4.1
5	0.456	96.5	29.7	3.5		
6	0.412	94.5	26.1	5.5		
7	0.450	97.5	27.1	2.5		

It is evidence, that proton relaxation rate, R_1 in such heterogeneous systems, related to water state and biopolymer matrix should be very complicated. NMR analysis has shown two proton subsystems with different molecular mobility in nearly all starch probes and three proton fractions in starch paste no 4. It should be noticed that in the case of starch paste no 4, complexing agent was incorporated to the starch solution just after 1 hour of cooling down the solution to the room temperature. During this time a spatial network of polymer could be formed as well as high temperature conformational transitions occurred. Rose Bengal molecules were incorporated „too late" for amylose conformation changes input. The effectiveness of complexing process in starch solution highly depends on temperature. In starch paste, cooled down to the room temperature amylose – RB interactions were significantly restricted. In this starch paste (no 4) three protons fractions were observed in NMR measurements, which proved polydispersity of the system.

Three used hydrothermal processing of potato starch paste with a complexing agent are schematically presented in Fig. 1.

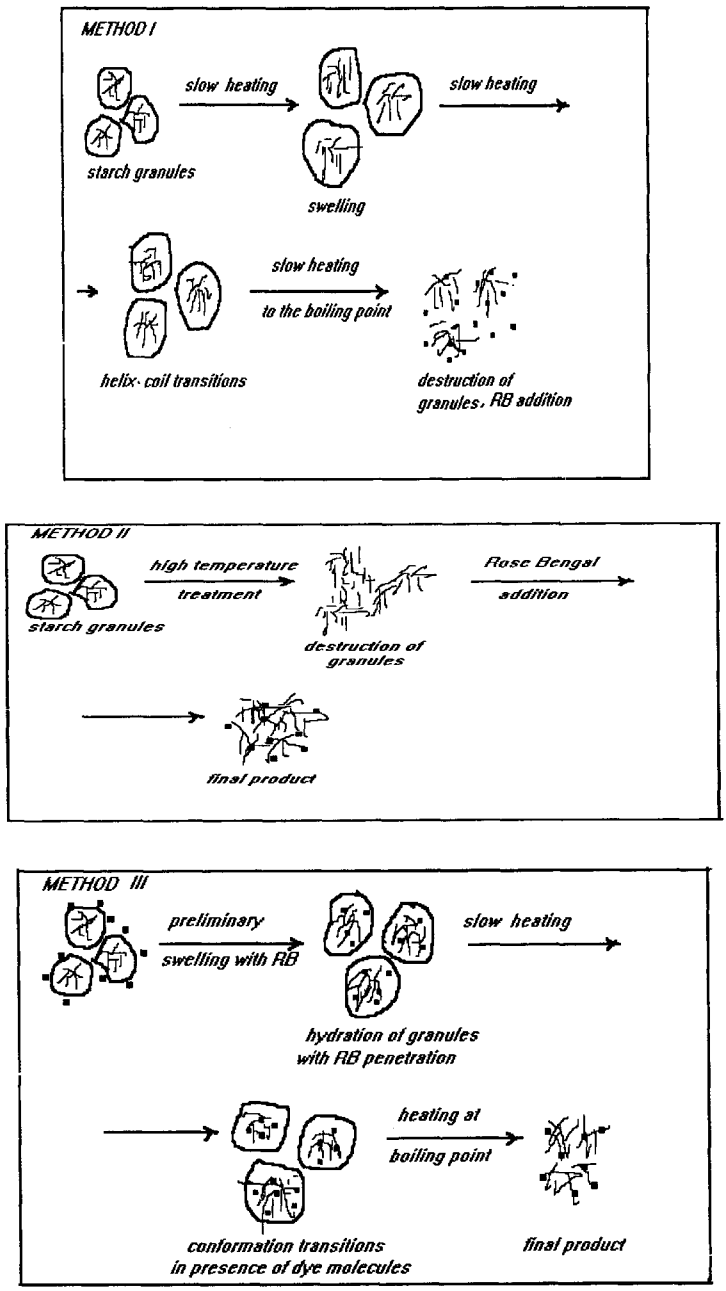


Fig. 1. Diagrams illustrating three hydro-thermal processing used in potato starch paste with complexing agent, Rose Bengal (•).

During slow heating of a starch suspension (Fig. 1 – Method I), a gradual disruption of starch granules takes place, accompanying with swelling, conformational transitions of amylose and amylopectin chains within. It is known, that the total granule disruption arises at temperature above 90°C [11]. Therefore, in starch paste obtained according to method I, some "trace" of granule disordering remains, which unfavourable effects on polymer chains entaglement in the whole system (probes 1, 2, 3, 4).

Quite different molecular conditions for starch pasting are in a starch suspension treated immediately with high temperature, above 90°C (Fig. 1 – Method II). Instantaneous starch granule disruption facilitates mutual polymer chains penetrating. It should influence structural homogeneity of starch paste at a molecular level. The interaction of Rose Bengal with starch may result in a more stable network (probe 7).

Preliminary starch swelling in presence of complexing agent (Fig. 1 – Method III) causing Rose Bengal penetration into starch granules, leads to better "prepare" of granules to form polymer spatial network with Rose Bengal. After high temperature treatment of such prepare starch with complexing agent within granules, starch paste obtained exhibit more resistance on ageing changes (probe no 5).

Conclusions

The study of the effect of dye – polymer interaction on the stability of starch colloidal solutions revealed that:

- the stability of potato starch spatial network modified with a complexing agent, Rose Bengal, is related with the method of hydrothermal processing of starch solutions,
- two agents may play a main role in forming a starch spatial network stable: preliminary swelling of the starch granules with a complexing agent, and – starting the process at high temperature, above gelatinisation temperature, in which random coil – helix conformation transition is observed.
- the interaction between amylose chains and RB molecules is not affected by temperature below the helix-coil transition temperature. When a complexing agent is added to starch solution at lower temperatures, intermolecular interaction is highly restricted, due to unfavourable amylose chain conformation.

REFERENCES

[1] Abia R., Buchanan C.J., Saura-Calixto F., Eastwood M.A.: J. Agric. Food Chem., **41,** 11, 1993, 1856-1863.

[2] Bohlin L., Eliasson A.-C.: Starch/Staerke, **38,** 4, 1986, 120-124.

[3] Hizukuri S.: Carbohydr. Res., **147**, 1986, 342-348.

[4] Hizukuri S., Tagaki T.: Carbohydr. Res., **134**, 1984, 134-140.

[5] Kalichevsky M.T., Orford P.D., Ring S.G.: Carbohydr. Res., **49**, 1990, 198-203.
[6] Lu T.J., Jane J.L., Keeling P.L.: Carbohydr. Polym., 1997, **33**(1), 19.
[7] Miles M.J., Morris V.J., Orford P.D., Ring S.G.; Carbohydr. Res., **135**, 1985, 271-281.
[8] Mizuno A., Mitsuiki M., Motoki A.: J.Agric. Food Chem., **46**, 1998, 98-103.
[9] Moravetz H.: Fizykochemia roztworów makrocząsteczek, PWN, W-wa 1970.
[10] Napierała D.: Materiały XXX Ogólnopolskiego Sem. MRJ, 1998, 51-54.
[11] Nowotna A.: Zeszyty Naukowe AR Kraków, Rozprawy nr 214, ed. A.R., Kraków 1996.
[12] Orford P.D., Ring S.G., Carroll V., Miles M.J., Morris V.J.: J. Sci. Food Agric., **39**, 1987, 169-177.
[13] Russell P.L.: J. Cereal Sci., **6**, 1987, 147-158.
[14] Van Soest J.J.G., Tournois H., de Wit D., Vligenthart J.F.G.: Starch/Staerke, **46**, 1995, 453.
[15] Węglarz W., Harańczyk H.: Mat. XXVI Ogólnopolskiego Sem. MRJ, Kraków, 1993, 137-140.
[16] Whistler R.L., Be Miller J.N., Paschall F.: Starch Chemistry and Technology, 1984, Orlando/Florida, Academic Press, 289.

BADANIE STARZENIA PAST ZE SKROBI ZIEMNIACZANEJ MODYFIKOWANYCH CZYNNIKAMI KOMPLEKSUJĄCYMI

Streszczenie

W pracy badano wpływ warunków kleikowania i sposobu wprowadzania czynnika kompleksującego na trwałość kleików skrobi ziemniaka. Próby skrobi o tej samej koncentracji 0.02g/cm^3 i tej samej ilości Rose Bengal, 0.25μM, zastosowanego jako barwnikowy czynnik kompleksujący, obserwowano przez okres ponad dwóch tygodni. Kleiki przechowywano w stałej temperaturze i stałej wilgotności. Znaczne różnicowanie w zachowaniu kleików w czasie starzenia, spowodowane jedynie modyfikowaniem sposobu kleikowania, wskazuje na istotny wpływ tego czynnika na trwałość skompleksowanej skrobi w roztworze. Najbardziej efektywnym na zahamowanie zmian w procesie starzenia, sposobem kleikowania skrobi w obecności RB jest natychmiastowe potraktowanie zawiesiny skrobi w wodzie wysoką temperaturą, przewyższającą charakterystyczną dla skrobi temperaturą przejść konformacyjnych łańcucha polimerowego. Wstepne pęcznienie skrobi wraz z czynnikiem kompleksującym przed kleikowaniem wpływa korzystnie na sieciowanie przestrzenne kleiku i jego trwałość w czasie przechowywania.

E. NEBESNY, J. ROSICKA, D. SUCHARZEWSKA

THE EFFECT OF PROCESS CONDITIONS OF ENZYMATIC HYDROLYSIS ON THE PROPERTIES OF WHEAT STARCH HYDROLYZATES

Abstract

In the study, glucose hydrolyzates were obtained by acting on wheat starch, which was liquefied with the aid of bacterial α-amylase, with Spezyme GA 300W Y553, an enzymatic preparation composed of glucoamylase and lysophospholipase, as well as with Amyloglucosidase AMG 300L, a glucoamylase preparation. Parallel to saccharifying enzymes, Gammazym CX 4000L, Shearzyme 500L and proteinaze Neutrase 0.5L, cellulolytic enzymes, were used.

The best physical and chemical properties of the obtained glucose hydrolyzates: the colour factor, the transparency factor, the filtration power, and the highest reductivity were obtained in the process of saccharification using, beside Spezyme, the xylanase preparations: Shearzyme 500L and proteinaze Neutrase 0.5L.

Introduction

The technology of starch hydrolyzates production depends on the origin of starch which is connected with the shape and size of starch grains, amylose-to-amylopectin ratio and chemical composition. Differences observed in the content of protein, fat and non-starch polysaccharides, e.g. pentosanes in raw starch subjected to hydrolysis, depending on its origin, affect the quality of hydrolyzates obtained.

In our country potato starch was the basic raw material in starch industry in the past. Now, for economic reasons grain starch is used to produce hydrolyzates. Wheat and corn starch reveals different physical and chemical properties than potato starch. Particularly, the presence of a larger amount of fat – lysophospholipids (wheat starch) and faty acids (corn starch) which form gelating complexes with amylose, and proteins and non-starch polysaccharides (arabinoxylanes) in wheat starch has a negative effect on the colour, transparency and aroma of the hydrolyzates obtained.

Institute of Chemical Technology of Food, Technical University of Łódź, Stefanowskiego Street 4/10, 90-924 Łódź, Poland

Wheat starch hydrolyzates are characterised by high resistance during the process of filtration. This calls for adding enzymes enhancing the hydrolysis of the above mentioned compounds to the process of grain starch hydrolysis [2, 4, 8, 9, 11].

Grain starches contain considerably more fat impurities (wheat 1.12% in this number 62% of lysolecithin, corn 0.87% with 17% lysolecithin) as compared to potato starch (0.05%) or tapioca starch (0.1%) [1, 9]. The lipid fraction of wheat starch is mainly composed of lysophospholipids, whereas that of corn starch, of free fat acids.

During heating of starch suspension at gruelling temperature or higher, fats form two types of amylose-fat complexes: amylose-lysolecithin and amylose-faty acids (palmitin acid, lynolene acid) [4, 9]. Each percent of fat forms from 10 to 20% of starch-fat complexes, the percentage depending on the composition of fat. [2].

Amylose in this state is resistant to the action of amylolytic enzymes and swells only slightly during gruelling, due to reduced water-fixing power. Lipids account also for clouding of hydrolyzates, an unpleasant taste and odour caused by oxidation of fats, and a decrease in the filtration rate of hydrolyzates [9].

A disadvantageous influence exerted by fat on the production process of corn and wheat hydrolyzates may be diminished by utilising the action of a lysophospholipase enzyme which catalyses the reaction of decomposition of amylose-fat complexes [4,5]. It decomposes lysophospholipids to fatty acids and glycerophosphocholine (with the formation of lysolecithins). In this way the ability of lysophospholipids to form emulsions is destroyed. Due to this the colour and clarity of hydrolyzates is improved, the filtration rate increases and the quantity of filtration assistance used is reduced [4].

From the point of view of technology, contamination of the starch with proteins which can produce unpleasant flavour and contribute to foaming during gruelling and stain starch hydrolyzates, is also disadvantageous.

A specially large quantity of proteins accompany grain starches, i.e. wheat starch (0.4%) and corn starch (0.35%), in comparison with potato starch (0.06%) [8, 9].

In water media proteins form difference complexes with polysaccharides. In these complexes there are ionic bonds. However, inert polysaccharides form with proteins hydrogen and hydroxyl bonds [3].

At the concentration of macromolecules exceeding 1%, in the biopolymer systems a division of the system into two liquid phases was observed: one containing only protein, while the other one only polysaccharide. This is a generally observed phenomenon, however not common to all types of starch. It refers only to grain starch and not to potato starch which irrespective of the pH value, yields a one-phase system. Thermodynamic adjustment of proteins and inert polysaccharides reduces with pH approaching the isoelectric point of proteins and with an increasing molecular weight of polysaccharide [12].

As distinguished from potato starch and corn starch, the chemical composition of wheat starch is characterised by the presence of non-starch polysaccharides – these are mainly pentosanes, i.e. the polymers of β-1,4-xylose with β-1,3-arabinose branchings.

These substances are characterised by high water absorption in the aqueous solution forming gel substances of high viscosity, and also by the ability to form complexes with proteins which cannot be removed using physical procedures only. They are a special obstacle in the process of filtration of hydrolyzates obtained by enzymatic hydrolysis [2].

Disadvantageous properties of pentosanes in the production process of enzymatic starch hydrolyzates can be reduced by applying pentozanase enzyme. It decomposes arabinoxylanes, bringing about a reduction of viscosity and an increase of the filtration rate. It also constrains clouding in the process of cooling the hydrolyzate (as a result of precipitation of xylanes at low temperature) and changes the structure of residue by transforming non-soluble pentosanes into soluble ones [2, 6].

The aim of this study was to obtain from wheat starch such glucose hydrolyzates which would have the optimum physical and chemical properties, i.e. good filtration ability, transparency, and lack of colour, the features reached due to the additional application of lysophospholipase, proteinase and cellulolytic enzymes in the process of enzyme hydrolysis. They enhance the hydrolysis of fat, proteins and arabinoxylanes.

Materials and analytical methods

The substrate used for research purposes was wheat starch containing 88.2% of dry matter, of pH = 6.4, of acidity equal to 1.3°N, alkalinity equal to 1.1°N, fat content equal to 1.0%, protein content equal to 1.1% and ash content equal to 0.2%, in relation to dry matter.

For research purposes, the following enzymatic preparations were used in the tests:

- α-amylase TERMAMYL 120L manufactured by Novo Nordisk A/S, of an activity equal to 240 KNU/g, (pH = 5.0-6.5, 80-105°C),
- glucoamylase AMG 300L manufactured by Novo Nordisk A/S, of an activity equal to 300 AG/cm^3, (pH = 3.5-5.5, 25-60°C),
- SPEZYME GA 300W Y553, an enzymatic preparation manufactured by Gamma Chemie GmbH, containing glucoamylase and lysophospholipase in its composition, of an activity equal to 300 SGU/ml, (pH = 3.8-4.5, 60°C),
- xylanase-cellulase GAMMAZYM CX 4000L Y552 manufactured by Gamma Chemie GmbH, (pH = 4.0-6.0, 40-55°C),
- xylanase SHEARZYME 500L, manufactured by Novo Nordisk A/S, of an activity equal to 500 FXU/g, (pH = 4.0-5.0, 50-70°C),

- proteinase NEUTRASE 0.5L, manufactured by Novo Nordisk A/S (pH = 5.5-7.0, 25-55°C).

Analytical methods

The analysis of enzymatic wheat hydrolyzates involved carrying out the following determinations:

- the content of reductive sugars, using the Lane-Eynon method [10], after a previous elimination of proteins from hydrolyzates;
- the filtration rate of wheat hydrolyzates;

(For determination purposes, always the same volume of hydrolyzate, equal 90 cm^3, was drawn and the concentration of each of the samples brought to the value of 37°Bx. At the temperature of 60°C, the hydrolyzate was filtered trough fluted filter paper of a diameter that was constant for all samples and equal to 205 mm and of the area of 33011 mm^2. The filtration area was equal to 31420 mm^2. Measurements of the filtrate volume obtained after 5, 10, 15, 20 and 25 minutes were taken.)

- the colour factor of the solution [7];

(Measurements of solution absorbency were taken at the length of the light wave equal to 400 and 720 nm and calculated in terms of 1 g of product and a layer of 1 cm in thickness. Absorbency measurements were taken using a "Spekol" spectrophotometer, after filtering, at pH equal to 5.6-5.7 and the concentration of hydrolyzates equal to 30° Bx.)

- the transparency factor of the solution [7].

(The determination was carried out by preparing wheat hydrolyzate solutions as above and using a "Spekol" spectrophotometer, absorbency measurements were taken, at the length of the light wave equal to 720 nm, in relation to distilled water.)

The process of hydrolysis

Production of glucose hydrolyzates out of wheat starch was carried out in the two following stages: liquefaction and saccharification of starch. For this purpose, the hydrolysis was carried out using the enzyme-enzyme method.

Liquefaction of a suspension of starch, of a concentration equal to 33%, was carried out at pH = 6.5, at the temperature of 95°C and the concentration of α-amylase enzyme equal to 0.15%/d.m.

During saccharification the enzymatic preparation Spezyme GA 300W Y552 of concentration 0.3%/d.m., containing glucoamylase and lysophospholipase was used. For comparison the glucoamylase preparation AMG 300L (concentration 0.3%/d.m.) was applied.

Jointly with these preparations cellulolytic enzymes (xylanase-cellulase Gammazym CX 4000L – concentration 0.06%/d.m. and xylanase Shearzyme 500L – concentration 0.069%/d.m.) and proteolytic enzyme (Neutrase 0.5L – concentration 0.013%/d.m.) were used.

Results and discussion

Results of analysis of wheat starch hydrolyzates obtained by acting on liquefied wheat starch with Spezyme GA 300W, containing glucoamylase and lysophospholipase and with cellulolytic enzymes and proteinase as compared to glucoamylase preparation Amyloglucosidase AMG 300L, are illustrated in Graphs 1 through 5.

With Spezyme (glucoamylase and lysophospholipase complex) used in the process of saccharification jointly with various cellulolytic preparations and proteinase, a much higher degree of saccharification was obtained (of the order of 99-99.7DE) than when using the same system with the application of glucoamylase AMG 300L, a saccharifying enzyme, where reductivity reached 97.9 to 98.4 DE. Lysophospholipase, due to the decomposition of amylase-lipid complexes, and cellulolytic enzymes, due to the destruction of cell walls of starch grains, enhance the access of amylolytic enzymes to starch. As a result, higher reductivity is obtained when Spezyme and assisting enzymes are used.

The dependence of saccharification degree of glucose hydrolyzates on the time of hydrolysis, and on the system of enzymes used, is shown in Graph 1.

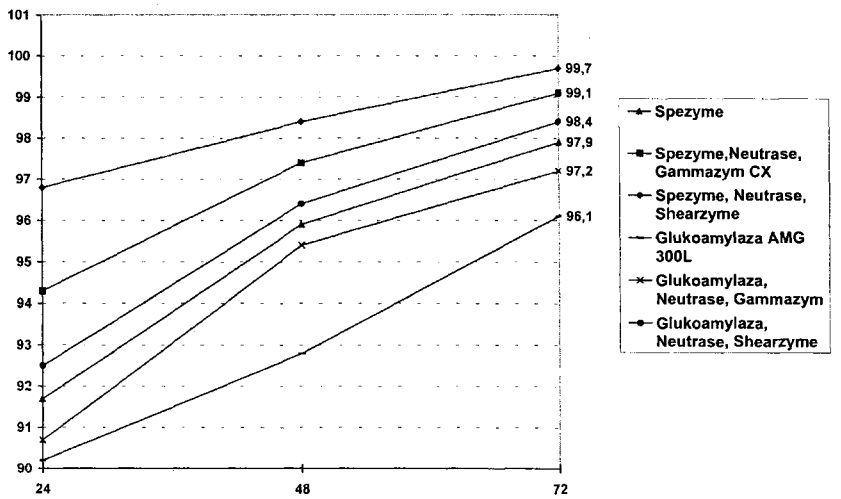


Diagram 1. Reducibility of a glucose hydrolyzate obtained in the process of saccharification, using either Spezyme preparation or glucoamylase AMG 300L, with on additive of various cellulolytic preparations and of a proteolytic preparation.
X: Duration of hydrolysis [h], Y: Degree of saccharification [DE]

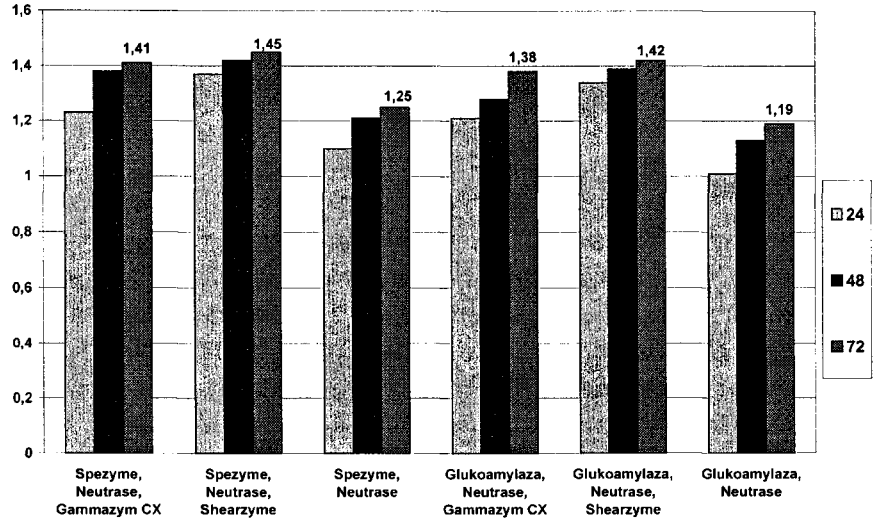


Diagram 2. Filtration rate of a glucose hydrolyzate obtained in the process of saccharification, using either Spezyme preparation or glucoamylase AMG 300L, with an additive of various cellulolytic preparations and of a proteolytic preparation.
X: Duration of hydrolysis [h]; Y: Filtration rate [cm^3/m^2s]

Due to the decomposition of amylase-lipid complexes, lysophospholipase contained in Spezyme preparation improves filtration ability of wheat glucose hydrolyzates.

The hydrolyzates obtained using in the process of saccharification Spezyme preparation with the addition of xylanase Shearzyme and proteinase, were characterised by the best filtration ability – 1.45 cm^3/m^2s. For the same system with the use of glucoamylase AMG 300L the hydrolyzates were filtered at a slower rate – 1.42 cm^3/m^2s.

Filtration ability of wheat glucose hydrolyzates is shown in Graph 2.

The application of both saccharifying preparations, cellulolytic enzymes and proteinase makes it possible to obtain glucose hydrolyzates which are less coloured and more transparent than those obtained without the addition of the enzymatic preparations.

The best appeared to be the system in which glucoamylase with lysophospholipase (Spezyme GA 300W), xylanase Shearzyme 500L and proteinase Neutrase 0.5L were used; the colour factor was then 206 and transparency factor – 122. For hydrolyzates obtained with the use of glucoamylase AMG 300L these factors were worse: 297 and 115, respectively.

The colour and transparency factors are shown in Graphs 3 and 4.

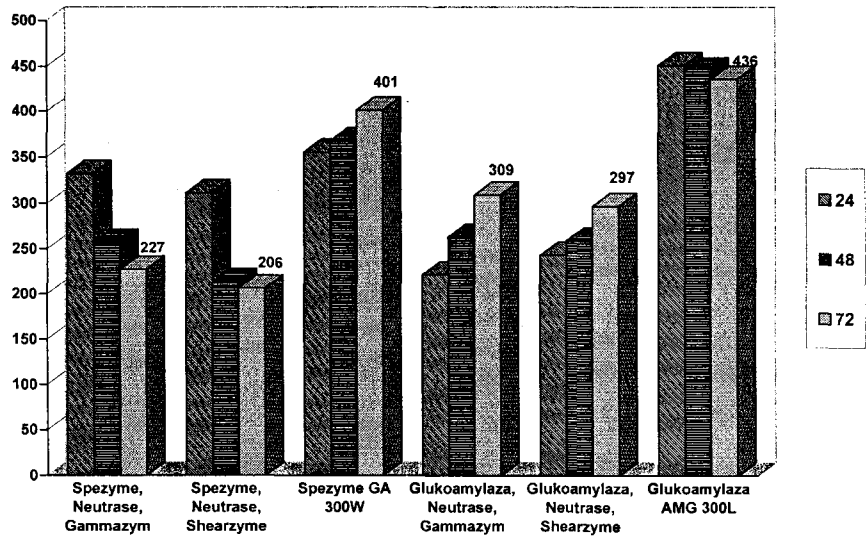


Diagram 3. The colour factor of a glucose hydrolyzate obtained in the process of saccharification, using either Spezyme preparation or glucoamylase AMG 300L with an additive of various cellulolytic preparations and of a proteolytic preparation.
X: Duration of hydrolysis [h]; Y: Colour factor

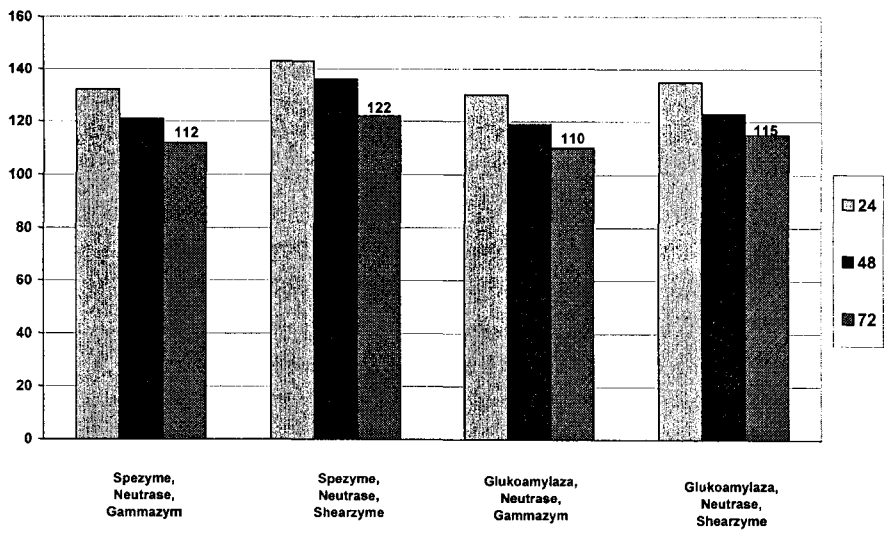


Diagram 4. The transparency factor of a glucose hydrolyzate obtained in the process of saccharification, using either Spezyme preparation or glucoamylase AMG 300L, with an additive of various cellulolytic preparations and of a proteolytic preparation.
X: Duration of hydrolysis [h]; Y: Transparency factor

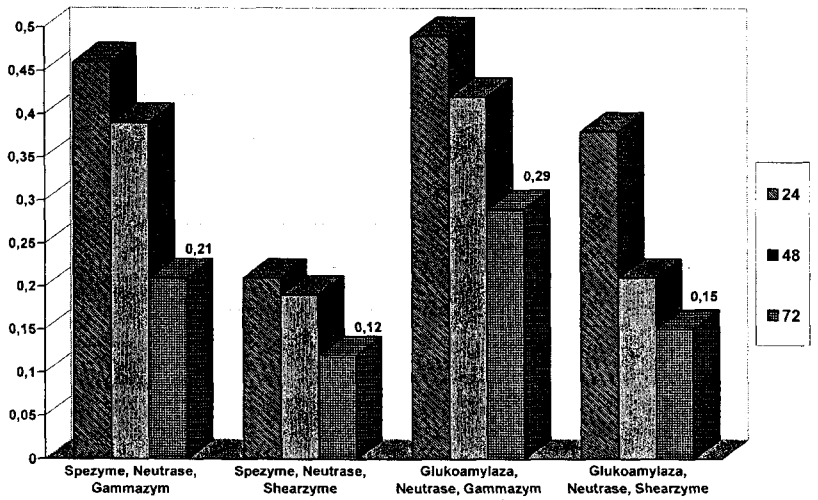


Diagram 5. The protein content in a glucose hydrolyzate obtained in the process of saccharification, using Spezyme preparation and glucoamylase AMG 300L, as well as Neutrase, a proteolytic preparation. Protein content: liquefied hydrolyzate-0,79% d.m.
X: Duration of hydrolysis [h]; Y: Protein content [%s.s.]

Investigations of protein content in glucose hydrolyzates (Graph 5) showed that much less protein is transferred to the solution when in the process of saccharification, beside glucoamylase and lyzophospholipase (Spezyme GA 300W), cellulolytic preparations and proteinase are used, than in the case of application of glucoamylase preparation AMG 300L with the above mentioned assisting enzymes.

Conclusions

1. Using cellulolytic enzymes and proteinase, simultaneously with saccharificating preparations, in the process of saccharification of wheat starch has an affect of an additional icrease of glucose hydrolyzates.
2. Better filtration ability is revealed by glucose hydrolyzates obtained in the presence of glucoamylase and lysophospholipase (Spezyme) with the addition of cellulolytic preparations and proteinase, than by those obtained when glucoamylase AMG 300L is used with these enzymes.
3. The application of Spezyme and, additionally, cellulolytic enzymes and proteinase in the process of saccharification results in formation of glucose hydrolyzates which are less coloured and more transparent than in the case when these enzymes are used jointly with glucoamylase AMG 300L.
4. The best physical and chemical properties of glucose hydrolyzates, i.e. colour, transparency, filtration ability and the highest reductivity are obtained using for

saccharification both Spezyme, the enzymatic preparation of xylanase Shearzyme 500L and proteinase Neutrase 0.5L.

REFERENCES

[1] Acker L., Schmitz H. J.: Über die Lipide der Weisenstärke. III. Mit. Die übrigen Lipide der Weisenstärke sowie die Lipide anderer Stärkearten. Starch/Stärke, **19**, 1967, 275.

[2] Bowler P., Towersey P.J., Galliard T.: Some effects of the minor components of wheat starch on glucose syrup production. Starch/ Stärke, **37**, 1985, 351.

[3] Chmiel A.: Biotechnology: Microbiological and Biochemical Fundamentals. PWN, Warszawa 1994, 37-39 (in Polish).

[4] Konieczny-Janda G., Richter G.: Progress in the enzymatic saccharification on wheat starch. Starch/Stärke, **43**, 1991, 308.

[5] Lionel A., Ghiskin W.: Carbohydrate refining process and enzyme compositions suitable for use therein. BE Pat. 86 307449.5, 1986.

[6] McCleary B.V., Gibson T.S., Allen H., Gams T.C.: Enzymic hydrolysis and industrial importance of barley β-glucans and wheat flour pentosans. Starch/ Stärke, **38**, 1986, 433.

[7] Polish Standard: Starch hydrolyzates. Methods of investigations. PN-78/A-74701 (in Polish).

[8] Słomińska L.: Starch hydrolyzates obtained from corn products. Przemysł Spoż., **51**, 1997, 9 (in Polish).

[9] Swinkels J.J.M.: Composition and properties of commercial native starches. Starch/Stärke **37**, 1985, 1.

[10] Świechowski Cz.: Technical analysis in food industry. WPLiS, Warszawa 1968, 350 (in Polish).

[11] Tegge G., Richter G.: Zur enzymatischen Hydrolyse verschierdener Stärkearten. Starch/Stärke **38**, 1986, 329.

[12] Trzmiel T.: Properties of enzymes and their applications. Przemysł Spoż., **9**, 1995, 338 (in Polish).

WPŁYW WARUNKÓW PROCESU ENZYMATYCZNEJ HYDROLIZY NA WŁAŚCIWOŚCI HYDROLIZATÓW SKROBI PSZENNEJ

Streszczenie

W przedstawionej pracy otrzymywano hydrolizaty glukozowe działając na upłynnioną za pomocą bakteryjnej α-amylazy skrobię pszenną preparatem enzymatycznym Spezyme GA 300W, w skład którego wchodzi glukoamylaza i lizofosfolipaza oraz preparatem glukoamylazy Amyloglucosidase AMG 300L. Jednocześnie z enzymami scukrzajacymi stosowano enzymy celulolityczne Gammazym CX 4000L i Shearzyme 500L oraz proteinazę Neutrase 0,5L.

Najlepsze właściwości fizykochemiczne hydrolizatów glukozowych, tj. barwę, przezroczystość, zdolność filtracji i najwyższą redukcyjność uzyskuje się, stosując w procesie scukrzania łącznie z preparatem Spezyme preparat ksylanazy Shearzyme 500L i proteinazy Neutrase 0,5L.

T.R. NOEL, R. PARKER, S.G. RING

THE EFFECT OF THE PHASE BEHAVIOUR AND DYNAMICS OF STARCH ON ITS FUNCTIONALITY

Abstract

The phase behaviour and dynamics of starch, and its dextrins, are reviewed. Topics discussed include the effect of diluents on the melting and glass transition behaviour of starch, and factors affecting the dynamics of the starch chain in concentrated systems. The approach adopted is to apply concepts developed in the synthetic polymer area to the biopolymer starch. Finally the relationship between molecular properties and aspects of functionality are examined.

Introduction

Granular starch is usually processed by heating, to achieve disruption of the native, partially crystalline structure. Water and other low molecular weight diluents may be added to facilitate processing or to improve product characteristics. Behaviour is influenced by equilibrium aspects, including how diluents modify the melting and phase behaviour of starch, and non-equilibrium factors, such as the effect of diluents both on the glass transition and plasticization of starch, and on time-dependent changes in properties. The latter may occur, either as a result of crystallization of starch chains, or structural relaxation in amorphous regions. In this review we will not focus entirely on polymeric behaviour as we think that useful insights can be gained by examining behaviour as a function of chain length. We will start by examining phase behaviour.

Phase behaviour

Melting and dissolution

For starch and its dextrins there is a body of experimental data on their crystalline conformations and dissolution behaviour. Even so, the amount of information available

Department of Biochemistry, Institute of Food Research, Norwich Research Park, Colney Lane, Norwich, UK NR4 7UA

is not as extensive as for a synthetic polymer. Although the existence of polymorphic crystalline forms of starch has been known for a number of years it is only comparatively recently that it has been possible to obtain single crystals (Imberty and Perez, 1988; Imberty et al., 1988; Buleon *et al.*, 1990; Helbert *et al.*, 1993). The A and B forms, which are present in the native granule, are based on the packing of double helices into monoclinic and hexagonal arrays, respectively.

Although polysaccharides can be crystallized from aqueous solution, it is generally easier to prepare single crystals from oligomeric fragments. The minimum chain length required for the formation of the A and B crystalline forms of starch is greater than 9 units (Pfannemuller, 1987; Gidley *et al.*, 1987). Less information is available on the crystallization behaviour of maltooligomers ranging in degree of polymerisation from 3-9 units. These oligomers readily form stable glasses on drying concentrated aqueous solutions at room temperature (Jeffey and Saenger, 1991; Orford *et al.*, 1989). Methyl α-maltotrioside crystallizes as a tetrahydrate, in which the chain conformation adopted is similar to that found in the A and B forms of amylose (Pangborn *et al.*, 1985). Similarly *p*-nitrophenyl α-maltohexaoside was crystallized as a hydrated molecular complex with $Ba(I_3)_2$, with the carbohydrate chain forming a double helical arrangement (Hinrichs *et al.*, 1987). Recently maltopentaose was crystallized as a hepta or octahydrate (Moates *et al.*, 1997). A characteristic of oligosaccharide hydrates (Jeffey and Saenger, 1991) is that the water forms a network structure, with the sites of occupancy having different affinities for water molecules, and the extent of occupancy depending on ambient conditions of water vapour pressure and temperature.

For synthetic polymers, in extended chain crystals, the melting temperature T_m increases with increasing chain length (Mandelkern *et al.*, 1990). T_m decreases with chain folding, or if the polydispersity of the polymer introduces defects in the crystalline lattice. In contrast to most crystalline synthetic polymers, it is not possible to observe directly the melting of the isolated starch crystals. Even the T_m of the "monomer", anhydrous β-D-glucose, is 150°C (Parks *et al.*, 1928), and close to the temperature at which thermal degradation of carbohydrates is observed. One option for the study of the melting of crystalline forms of starch is to use a diluent to depress T_m (Donovan, 1979). The classical description (Flory, 1953) of the compositional dependence of polymer melting in the presence of a diluent is given by

$$1/T_m = 1/T_m^0 + (R/\Delta H_u).(V_u/V_1).[\nu_1 - \chi\nu_1^2] \quad (1)$$

where T_m^0 is the melting temperature of the pure polymer, V_u and V_1 are the molar volumes of polymer repeating unit and diluent, respectively, ν_1 and ν_2 are the diluent and polymer volume fractions, respectively. ΔH_u is the enthalpy of fusion per repeating unit and χ is the Flory-Huggins interaction parameter characterising the interaction energy per solvent molecule. The above relationship predicts that the smaller the vol-

ume of diluent relative to that of the polymer segment, and the more favourable the interaction between diluent and polymer, the greater the effect of the diluent in depressing T_m.

Calorimetric studies (Taylor and Rowlinson, 1955) on carbohydrate/water mixtures show a favourable energetic interaction between the components. With increasing carbohydrate chain length, an opposing entropic contribution to the interaction free energy becomes evident (Moates *et al.*, 1997).

Water at room temperature can be classified as a relatively poor solvent for the starch chain. At the limit of infinite molecular weight, χ is 0.5 for a dilute amylose solution, rising to ~0.8 at a polymer concentration of 80% w/w. These values of χ are qualitatively consistent with the aqueous solution behaviour of the amylosic chain. Although lower maltooligomers form stable aqueous solutions at room temperature, the effect of increasing chain length leads to instability, this is revealed through the gelation and aggregation from concentrated aqueous solution. The solvent quality of water increases with increasing temperature, although as yet there is limited data on the temperature dependence of χ.

The relatively small molecular volume of the water molecule compared to the anhydrohexose unit of the starch chain indicates that it can be an effective diluent in depressing T_m. This is indeed observed in recent studies (Moates *et al.*, 1997) which have examined both the chain length and composition dependence of the dissolution of amylose crystals. The dissolution in water of the B-type crystalline polymorph of amylose, crystallized from fractions of limited polydispersity, ranging in chain length from 12 to 55 residues, was examined by scanning calorimetry. With increasing chain length in this range, the dissolution temperature, at a volume fraction of water of 0.8, increased from 57 to 119°C. The extrapolated dissolution temperature for the high molecular weight polymer at this water content was 147°C. From equation 1 it is possible to obtain a prediction of a T_m of ~250°C at a water content of 26% w/w rising to ~480°C for T_m^0. For the same chain length it was found that the dissolution of the A-polymorph in water occurred at temperatures approximately 20°C higher than the B-polymorph (Whittam *et al.*, 1990). Some of the data on the composition and chain length dependence of melting is summarised schematically in Figure 1. The data is relevant to the stability and processability of starch and its products, and it gives insight into the potential links between starch structure and gelatinization behaviour. It also forms a framework to help interpret the observed retrogradation behaviour of amylose and amylopectin. For example, synthetic polymers in a poor solvent, at a temperature which is far below the T_m of the crystalline solid, often form turbid gels – as does amylose. Similarly, the association between increasing the length of unsubstituted amylopectin constituent chain, and the tendency for amylopectin to recrystallize at ambient temperatures.

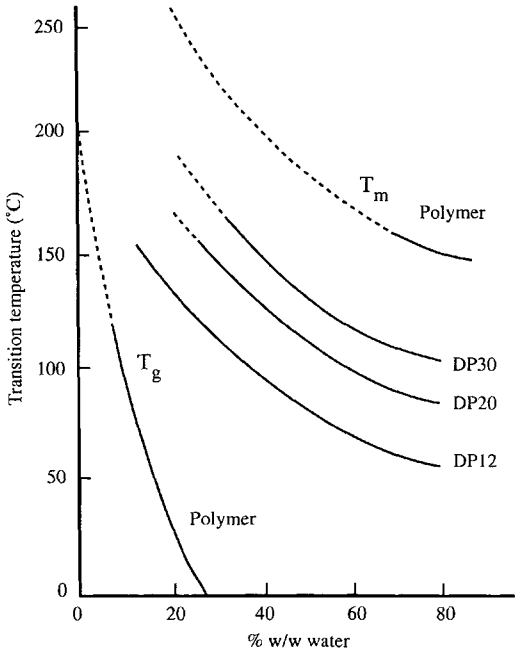


Fig. 1. Composition dependence of the T_m of the B crystalline polymorph of starch in water and T_g of a starch/water mixture. Solid lines denote current limit of observations.

Ternary systems – one polymer and two diluents

Although water is the most common diluent encountered in starch systems, other diluents may be added to further modify behaviour. As water is invariably present, the next step in complexity is to consider behaviour in ternary mixtures, containing two diluents, one of which is water. As before, the balance of interactions between species influences behaviour. When these interactions are sufficiently large, phase separation is obtained, with a polymer-rich phase containing predominantly one diluent, and a polymer-deficient phase containing the other. Even before a phase separation is observed, the partial replacement of one diluent by another can have a dramatic effect on polymer behaviour.

The physicochemical analysis of this phenomenon is dependent on the system, differing somewhat depending on whether a synthetic polymer (Flory, 1953; Altena and Smolders, 1982) or a biopolymer (nucleic acid, protein) is being studied (Arakawa and Timasheff, 1982; Lee *et al.*, 1979; Eisenberg, 1994). An important parameter in both approaches is the pairwise interaction free energy between components. The 'classical approach' as given in equation 1 can be developed to predict phase behaviour in these ternary mixtures (Flory, 1953; Altena and Smolders, 1982). It can also be

modified to predict the dissolution of a crystalline polymer in the presence of a diluent and low molecular weight solute (Lelievre, 1976)

$$1/T_m - 1/T_m^0 = R/\Delta H_u \cdot V_u/V_1 \, (v_1 + v_3/x_3 + \chi_{13}v_1v_3 - [\chi_{12}v_1 + (\chi_{23}v_3)/x_3] \, (v_1 + v_3)) \qquad (2)$$

where v_i is the volume fraction of component i and the subscripts 1, 2, 3 refer to diluent, polymer and low molecular weight solute respectively, with the quantity x_3 the ratio of the molar volume of this solute to the molar volume of diluent. The effect of diluents on depression of T_m is therefore dependent on the various interaction parameters, χ_{12}, χ_{13}, χ_{23}, and the relative sizes of the diluents. As before, the interaction between diluent and polymer is characterized as the interaction per polymer segment. In the dissolution of crystalline forms of starch, at a fixed volume fraction of polymer, the replacement of water by D-glucose or sorbitol is predicted to lead to an increase in T_m, simply as a result of the replacement of a relatively small diluent (water) by a larger one which can only interact weakly with the starch chain. Conversely, to observe a depression in T_m, a relatively strong attractive interaction, χ_{23}, is needed to counteract the replacement of water by a larger solute. An illustration of these effects is given in Figure 2 which shows the effect of added diluents on the T_m of a crystalline amylose fraction. For diluents which interact weakly with the starch chain (glycerol, D-glucose, sorbitol) the addition of a larger diluent elevates T_m. For diluents which have a stronger attractive interaction (urea, guanidinium thiocyanate) a depression in T_m is observed. The validity of the approach requires more research.

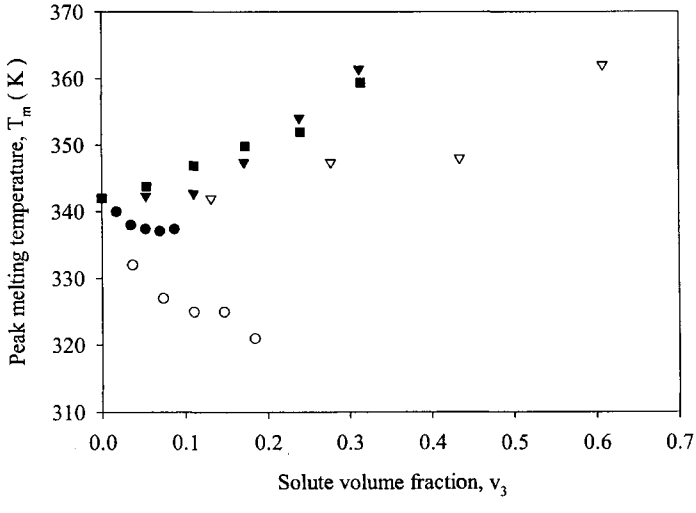


Fig. 2. T_m versus solute volume fraction for the dissolution of amylose crystallites in a ternary aqueous mixture for the solutes, glycerol (▼); sorbitol (■); D-glucose (▽); urea (●); guanidinium thiocyanate (○).

Dynamics

Glass transition behaviour of starch

A glass has a liquid-like structure without obvious mobility. As a material is undercooled below T_m, and providing crystallization does not intervene, its viscosity will progressively increase. If the viscosity is so large, that the material cannot flow within a practical timescale, it will have solid-like characteristics. Typically this occurs at viscosities in excess of 10^{12} Pas when the shear stress relaxation time is of the order of 100s. The transition from solid-like to liquid-like behaviour occurs at the glass transition temperature, T_g. As the heat capacities of glasses and supercooled liquids differ, calorimetry is a convenient method for the measurement of T_g from the 'step' change in heat capacity. The solid-like to liquid-like transition will occur at different temperatures depending on the timescale imposed by the experimental procedure. The glass transition behaviour of D-glucose was examined over 60 years ago when a T_g of 7°C was found (Parks *et al.*, 1928, 1934). More recently, the glass transition behaviour of a maltooligomer series was examined (Orford *et al.*, 1989). T_g increased with increasing degree of polymerization and reached 173°C for maltohexaose. Again this is close to the temperature at which thermal degradation of carbohydrates becomes apparent, with a result that the T_g of amorphous starch cannot be determined directly. A plasticizer has to be used to depress T_g and the glass transition behaviour examined as a function of composition. In starchy materials, water has a very marked effect on depressing T_g. For example, the addition of just 6% w/w water to dry maltohexaose (about 0.8 water molecules per anhydrohexose unit) depresses T_g by about 100°. The composition dependence of T_g for a high molecular weight starch polysaccharide is summarised schematically in Figure 1. The T_g of starch/dextrin water mixtures shows, compared to T_m, a weak dependence on chain length.

There is more limited information on the effect of other plasticizers on the T_g of starch. One of the more widely studied is glycerol. A 88% starch/water mixture had a T_g of ~ 70°C. To achieve the same depression in T_g it was necessary to add > 29% w/w glycerol to starch, glycerol can therefore be considered to be a less effective plasticizer than water. As water was added to these mixtures, the behaviour became more complex, with the appearance of two separate glass transitions. The major component involved in one was thought to be starch, for the other glycerol. The most likely explanation was that there had been some sort of phase separation with the formation of starch-rich and glycerol-rich phases (Forssell *et al.*, 1997). Further research is necessary to confirm this suggestion.

Structural relaxation and localised motions

The relationship between viscosity and molecular mobility is relevant to practical usage of starch in several ways, including mechanical properties; the stability of the material to crystallization; the encapsulation performance of starch and maltodextrin matrices; and the ageing of amorphous materials. As a background to these phenomena, it is useful to examine the dependence of viscosity, η, on temperature, T, as the liquid is undercooled. For simplicity we only describe the behaviour of a low molecular weight non-entangling system which exhibit Newtonian viscous behaviour. Higher molecular weight materials have more complex non-Newtonian rheologies, however broadly similar concepts still apply. The behaviour of many organic liquids, (including the carbohydrates - D-glucose (Parks *et al.*, 1934), glucitol and maltose (Angell *et al.*, 1982; Noel *et al.*, 1991)) can be described by an empirical expression of the type

$$\eta = \eta_\infty \exp (B/(T-T_0)) \quad (3)$$

where η_∞, B and T_0 are constants. As T_g is approached there is a very strong dependence of viscosity on temperature.

The enormous viscosity of glassy carbohydrates has led to their use as matrices for the encapsulation of active agents, including flavours and pharmaceutical products. While the glass is often mechanically stable for months, deterioration can occur through moisture sorption and plasticization of the matrix, which can accelerate the rate of crystallization. Crystallization of the carbohydrate can concentrate the active agent leading to loss. A topic of current interest is the extent to which mobility persists in the glass, and the supercooled region above T_g, and the effect of this mobility on chemical and enzymic reaction. To start to tackle this topic it is necessary to examine in more detail the dynamics of these undercooled liquids.

The reorientational dynamics of the carbohydrate in the undercooled liquid can be probed by NMR (Girlich and Ludemann, 1993) and dielectric techniques (Noel *et al.*, 1996a). For pure maltose two main dielectric relaxations are observed. The primary relaxation, at the higher temperature, is ascribed to reorientation of the whole molecule, at a rate influenced by the bulk shear viscosity. This relaxation is therefore a direct probe of viscosity, and is intimately linked with the glass transition. The secondary relaxation, at the lower temperature, is ascribed to a more localised motion. NMR and further dielectric experiments on other carbohydrates suggest that this localised motion is linked with the reorientation of the pendant hydroxymethyl group at C-6. Addition of water, with its plasticizing action, to the maltose, shifts the primary and secondary relaxations to lower temperatures, with a marked increase in the strength of the secondary relaxation. This suggests that water is relatively free to reorient within the maltose matrix and is consistent with the observed diffusive behaviour of water in these systems.

In addition to identification of the link between molecular structure and dynamics there is also important to consider in more detail the structure of the amorphous liquid and its dependence on temperature. Such information can be obtained by wide-angle neutron scattering combined with H/D isotopic substitution. In a recent study (Tromp *et al.*, 1997) on amorphous D-glucose, it was found that the number of hydrogen bonds in the glass were more or less similar to that in the crystal. On heating the undercooled liquid, there was a fall in number of hydrogen bonds coupled with a restructuring of the hydrogen bond network. Presumably one potential effect of any added plasticizer is disruption of this network.

For species which are large relative to the molecular length scale, an increase in viscosity will lead to a comparable reduction in diffusion. As size is reduced and approaches molecular dimensions, an uncoupling of diffusion from viscosity can be observed. As yet there are few measurements on carbohydrate liquids. One example is shown schematically in Figure 4 where the mutual diffusion in maltose/water mixtures is compared to the viscosity increase on undercooling. As T_g is approached, a dramatic uncoupling of diffusive and viscous behaviour is observed, with diffusion in the undercooled liquid being much more rapid than expected (Parker and Ring, 1995). Other ways of probing transport processes, such as the measurement of ionic conductivity, yield comparable data (Noel *et al.*, 1996b). There is a need for further measurements which determine the effect of molecular size on the observed uncoupling.

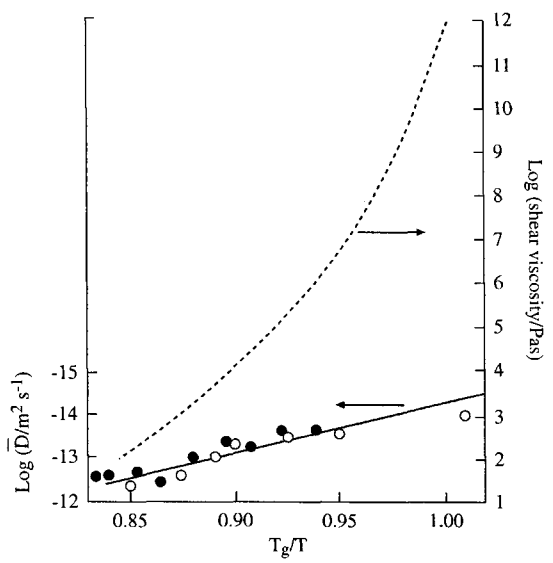


Fig. 4. Comparison of viscous and diffusive behaviour in maltose/water mixtures as the glass transition is approached.

Although this discussion has concentrated on low molecular weight carbohydrates, for which data is emerging, it is to be expected that studies will be extended to polysaccharides such as starch.

Functionality of starch

As a texture modifier

Starch often makes a major contribution to food texture. Several types of microstructure can be produced. For example after heating a moderately concentrated starch suspension (~ 6% w/w) above its gelatinization temperature a starch paste is formed consisting of swollen gelatinized starch granules dispersed in a liquid containing solubilised starch polysaccharides, chiefly amylose (Evans and Haisman, 1979; Doublier *et al.*, 1987; Ellis *et al.*, 1989; Svegmark and Hermansson 1991). The rheological properties of this material is influenced by the volume that the granules occupy and their deformability and shape. The contribution of the solubilised material to the rheological properties is, at this stage, rather small.

If the paste is cooled the solubilised amylose can, if sufficiently concentrated, aggregate to form a gel network (Miles *et al.*, 1985a; Gidley, 1989; Gidley and Bulpin, 1989). As the stiffness of this network is very dependent on amylose concentration, the amylose can make a major contribution to the elastic properties of the starch gel. The swollen granules reinforce this gel by acting as a filler (Miles *et al.*, 1985b). The crystallization of amylopectin on aging (Ring *et al.*, 1987; Shi and Seib, 1992; Wursch and Gumy, 1994) increases the stiffness of the granules and the overall stiffness of the starch gel.

As a nutrient

As well as making an important contribution to food texture starch is a major dietary polysaccharide. There is a need to understand the factors affecting digestibility in the gastrointestinal tract. Although there is a substantial literature on the physical chemistry of how enzymes attack polymers in solution, other factors influence the attack of solid and semi-solid materials. For dense solids, attack can only occur at the solid liquid interface. An important factor relevant to the rate of enzymolysis is therefore the amount of interface rather than the concentration of substrate. For the enzyme to attack the solid it needs to bind to the surface (Leloup *et al.*, 1991,1992a). Access to the surface substrate may be somewhat restricted reducing the affinity of the enzyme for its substrate. This has two effects. Firstly, to achieve surface saturation, and therefore maximal rate of attack, relatively high concentrations of enzyme in solution may be required. Secondly, in mixed systems, with some of the substrate in soluble form and some as a solid, the higher affinity of the enzyme for the soluble substrate will lead

to its preferential attack. An additional factor is the difference between starch conformation in the solid and solution. Starch in solution has a flexible conformation which can fit into the active site of the enzyme. In the solid state, flexibility is reduced and other conformations e.g. double helical, may be present which reduce enzyme substrate binding and catalysis. For processed starch which has been allowed to partially crystallize, the amylose double helix is resistant to amylolysis (Colquhoun *et al.*, 1992). This resistant fraction can modify the digestibility of the starch substrate. For network solids which are to some extent porous to the enzyme, additional factors affecting the rate of enzymolysis include the fraction of the network which is accessible to molecules the size of the enzyme, and the effect of the network on the diffusion of the enzyme (Leloup *et al.*, 1990, 1992b).

REFERENCES

[1] Altena F.W., C.A. Smolders: Calculation of liquid-liquid phase separation in a ternary system of a polymer in a mixture of a solvent and a non-solvent. Macromolecules, **15**, 1982, 1491-1497.

[2] Angell C.A., Stell R.C., Sichina W.: Viscosity-temperature function for sorbitol from combined viscoity and differential scanning calorimetry studies. Journal of Physical Chemistry, **86**, 1982, 1540-1542.

[3] Arakawa T., Timasheff S.N.: Stabilization of protein structure by sugars. Biochemistry, **21**, 1982, 6536-6544.

[4] Buleon A., Delage M.M., Brisson J., Chanzy H.: Single-crystals of V-amylose complexed with isopropanol and acetone. International Journal of Biological Macromolecules, **12**, 1990, 25-33.

[5] Colquhoun I.J., Parker R., Ring S.G., Sun L., Tang H.R.: "An NMR spectroscopic characterization of the enzyme-resistant residue from alpha-amylolysis of an amylose gel. Carbohydrate Polymers, **27**, 1995, 255-259.

[6] Donovan J.W.: Phase transitions in the starch-water system. Biopolymers, **18**, 1979, 263-268.

[7] Doublier J.-L., Llamas G., Le Meur M.: A rheological investigation of cereal starch pastes and gels. Carbohydrate Polymers, 7, 1987, 251-275.

[8] Eisenberg H.: Protein and nucleic acid hydration and cosolvent interactions: establishment of reliable baseline values at high cosolvent concentrations. Biophysical Chemistry, **53**, 1994, 57-68.

[9] Ellis H.S., Ring S.G., Whittam M.A.: A Comparison of the viscous behaviour of wheat and maize starch pastes. Journal of Cereal Science, **10**, 1989, 33-44.

[10] Evans I.D., Haisman D.R.: Rheology of gelatinised starch suspensions. Journal of Texture Studies, **10**, 1979, 347-370.

[11] Flory P.J.: Principles of polymer chemistry. First edn, Cornell: Cornell University Press, 1953.

[12] Forssell P.M., Mikkila J.A., Moates G.K., Parker R.: Phase and glass transition behaviour of concentrated barley-starch-water mixtures, a model for thermoplastic starch. Carbohydrate Polymers, **34**, 1997, 275-282.

[13] Gidley M.J., Bulpin P.V.: Crystallization of malto-oligosaccharides as models of the crystalline forms of starch - minimum chain-length requirement for the formation of double helices. Carbohydrate Research, **161**, 1987, 291-300.

[14] Gidley M.J., Bulpin P.V.: Aggregation of amylose in aqueous systems: the effect of chain length on phase behaviour and aggregation kinetics. Macromolecules, **22**, 1989, 341-6.

[15] Gidley M.J.: Molecular mechanisms underlying amylose aggregation and gelation. Macromolecules **22**, 1989, 351-8.

[16] Girlich D., Ludemann H.-D.: Molecular mobility of sucrose in aqueous solution studied by ^{13}C NMR relaxation. Zeitschrift fur Naturforschung, **48c**, 1993, 407-413.

[17] Helbert W., Chanzy H., Planchot V., Buleon A., Colonna P.: Morphological and structural features of amylose spherocrystals of A-type. International Journal of Biological Macromolecules, **15**, 1993, 183-187.

[18] Hinrichs W., Buttner G., Steifa M., Betzel C., Zabel V., Pfannemuller B., Saenger W.: An amylose antiparallel double helix at atomic resolution. Science, 238, 1987, 205-208.

[19] Imberty A., Perez S.: A revisit to the 3-dimensional structure of B-type starch. Biopolymers, **27**, 1988, 1205-1221.

[20] Imberty A., Chanzy H., Perez S., Buleon A., Tran V.: The double-helical nature of the crystalline part of A-starch. Journal of Molecular Biology, **201**, 1988, 365-378.

[21] Jeffrey G.A., Saenger W.: Hydrogen Bonding in Biological Structures. 1st Ed. Springer-Verlag, Berlin, 1991.

[22] Lee J.C., Gekko K., Timasheff S.N.: Measurements of preferential solvent interactions by densimetric techniques. Methods in Enzymology, **61**, 1979, 26-49.

[23] Lelievre J.: Theory of gelatinization in a starch-water-solute system. Polymer, **17**, 1976, 854-858.

[24] Leloup V.M., Colonna P., Ring S.G.: Studies on probe diffusion and accessibility in amylose gels. Macromolecules, **23**, 1990, 862-866

[25] Leloup V.M., Colonna P., Ring S.G.: Alpha-amylase adsorption on starch crystallites. Biotechnology and Bioengineering, **38**, 1991, 127-134.

[26] Leloup V.M., Colonna P., Ring S.G.: Physicochemical aspects of resistant starch. Journal of Cereal Science, **16**, 1992a, 253-266.

[27] Leloup V.M., Colonna P., Ring S.G., Roberts K., Wells B.: Microstructure of amylose gels. Carbohydrate Polymers, **18**, 1992b, 189-197.

[28] Mandelkern L., Prasad A., Alamo R.G., Stack G.M.: Melting temperature of the n-alkanes and the linear polyethylenes. Macromolecules, **23**, 1990, 3696-3700.

[29] Miles M.J., Morris V.J., Ring S.G.: Gelation of amylose. Carbohydrate Research, **135**, 1985a, 257-269.

[30] Miles M.J., Morris V.J., Ring S.G.: Roles of amylose and amylopectin in the gelation and retrogradation of starch. Carbohydrate Research, **135**, 1985b, 269-275

[31] Moates G.K., Noel T.R., Parker R., Ring S.G., Cairns P., Morris V.J.: Observations on the crystallization and melting of maltopentaose hydrate. Carbohydrate Research, **299**, 1997, 91-94.

[32] Moates G.K., Noel T.R., Parker R., Ring S.G.: The effect of chain length on the dissolution of the B-crystalline polymorph of starch. Carbohydrate Research, **298**, 1997, 327-333.

[33] Noel T.R., Ring S.G., Whittam M.A.: Kinetic Aspects of the Glass-Transition Behavior of Maltose-Water Mixtures. Carbohydrate Research, **212**, 1991, 109-117.

[34] Noel T.R., Parker R., Ring S.G.: Comparative study of the dielectric relaxation behaviour of glucose, maltose and their mixtures with water in the liquid and glassy states. Carbohydrate Research, **282**, 1996a, 193-206.

[35] Noel T.R., Parker R., Ring S.G.: Conductivity of maltose-water-KCl mixtures in the supercooled liquid and glassy states. Journal of the Chemical Society : Faraday Transactions 1, **92**, 1996b, 1921-1926.

[36] Orford P.D., Parker R., Ring S.G., Smith A.C.: Effect of water as a diluent on the glass-transition behavior of malto-oligosaccharides, amylose and amylopectin. International Journal of Biological Macromolecules, **11**, 1989, 91-96.

[37] Pangborn W., Langs D., Perez S.: Regular left-handed fragment of amylose: crystal and molecular structure of methyl-α-maltotrioside, $4H_2O$. International Journal of Biological Macromolecules, **7**, 1985, 363-369.

[38] Parker R., Ring S.G.: Diffusion in maltose-water mixtures at temperatures close to the glass-transition. Carbohydrate Research, **273**, 1995, 147-155.

[39] Parks G.S., Huffman H.M., Cattoir F.R.: Studies on glass - the transition between the liquid and glassy states of glucose. Journal of Physical Chemistry, **32**, 1928, 1366-1379.

[40] Parks G.S., Barton L.E., Spaght M.E., Richardson J.W.: The viscosity of undercooled liquid glucose. Physics, **8**, 1934, 193-199.

[41] Pfannemuller B.: Influence of chain length of short monodisperse amyloses on the formation of A-type and B-type x-ray diffraction patterns. International Journal of Biological Macromolecules, **9**, 1987, 105-108.

[42] Ring S.G., Colonna P., Ianson K.J., Kalichevsky M.T., Miles M.J., Morris V.J., Orford P.D.: The gelation and crystallization of amylopectin. Carbohydrate Research, **162**, 1987, 277-293.

[43] Shi Y.C., Seib P.A.: The structure of 4 waxy starches related to gelatinization and retrogradation. Carbohydrate Research, **227**, 1992, 131-145.

[44] Svegmark K., Hermansson A.-M.: Changes induced by shear and gel formation in the viscoelastic behaviour of potato, wheat and maize starch dispersions. Carbohydrate Polymers, **15**, 1991, 151-169.

[45] Taylor J.B., Rowlinson J.S.: The thermodynamic properties of aqueous solutions of glucose. Transactions of the Faraday Society, **51**, 1955, 1183-1192.

[46] Tromp R.H., Parker R., Ring S.G.: A neutron scattering study of the structure of amorphous glucose. Journal of Chemical Physics, **107**, 1997, 6038-6049.

[47] Whittam M.A., Noel T.R., Ring S.G.: Melting behavior of A- and B-type crystalline starch. International Journal of Biological Macromolecules, **12**, 1990, 359-362.

[48] Wursch P., Gumy D.: Inhibition of amylopectin retrogradation by partial β-amylolysis. Carbohydrate Research, **256**, 1994, 129-137.

WPŁYW ZACHOWANIA FAZOWEGO I DYNAMICZNEGO SKROBI NA JEJ FUNKCJONALNOŚĆ

Streszczenie

Dokonano przeglądu fazowego i dynamicznego zachowania się skrobi i dekstryn z nich. Omawia się wpływ rozcieńczania na topnienie i zeszklenie skrobi oraz czynniki wpływające na dynamikę łańcuchów skrobiowych w układach stężonych. Przyjęto podejście stosowane do polimerów syntetycznych. Sprawdzono zależność pomiędzy właściwościami molekularnymi i pewnymi cechami funkcjonalności.

W. PRAZNIK*, A. HUBER**

MODIFICATION OF BRANCHING PATTERN OF POTATO MALTODEXTRIN WITH Q-ENZYME

Abstract

Pure branching enzyme (Q-enzyme [(1→4)-α-D-glucan: (1→4)-α-D-glucan 6-glucosyltransferase, EC 2.4.1.18]) could be isolated from a crude potato tubers extract by means of a sequence of LC-techniques (HIC and IEC); Q-enzyme accepts native and synthetic non-branched (amylose-type) glucans as substrates and increased the percentage of branching by a combined hydrolytic and transfer-activity. Q-enzyme accepts technical-grade potato-maltodextrin as substrate: the resulting branching patterns depend strongly on the incubation temperature. Determined molecular-level characteristics clearly show the influence of modified branching pattern on glucan-coil dimensions, conformation and interactive properties. In particular, modification of interactive characteristics on the molecular level is strongly suspected to control macroscopic/technological qualities of starch-based materials such as gelation potential or freeze/thaw-stability.

Introduction

In the recent years an enhanced variability of starches on the molecular level was developed by new and improved breeding-techniques of starch containing plants, gene technology and enzymatically catalyzed modification of starches. Application of hydrolases for instance, produces a wide range of different starch hydrolizates with characteristics primarily controlled by the amount of applied enzymatic activity. Activity of transferases, such as Q-enzyme, is not tested yet, because no pure enzyme was available up to now.

A first step to improve understanding of the background of technological properties of starch containing goods is the development of analytical strategies to obtain reliable information about molecular-level characteristics of starch. Therefore, laboratory-made glucans of specific and varying molecular characteristics with respect to

* *Institut für Chemie, Muthgasse 18, A-1190 Wien and Interuniversitäres Forschungsinstitut für Agrarbiotechnologie (IFA), Center f.Analytical Chemistry, Konrad Lorenz-Str. 20, A-3430 Tulln / Austria*

** *Institut für Physikalische Chemie, Heinrichstrasse 28, A-8010 Graz / Austria*

branching pattern, molecule dimension, conformation and interactive potential need to be established to investigate correlations between the molecular and the macroscopic/technological level.

A first kind of laboratory-made glucans are synthetic amyloses, non-branched α(1→4)-linked glucans, which are studied now for years already. *In vitro* synthesis of amylose by means of phosphorylase was introduced by Pfannemüller who investigated functionality of non-branched starch-glucans [1-3]. Sshe isolated highly active phosphorylase from potato and stabilized it to maintain constant substrate turnover during synthesis. Such Phosphorylase-catalyzed syntheses with glucose-1-phosphate as substrate and maltooligomers of dp≥3 as starters provide quite uniform polymers with a degree of polymerization which simply is controlled by the concentration of the starter-oligomers.

But non-branched glucans are just one kind out of a wide range of starch glucans if the branching pattern is considered as criterion for discrimination. Thus, for an appropriate correlation of molecular glucan-characteristics with macroscopic level starch-properties, additionally branched 'amylopectin'-type glucans with α(1→4) and more or less α(1→6) linked branches need to be investigated.

For the modification of amylose-type, purely α(1→4)-linked non-branched, glucans into amylopectin-type, short-chain branched glucans, branching-enzyme Q-enzyme [(1→4)-α-D-glucan: (1→4)-α-D-glucan 6-glucosyltransferase, EC 2.4.1.18] needs to be isolated from plants and stabilized after purification and during application [4-6]. Complexity is even increases as Q-enzyme owns a twofold activity: hydrolytic and transfer activity. As a result, depending on reaction conditions and amylose-characteristcs, a wide range of differently branched glucans may be achieved by the application of Q-enzyme. The scheme of transfer-activity of Q-enzyme is illustrated in Fig. 1 with non-branched glucan (nb-Glc) as substrate for the formation of short-chain branched glucans (scb-Glc).

$$\text{nb-Glc}_n + \text{nb-Glc}_m \longrightarrow [\alpha(1\rightarrow 4)_n\ \alpha(1\rightarrow 6)_n]\text{-scb-Glc}_{n+m}$$

Fig. 1. Scheme of modification of non-branched glucans (nb-Glc) into short-chain branched glucans (scb-Glc) by the transfer-activity of Q-enzyme.

Isolation and purification of Q-enzyme from potato and application of the stabilized branching enzyme [(1→4)-α-D-glucan: (1→4)-α-D-glucan 6-glucosyltransferase, EC 2.4.1.18] on technical-grade potato maltodextrin as substrate will be presented and discussed. Additionally, the consequences of modification of the

maltodextrin branching pattern on the physico-chemical characteristics will be illustrated.

Experimental

Material

Potato tubers, *vs.* Fambo, which were utilized to extract Q-enzyme, were grown in Großenzersdorf, Lower Austria/Austria at controlled conditions. Technical-grade potato maltodextrin (C-Pur 1906), which was used as substrate for the purified Q-enzyme, was provided by Cerestar/Belgium.

Preparation of the crude Q-enzyme extract from potato tubers

500 g freshly harvested potatoes were washed, peeled, cut in small pieces and homogenized after mixing with 20 mL of 4°C Cleland-buffer pH 7.3 [50 mM Tris (2-amino-2-(hydroxymethyl)-1,3-propandiol), 2.5 mM DTT (1,4-dithiothreitol), 5 mM EDTA (etylendiamintetraaceticacid), set to pH 7.3 by means of citric acid]. Particles exceeding the millimeter range were removed from the cooled (4°C) suspension by filtration, starch and starch-accompanying materials by centrifugation (20 min, 4°C, 30000 g). The clear filtrate was mixed with ammonium sulfate to yield a solution percentage of 20% and was stored over night for protein precipitation. The resulting precipitate was removed from the solution by centrifugation (20 min, 4°C, 30000 g). Then, ammonium sulfate concentration in the supernatant was increased to 50% and left once again over night at 4°C. This second precipitate again was removed from the solution by centrifugation (20 min, 4°C, 30 000 g), resuspended in a small volume of 0.01 M Cleland buffer and stored at -80°C under N_2 for final purification.

Purification of the crude Q-enzyme extract by means of Hydrophobic Interaction Chromatography (HIC)

15 mL of the -80°C/N_2-stored crude protein extract is applied to the Fractogel TSK Butyl-650 (M) HIC-system (Merck/FRG; 70x50 mm) which was equilibrated with Cleland-buffer dissolved 30% ammonium sulfate. Elution of different protein fractions at a flow rate of 4 mL/min was achieved by a step-gradient of decreasing ammoniumsulfate concentration: 30% → 20% → 15% → 10% → 0%. The 10%-fraction was collected and proteins precipitated over night by increasing ammonium sulfate concentration to 50%. The precipitate was separated from the supernatant by centrifugation (30 min, 4°C, 13000 g) and resuspended in a small volume of 0.01 M Cleland-buffer. The obtained enzyme could be stored without significant loss of activity for several days at 4°C.

Purification of an HIC-fraction of the crude Q-enzyme extract by means of Ion-Exchange Chromatography (IEC)

Before the proteins of the HIC-10%-eluted pool were applied on the ion-exchange matrix, the solution was de-salted by means of Centriprep-vials (Amicon, No.4306, cut-off 30 kDa) by two-times adding 0.01 M Cleland-buffer. Then, the obtained 15 mL of de-salted protein-solution was applied to a DEAE (diethylamionoethyl-cellulose)-matrix (Merck/FRG; Fractogel EMD DEAE 650 (S); 150x26 mm) and eluted with 0.01M Tris buffer and a NaCl step-gradient: 0.00 M → 0.30 M → 0.35 M. The major amount of Q-enzyme is eluted at 0.35 M NaCl conditions and proofed to be free of any kind of side-activities at electrophoretic tests.

PAGE of branching- and hydrolytic-enzyme: activity-staining [7]

For verification of branching activity of the obtained enzyme and to distinguish branching activity from purely hydrolytic activities, PAGE (polyacryl gel electrophoresis) with activity-staining was performed with a 12% PA-gel and 1% starch-containing gel on a Mini-Protean II (Biorad/FRG; P_{max}: 20 W, I_{max}: 70 mA, U_{max}: const. 200 V, gel thickness: 1mm); collector-gel: 4% PA ; collector-gel-buffer: 0.5M Tris with citrate acid at pH 6.8; separation-buffer: Tris/glycin pH= 8.3 (3.0g Tris and 14.4g glycin in 2 L Deionat). For activity staining after separation the gels were carefully washed with pure water and then equilibrated over night with incubation-buffer (50 mM Tris, 2 mM ascorbic acid set with citric acid to pH 7.5). The surface-cleaned equilibrated gel then is put for 30 min into an iodine-solution (0.1 g iodine, 1.5 g KJ per 1 L Deionat) for staining of reaction products.

Photometric test of Q-enzyme activity with iodine staining

1-2 mg of long-chain branched (lcb) starch glucan (Sigma S-4501) is dissolved in 1 mL Cleland buffer and mixed with an aliquot of enzyme-solution and stored for 48 hours at room temperature. Enzymatic activity is determined for test- and blind-mixtures by photometric scanning of the maxima of formed iodine/starch-complexes. The photometrically investigated solutions contain: 500 µL test-solution, 2 mL Deionat, 200 µL iodine solution (0.1 g iodine, 1.5 g KJ for 1 L Deionat).

Incubation of Q-enzyme with aqueous dissolved potato maltodextrins

A 40mg/mL solution of potato maltodextrin in 0.01M Cleland-buffer pH 7.3 was obtained by slightly raising temperature. 25 mL of this solution was diluted with buffer (blank) or Q-enzyme solution to yield a volume of 50 mL. A small amount of NaN_3 was added to the glucan-solution to prevent microbial growth; oxidation of Q-enzyme is prevented by N_2-atmosphere and reductive conditions in the solution. The batches were kept at two reaction-temperatures: 4°C and 20°C. After 5 days of incubation the

samples were analyzed with respect to the absorption spectra of glucan/iodine-complex and provided for further and more detailed destructive and non-destructive investigations.

Destructive analysis of Q-enzyme modified maltodextrin: controlled debranching with pullulanase and isoamylase combined with LC-fragment analysis

3 mL of each enzyme/substrate-solution was set to pH 3.7 with acetic acid/acetate-buffer, mixed with 10 µL isoamylase-suspension (Hayashibara Biochem. Lab./Japan, Lot No. 30600) and kept at 50°C for 24 hours. Then the solution was set to pH 5.5 with 0.1 M NaOH and mixed with 5 µL Pullulanase-suspension (Hayashibara Biochem. Lab./Japan, Lot No. 002232). After 6 hours at 37°C once again 5 µL of Pullulanase-suspension was added. After 24 hours at 37°C the solution was short-time boiled to denaturate proteins completely and then diluted 1:4 with Deionat for LC-analyses of obtained glucan-fractions.

50 µL of the completely debranched glucan fractions, representing the constituting glucan-chain length distribution, were applied to a Carbopack PA 100 (Dionex, 4x200 mm) and eluted at a flow rate of 0.8 mL/min with a continuos gradient starting from H_2O/1 M NaOH $\rightarrow$ 1 M NaOH/1 M NaAc. Detection of eluted carbohydrates was done with an electrochemical detector.

Non-destructive analysis of Q-enzyme modified starch-glucans: SEC-DRI/LALLS

Absolute molecular weight of the glucan-chain distributions was determined by means of size-exclusion chromatography combined with dual detection of scattering intensity (low angle laser light scattering device) and mass (differential refractive index detector (SEC-DRI/LALLS). 200 µL of each sample solution was separated on a series of SEC-columns (TSK/Japan; PW6000, 5000G, 4000G, 3000G: 300+300+300+300 x 7.5 mm) at a flow rate of 0.8 mL/min with 0.1 M aqueous NaCl as eluent. Individual SEC-separated fractions were detected with respect to their scattering intensity at a scattering angle of 5° (TSP/USA; KMX-6; λ=632 nm) and with respect to their mass (Wyatt/USA; Optilab 903, interferometric differential refractometer λ=630 nm). Data acquisition was performed with software package CODAwin, data processing and documentation with software package CPCwin (both: A.H group /Austria).

Results and discussion

For successful *in vitro* modification of branching patterns of starch polymers, branching enzyme (Q-enzyme) needs to be applied in an active form and in sufficient amounts. Q-enzyme, isolated from amyloplasts of storage cells of green plants, is an

oxidation-sensitive SH-enzyme and thus, requires a permanent reductive medium to stand the purification procedure.

Isolation and purification of Q-enzyme from potato

Pure and active branching enzyme (Q-enzyme) was isolated by a sequence of liquid chromatographic techniques from potato tubers and by fractionated precipitation with ammonium sulfate from the crude-extract obtained from the initial homogenate. To prevent oxidation, reducing agents such as sodiumdithionit or 1,4-dithiothreitol, were applied at each single step of purification.

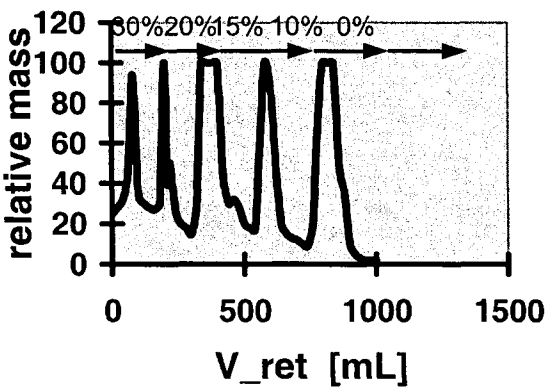


Fig. 2. Hydrophobic Interaction Chromatography (HIC) of crude enzyme extract from potato tubers with varying $(NH_4)_2\ SO_4$ content as step-gradient 30–0%; in the eluent: Clelandpuffer, pH 7.4; (pre-fractionated by $(NH_4)_2\ SO_4$-precipitation); Phosphorylase was identified in the 15% $(NH_4)_2\ SO_4$ pool; amylases were identified in the 0% $(NH_4)_2\ SO_4$ pool; Q-enzyme was eluted at 10% $(NH_4)_2\ SO_4$;

By means of hydrophobic interaction chromatography (HIC) phosphorylase, amylases, R-enzyme and Q-enzyme could be separated and eluted at different ammoniumsulfate molarities (Fig. 2). The PAGE-test of the different ammoniumsulfate-pools results in low amounts of hydrolytic side-activities for the Q-enzyme-pool (10% ammoniumsulfate). After purification of this pool with ion exchange chromatography (IEC), pure Q-enzyme, free of phosphorylase, amylases and R-enzymes, in appropriate amounts for application in modification reactions of glucan branching patterns could be achieved.

Branching activity of isolated and purified Q-enzyme was controlled by means of gel-electrophoresis (PAGE) combined with activity-staining to distinguish between amylases, R-enzyme and Q-enzyme.

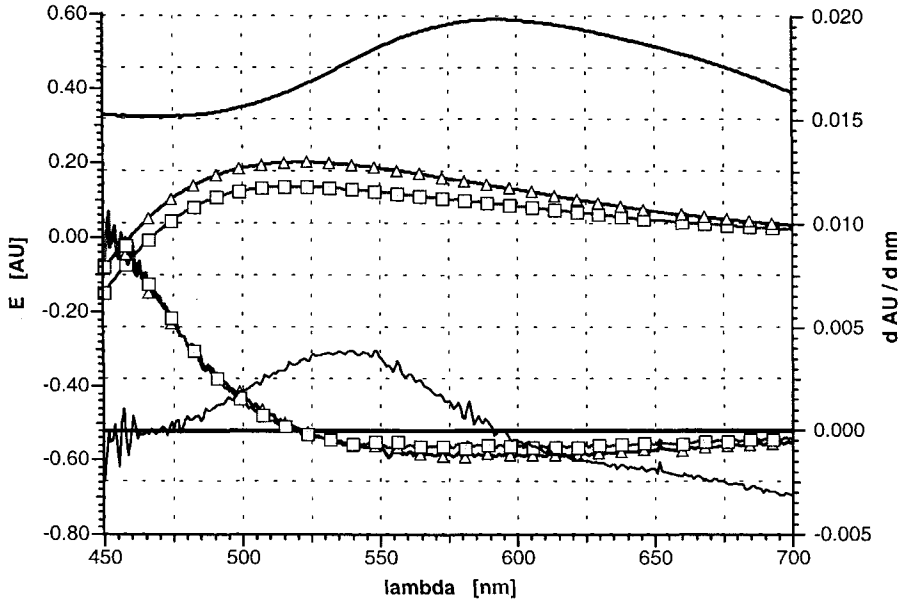


Fig. 3. VIS spectrum of long-chain branched (lcb) starch glucan, Sigma S–4501 (———), Q-enzyme modified starch glucan at 20°C (—□—), Q-enzyme modified starch glucan at 4°C (—Δ—); absorbance maxima: initial starch glucan: 590 nm, Q-enzyme modified at 20°C: 520 nm, Q-enzyme modified at 4°C: approx. 520 nm.

The gel-incorporated starch/iodine-complex comes up with a blue color, whereas at the Q-enzyme-position the gel-incorporated starch-complex turns red on a blue background, as it is a substrate for the Q-enzyme and becomes more branched than the initial sample. For purely hydrolytic enzymes, such as for amylases, either no color will found as the incorporated starch gets degraded and the glucan oligomers will be lost by diffusion, or a bright blue color comes up, such as for R-enzyme which hydrolizes branching positions. As a matter of fact, activity staining provides no quantitative information about enzyme Q-enzyme activity and about the formed products, however, it is a sensitive tool to identify presence/absence of pure Q-enzyme in protein fractions.

To investigate the activity of branching enzyme, long-chain branched (lcb) starch glucans ('amylose') was applied as substrates for the Q-enzyme. Fig. 3 shows a significant shift of the maximum to lower wavelengths in the absorption spectra of the glucan/iodine-complexes due to Q-enzyme activity. However, quantification of Q-enzyme activity due to the magnitude of the absorption maximum of glucan/iodine-complexes might be erroneous as the reducing conditions may interfere.

Modification of potato maltodextrin with Q-enzyme

Q-enzyme hydrolyzes α(1→4)-glycosidically linked glucans and transfers the resulting fragments inter- and/or intramolecular by formation of α(1→6)-glycosidic branching positions. For successful transfer the acceptor-glucan needs a non-branched α(1→4)-segments of at least dp 20-30. Degree of polymerization (dp) of hydrolytically formed glucans strongly depends on reaction temperature, however, the minimum for transfer is dp 6 [6, 8, 9].

Q-enzyme was applied to modify an water-soluble technical-grade potato maltodextrin with a high percentage of low-dp short-chain branched glucans (C-Pur 1906, Cerestar/B). In a first attempt Q-enzyme was incubated to aqueous 20 mg/mL potato starch maltodextrin solutions for 5 days at 20°C and at 4°C. Investigations of the formed products were focused on two major questions:

- is maltodextrin accepted as substrate: if yes, how will Q-enzyme modify these primarily low-dp short-chain branched glucans?
- if there is a modification of the branching pattern, will there be a correlated significant modification of physico-chemical characteristics?

As a first and qualitative indicator for the maltodextrin to be accepted as a substrate by the Q-enzyme, a shift of the maximum of glucan/iodine-absorption spectrum was observed (Fig. 4): the magnitude of the shift obviously depends on the reaction-temperature. The absorption maximum of the glucan/iodine-complex shifted from 540 nm to 520 nm at 20°C and close to 460 nm at 4°C.

Simultaneously, SEC-elution-profiles proof, that at both incubation temperatures molecular composition of potato maltodextrin was significantly modified by the Q-enzyme (Fig. 5). High-dp glucans of the initial maltodextrin were eliminated by the hydrolitic activity of Q-enzyme (V_{ret} 30 ... 36 mL), whereas compact low-dp glucan-coils were formed. Q-enzyme-activity is higher at 4°C than at 20°C.

To obtain molecular weight (degree of polymerization) distributions, molecular weight averages and additional molecular characteristics, SEC combined with dual-detection of scattering intensity and fraction masses was applied [10-13] (Fig. 6). Excluding the extremely high-dp 5% of observed components which most probably are due to aggregation phenomena, for both reaction temperatures average degree of polymerization decreased significantly due to the Q-enzyme action: $dp_w = 54$ for 4°C and $dp_w = 89$ for 20°C.

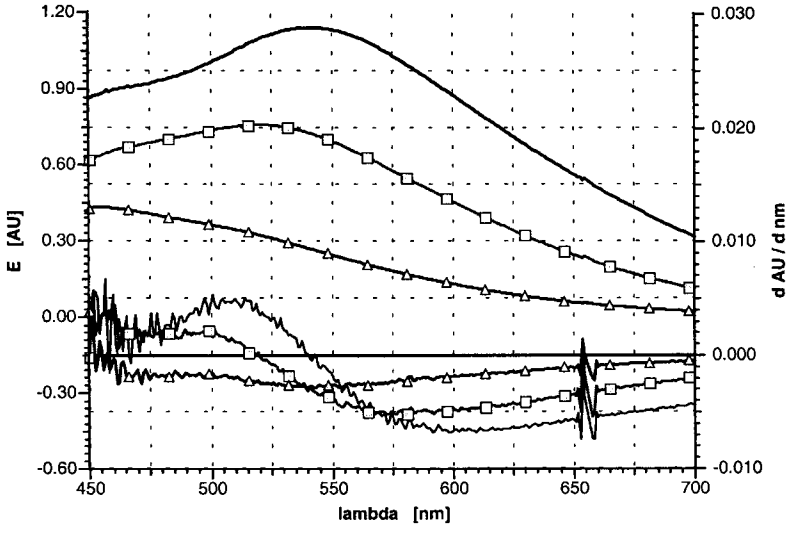


Fig. 4. VIS spectrum of potato maltodextrin (———), Q-enzyme modified potato maltodextrin at 20°C (—□—), Q-enzyme modified potato maltodextrin at 4°C (—Δ—); the first derivative of these spectra illustrate a shift of the absorption maximum (zero-intercept) and a broadening of absorbance in the wavelength-range below 550 nm; absorbance maxima: potato maltodextrin: 540 nm, Q-enzyme modified at 20°C: 520 nm, Q-enzyme modified at 4°C: approx. 460 nm.

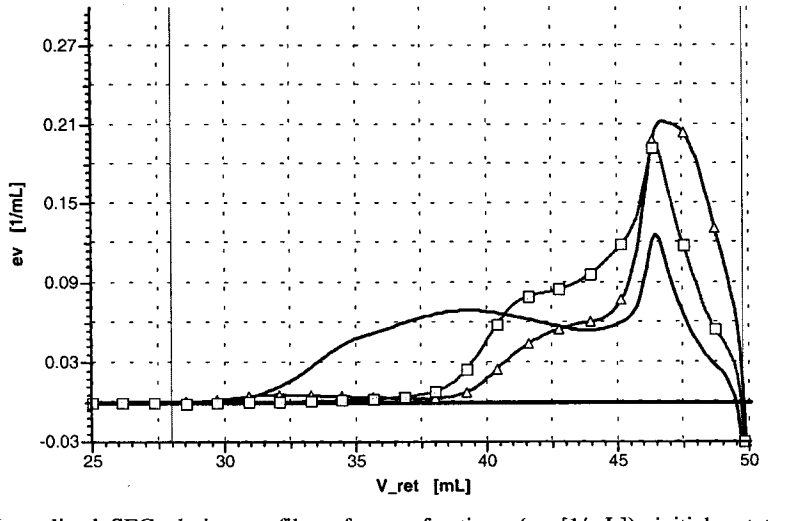


Fig. 5. Normalized SEC elution profiles of mass fractions (ev [1/mL]): initial potato maltodextrin (———); Q-enzyme modified potato maltodextrin at 20°C (—□—); Q-enzyme modified potato maltodextrin at 4°C (—Δ—).

For detailed investigations on Q-enzyme caused modifications in branching characteristics, the modified maltodextrins were selectively debranched with isoamylase and pullulanase. The obtained glucan-fragments then were analyzed by means of HPAEC-PAD (high performance anionic exchange chromatography – pulsed amperometric detection: DIONEX-system) and by analytical SEC. Component-composition of maltodextrins before and after debranching differ significantly and proof the increase of short-chain branching by Q-enzyme (Fig. 7). Hydrolysis by amylases can be excluded as the increase of low-dp glucan-chains is more pronounced at 4°C than at 20°C which is just the opposite of temperature dependence of amylase-activity. Results of average molecular weights and degree of polymerization, obtained from analytical SEC, are listed in Tab.1.

Table 1

Weight and number average molecular weight (M_w, M_n), weight and number average degree of polymerisation (dp_w, dp_n) and polydispersity (M_w/M_n) of initial - and Q-enzyme modified maltodextrin after debranching

	initial potato maltodextrin	potato maltodextrin Q-enzyme modified at 20°C	potato maltodextrin Q-enzyme modified 4°C
M_w [g/M]	7566	3045	1873
M_n [g/M]	2891	1382	1034
dp_w [Glc]	46	19	12
dp_n [Glc]	18	9	6
M_w/M_n	2.6	2.2	1.8

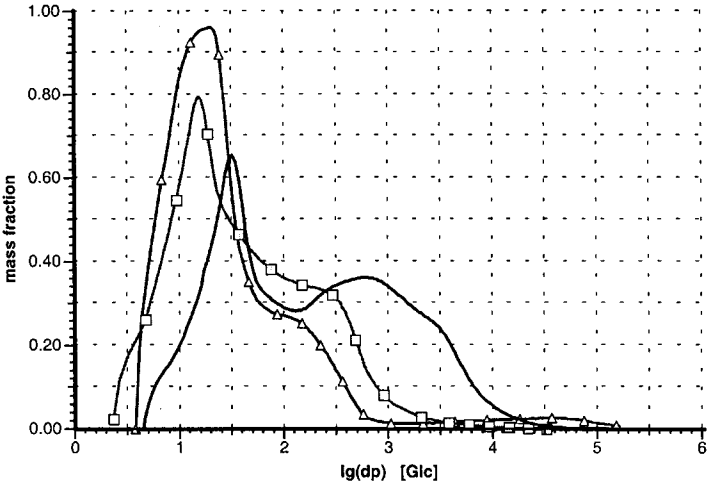


Fig. 6. Normalized differential degree of polymerization distribution: mass fractions (m_dpD_d, area = 1.0); initial potato maltodextrin (———);Q-enzyme modified potato maltodextrin at 20°C (—□—); Q-enzyme modified potato maltodextrin at 4°C (—Δ—).

Mass fractions of degree of polymerization distribution indicate that initially high-dp glucans have been transformed preferably into midrange-dp glucans by the Q-enzyme. Simultaneously, packing density and scb-characteristics of these midrange-dp glucan coils increased.

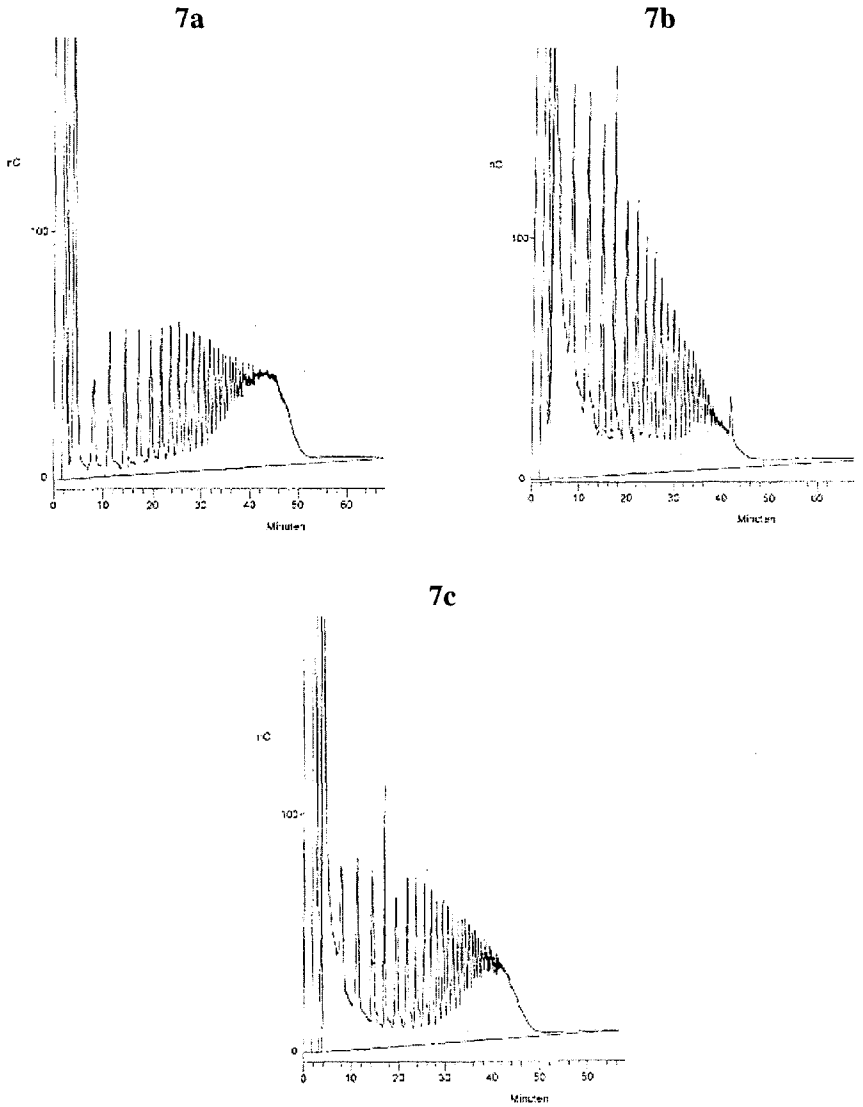


Fig. 7. HPAEC-PAD analysis of debranched potato maltodextrin; glucan chain length distribution of initial potato maltodextrin (a), Q-enzyme modified potato maltodextrin at 20°C (b) and Q-enzyme modified maltodextrin at 4°C (c).

Dionex- and SEC-profiles clearly show the differences in the activity of Q-enzyme at 20°C and at 4°C:
at both temperatures Q-enzyme eliminates high-dp components more or less completely by transforming them into short-chain branched glucans;
transferase activity obviously is more pronounced at 4°C than at 20°C; packing density of glucan-coils is higher at 4°C than at 20°C which strongly indicates more pronounced scb-characteristics for glucans formed at 4°C compared to those formed at 20°C;

Detailed physico-chemical analysis of initial and Q-enzyme modified potato maltodextrins then was achieved from analysis of SEC-DRI/LALLS-data [14]. Distribution profiles of intrinsic viscosity, Staudinger/Mark/Houwink-constants K (dissolution status of glucan coils) and a (coil conformation) were calculated (Fig. 8-10). Average values and occupied ranges of these parameters are listed in Tab. 2.

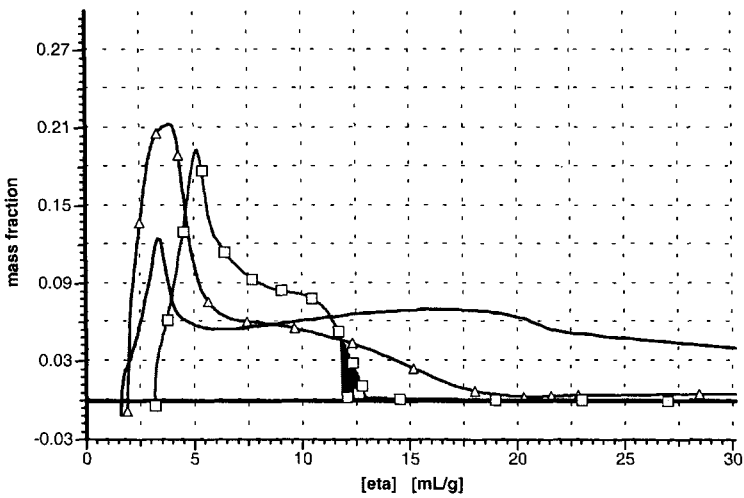


Fig. 8. Intrinsic viscosity distribution (m_IVD_d); initial potato maltodextrin (———);Q-enzyme modified potato maltodextrin at 20°C (—□—); Q-enzyme modified maltodextrin at 4°C (—Δ—).

A complex but nevertheless significant structure-sensitive parameter on the molecular level, intrinsic viscosity in terms of occupied volume per mass-unit of individual maltodextrin-components, can be achieved from absolute molecular weight data (SEC-DRI/LALLS) and universal SEC-calibration. Obviously Q-enzyme-activity stabilizes the glucan-coils by introducing branches which causes reduction of intermolecular polymer/polymer-interaction. Intrinsic viscosity monitors this modification quite sensitively and decreases significantly after Q-enzyme action. Of course, reduced occupied volumina partially are caused by reduced geometric coil dimensions but also

by reduced 'interaction-radii' of the modified glucans. Intrinsic viscosity according the power law $[\eta]=K.M^a$ can be splitted into contributions correlated with molecular dimensions (M: molecular weight), polymer-coil conformation (*a*) and contributions correlated with interactive polymer-characteristics.

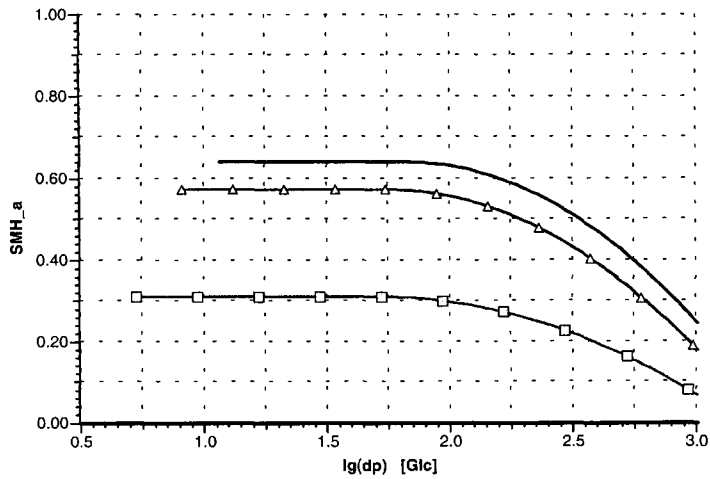


Fig. 9. Dependence of Staudinger/Mark/Houwink (SMH) exponent *a* of power low $[\eta]=K.M^a$ on degree of polymerization of potato maltodextrin (initial), Q-enzyme modified at 20°C and Q-enzyme modified at 4°C.

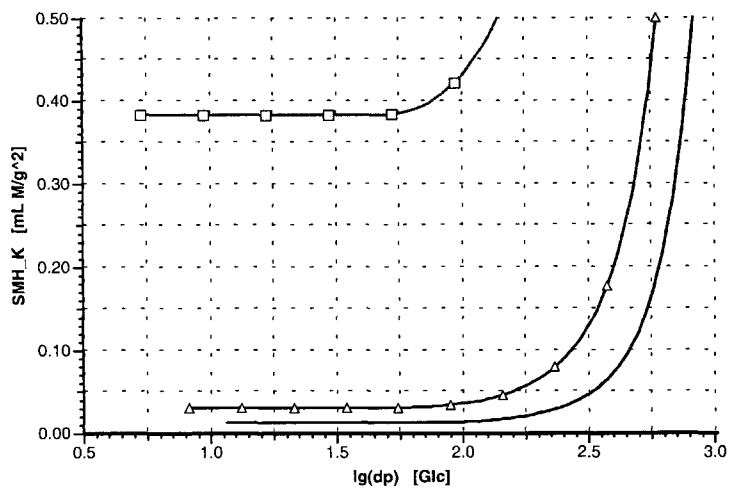


Fig. 10. Dependence of Staudinger/Mark/Houwink (SMH) factor *K* of power low $[\eta]=K.M^a$ on degree of polymerization of potato maltodextrin (initial), Q-enzyme modified at 20°C and Q-enzyme modified at 4°C.

Different *K*-values for Q-enzyme modified maltodextrins at 4°C and at 20°C strongly indicate different dissolution-states for the glucans: more pronounced polymer/solvent interaction for the glucans formed at 20°C than for those formed at 4°C. Although at both investigated reaction temperatures – 4°C and 20°C – glucan-coil dimensions decreased, a significant difference for products formed at 4°C and at 20°C was found: at 20°C the glucan-coils were even more compact than those formed at 4°C. Obviously and maybe primarily, Q-enzyme modifies interactive glucan-characteristics and therefore is suspected to be a key-tool to control interaction-correlated macroscopic/technological material properties such as gelation-potential, freeze/thaw-stability, etc. on molecular level.

Table 2

Molecular characteristics of initial potato maltodextrin, Q-enzyme modified at 20°C potato maltodextrin and Q-enzyme modified at 4°C potato maltodextrin

Characteristics	Initial potato MD	Q-enzyme modified at 20°C	Q-enzyme modified at 4°C
molecule dimension glucan coil radius [nm]	1 .. 45 10.1	1 .. 38 7.7	1 .. 36 4.0
molecule conformation SMH *a*	0.64	0.56	0.32
molecular interactive potential SMH *K* [mL M g^{-2}]	0.01	0.03	0.38
occupied molecule volume due to dimension, conformation and interactive potential $[\eta] = K.M^a$ [mL g^{-1}]	2 .. 120 23	2 .. 90 17	3 .. 31 8

Thus, some first answers to the initial questions concerning Q-enzyme activity were obtained:

Q-enzyme accepts short-chain branched and primarily low-dp starch glucans as substrates and modifies the branching pattern such, that primarily the interactive potential of the glucan-coils is modified. Extent of modification, i.e. intensity of scb-branching and actual packing density of glucan-coils, strongly depends on external conditions, such as reaction-temperature.

Modification of interactive characteristics on the molecular level is strongly suspected to control macroscopic/technological qualities of starch-based materials – at least such qualities, which obviously are correlated with interactive characteristics such as gelation potential or freeze/thaw-stability.

Summarizing, controlled modification of branching pattern of starch-glucans with Q-enzyme is equal to controlled modification of interactive starch-glucan properties on the molecular level with consequences on macroscopic/technological material qualities.

Thus, Q-enzyme seems to be a promising tool to improve processing-efficiency e.g. by 'tailoring' the raw material before it is transferred to specific traditional technological processing.

Acknowledgement

This work was partially supported by the Austrian 'Fonds zur Foerderung wissenschaftlicher Forschung (FWF)', project number P-12498-CHE.

REFERENCES

[1] Pfannemüller, B. and Burchard, W.: Makromol. Chem., **121**, 1969, 1.
[2] Pfannemüller, B., Maierhöfer, H. and Schulz, R. C.: Biopolymers, **10**, 1971, 242.
[3] Pfannemüller, B., In: Polysaccharide, W. Burchard (Ed.), Springer-Verlag, Berlin, ISBN 3-540-13931-1, 1985, 25.
[4] Praznik, W., Rammesmayer, G. and Spies, Th.: Carbohydr. Research, **227**, 1992, 171.
[5] Borovsky, D., Smith, E.E. and Whelan, W.J.: Eur. J. Biochem., **59**, 1975, 615.
[6] Manners, D.J.: Carbohydr. Polym., **11**, 1991, 87.
[7] Rammesmayer, G. and Praznik, W.: J. Chromatogr., **623**, 1992, 399.
[8] Whelan, W. J.: Biochem. J., **122**, 1971, 609.
[9] Borovsky, D., Smith, E.E. and Whelan, W.J.: FEBS Letters, **54**, 1975, 201.
[10] Harding S.E., Sattelle D.B., Bloomfield V.A. (Eds.), Laser light Scattering in Biochemistry, RSC, Cambridge, 1992.
[11] Cooper A.R. (Ed.), Determination of Molecular Weight, **103**, Chemical Analysis,John Wiley & Sons, New York, 1989.
[12] Barth H.G. (Ed.), Modern Methods of Particle Size Analysis, John Wiley & Sons, New York, 1984. Barth H.G. (Ed.), Modern Methods of Particle Size Analysis, John Wiley & Sons, New York, 1984.
[13] Huber A., In: Analysis of Polymers / Molar-mass and molar-mass distribution of polymers, polyelectrolytes and latices, W.-M.Kulicke (Ed.), Hüthig & Wepf Verlag, **61**, 1992, 248.
[14] Huber, A., Praznik W., in: Carbohydrates as Organic Raw Materials IV (Ed.: Praznik W., Huber A.), WUV-Universitätsverlag Wien, **19**, 1998, 230.

MODYFIKOWANIE SPOSOBU ROZGAŁĘZIEŃ W ZIEMNIACZANEJ MALTODEKSTRYNIE ZA POMOCĄ ENZYMU Q

Streszczenie

Czysty enzym rozgałęziający (enzym Q [(1→4)-α-D-glukan: (1→4)-α-D-glukano 6-glukosylotransferaza, EC 2.4.1.180] został wyizolowany z ekstraktu z surowych bulw ziemniaczanych za pomocą kolejnych rozdziałów chromatograficznych (HIC i IEC). Enzym Q wykorzystuje jako substraty natywne i syntetyczne nierozgęzione glukany typu amylozowego i zwiększa procentową zawartość rozgałęzień przez połączoną aktywność hydrolityczną i transferową. Jako substrat enzym Q wykorzystuje też techniczną maltodekstrynę ziemniaczaną. Tworzenie rozgałęzień bardzo zależy od temperatury inkubacji. Znaleziona charakterystyka na poziomie molekularnym wyraźnie wskazuje na zależność sposobu rozgałęziania od rozmiaru zwoju w glukanie, konformacji i jego możliwości oddziaływań z otoczeniem. Szczególnie ten ostatni czynnik w wyraźnym stopniu wpływa na mnakroskopowe i technologiczne właściwości materiału skrobiowego, np. na zdolność do kleikowania i odporność na niskie temperatury.

C.H. SCHILLING*, P. TOMASIK**, M. SIKORA***, J.C. KIM+,
V.J. GARCÍA*, C.P. LI++

MOLDING TECHNICAL CERAMICS WITH POLYSACCHARIDES

Abstract

Technical ceramics represent a large, international market dominated by electronic applications, such as insulators, substrates, integrated circuit packages, capacitors, and magnets. Typical manufacturing operations involve blending ceramic powder with organic liquids (e.g., polyethylene wax, organic solvents) to form a slurry that is molded into a three-dimensional shape before it is dried and kiln-fired. There are serious problems with the pyrolysis of these organics prior to kiln firing: (*i*) slow and costly heating (e.g., 200 °C for one week) is required to avoid the formation of cracks and gas bubbles, (*ii*) toxic fumes are emitted, and (*iii*) residual carbon contaminates the final microstructure. Aqueous suspending media are needed to eliminate these organic carrier liquids and evaporate safely without causing cracks, shape distortion, and microstructure contamination in sintered parts. Our experiments indicate that various dextrins and maltodextrins are useful to achieve this goal because of their natural tendency to sorb to oxide powders in aqueous suspensions. Small concentrations of these starch hydrolysis products (< 5 wt%) significantly improve molding paste rheology and enable clean pyrolysis with minimal carbon contamination of microstructures. In addition, these polysaccharides form strong, interparticle bonds after water evaporation, which enables processing of strong, crack-free ceramics before they are kiln-fired. In this paper, we begin by discussing background information on surface chemical aspects of controlling the rheology of ceramic molding slurries. Experiments involving sedimentation, filtration, extrusion, rheology, and surface chemical analysis are then presented which illustrate the practical potential of maltodextrins and dextrins as rheological modifiers in ceramic manufacturing.

* *Ames Laboratory# and Department of Materials Science and Engineering, Iowa State University, Ames, USA*

** *Department of Chemistry and Physics, University of Agriculture, Krakow, Poland*

*** *Department of Carbohydrate Technology, University of Agriculture, Krakow, Poland*

+ *HanYang University, Ceramic Materials Research Institute, 17 Hanengdang-Dong, Seongdong-Gu Seoul 133-791, South Korea*

++ *Materials Research and Testing Center, Hunan Light Industry College, Changsha, Hunan 410007, People's Republic of China*

Ames Laboratory is operated by Iowa State University under the contract number W-7405-eng-82 with the U.S. Department of Energy.

Introduction

Technical ceramics: market overview

In the past two decades, significant advances have been made in the synthesis of technical ceramic powders, which have an unprecedented degree of control over particle size, shape, and chemistry (Segal 1989 and 1996). In 1994, the market value of technical ceramic powder for electronic applications was estimated at 613 million U.S. dollars. By the year 2000, the market value of ceramic powders used in electronic applications is estimated to reach 977 million U.S. dollars as a result of 8.1 percent average annual growth (Abraham 1996).

Oxide ceramics have as much as 97% of the market for electronic ceramics. Aluminum oxide has the largest market for substrate materials that are utilized in making insulators and integrated circuits. Ceramic ferrite powders are used for making permanent and soft magnetic materials and constitute the second largest market. Ceramic titanates are the basis for electronic capacitors as well as piezoelectric components and constitute the third largest market (Abraham 1996).

Technical ceramic powders are also utilized in structural applications such as wear-resistant parts, mechanical seals, sliding bearings, cutting tool inserts, and surgical implants. Oxides, silicon carbide, and silicon nitride are three of the most common technical ceramics for structural applications. The market for technical ceramics powder for structural applications is smaller than that of electronic ceramics and was estimated at 45 million U.S. dollars in 1994 (Abraham 1996).

Plastic molding of ceramics: why polysaccharides?

Technical ceramics are typically produced by a sequential process of (*i*) mixing ceramic powder with an organic liquid carrier (e.g. alcohols, ketones, polyethylene wax, vinyl additives) to form a moldable slurry, (*ii*) forming the slurry into a three-dimensional shape (e.g., by injection molding or plastic shaping), (*iii*) thermal treatment to evaporate or pyrolyze the liquid carrier, and (*iv*) kiln firing. Large concentrations (up to 60 to 70 vol%) of the organic carrier liquid are typically needed to maintain plasticity during shape-forming. A transition to brittle, dilatant behavior occurs at lower concentrations of organic liquid (Pujari 1989; Franks and Lange 1996). Aside from the associated environmental hazards, another concern is that the diffusion of hydrocarbon liquids during heating and the gases produced during pyrolysis cause unwanted cracks and shape distortion in pre-sintered parts. Relatively long heat treatments (e.g., up to one week at $\approx$ 200°C) are typically needed to complete pyrolysis; more rapid heating produces internal stresses that can cause cracks and shape distortion (Stangle and Aksay 1990). In addition, evaporation and gaseous diffusion typically

remove not all of the decomposition products of pyrolysis; carbonaceous residues are frequently left behind which contaminate microstructures.

Aqueous suspending media are needed to eliminate these organic carrier liquids and evaporate safely without causing cracks, shape distortion, and microstructure contamination in sintered parts. Several industrial firms and academic researchers are currently working towards this goal. Much of this research entails fundamental studies of the surface chemical origins of the rheology of ceramic powder suspensions. In the paragraphs below, we shall review important aspects of these studies to help build an understanding of the advantages of dextrins and maltodextrins in formulating molding pastes for the ceramic industry.

Experience indicates that the simple addition of deionized water to ceramic powder results in essentially the same problem mentioned above: a dramatic reduction of slurry plasticity if the water concentration is beneath a critical value. As a result, molding becomes impossible because insufficient water tends to produce a stiff, brittle consistency. Experience indicates that this critical water concentration is usually much smaller for aqueous, clay suspensions as opposed to aqueous suspensions of oxide ceramics. Therefore, shapes that are molded from oxides are often too porous, which results in cracks, warpage, and excessive shrinkage during drying and kiln firing. In contrast, clay-based ceramics are much easier to manufacture at smaller concentrations of water, which in turn makes them less susceptible to warpage and cracks during drying. Unfortunately, clay-based ceramics are unsuitable for most consumer products that are made of technical ceramics, because they do not possess the high performance electrical, magnetic, and mechanical properties that are required.

The remarkable difference in rheology between oxides and clay minerals can be attributed to a large extent to inherent differences in particle morphology, the nature of the surface charge, and the adsorption of structured water molecules on clay surfaces (van Olphen 1977; Pashley and Israelachvili 1984; Lawrence 1978). The stacked-platelet morphology of clays, along with a superposition of long range, interparticle van der Waals (attractive) and short range, interparticle hydration (repulsive) forces, are thought to contribute to the high degree of plasticity of clays. In contrast, nonclay, technical ceramic powders do not generally have this same shape and surface-repulsion characteristic that aid particle rearrangement in clay-based systems.

Several researchers have recently shown that the key to improving the plasticity of concentrated suspensions of nonclay ceramic powders is to develop a weakly-flocculated state by coating powders with a substance producing a „clay-like" superposition of interparticle, long-range attraction and short-range repulsive forces. Two general methods are reported: (*i*) the hydration-layer approach (Velamakanni et al. 1990 and 1994; Chang et al. 1991 and 1994 a and b; Luther et al. 1994; Franks et al. 1995; Franks and Lange 1996) and (*ii*) adsorbate-mediated steric-hindrance (i.e., the creation

of a steric adlayer that inhibits complete mutual approach of individual particles). Before presenting our research, we shall briefly mention previous studies on rheological effects of adsorbate-mediated steric hindrance, studies that have led to the current interest in the use of polysaccharides for ceramic molding.

Yin et al. (1988) first introduced the method of weak-flocculation by adsorbate-mediated, steric-hindrance and reported the formation of high-density, low-viscosity suspensions with polymethacrylates adsorbed on alumina powders suspended in heptane or paraffin oil. Schilling (1992) and Bergström et al. (1992) subsequently reported significant improvements in the packing densities of centrifuged suspensions in which short-range, steric-repulsion forces were established by the adsorption of fatty acids to alumina powders suspended in decalin. Kramer and Lange (1994) performed similar experiments with alcohols adsorbed on silicon nitride powders suspended in an organic solvent.

More recent studies focused on developing aqueous analogs to the method of weak-flocculation by adsorbate-mediated steric-hindrance, analogs producing highly-concentrated and highly-plastic suspensions of oxide powders without the practical concerns of the organic solvents used in the studies mentioned above. For example, Leong et al. (1993) reported significant reductions in the viscosities of aqueous suspensions of concentrated zirconia by establishing short-range, steric repulsion through the adsorption of anionic molecules (sulfate, various phosphates) and simple, organic-acid anions (lactate, malate, and citrate). Luther et al. (1995) reported similar results by the use of ammonium citrate additives in aqueous alumina suspensions. Although focused on interparticle dispersion and not weak flocculation per se, there are also excellent reports on the use of sorbed-polyelectrolytes in the preparation of highly concentrated and dispersed, aqueous alumina suspensions (Shanefield 1995; Hidber et al. 1996). In addition, Chan and Lin (1995) reported significant reductions in viscosity by the adsorption of steric acid onto alumina powder surfaces in paraffin/polypropylene suspensions.

More recently, Schilling and co-workers (Schilling et al. 1995; Goel et al. 1996) reported significant improvements in the consolidation and rheology of aqueous, filter-pressed suspensions of submicron alumina powder, weakly-flocculated suspensions that were prepared with maltodextrin. It was reported that these suspensions exhibited a high degree of „clay-like" plasticity based on measurements of equibiaxial extensional rheometry. Schilling et al. (1998a) then performed surface chemical experiments to analyze the mechanism of enhanced fluidity caused by one, model polysaccharide: a commercially available maltodextrin having an average molecular weight of 3,600 Daltons. It was concluded that this mechanism primarily entails reduction of interparticle attraction by adsorbate mediated steric hindrance rather than double-layer, interparticle repulsion. Another benefit of this maltodextrin is that, during drying, it acts as

a binder to strengthen ceramic bodies (Schilling et al. 1998 b). We will highlight some of the results of these earlier studies in the paragraphs below. In addition, we will present new experimental data on the effects of polysaccharide molecular weight and concentration on suspension rheology and the strength of dried ceramics.

Finally, we should mention that there are a few publications on the use of different polysaccharides for ceramic processing (Sarkar and Greminger 1983, Fanelli et al. 1989; Shanefield 1995; Dmitriev et al. 1990; Bonomi et al. 1989; Mach et al. 1988; Panda et al. 1988). In fact, a broad range of polysaccharides are commonly utilized as additives for aqueous suspensions of colloidal, oxide powders in other applications including papermaking, mineral separation, and treatment of chemical waste (Tomasik and Schilling 1998 a and b).

Experimental procedure

Suspensions were prepared with deionized water and α- Al_2O_3 powder having an equiaxed particle shape, an average particle size of 0.4 μm, and a specific surface area of 8.5 m^2 per gram (A-16 SG, Alcoa Corporation, Bauxite, Arkansas, U.S.A.). Kaolin suspensions were prepared with deionized water and Pioneer Airfloated Clay (Dry Branch Kaolin Company, Dry Branch, Georgia, U.S.A.), having an average particle size of 1.0–1.2 microns according to the supplier. The majority of the experiments below were conducted using a single, commercially-available maltodextrin, which we shall refer to as maltodextrin 040 (Maltrin 040, Grain Processing Corp., Muscatine, Iowa, U.S.A., average molecular weight 3,600 g/mole; average degree of polymerization 22.1). Chromatography measurements by the manufacturer revealed the following composition of maltodextrin 040: (*i*) 85% maltodextrins having a degree-of-polymerization greater than 10 and (*ii*) a 15% concentration of shorter chain maltodextrins, oligo- and monosaccharides. The manufacturer also reported that the dextrose equivalent value of maltodextrin 040 was 5.

Commercially-available maltodextrins and dextrins having a range of average molecular weights from 900 to 63,000 Daltons were used in the rheology experiments below (Table 1). Maltodextrin 100 and 200 were provided by the Grain Processing Corporation as described above. Four different dextrins, D1, D2, D3, and D4, were provided by Sigma Chemical Company of St. Louis, Missouri, U.S.A. The average molecular weight of these polysaccharides is listed in Table 1 and is based on chromatography measurements reported by each manufacturer. Dextrans, pullulan (Sigma Chemical Company, St. Louis, Missouri, U.S.A.), and soluble starch (Difco Corporation, Detroit, Michigan, U.S.A.) were used in the mechanical properties experiments below.

Suspensions were prepared by simply adding alumina powder to an aqueous solution of a given polysaccharide. In some instances, the solutions contained 0.01 M

NaCl. Each suspension was then sonicated for approximately two minutes using a sonifier with a 0.25 inch horn (CV17 Vibracell, Sonics and Materials Inc., Danbury Connecticut, U.S.A.). The sonicator was operated at between 60 to 80% of the maximum power output of 600 W. Suspensions were then poured into sealed, plastic bottles and placed on a shaker for 24 hours.

Table 1

Average molecular weights of polysaccharides

Specimen	Average Molecular Weight (Daltons)
Maltodextrin 200	900
Maltodextrin 100	1800
Maltodextrin 040	3600
Dextrin D1 (potato)	6650
Dextrin D2 (corn)	6450
Dextrin D3 (corn)	15,000
Dextrin D4 (corn)	63,000

Sedimentation, filtration, and extrusion

Gravity sedimentation experiments were performed to analyze the effects of the maltodextrin 040 concentration on the degree of consolidation (Schilling et al. 1998a). Each suspension was prepared with an initial volume fraction of alumina of $\phi_o = 0.15$ and a zero total concentration of NaCl. One hundred milliliters of a given suspension were added to a 100 ml graduated cylinder (Pyrex glass), and the sediment height was recorded as a function of time. Each graduated cylinder was sealed to minimize evaporation.

We also compared the filtration behavior of the following suspensions: (*i*) alumina with 0.03 grams of maltodextrin 040 per gram of Al_2O_3,(*ii*) flocculated alumina near the isoelectric point without maltodextrin and (*iii*) kaolin without maltodextrin. Each suspension was prepared with a solids volume fraction of $\phi_o = 0.2$ and a zero total concentration of NaCl. In case (*ii*) above, NH_4OH was added to suspensions to raise the pH to 8.6. In case (*iii*) above, kaolin powder was simply added to deionized water and then stirred for 2 hours before filtration (Schilling et al. 1998a).

Each suspension was poured into a filter-press that was precision machined from an acrylic tube (5.08 cm inside diameter) fitted with filter paper membranes and a porous, polyethylene piston. The applied pressure remained constant, and the movement of the piston was monitored as a function of time. In each filtration experiment, a single, liquid-saturated cake was removed from the filter press after the piston stopped

moving, and its green density was immediately analyzed by an oil-immersion technique based on the Archimedes principle (Schilling et al. 1995; Goel et al. 1996). Three specimens of each composition were filter-pressed and evaluated by this procedure in order to confirm repeatability of the packing-density measurements.

Liquid-saturated filter-cakes were examined by extrusion measurements and Benbow analysis (Schilling et al. 1998a; Benbow et al. 1987 and 1989). We performed these experiments with a stainless steel, piston extruder having a barrel diameter, D_o, of 12.7 mm. Square-entry dies of circular crossection were used with die-land diameters, D, of 1 and 2 mm and die-land lengths, L, of 10 and 15.8 mm. L/D ratios of 5:1, 7.9:1, 10:1, and 15.8:1 were obtained.

The extruder was manually filled with a given filter cake and placed in mechanical testing machine, which operated in the compression mode and subjected the piston to a constant axial velocity. Extrusion data were fitted to the Benbow equation, which describes the relationship between the piston velocity and the pressure drop, both in the die-entry region and in the die land (Benbow et al. 1987 and 1989):

$$P_{tot} = \frac{4F}{\pi D^2} = P_{de} + P_{dl} = 2\ln\frac{D_o}{D}\left[\tau_b + k_b V^n\right] + 4\frac{L}{D}\left[\tau_f + k_f V^m\right]$$

In this expression, P_{tot} is the total extrusion pressure, P_{de} is the pressure drop in the die entry region, P_{dl} is the pressure drop in the die land, V is the velocity, τ is the yield strength, k is a constant, the subscripts b and f refer to the body and the die-land slip film, respectively, and n and m are the shear-thinning exponents for the body and film, respectively. In all experiments, we used the Benbow assumption that $m = n = 1.0$ (Benbow et al. 1989).

Rheology studies

Experiments were performed to determine the effects of the polysaccharide concentration and molecular weight on rheological properties (Sikora et al. 1998). Stock solutions of deionized water, 0.01 M NaCl, and varying concentrations of a given polysaccharide were initially prepared. A weighed amount of alumina powder (ϕ_o = 0.2) was then added to each solution, followed by 24 hours of shaking in sealed, plastic containers.

Rheological measurements were performed at room temperature with a computer-controlled rheometer (RheoStress RS 75, Gebrüder Haake GmbH, Karlsruhe, Germany) having a double-gap cylinder (DG 41; DIN 54453). Each specimen was subjected to an increasing shear rate starting at 1 s^{-1} and ending at 500 s^{-1}. The shear rate was subsequently swept back to 1 s^{-1}. This process of sweeping the shear rate up and down was subsequently repeated on the each specimen two more times in order to ver-

ify repeatability. In addition, we verified repeatability by performing rheological measurements on two additional specimens of each composition. A total of 87, separate specimens were analyzed in the rheometer.

Rheological measurements were expressed in terms of the shear stress τ as a function of the shear rate $\dot{\gamma}$. These measurements were fitted to the Herschel-Bulkley model $\tau = \tau_o + K\dot{\gamma}^n$ using a computer (Steffe, 1996). In this expression, τ_o is the yield stress, K is the consistency coefficient, and n is the flow behavior index. This model is convenient, because it describes the rheological behavior of a broad range of fluids that are either Newtonian ($n = 1$), shear-thinning ($0 < n < 1$), or shear-thickening ($1 < n$). A computer was used to statistically analyze the parameters τ_o, K, and n for as a function of the polysaccharide concentration and molecular weight (Sikora et al. 1998).

Surface chemical analysis

In an earlier study, we performed sorption and acoustophoresis measurements to study whether the enhanced rheological behavior of maltodextrin-alumina suspensions was attributed to adsorbate-mediated steric hindrance, electrostatic, interparticle repulsion, or both (Schilling et al. 1998a). Sorption isotherms measurements entailed centrifugation of aqueous suspensions prepared with varying concentrations of maltodextrin 040. Maltodextrin concentrations in centrifuged supernatants were measured spectrophotometrically by the addition of 1 ml of 5% phenol and 5 ml of concentrated sulfuric acid to 1 ml of the maltodextrin solution to form hydroxymethyl furfural, which strongly adsorbed at 488 nm (Dubois et al. 1956). The Smoluchowski zeta potentials of several suspensions were calculated using measurements of the electrokinetic sonic amplitude as a function of frequency (Acoustosizer^{TM}, Matec Applied Sciences Corp., Hopkinton, Massachusetts, U.S.A.). Suspension preparation entailed use of procedures where we varied the maltodextrin 040 concentration and the pH by the dropwise addition of reagent grade HCl or NH_4OH. All suspensions were prepared at 0.01 M NaCl and $\phi = 0.2$.

Mechanical properties

Experiments were performed to determine the effects of the polysaccharide molecular weight and concentration on the tensile strength of molded alumina specimens. These specimens were prepared using suspensions containing $\phi_o = 0.2$ alumina, 0.01 M NaCl, and varying concentrations of a single type of polysaccharide. Several types of polysaccharide were investigated, including soluble starch, pullulan, dextrans, and maltodextrins. Each slurry underwent 24 hours of shaking in a sealed, plastic bottle. Slip-casting was subsequently used to prepare disc-shaped specimens (diameter ~ 1.3 cm and thickness ~ 0.25 cm) for mechanical strength measurements. Slip casting is

a common, ceramic molding process that entails pouring a suspension onto gypsum mold (Aksay and Schilling 1984). Capillary suction of the gypsum serves to consolidate the suspensions by filtration (gypsum contains fine pores that are much smaller than the alumina powder). Each cast specimen was dried by storing at room temperature for one week prior to mechanical testing. The tensile strength of each specimen was measured by the diametric compression method (Bortzmeyer 1992). At least 5 measurements of tensile strength were performed on specimens of each type.

Experimental results

Sedimentation, filtration, and extrusion

Significant increases in sediment density resulted after adding small amounts of maltodextrin 040 to suspensions of alumina powder (ϕ_o = 0.15) and deionized water (Table 2). The simple addition of alumina powder to deionized water without maltodextrin resulted in a strongly flocculated condition and the formation of a low-density sediment (ϕ = 0.18). The alumina volume fraction sharply increased from ϕ = 0.25 to 0.47 as the maltodextrin concentration increased from 0.01 to 0.06 grams of maltodextrin 040 per gram of Al_2O_3.

Volume-averaged densities of liquid-saturated filter-cakes are shown as a function of the consolidation pressure in Table 3. Suspensions of strongly-flocculated alumina near the isoelectric point without maltodextrin (pH 8.6) exhibited the lowest, alumina volume-fraction of ϕ = 0.49 when consolidated at a pressure of only 0.54 MPa. A much higher pressure of 3.5 MPa was needed to raise the alumina volume-fraction of this same slurry system to ϕ = 0.57. In contrast, kaolin cakes exhibited a solid-volume-fraction of ϕ = 0.6 when consolidated at a pressure of only 0.54 MPa. At the same, low consolidation pressure of 0.54 MPa, maltodextrin-alumina cakes exhibited a slightly lower, alumina volume-fraction of ϕ = 0.57.

Table 2

Alumina sediment densities (ϕ_o = 0.15)

Maltodextrin 040 Concentration (g/g Al_2O_3)	Volume Fraction Alumina ϕ
0	0.18
0.01	0.25
0.03	0.43
0.06	0.47
0.09	0.40

Table 3

Filter-cake properties ($\phi_o = 0.2$)

Suspension type	Solids volume fraction, ϕ	Consolidation pressure, MPa
Al_2O_3, pH 8.6	0.49	0.54
Al_2O_3, pH 8.6	0.57	3.5
0.03 g maltodextrin / g Al_2O_3	0.57	0.54
Kaolin	0.60	0.54

As shown in Table 4, strongly-flocculated alumina suspensions without maltodextrin at pH 8.6 displayed the most „clay-like" extrusion at an alumina volume-fraction of $\phi = 0.49$: they exhibited yield stresses and velocity factors that were similar to those of the kaolin suspensions (Table 4). Raising the alumina concentration of the pH 8.6 suspensions to $\phi = 0.57$ produced specimens that were too stiff to be extruded. Alumina specimens that were prepared with 0.03 grams of maltodextrin 040 per gram of Al_2O_3 (at the same alumina concentration of $\phi = 0.57$) were easily extruded, although they exhibited higher yield stresses than all of the other systems in Table 4. In addition, the alumina specimens containing maltodextrin had yield stresses and velocity factors that were several times higher than the corresponding values for kaolin.

Table 4

Extrusion summary

Specimen type	Solids volume fraction, ϕ	Benbow parameters			
		τ_b (MPa)	k_b (MPa·s·m^{-1})	τ_f (MPa)	k_f (MPa·s·m^{-1})
Kaolin	0.60	0.42	3.7	0.03	0.69
Al_2O_3, pH 8.6	0.49	0.34	2.8	0.05	1.0
Al_2O_3, pH 8.6	0.57	*	*	*	*
Al_2O_3 + 0.03 grams maltodextrin/gram Al_2O_3	0.57	1.72	16.4	0.13	4.26

* These samples were too stiff to be extruded.

Rheology studies

In the absence of polysaccharide, alumina suspensions near the isoelectric point commonly exhibited pseudoplastic behaviour (Figure 1). Herschel-Bulkley parameters for these suspensions are: $0.2 < \tau_o < 3$ Pa, $6.1 < K < 10.18$ Pa.s^n, and $0.18 < n < 0.25$. The addition of nearly all of the polysaccharides in this study dramatically suppresses this pseudoplastic behaviour. Small amounts (a few weight per cent) of these polysac-

charides typically produce a major reduction in the flow stress along with a transition from pseudoplastic to Newtonian-like behaviour. For example, Figure 1 illustrates this trend for a suspension containing 0.03 grams of maltodextrin 040 per gram of alumina. In this case, Herschel-Bulkley parameters are as follows: $0.01 < \tau_o < 0.03$ Pa, $0.004 < K < 0.005$ Pa.s^n, and $0.89 < n < 0.95$.

As shown in Figure 2, the consistency coefficient K rapidly decreased upon the addition of each of the polysaccharides in this study. For example, all of the polysaccharides except D4 exhibit a sharp reduction in K as the solution concentration increased from 0 to 0.01 gram of polysaccharide per gram of alumina. For these specimens, $K \sim 0$ for all of the higher concentrations of polysaccharide. In contrast, the addition of the polysaccharide with the largest molecular weight (D4) produces more of a gradual decrease in K as the polysaccharide concentration increases. In this case, K approaches zero only when the concentration of D4 exceeds 0.05 g/g alumina.

Surface chemical analysis

Let us define c_o as the maltodextrin 040 concentration of a given, stock solution without adding $\phi_o = 0.2$ alumina powder, c_a as the equilibrium concentration of maltodextrin 040 sorbed to alumina after adding $\phi_o = 0.2$ alumina to the stock solution, and c_f as the equilibrium concentration of free maltodextrin 040 in solution after adding $\phi_o = 0.2$ alumina to the stock solution. We showed in an earlier publication that maximum sorption is achieved at a minimum c_o of 0.02 grams of maltodextrin 040 per gram of alumina (Schilling et al. 1998). Under these conditions when $c_o = 0.02$ grams of maltodextrin 040 per gram of alumina, approximately 50% of c_o sorbs to alumina, whereas the other 50% remains in solution.

At pH 10 and $c_o = 0.01$ grams of maltodextrin 040 per gram of alumina, acoustophoresis indicated a Smoluchowski zeta potential of -7.6 mV (Table 5). At the same pH of 10, a larger c_o of 0.03 grams of maltodextrin 040 per gram of alumina increased the zeta potential to -3.6 mV. At a lower pH of 7, we observed a Smoluchowski zeta potential of +8.7 mV without maltodextrin. Also at pH 7, c_o of 0.01 grams of maltodextrin 040 per gram of alumina resulted in a Smoluchowski zeta potential of +2.5 mV. Also at pH 7, a larger c_o of 0.03 grams of maltodextrin 040 per gram of alumina reduced the Smoluchowski zeta potential to +1 mV.

Since we previously observed maltodextrin sorption to alumina, it is not surprising that acoustophoresis revealed a decreasing surface charge at pH 10 upon raising c_o from 0.01 to 0.03 grams of maltodextrin 040 per gram of alumina. We should mention that a pH of 9.7 was routinely observed in alumina – maltodextrin 040 slurries that were used in all the sedimentation, filtration, and rheology experiments above. Acoustophoresis revealed a relatively small zeta potential of -3.6 mV under similar conditions (pH 10, $\phi = 0.2$, 0.01 M NaCl, $c_o = 0.03$ grams of maltodextrin 040 per gram of

alumina). This small potential suggests that electrostatic, interparticle repulsion is not a primary mechanism for the increased consolidation and fluidity upon adding maltodextrin to alumina. Instead, sorption data suggest that sorbate-mediated steric hindrance appears to play a major role in this regard.

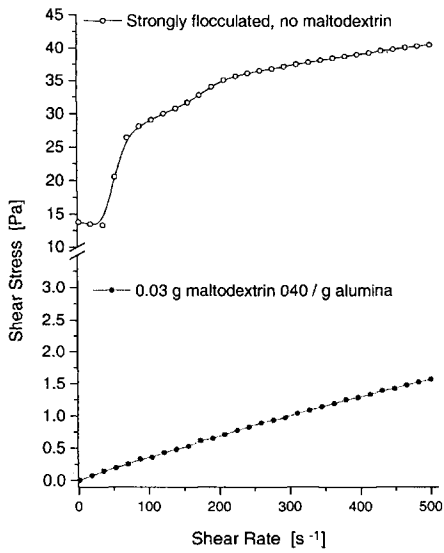


Fig. 1 Aqueous suspensions of 20 vol% alumina exhibit a transition from strongly-flocculated, pseudoplastic behavior to a Newtonian-like state upon the addition of 0.03 grams of maltodextrin 040 per gram of alumina (Sikora et al. 1998).

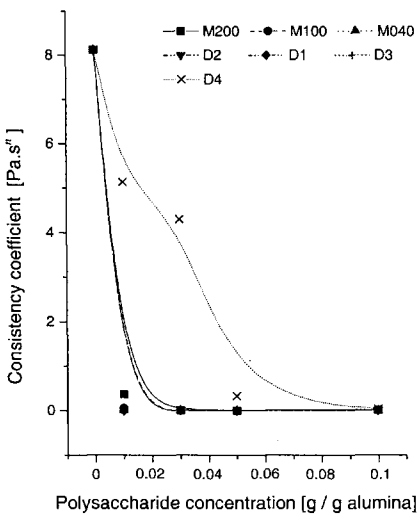


Fig. 2 Increasing the polysaccharide concentration significantly enhanced suspension fluidity, as apparent from the reductions in consistency coefficient. The largest molecular weigh dextrin (D4) was least effective in this regard (Sikora et al. 1998).

Table 5

Acoustophoresis measurements

Maltodextrin concentration (g/g Al_2O_3)	pH	Mean Smoluchowski zeta potential (mV)
0.01	10	-7.6
0.03	10	-3.6
0.01	7	2.5
0.03	7	1.0
0	7	8.7

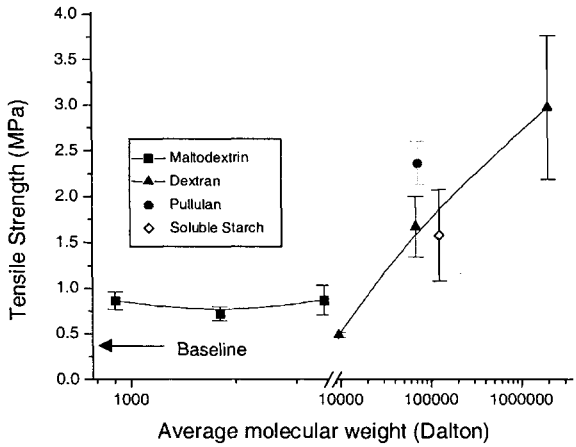


Fig. 3. Tensile strength of slip cast and dried alumina as a function of the polysaccharide molecular weight. Baseline specimens were prepared without polysaccharide and had the lowest strength. All other specimens were prepared with 0.03 grams of a given polysaccharide per gram of alumina.

Mechanical properties

Diametric compression measurements indicated tensile strengths between 0.2 to 0.5 MPa for baseline alumina specimens that were prepared without polysaccharide (Figure 3). The strength increased to approximately 0.75 MPa upon the addition of 0.03 grams of maltodextrin per gram of alumina. Figure 3 illustrates the general trend of increasing the tensile strength upon increasing the molecular weight of the polysaccharide above 10,000 Daltons. Below 10,000 Daltons, the strength does not appear to be influenced by the molecular weight. Specimens containing pullulan and the largest-molecular-weight dextran were approximately 10 times stronger than that of the baseline specimens made without polysaccharide. We should mention that we also observed systematic increases in tensile strength as the concentration of each polysaccharide increased. Finally, we should mention that we had previously reported ultrasonic velocity measurements illustrating that the simple addition of 3 grams of maltodextrin 040 per gram of alumina results in dried filter cakes with high elastic stiffness (13.8

GPa as opposed to 8.2 GPa for specimens prepared without maltodextrin 040) (Schilling et al. 1998b).

Conclusions

Aqueous suspensions of submicron alumina powder exhibited striking improvements in consolidation and rheological properties after adding small amounts of various maltodextrins and dextrins. These results provide strong support for the use of these additives in technical ceramic manufacturing.

High-density sediments (47 vol%) and high-density filter-cakes (57 vol%) were produced at low filtration-pressures (0.54 MPa). In contrast, alumina filter-cakes that were flocculated at the isoelectric point without maltodextrin required an order-of-magnitude greater filtration pressure to achieve the same 57 vol% density.

Maltodextrin-alumina filter-cakes were easily extrudable with Benbow parameters comparable to but higher than those of kaolin at approximately the same packing density of 57 vol%. Alumina filter-cakes without maltodextrin at the same 57 vol% density were too stiff to be extruded.

Rheometry experiments indicated a strongly-flocculated, Bingham-plastic response upon adding 20 vol% alumina powder to aqueous solutions of 0.01 M NaCl without maltodextrin. In contrast, the addition of 3 wt% of various dextrins and maltodextrins resulted in low viscosities, Newtonian-like behavior, and Bingham yield stresses of approximately zero.

Sorption measurements indicated that maltodextrin sorption to alumina enhances the consolidation and flow behaviour of these specimens. Acoustophoresis data support the hypothesis that sorbate-mediated steric-hindrance, rather than electrostatic, interparticle repulsion, plays a significant role enhancing the consolidation and plastic flow behavior.

The addition of 0.03 grams of a given polysaccharide per gram of alumina significantly increased the tensile strength of slip cast and dried alumina. Specimens containing pullulan and dextran were approximately 10 times stronger than that of the baseline specimens made without polysaccharide.

Acknowledgements

The authors wish to thank the U.S. National Science Foundation, the Polish Research Council, and the Office of Basic Energy Sciences at the U.S. Department of Energy for supporting this research. We also wish to thank H. Goel, R. Smith, J. Jane, L. Ukrainczyk, and R. Bellman for valuable assistance.

REFERENCES

[1] Abraham T.: „Advanced Ceramic Powder and Nano-Sized Ceramic Powder: An Industry and Market Overview," in Ceramic Transactions Volume 62, Science, Technology, and Commercialization of Powder Synthesis and Shape Forming Processes, edited by J.J. Kingsley, C.H. Schilling, J.H. Adair (American Ceramic Society, Westerville, Ohio, U.S.A., 1996), 3-14.

[2] Aksay A., Schilling C.H.: „Colloidal Filtration Route to Uniform Microstructures," in Ultrastructure Processing of Ceramics, Glasses, and Composites, edited by L.L. Hench, D.R. Ulrich (John Wiley and Sons, New York, 1984), 439-447.

[3] Benbow J.J., Lawson T.A., Oxley E.W., Bridgwater J.: „Prediction of Paste Extrusion Pressure," Am. Ceram. Soc. Bull., **68**, 10, 1989, 1821-24.

[4] Benbow J.J., Oxley E.W., Bridgwater J.: „The Extrusion Mechanics of Pastes - The Influence of Paste Formulation on Extrusion Parameters," Chem. Eng. Sci. **42** [9] 2151-62 (1987).

[5] Bergström L., Schilling C.H., Aksay I.: „Consolidation Behavior of Flocculated Alumina Suspensions," J. Am. Ceram. Soc., **75**, 12, 1992, 3305-14.

[6] Bonomi Z., Persay G., Schleiffer E.: „Manufacture of Alumina-Based Refractory Materials," Hungarian Patent, Hung. Teljes HU 49, 103, 28 August 1989.

[7] Bortzmeyer D.: „Tensile Strength of Ceramic Powders," J. Mater. Sci., **27**, 1992, 3305-8.

[8] Chan T.-Y., Lin S.-T.: „Effects of Stearic Acid on the Injection Molding of Alumina," J. Am. Ceram. Soc., **78**, 10, 1995, 2746-52.

[9] Chang J.C., Velamakanni B., Lange F., Pearson D.: „Centrifugal Consolidation of Al_2O_3 and Al_2O_3/ZrO_2 Composite Slurries vs Interparticle Potentials: Particle Packing and Mass Segregation," J. Am. Ceram. Soc., **74**, 9, 1991, 2201-04.

[10] Chang J.C., Lange F.F., Pearson D.S.: „Viscosity and Yield Stress of Alumina Slurries Containing Large Concentrations of Electrolyte," J. Am. Ceram. Soc. **77** (1) 19-26 (1994 a).

[11] Chang J.C., Lange F.F., Pearson D.S., Pollinger J.P.: „Pressure Sensitivity for Particle Packing of Aqueous Alumina Slurries vs. Interparticle Potential," J. Am. Ceram. Soc., **77**, 5, 1994b, 1357-60.

[12] Dmitriev A., Podkovyrkin M.I., Beloborodova L.G., Kleshcheva T.M., Gorskaya E.S.: „Molding of Nonplastic Corundum Bodies," Ogneupory, **9**, 1990, 30-33.

[13] Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A., Smith F.: „Colorimetric Method for Determination of Sugars and Related Substances," Analytical Chemistry, **28**, 1956, 350-56.

[14] Fanelli J., Silvers R.D., Frei W.S., Burlew J.V., Marsh G.B.: „New Aqueous Injection Molding Process for Ceramic Powders," J. Am. Ceram. Soc., **72**, 10, 1989, 1833-1836.

[15] Franks G.V., Velamakanni B.V., Lange F.F.: „Vibraforming and In-Situ Flocculation of Consolidated, Coagulated, Alumina Slurries," J. Am. Ceram. Soc., **78**, 5, 1995, 1324-28.

[16] Franks G.V., Lange F.F.: „Plastic-to Brittle Transition of Saturated, Alumina Powder Compacts," J. Am. Ceram. Soc., **79**, 12, 1996, 3161-68.

[17] Goel H., Schilling C.H., Biner S.B., Moore J.A., Lograsso B.K.: „Plastic Shaping of Aqueous Alumina Suspensions with Saccharides and Dicarboxylic Acids," in Science, Technology, and Commercialization of Powder Synthesis and Shape Forming Processes, edited by J. J. Kingsley, C. H. Schilling, and J. H. Adair (American Ceramic Society, Westerville, Ohio 1996), 241-54.

[18] Hidber P.C., Graule T.J., Gauckler L.J.: „Citric Acid - A Dispersant for Aqueous Alumina Suspensions," J. Am. Ceram. Soc., **79**, 7, 1996, 1857-67.

[19] Kono T., Otsuka T.: „Concrete Compositions Suitable for Application by Compacting," Jpn. Kokai Tokyo Koho JP 02,239,144, 21 September 1990.

[20] Kramer T., Lange F.F.: „Rheology and Particle Packing of Chem- and Phys-Adsorbed Alkylated Silicon Nitride Powders," J. Am. Ceram. Soc., **77**, 4, 1994, 922-928.

[21] Lawrence W.G.: „The Structure of Water and its Role in the Clay-Water System," in Ceramic Processing Before Firing, edited by G. Y. Onoda, Jr. and L. L. Hench (John Wiley and Sons, New York, 1978), 193-210.

[22] Leong Y.K., Scales P.J., Healy T.W., Boger D.V., Ruscall R.: „Rheological Evidence of Adsorbate-mediated Short-Range Steric Forces in Concentrated Dispersions," J. Chem. Soc., Faraday Trans., **89**, 1993, 2473-78.

[23] Luther E.P., Kramer T.M., Lange F.F., Pearson D.S.: „Development of Short-Range Repulsive Potentials in Aqueous, Silicon Nitride Slurries," J. Am. Ceram. Soc., **77**, 4, 1994, 1047-51.

[24] Luther E.P., Yanez J.A., Franks G.V., Lange F.F., Pearson D.S.: „Effect of Ammonium Citrate on the Rheology and Particle Packing of Alumina Slurries," J. Am. Ceram. Soc., **78**, 6, 1995, 1495-5000.

[25] Mach Z., Wendler L., Plucar A., Kostkova A.: „Compositions for Cordieritic Kiln Furniture," Czech. Patent CS 248,912, 15 January 1988.

[26] Panda J., Sahoo N., Agarwal S.: „Manufacture of High-Alumina Gas-Permeable Refractory Shaped Articles," Indian Patent IN 162,517, 04 June 1988.

[27] Pashley R.M., Israelachvili J.N.: „DLVO and Hydration Forces Between Mica Surfaces in Mg^{+2}, Ca^{+2}, Sr^{+2}, and Ba^{+2} Chloride Solutions," J. Colloid Interface Sci., **97**, 1984, 446-55.

[28] Pujari V.K.: „Effect of Powder Characteristics on Compounding and Green Microstructure in the Injection Molding Process," J. Am. Ceram. Soc., **72**, 10, 1989, 1981-1984.

[29] Sarkar N., Greminger G.K. Jr.: „Methylcellulose Polymers as Multifunctional Processing Aids in Ceramics," Bull. Am. Ceram. Soc., **62**, 11, 1988, 1280-1284.

[30] Schilling H.: „Plastic Shaping of Colloidal Ceramics," Ph.D. thesis, University of Washington, Seattle, Washington, 1992).

[31] Schilling H., Biner S.B., Goel H., Jane J.L.: „Plastic Shaping of Aqueous Alumina Suspensions with Sucrose and Maltodextrin Additives," J. Environmental Polymer Degradation, **3**, 3, 1995, 153-60.

[32] Schilling H., Bellman R.A., Smith R.M., Goel H.: „Plasticizing Aqueous Suspensions of Concentrated Alumina with Maltodextrin Sugar," accepted in J. Am. Ceram.Soc., 1998a.

[33] Schilling C.H., Garcia V.J., Smith R.M., Roberts R.A.: „Ultrasonic- and Mechanical Behavior of Green- and Partially-Sintered Alumina: Effects of Slurry Consolidation Chemistry," accepted in J. Am. Ceram. Soc., 1998b.

[34] Segal: „Chemical Preparation of Powders," in Materials Science in Technology, Volume 17A, Processing of Ceramics, Part 1, edited by R. J. Brook, R. W. Cahn, P. Haasen, and I. J. Kramer (VCH Verlagsgesellschaft mbH, Weinheim, Federal Republic of Germany, 1996), 70-98.

[35] Segal D.: Chemical Synthesis of Advanced Ceramic Materials (Cambridge University Press, Cambridge 1989).

[36] Sikora M., Schilling C.H., Garcia V.J., Li C.P., Tomasik P.: „Effects of Polysaccharide Concentration and Molecular Weight on the Rheology of Alumina Suspensions," submitted to J. Am. Ceram. Soc., 1998.

[37] Shanefield D.J.: Organic Additives and Ceramic Processing (Kluwer Academic Publishers, Boston, 1995).

[38] Stangle C., Aksay I.A.: "Simultaneous Momentum, Heat and Mass Transfer with Chemical Reaction in a Disordered Porous Medium: Application to Binder Removal from a Ceramic Green Body," Chem. Engr. Sci., **45**, 7, 1990, 1719-31.

[39] Steffe J.F.: Rheological Methods in Food Process Engineering (Freeman Press, East Lansing, Michigan, 1996), 19-23.

[40] Tomasik P., Schilling C.H.: "Starch Complexes, Part 1: Complexes with Inorganic Guests," Advances in Carbohydrate Chemistry and Biochemistry, **52**, 1998a, in press.

[41] Tomasik P., Schilling C.H.: "Starch Complexes, Part 2: Complexes with Organic Guests," Advances in Carbohydrate Chemistry and Biochemistry, **52**, 1998b, in press.

[42] van Olphen: An Introduction to Clay Colloid Chemistry, Second Edition (John Wiley and Sons, New York, 1977).

[43] Velamakanni B.V., Chang J.C., Lange F.F., Pearson D.S.: „New Method for Efficient Colloidal Particle Packing via Modulation of Repulsive Lubricating Hydrating Forces," Langmuir, **6**, 1990, 1323-25.

[44] Velamakanni B.V., Lange F.F., Zok F.W., Pearson D.S.: „Influence of Interparticle Forces on the Rheological Behavior of Pressure-Consolidated Alumina Particle Slurries," J. Am. Ceram. Soc., **77**, 1, 1994, 216-20.

[45] Yin T.K., Aksay I.A., Eichinger B.E.: „Lubricating Polymers for Powder Compaction," in Ceramic Transactions Volume 1, Ceramic Powder Science II, edited by G. L. Messing, E. R. Fuller, Jr., and H. Hausner (American Ceramic Society, Westerville, Ohio, 1988), 654-662.

FORMOWANIE CERAMIKI TECHNICZNEJ Z UŻYCIEM POLISACHARYDÓW

Streszczenie

Techniczna ceramika stanowi olbrzymi międzynarodowy rynek zbytu zdominowany przez zastosowania elektroniczne. Wyrobami do tego celu są izolatory, pakiety zintegrowanych obwodów, kondensatory i magnesy. Typową operacją w produkcji tego rodzaju wyrobów jest mieszanie proszku ceramicznego z ciekłą substancją organiczną (np. smar polietylenowy, rozpuszczalniki organiczne) w celu uzyskania masy konsystencji plasteliny, której nadaje się trójwymiarowy kształt który następnie się suszy i wypala. Piroliza dodatków organicznych stwarza problemy przed ostatecznym wypalaniem wyrobu. Najpierw trzeba taki wyrób powoli (ok. tygodnia) ogrzewać w 200°C aby uniknąć pęknięć wyrobów i tworzenia się pęcherzyków gazowych. W trakcie tego, związanego z wysokimi kosztami ogrzewania tworzą się trujące wyziewy. Wyrób zostaje przy tym zanieczyszczony mikrokrystalicznymi cząsteczkami węgla. Chcąc uniknąć pęknięć, odkształceń oraz zanieczyszczeń węglowych w wyrobach spiekanych przy równoczesnym wyeliminowaniu dodatków organicznych należy posługiwać się szlamem proszku w zawiesinie wodnej, do którego dodaje się rozpuszczalnych w wodzie dodatków wiążących. Z naszych badań wynika, że do tego celu nadają się różne dekstryny i maltodekstryny, gdyż w roztworze wodnym wykazują one naturalną zdolność do sorbowania się na powierzchni cząsteczek tlenków metali. Niewielki ich dodatek (<5 wag%) znacznie ułatwia proces formowania i zapewnia łatwą pirolizę z minimalnym zanieczyszczeniem drobinami węgla. Ponadto po odparowaniu wody te polisacharydy mocno wiążą ze sobą ziarenka tlenków zapewniając otrzymywanie mocnych, wolnych od pęknięć wyrobów ceramicznych przed ich wypalaniem. Niniejsza praca przedstawia omówienie podstawowych elementów wpływających na reologię wodnych zawiesin proszków ceramicznych w oparciu o chemiczną naturę oddziaływań międzycząsteczkowych. Przedstawiono wyniki badań nad sedymentacją, sączeniem , ekstruzją i i chemiczną analizą powierzchni obrazujące praktyczne możliwości zastosowania maltodekstryn i dekstryn jako reologicznych modyfikatorów w produkcji wyrobów ceramicznych.

M. SORAL-ŚMIETANA, M. WRONKOWSKA, G. LEWANDOWICZ*,
J. SADOWSKA

TECHNOLOGICAL AND SENSORY ASPECTS OF NEW RESISTANT STARCH PREPARATIONS USED IN BAKING PROCESS

Abstract

Among polysaccharides occurring in food products, only starch and glycogen are completely digested. Rich sources of resistant starch are starchy food products after hydrothermal treatments, being obtained with share of chemically-modified starch, or thermally-dehydrated foods. Bread is also a source of resistant starch. Because of high consumption of bread in our climatic zone, it was interesting to know how the addition of new preparations of resistant starch, being obtained from physically-modified wheat, potato, maize and tapioca starches, would affect the technological and sensory qualities of the final product and its level of RS.

Based on the results of experiment, in which wheat dough was prepared with 10% share of RS-preparations from different botanic origin, it could be observed that the water absorption of flour mixed with RS-preparations increased from 4 to 7%. The rheological properties of dough from commercial wheat flour of poor technological quality with the share of RS-preparations were slightly changed since the time of dough development was lengthened, consistency of dough was improved, and its structure stability was weakened during kneading. Farinographic quality number (FQN) decreased, as compared to control in the same degree irrespective of the type of investigated RS-preparation.

On a basis of the results of panel evaluation by profile method, in which 16 quality factors and total desirability in hedonic terms were considered, it was found that the wheat RS-preparation affected most favourably the taste and smell qualities. Tapioca and maize RS-preparations favoured less advantageous quality factors such as plain and floury.

The examination of rheological properties of bread crumb showed that hardness of fresh breads, 1h after baking, was higher for breads with RS-preparations compared to control bread. The instrumental measurements confirmed the expected decrease of elasticity and cohesiveness in fresh and 24- and 72h-stored breads. These results suggest lower staling of bread, particularly with wheat and potato RS-preparations.

The RS contents measurements as determined with involvement of salivary α-amylase during chewing, show the increasing tendency for all RS preparations.

Division of Food Science, Institute of Animal Reproduction & Food Research Polish Academy of Sciences, Olsztyn, Poland

* *Starch and Potato Products Research Laboratory, Poznań, Poland*

Introduction

Starch is quantitatively the most important food carbohydrate and along with glycogen is the only digestible food polysaccharide. The way and rate of enzymatic hydrolysis of dietary starch depend to a considerable degree on starch interaction with other food components and conditions of technological process. Hydrolysis of starch is initiated by salivary amylase and is completed in the small intenstine. Thus, in starchy foods the proportion between starch in solution, partially degraded starch, and glucose gives the same glycemic and insulin responses as their equivalent amounts (Wahlqvist et al, 1978). However glycemic index of starchy food varies widely because of food properties that affect the availability of starch for enzymatic degradation (Björck et al, 1994). One of the richer sources of resistant starch are food products after hydrothermal treatment which were obtained with the share of chemically modified or retrograded starches. Resistant starch was first discovered as a starch fraction that was associated with the non-starch polysaccharides in dietary fibre. Most frequently it is retrograded amylose (Siljeström et al, 1989), which is one of many forms of physically resistant starch that pass through the small intestine (Englyst et al, 1992).

The extent the resistant starch is present in foods depend on botanical source of starch and number of factors, including the type of processing. The most important sources of dietary starch are cereal products. As Liljeberg et al. (1996) report, the estimated annual intake of bread in European countries ranges from 46 to 100 kg per person. Attempts to change the resistant starch (RS) intake in a mixed diet should thus focus on optimizing the RS content in rolls or bread. Higher resistant starch content in food, escapes digestion in small intestine, improves long-term glycaemic and lipid metabolism through the short-chain fatty acids produced during its fermentation (Muir et al., 1993). Resistant starch is particularly prone to generating butyric acid upon fermentation in colon (Scheppach et al., 1988) and might have a protective effects against colonic diseases.

The purpose of present study was to see if it is possible to increase the amount of resistant starch in wheat bread by including the RS-preparations from different botanic sources to dough before fermentation and to assess the technological and sensory effects.

Material and methods

Material

Basic material for baking experiment was commercial wheat flour with characteristics as shown in Table 1. Resistant starch preparations were obtained from commercial wheat (RSW), potato (RSP), maize (RSM) and tapioca (RST) starches pro-

duced by the physical processes acc. to Polish patent specification No. P.325981. The preparations were made of particles smaller than 200 μm. Chemical and functional characteristics of these RS-preparations is shown in Table 2.

Table 1

Chemical components and technological properties of commercial wheat flour

Wheat flour	
Chemical components:	
Moisture [%]	15.0
Starch [%d.m.]	84.2
Proteins [% d.m.]	12.1
Ash [% d.m.]	0.6
Quality and quantity of gluten proteins:	
Sedimentation index	32
Amount of wet gluten [%]	37.2
Elasticity of gluten [degree]	3
Deliquescence of gluten [mm]	22
Moisture of wet gluten [%]	33.8

Table 2

Granulation of commercial wheat flour

Eyelet [μm]	**Amount of flour [%]**
>265	0.03
>150	6.44
>120	3.48
>105	17.96
>95	11.37
<95	59.34
sum	98.62

Experiment

One-phase baking experiment was carried out after 1h fermentation.

The control sample contained: 100g commercial wheat flour, 3g yeast, 1g salt and the addition of water that enabled to kneed the dough, after determining the flour water absorption from farinographic curves for the dough consistency at 350 B.U. The experimental samples were prepared on a basis of the same as above composition, using instead the wheat flour mixture of 90g wheat flour and 10g addition of resistant

starch preparations from wheat (+RSW), potato (+RSP), maize (+RSM), and tapioca (+RST). Baking was carried out at 220°C for 30 min. The size of pieces was 250g.

Methods

Chemical composition was analysed by standard AOAC methods.

The *in vitro* resistant starch content was determined acc. to modified Berry method described by Champ (1992). The content of resistant starch in *in vivo* simulated conditions was determined acc. to Asp et al (1992) in modification by Granfeidt (1992) method.

Functional properties:

- water holding capacity (WHC) was determined for 5g d.m. of starch suspended in 75 cm^3 of distilled water at 20°C. Suspension was shaken for 1 h. Centrifugation was made at 2200 x g for 10min and the mass of water holding per 1g starch d.m. was determined.
- oil absorption was determined after mixing 0.5g d.m. of starch with 3 cm^3 sunflower oil for 1min at 200 rpm, standing 30 min at 20°C and centrifuging at 1700 x g for 30 min. The supernatant was discarded by turning the tube for 30min. The increase in weight per 1g starch d.m. was measured for oil absorption.

***Technological characteristics*:** amount and quality of gluten proteins, including sedimentation test was determine acc. to Polish Standards No PN-77/A-74041 and PN-77/A-74019. It was measured water absorption of flour acc. to farinographic curves at 500B.U. and its technological value from farinographic curves acc. to AACC method, and farinographic quality number (FQN) acc. to ICC methods (Schogll, 1995; ICC Standard no. 155).

Sensory analysis was carried out by an 11 member panel using profile sensory method – Quantitative Description Analysis (QDA). The panelists evaluated 16 quality factors: colour, taste, smell, texture and desirability in hedonic scale degree of liking. Computer program ANALSENS was used to prepare test, record individual scores, and make statistical analysis of results.

***Texture analysis*:** texture properties of crumbs were measured using compression device of Instron 1011 (Juston, England). The samples, size 20 x 20 x 20 mm, were twice compressed to 70% strain at 10 mm/min crosshead speed. Hardness expressed as maximum force during first compression, F_1 [MPa], elasticity expressed as ratio of maximum forces determined in second and first compression, F_2/F_1 [-], cohesiveness expressed as ratio of energies determined in second and first compressions, E_2/E_1 [-], and gumminess characterized by expression, $F_1 \times (E_2 / E_1)$ [MPa], were calculated according to Mohan Rao and Skiner (1986). Four replications were made for each loaf.

Results and discussion

Commercial wheat flour, the basic raw material in technological experiment, contained a standard amount of total protein, including a considerable amount of gluten, as wet gluten (Table 1). On a basis of sedimentation test, indicative of hydration properties of gluten fractions, gliadine and glutenin, it can be however said that the quality of proteins responsible for dough structure was poor. This would also indicate two other quality factors of hydrated and isolated gluten; elasticity of gluten and deliquescence of gluten. The quality of starch in wheat flour was indirectly determined by the analysis of flour granulation, according to which the particles below 100μm made over 70% of the mass (Table 2). Thus, the amount of particles with the granulation between 265μm to 100μm was too low, proving highly developed surface of particles in the flour and a possible mechanical damage of a certain amount of starch granules during milling.

The characteristics of resistant starch preparations showed that the greatest amount of resistant starch was present in potato starch preparation (~32%). The other preparations followed the order: wheat>maize>tapioca (Table 3). Analysing the functional properties for water holding capacity (WHC) of the examined RS-preparations it was found that all of them absorbed at least 3.5g water per 1g d.m. of sample. Slightly higher absorption of water showed tapioca and wheat RS-preparations. The affinity for oil was characteristically higher for potato starch preparation, and wheat starch preparation had the smallest oil absorption ability.

Such behaviour of RS-preparations against oil was determined by native structure of the starches, particularly B-type, inclusive fat-free potato starch and A-type wheat starch containing bound fat, mostly polar glyco-and phospholipids (Soral-Śmietana, 1992, Soral-Śmietana et al, 1997).

Table 3

Chemical and physico-chemical characteristics of investigated resistant starch preparations

Components / Properties	Preparation from starches:			
	Wheat (RSW)	Potato (RSP)	Maize (RSM)	Tapioca (RST)
Components:				
resistant starch [% d.m.]*	28.4	31.8	22.5	18.9
ash [% d.m.]	0.3	0.5	0.3	0.3
moisture [%]	10.0	11.5	10.5	11.0
Properties:				
water holding capacity [g H_2O/g d.m. sample]	3.8	3.6	3.5	3.9
oil absorption [g oil/g d.m. sample]	1.1	1.5	1.2	1.0

* measured modified Berry method described by Champ

Based on farinographic measurements of water absorption of wheat flour and experimental mixtures of wheat flour with the preparations containing starch resistant to amylases from different sources, it was observed that the incorporation of RS-preparations to dough required to increase the addition of water to obtain the farinograph curve of consistency 500 B.U. (Table 4). Inclusion of the RS-preparations obtained from cereal starch, potato starch and tapioca starch required to increase the addition of water by 5%, 4% and 7%, respectively, as compared to control flour.

Table 4

Technological values of wheat flour and mixed with resistant starch preparations to farinographic curves acc. to AACC method

Farinographic parameters from normal curve (500 B.U.)	Samples of dough				
	Control	with RSW	with RSP	with RSM	with RST
Water absorption [%]	54.8	60.0	58.6	60.0	61.6
Arrival time [min.]	0.5	0.7	0.5	0.5	1.0
Stability time [min.]	4.8	3.2	3.5	3.0	2.8
Resistance of dough to kneeding [min.]	5.3	3.9	4.0	3.5	3.8
Peak time [min.]	1.5	1.6	1.5	1.5	1.8
Time to breakdown [min.]	6.9	4.8	5.0	5.0	5.0
Tolerance index [B.U.]	120.0	190.0	200.0	180.0	150.0
Farinographic quality number [FQN]	69.0	48.0	50.0	50.0	50.0

Table 5

The resistant starch contents in bread crumb

Bread crumb	Resistant starch content* [% d.m.]
Control	4.39 ± 0.47
with RSW	4.27 ± 2.17
with RSP	4.64 ± 1.49
with RSM	4.72 ± 1.45
with RST	4.75 ± 2.23

* measured acc. to Asp modified by Granfeidt method.

Analysis of farinographic curves (Table 4) showed that arrival time was lengthened the most when tapioca RS-preparation was used, whereas it lengthened slightly for wheat RS-preparation. The contribution of RS-preparations in the formation of wheat dough structure generally shortened the time of dough stability and resistance time to kneeding. Both these parameters were distincly different using RSM and RST. Although the time to breakdown was similar for all RS-preparations, it was shorter by about 2 min. than for control sample. The tolerance index had a bigger value for all

mixtures than for control, but the smaller difference was for RST. Comparing new parameter acc. to ICC, fariographic quality number (FQN), is the lenght from the first addition of the water to the time at which the consistency has decreased 30 B.U. from the peak point, it could be observed a similar tendency as for the measurements of time to breakdown.

Based on characteristics of the farinographic curve it can be said that during dough formation the participation of the examined RS-preparations produced a competition for water with native structure-forming flour polymers proteins and starch. This is confirmed by the results in table 3 and table 4. Elongation of arrival time in the case of RST and RSW preparations suggests that higher affinity of both these preparations to water disturbed the hydration of gluten proteins and then the formation of spatial gluten network. This phenomenon caused the formation of a weaker quaternary structure, less resistant to mechanical action of mixers. It may be also supposed that competitive and diversified water affinity of all polymeric structures in the mixture allowed the interactions between wheat protein and RS-preparation to be occurred more quickly and decreased the possibility for an overtaking hydration and stabilization of protein within the structure of spatial network.

Additionally, the 10g share of RS-preparation relative to 90g of wheat flour causes a certain dilution of the proteins able to form gluten network of dough. It may be also supposed that included RS-preparation can be located due to water absorption and swelling in the gluten network on inert supporting-filling structures being caused by wheat starch – wheat protein – RS-preparation interactions.

Based on *in vivo* simulated determination of resistant starch content in experimental bread crumb it was observed a tendency that the average resistant starch content increased with the addition of all RS-preparations (Table 5), although the most labile enzymatic susceptibility (standard deviation value) of resistant starch had the bread crumb with RSW and RST preparations. The assumption then that wheat starch non-susceptible for amylases, formed upon reactions of colloids from the same botanic source, may be formed via physical forces or physico-chemical interactions, showing a considerable amylolytic lability. A similar behaviour of variable susceptibility was also observed in the case of RST-preparation, however, in this case the source of resistant starch was starch with a small amount of amylose. It is assumed that the co-formation of resistant starch within the fraction amylose/amylopectin of wheat flour and amylopectin of tapioca starch gives structures less resistant to hydrolytic action of amylases. The ability for the formation of resistant starch structures during production of this preparation also appeared the smallest (Table 3).

Profile sensory analysis performed for 16 factors of colour, smell, taste and hedonic sensations showed no statistically significant differences between the share of resistant starch preparations (Table 6, Fig. 1). Among the sensory factors analysed for

bread samples with RS preparations, the following ones dominated, as compared to control bread: crumb color and elasticity, smell and taste of bread, and mastication. Unfavourable smells, like floury, yeasty or sour and tastes, i.e. sweet, salty and insipid reached the low level in profile analysis. Distinctly higher intensity of measurements were also noted for mastication of samples with RS-preparations. It also turns attention that on a basis of evaluation of desirability, the control bread was scored the least (Fig. 2). Therefore, it can be said that despite the effect of RS-preparations on the compactness of gluten protein structure, the barrier for gases generated during fermentation, the products of smaller volume but with better dispersion of gases in the crumb structure can be obtained.

Table 6

Results of sensory profiling of bread with share of RS-preparations

Quality factors	Samples of bread (0-10 point)				
	Control	with RSW	with RSP	with RSM	with RST
C. crumb	5.95	5.06	6.47	6.07	6.43
C. peel	7.19	5.14	5.36	6.18	7.14
Porosity	6.02	5.54	4.42	3.93	5.51
Size of pore	5.46	3.84	5.14	4.52	6.30
Elasticity	6.20	8.12	6.09	7.35	6.68
S. bread	4.05	4.32	4.03	4.23	3.26
S. roll	4.00	3.54	3.95	3.80	4.33
S. floury	2.96	2.27	2.35	2.88	2.48
S. yeasty	1.77	1.89	2.14	1.58	1.66
S. sour	1.64	1.37	2.20	1.91	1.76
T. bread	3.12	4.15	4.90	4.18	3.98
T. roll	3.08	3.57	3.34	3.23	2.89
T. sweet	2.08	3.03	2.21	1.95	2.38
T. salty	1.00	0.96	1.35	0.97	0.81
T. insipid	2.57	1.25	1.05	1.23	2.10
Mastication	5.04	7.09	6.05	7.21	4.72

C – colour, S – smell, T – taste.

Instrumental measurements of texture of experimental bread crumbs included hardness, guminess, elasticity and cohesiveness, as determined after 1, 24 and 72h from the baking (Fig. 3A and 3B). The analysis of hardness after 1h from baking showed that all bread crumbs with

RS-preparations were harder than control crumb. This phenomenon intensified during 72h storage, except breads with RSW- and RSP-preparations. A decrease of guminess was observed during storage in all breads with RS-preparations. Fresh bread

with RSW-preparation had the highest guminess that turned to be the lowest after 72h. Despite large differences in hardness, the values for crumb elasticity were within a narrow range. The changes of cohesiveness during 72h were smaller for experimental samples than control sample. The results for rheological properties of bread with RS-preparations, from wheat and potato starches allow to say that experimental breads remained fresh during 72h without the necessity for using improvers.

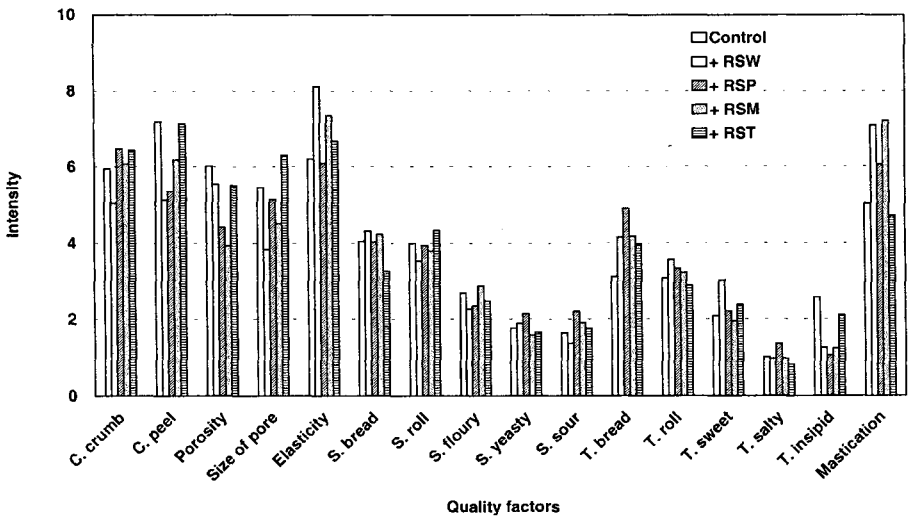


Fig. 1. Sensory profiling of bread with share of RS-preparations.

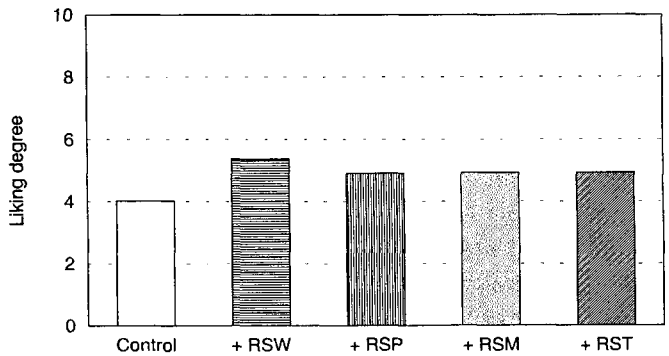


Fig. 2. Liking degree of bread with share of resistant starch preparations.

Gelatinization, which takes place in baking process, is the first step in many cases of starch utilization whereby native granular structure is partially or completelly disrupted. Swelling and solubility of starch granules is a function of temperature and indicate two levels of bonding forces within a granule structure. Donovan (1979) put for-

ward the hypothesis that the gelation process is one of disorder being aided by the swelling action of water in the amorphous phase. In the literature, this process has been referred to as water-mediated, and as solvation or hydration-assisted melting. Donovan further described the gelatinization phase transition as the disordering of individual chains being separated from ordered regions with the possibility that crystallites might not be left for melting at a higher temperature. He also alluded to the unfolding and hydration of helices as result of their being separated from crystallities. Subsequently, this author as cited in a book (Alexander and Zobel, 1992) described the process as that in which crystallites were being „pulled apart as increased thermal energy and swelling pressures overcame the internal binding forces of the crystallites".

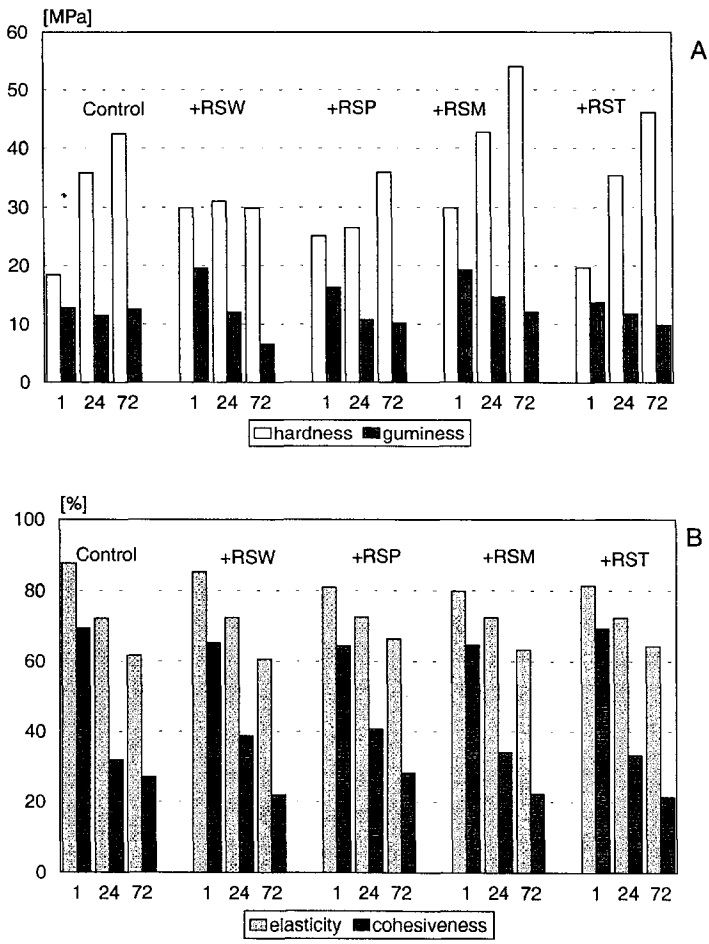


Fig. 3. Texture of bread crumbs 1-, 24- and 72 hours after baking;
A- Hardness and guminess measumements,
B- Elasticity and cohesiveness measurements.

Conclusions

The results obtained for bakery product during dough formation, fermentation and baking indicate that, because of hydratation of native polymers, the inclusion of starch preparations containing a certain amount of resistant starch is possible via hydrophilic interactions.

By replacing of 10% wheat flour with RS-preparations from wheat, potato, maize and tapioca, there are tendencies for the RS content in product to increase from 5 to 7%, with a weaker effect being observed for a homogenous botanic source of wheat starch.

Hydrophilic-hydrophobic affinity of included RS-preparations gives the possibility, through swelling and gelatinization of starch, for active participation in post-baking redistribution of water between polymers, wheat starch – wheat proteins – RS-preparations, resulting in prolonged freshness, particularly in the case of preparations obtained from wheat and potato starches.

Acknowledgement

The research was financed by grant No 5 PO6 G008 10 from the State Committee for Scientific Research (KBN)

The authors thanks M.Sc. ANNA BEDNARSKA for organizing Quantitative Description Analysis and discussion about sensory results.

REFERENCES

[1] Approved Methods of AACC, 1983, 54-21 AACC method, St. Paul, Minnesota.
[2] AOAC. Official Methods of Analysis. 15th ed., Arlington, Virginia, 1990.
[3] Björck I., Granfeldt Y., Liljeberg H., Tovar J., Asp N-G.,: Am. J. Clin. Nutr., 1994, **59**, 699-705.
[4] Champ M.: Europ. J. Clin. Nutr., **46**, 1992, 51-62.
[5] Donovan J.W., Biopolymers, **18**, 1979, 263.
[6] Englyst H.N., Kingman S.M., Cummings J.H., Eur. J. Clin. Nutr., **46**, 1992, 51-62.
[7] Granfeldt Y., Björck I., Drews A., Tovar J., Eur. J. Clin. Nutr., **46**, 1992, 649-660.
[8] ICC Standard no. 155. ICC Standards.
[9] Liljeberg H., Akerberg A., Björck I.: Food Chem., **56**, 1996, 389-394.
[10] Mohan Rao V.N., Skiner G.E. Rheological properties of solid foods. In Engineering Properties of Food., New York, 1986.
[11] Muir J.G., Young G.P., O'Dea K., Cameron-Smith D., Brown I.L., Brown I.L., Collier G.R.: Diet. Fiber Bibliogr. Rev., **1**, 1993, 33-47.
[12] Polish Standard No PN-77/ A-74041. Cereal grain and cereal products. Determination of quantiti and quality of gluten [in polish], 1977.
[13] Polish Standard No PN-93/ A-74019. Wheat. Determination of sedimentation index. Test Zeleny [in polish], 1993.
[14] Russel P.L., Berry C.S., Greenwell P.: J. Cereal Sci., **9**, 1989, 1-15.

[15] Scheppach W., Fabian C., Sachs M. Kasper H., Scand. J.: Gastroenterol., 1988, **23**, 755-759.
[16] Schogll G.: Modern analytical and rheological methods in quality control of cereals and cereal products. 9th World Congress of Food Science and Technology, Budapest, 1995.
[17] Siljeström M., Eliasson A-C., Björck I.: Starch/Stärke, **41**, 1989, 147-151.
[18] Soral-Śmietana M.: Acta Acad. Agricult. Tech. Olst., 1992, **24**, 3-57.
[19] Soral-Śmietana M., Śmietana Z., Fornal J.: Potato starch modified by HTST extrusion process. Proceedings on 4th International Workshop on Carbohydrates as Organic Raw Materials. Vienna, 1997
[20] Wahlqvist M.L., Wilmshurst E.G., Murton C.R., Richardson E.N., Am. J. Clin. Nutr., **31**, 1978, 1998-2001.
[21] Zobel H.F.: Starch Granule Structure; in: Developments in Carbohydrate Chemistry, ed. Alexander R.J., Zobel H.F., St. Paul, Minnesota, 1992, 1-37.

TECHNOLOGICZNE I SENSORYCZNE ASPEKTY ZASTOSOWANIA NOWYCH PREPARATÓW SKROBI OPORNEJ (RS) W PROCESIE WYPIEKU

Streszczenie

Skrobiowe produkty żywnościowe po procesach hydrotermicznych, mogą być znacznym źródłem skrobi opornej na działanie enzymów trawiennych ("resistant starch" – RS). Również chleb zawiera skrobię oporną. Ze względu na znaczące jego spożycie w naszej diecie, wydało się interesujące określenie jak wpłynie dodatek preparatów skrobi opornej, otrzymanych wg polskiego zastrzeżenia patentowego nr P.325981 ze skrobi pszennej, ziemniaczanej, kukurydzianej i tapiokowej, na jakość technologiczną i sensoryczną oraz na poziom RS w gotowym produkcie.

Wyniki doświadczenia, w którym zastosowano podczas tworzenia ciasta pszennego 10% udział preparatów skrobi opornej z różnych źródeł botanicznych wskazały wyraźne oddziaływanie na wodochłonność mąki, powodując jej zwiększenie podczas tworzenia ciasta od 4 do 7%. Cechy reologiczne ciasta otrzymanego z handlowej mąki pszennej o słabej jakości technologicznej z udziałem preparatów RS uległy zmianie. Wydłużył się nieznacznie czas rozwoju ciasta, poprawie uległa konsystencja tworzonego ciasta lecz osłabiła się trwałość jego struktury podczas miesienia. Farinograficzna liczba jakości (FQN) obniżała się w stosunku do próby kontrolnej w takim samym stopniu przy udziale wszystkich badanych preparatów skrobi opornej.

Na podstawie wyników panelowej oceny wykonanej metodą profilowania sensorycznego, w której brano pod uwagę 16 wyróżników jakościowych oraz ocenę pożądalności ogólnej w kategoriach hedonicznych stwierdzono, że najkorzystniej na wyróżniki smakowe i zapachowe wpłynął udział preparatu RS pszennej. Z kolei dodatek preparatów RS tapiokowej i kukurydzianej sprzyjał zaznaczeniu mniej korzystnych wyróżników, jak mdły czy mączny.

Badanie cech reologicznych miękiszu wskazało większą twardość miękiszu w porównaniu do próby kontrolnej, mierzoną po 1 godz. od wypieku. Natomiast w ciągu 72 godz. stwierdzono zmniejszenie elastyczności i kohezyjności. Stabilny poziom twardości miękiszu, przy zastosowaniu preparatów RS pszennej i ziemniaczanej podczas 72 godz., sugeruje minimalne objawy starzenia tego chleba.

Pomiary zawartości skrobi odpornej w symulowanych warunkach *in vivo* wykazały, w stosunku do próby kontrolnej, tendencję zwiększania zawartości RS w produkcie przy zastosowaniu badanych preparatów.

J. SZOSTAK-KOTOWA, J. WITALIS

THE COMBINED EFFECT OF UV-RADIATION AND SOIL MICROORGANISMS ON THE BIODEGRADABILITY OF POLYETHYLENE PACKAGE FILM WITH STARCH ADDITIVE

Abstract

Studies were carried out on the degradation of polyethylene package film with 5% of starch (MALEN E FABS 23 DO 22), which was exposed to UV rays (low pressure mercury lamps Philips UV-A TL/05, 15 W power, emission maximum 365 nm) and soil microorganisms. Film of the 0,04–0,1 mm thickness film was exposed to UV radiation for 60 h, 120 h, 180 h, 240 h, 300 h and 800 h. The exposed film was stripped up into 150 mm × 15 mm pieces and underwent the soil burial test for 24 weeks in the temp. of 28–30°C and relative humidity of 20–30%. The degradation was estimated based on the tensile strength. The impact of the UV-radiation exposure time and soil microorganisms on the mechanical properties of the polyethylene film with starch were analysed by the regression models. The 3-rd order polynomial model was fitted to empirical data. The tensile strength was turned out to be useful measure of the mechanical changes in the polyethylene film.

Introduction

Polyethylene is a polymer which, beside polyvinyl chloride, has the highest share in the production of plastics in the world [4] and at the same time is one of the most difficult degradable polymer in the environment [1]. It is attempted to enhance the degradability of polyethylene waste e.g. by the addition of a natural polymer like starch as a filling material [3]. The use of starch is based on an assumption that it is easily biodegradable giving rise to a decrease in weight and making the structure of the remaining part of the polymer looser. Such a porous material can be more easily saturated with oxygen and colonised by microorganisms which enhances its biodegradability.

The degradation of polymers can occur due to the process of photodegradation [5]. It takes place by the action solar radiation, especially the ultraviolet rays, which damage the polymers. In the process of photodegradation, three stages can be distinguished, namely:

Department of Microbiology, Cracow University of Economics, Sienkiewicza 5, 30-033 Cracow

destruction (the abstraction of hydrogen atoms from the chain), cross-linking (the formation of transverse bondings) and degradation (the cracking of main chains). It is believed to be the initial path for the degradation of plastics in the environment.

The aim of this work was the estimation of the degree of the degradation of a film of polyethylene with added starch exposed to UV-radiation and the action of soil microflora.

Material and methods

Material

The studies were carried out on LDPE FGNX 18-D O22 samples with the addition of a biodegradable concentrate BIO-50 (in the amount yielding 5% of potato starch in the film), manufactured in Chemical Works in Kędzierzyn-Koźle. The thickness of the film varied between 0.04 and 0.1 mm.

UV Irradiation

UV irradation was based on ASTM D5208-91 [2]. Superactinic low pressure mercury lamps Philips UV-A TL/05 (15 W power, UV-A radiation power 2.1 W, 300–500 nm spectrum range, emission maximum – 365 nm) were employed for the irradiation. Four lamps were mounted in equal distances on a wooden frame of 440 mm × 320 mm dimensions. A band of film stretched parallel to the plane of the lamps was irradiated from a distance of 140 mm. The films were irradiated in daily cycles: 12 h/12 h (lamps switched on 9 a.m. to 9 p.m., lamps switched off 9 p.m. to 9 a.m.). The total time of the irradiation of individual film samples was 60 h, 120 h, 180 h, 240 h, 300 h and 800 h. The irradiated film samples were cut along the band into strips of 150 mm × 15 mm dimensions (5 pieces for each measurement).

Soil burial test

The films irradiated previously by the UV lamp were incubated in containers with microbiologically active soil containing: peat, river sand, compost earth and manure, all in equal parts. The incubation of the film in the soil was carried out at the temperature of 28–30°C and relative humidity of 20–30% for 24 weeks.

The films subjected to the combined effect of the UV irradiation and the soil agents were compared with (1) sufficiently long UV irradiated films but not subjected to the soil burial test, (2) brand new films (subjected neither to irradiation nor to the soil burial test).

Determination of strength characteristics

The tensile strength was determined according to the standart PN-68/C-89034 [6] and using a device for the study of the mechanical strength – Zwick 1445. The initial distance of jaws was 50 mm and the testing rate 50 mm/min.

The tensile strength was calculated using the formula:

$$N = F \times P^{-1} \; [N \times m^{-1}],$$

where: F is the maximum measured force [N]
P is the sample cross section area [m^2]
The obtained results were subjected to variance analysis.

Results

The impact of the UV radiation exposure time and soil microorganisms on the mechanical properties of the polyethylene film with starch is analysed by the regression models. The tensile strength has turned out the useful measure of the mechanical changes in the polyethylene film. The theory of the photodegradation predicts the three-phase of the process [5]. The first phase, *the destruction*, leads to the lowering the tensile strength of the polyethylene film. In the next phase, *the cross-linking*, the tensile strength increases. In the last phase, *the degradation*, the UV-radiation decreases the tensile strength again. It follows that the polynomial of the 3-rd order seems to be the best model for the tensile strength. The results of the estimation of the model are presented in table 1.

Table 1

The results of the estimation of the 3-rd order polynomial for tensile strength of the polyethylene with starch (Variable: time of the UV radiation [hours])

Variable	Estimate	Standard error	Student t	p-value
Time	-.016804	.007324	-2.295	.01429
$Time^2$	$5.87284*10^{-5}$	$3.2562*10^{-5}$	1.804	.04096
$Time^3$	$-5.11442*10^{-8}$	$3.0236*10^{-8}$	-1.692	.06004
Intercept	7.591848	.394814	19.229	.00000

$R^2 = 0.30590$

The impact of the UV-radiation on the tensile strength is shown in fig.1.

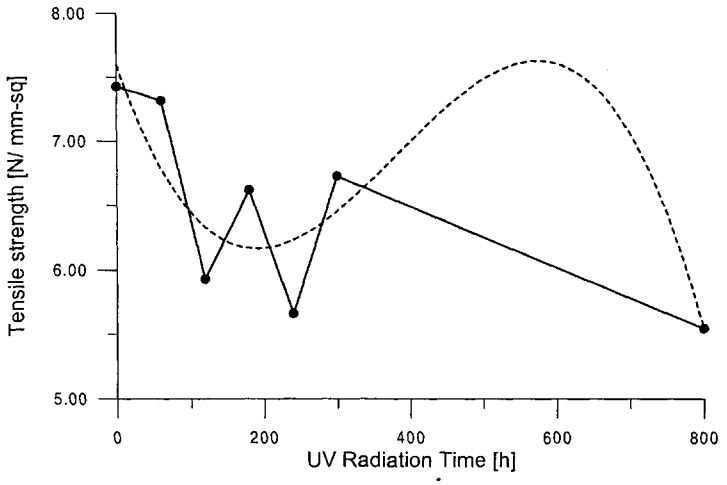


Fig. 1. Tensile strength of the polyethylene film with starch after 6 months soil burial test (empirical values and the 3rd order polynomial fit).

Conclusions

1. The soil microorganisms have statistically significant impact on the degradation of the polyethylene film with starch exposed previously to UV-radiation.
2. The polynomial of the 3-rd order is the best model of tensile strength of the polyethylene film with starch exposed to combined effect of UV-radiation and soil burial test.

REFERENCES

[1] Albertsson A.-Ch., Andersson, S. O., Karlsson, S.: The mechanisms of biodegradation of polyethylene. Polymer degradation and stability, **18**, 1987, 73-87.

[2] ASTM D 5208-91 Standart Practice for Operating Fluorescent Ultraviolet (UV) and Condensation Apparatus for Exposure of Photodegradable Plastics.

[3] Barenstedt C.: Environmental Degradation of Starch-modified Polyethylene: Degradation Products and Long-term Properties. Royal Institute of Technology, Stockholm, 1994, 10-18.

[4] Błędzki A.K.: Recykling materiałów polimerowych. WNT, Warszawa, 1997.

[5] Łączyński B.: Tworzywa wielkocząsteczkowe. Rodzaje i własności. WNT, Warszawa, 1982, 308-309.

[6] PN-68/C-89034 Tworzywa sztuczne. Oznaczanie cech wytrzymałościowych przy statycznym rozciąganiu.

POŁĄCZONY WPŁYW PROMIENI UV I MIKROORGANIZMÓW GLEBOWYCH NA BIODEGRADACJĘ OPAKOWANIOWEJ FOLII POLIETYLENOWEJ Z DODATKIEM SKROBI

Streszczenie

Celem pracy było zbadanie w warunkach laboratoryjnych połączonego wpływu fotodegradacji i biodegradacji na właściwości mechaniczne folii opakowaniowej z polietylenu z dodatkiem 5% skrobi (MALEN E FABS 23 DO 22 produkcji Zakładów Chemicznych w Kędzierzynie Koźlu). Folię o grubości 0,04-0,1 mm naświetlano promieniami UV przez okres 60 h, 120 h, 180 h, 240 h, 300 h i 800 h przy użyciu niskoprężnych lamp rtęciowych Philips UV-A TL/05 (moc 15 W, moc UV-A 2,1 W, max. emisji 365 nm). Następnie poddano ją przez 24 tygodnie działaniu mikroorganizmów metodą testu glebowego w temp. 28–30°C i wilgotności względnej 20–30%. Stopień degradacji polietylenu oceniano na podstawie zmian naprężenia maksymalnego. Otrzymane wyniki poddano analizie wariancji. Z przeprowadzonych badań wynika, że: 1) mikroflora gleby wpływa w sposób statystycznie istotny na degradację folii polietylenowej z 5% dodatkiem skrobi, 2) właściwości mechaniczne badanej folii polietylenowej, poddanej działaniu promieni UV, ulegają trójfazowym zmianom, najlepiej opisanym przez model, w którym czas naświetlania występuje w postaci wielomianu stopnia trzeciego.

R. ZIOBRO, H. GAMBUŚ, A. NOWOTNA, A. BALA-PIASEK, R. SABAT

STARCH EXTRUDATES AS A SOURCE OF LOW MOLECULAR DEXTRINS SLOWING DOWN BREAD STALING

Abstract

It has been observed that low mass dextrins can prevent bread staling by modifying gluten-starch interaction. Extruded starch is significantly decomposed. Thus it could be used for an increase of level of low molecular mass dextrins in bread. Studies were carried out on effect of starch extrudates supplement on the quality and staling of the baked pup loaves.

Introduction

Bread staling includes all changes that take place after baking, unless they are caused by microorganisms. This includes organoleptic changes in taste, smell and texture (crumb hardens and loses its elasticity, crust softens and becomes chewy) but also structural and molecular changes, which include loss of water binding capacity of crumb, water solubility, enzymatic susceptibility of starch, microscopic changes in crumb and increase of starch crystallinity [Kim, D'Appolonia, 1977, D'Appolonia, Morad 1981].

Up-to date many different models of staling have been suggested. They are all based on a fact that bread dough consists of three main components: starch, water and proteins in proportion: 6:5:1. The models try to explain their role in establishing bread microstructure and staling. Martin and Hoseney [1991] in their model focused on the interaction between swollen starch granules and continuous gluten phase. They observed that weak hydrogen bonds make the structure elastic, but in time the bonds become stronger and more numerous, which is the cause of textural changes. This model is consistent with experiments which show that crumb hardening is higher when starch granules are more swollen. It also explains why only dextrins with long enough

Faculty of Food Technology, Agricultural University of Cracow, Poland

DP (12-15) could stabilize crumb structure thus increasing staling. Dextrins with DP less than 9 are to short for cross linking so they have an opposite effect. Many other models explain staling as an effect of starch recrystalization [Kim, D'Appolonia, 1977, Neukom, Rutz, 1981, Krog et al., 1989]. It seems that staling is a complex process, which depends on the gelatinization and swelling of starch granules, as well as on retrogradation, changes in moisture content and interactions between the components of crumb. We can't neglect any of these factors [Gambuś 1997].

Preparations of bacterial alpha-amylases have been shown to reduce the process of crumb hardening by supplying low molecular weight dextrins [Martin, Hoseney 1991, Lin, Lineback, 1990]. But such enzymatic preparation can also cause some uncontrolled changes in stored bread. An interesting alternative for such treatment is the modification of dough by decomposed starch supplement [Gambuś 1997]. To avoid chemical contamination, for our experiments we have chosen extrusion cooking- clean and rapid method of starch degradation.

Materials and methods

A single-screw extruder (Brabender 20DN) was used to extrude the starches: wheat, corn, potato. The starch moisture contents were adjusted to 16% or 24% (dry basis). The temperatures in 3 barrel sections of extruder were: 80, 120 and 150, the screw speed was maintained at 210 rpm.

For the SEC analysis we used four columns with Sephacryl gel: S-200 (1.6 x 50 cm), S-200 (1.6 x 82 cm), S-500 (1.6 x 90 cm) and S-1000 (1.6 x 88 cm). 2 cm^3 of DMSO solution containing 0.025 g of starch extrudates were eluted with aqueous 0.005 M NaOH. RI Detector was used for on-line detection.

To check if the dextrins present in the extrudates may have similar effect as those produced by enzymes, the extrudates were milled and used for baking of 40 g pup loaves. Laboratory bread baking was performed using straight method. The dough contained 80 g of starch, 20 g of gluten, 8 g of sugar, 3 g of salt, 1.5 g of yeast and 70 cm^3 of water. In supplemented pup-loaves 3% of starch was replaced with extrudates. On the day of baking: bread volume, organoleptic scores, and penetration (hardness) using PNR - 10 penetrometer were estimated.

In addition, the process of staling during 3 days of storage in plastic bags at 23-24°C at relative humidity 64% were assessed. Following parameters were considered: changes in crumb humidity, dry substances of crust and crumb penetration.

On each day water extract of crumb was prepared by modified method of Neukom and Rutz [1981]. Blue value was measured as an indicator of free amylose present in bread crumb [Morrison, Laignelet 1983].

SEC chromatography was applied to the water extracts of bread crumb. Total carbohydrate determination by anthron (540 nm) in 5 cm^3 fractions was done to measure low mass dextrins content.

Results and discussion

Due to extrusion molecular-level processes take place. Starch melts or gelatinize, depending on moisture content [Qu, Wang, 1994]. Starch granules reduce their size [Zheng et al., 1995] and change their shape. Some glycosidic bonds within glucans break [Davidson et al., 1984].The most important factors which affect the properties of extruded starch are barrel temperature, mechanical shear and moisture content in raw material [Owusu-Ansah et al., 1983, Diosady et al. 1985, Cai et al., 1995]. Generally, it's hard to predict which of these factors will be decisive in each situation [Cai, Diosady, 1993].

To establish at which moisture content level, at the same barrel temperature profile, starch was more decomposed, we have compared SEC profiles of the extruded products. The results (Fig. 1) were consistent with previously reported by other authors [Yamada et al., 1990, Chinnaswamy, Hanna, 1990, Davidson et al., 1984]. Starches extruded at lower moisture content contained glucans with lower DP. Thus they were chosen to be added to bread dough.

Our previous SEC experiments show that the extent of degradation during extrusion is significant and comparable for different botanical sources of starch [Ziobro R.

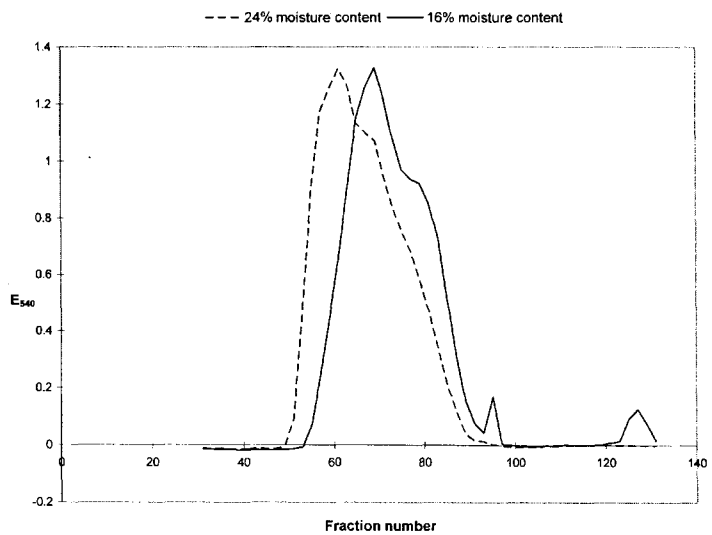


Fig. 1. SEC profile of wheat starch extruded at different moisture content.

et al., 1998]. Decomposed starch could be a good source of low molecular mass dextrins in bread, even if it wouldn't be much degraded. The processes (mainly enzymatic) that take place in bread during its preparation and baking cause some further degradation, and even after processing of a standard dough, where almost all starch glucans are intact, we could observe some oligosacharides in water extract of crumb (Fig. 2).

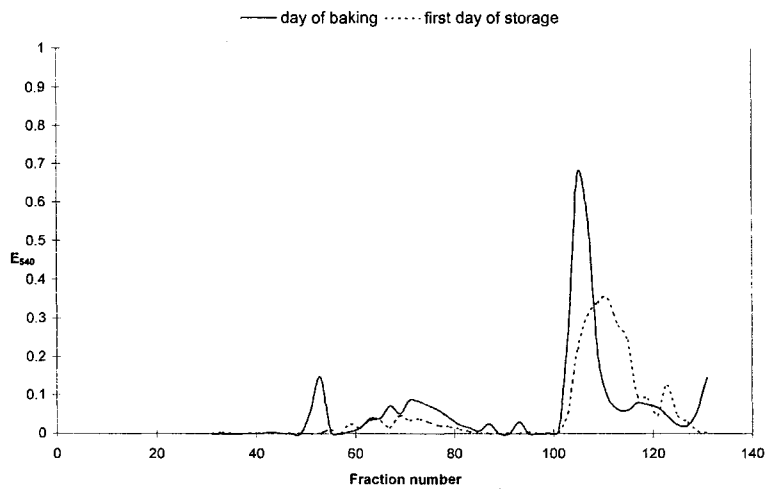


Fig. 2. SEC profiles of standard bread water extract.

Table 1 contains the data obtained on the day of baking and during storage of breads baked with supplement of extrudates. The volume of pup loaves was not affected by the addition of extruded starch. Organoleptic scores of the supplemented pup loaves were good, in two cases better than of one without extrudate. Crumb humidity of supplemented pup loaves was low in comparison with standard. Higher level of dry substance of crust suggests, that the extruded starches were converted into sugars, which produced more caramel.

The addition of extrudates reduced the hardening process as observed by penetration changes during storage period (Tab. 1). Changes in blue value, corresponding to soluble amylose content, were comparable for all breads. Therefore we think that in this case retrogradation was not responsible for the observed differences in penetration changes. Thus in our SEC experiments we concentrated on the range of low molecular masses. The results indicate that on the day of baking all pup loaves with the supplement of extrudates contained more dextrins and that these dextrins were shorter than in the standard pup loaf (Fig. 3).

Table 1

Quality assessment of model breads

Bread type	Volume	Organoleptic assessment		Day	Crumb humidity	Dry substance of crust	Penetration	Blue value
		Total points	Quality class		[%]	[%]	[mm]	
Standard bread	102.5	33	II	0	40.90	70.13	9.27	0.11
				1	40.60	72.25	5.10	0.04
				2	35.45	72.25	3.30	0.02
				3	34.20	70.85	2.69	0.02
Bread supplemented with corn starch extrudate	102.5	37	I	0	37.75	84.37	9.99	0.12
				1	33.00	75.30	4.36	0.04
				2	32.45	73.00	4.19	0.03
				3	32.20	70.85	3.45	0.04
Bread supplemented with wheat starch extrudate	109.2	38	I	0	37.43	82.09	12.40	0.14
				1	35.46	73.45	6.50	0.05
				2	33.25	72.67	4.36	0.03
				3	32.60	71.94	4.12	0.03
Bread supplemented with potato starch extrudate	118.5	31	II	0	40.04	81.54	12.30	0.12
				1	36.68	74.07	6.00	0.05
				2	32.31	73.15	5.70	0.05
				3	30.78	72.36	5.20	0.04

0 - day of baking; 1- first day after baking; 2- second day after baking; 3- third day after baking.

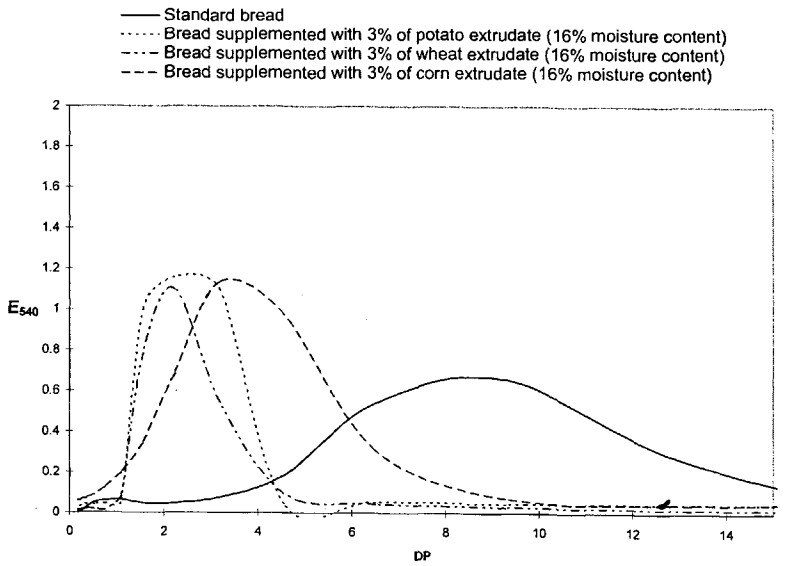


Fig. 3. Low molecular mass dextrins present in the water extracts of crumb prepared from pup loaves.

After storage the amount of dextrins with higher DP was reduced while the quantity of dextrins with lower DP was still the same or even greater. It is probably the result of interactions between dextrins with DP>9 with continous gluten phase, which could have an impact on crumb hardening (Fig. 4a,b,c,d).

Pup loaf supplemented with corn extrudate (Tab. 1) was characterized by parameters similiar to the standard one. It could be explained by the presence of dextrins larger than in other pup loaves (with potato and wheat extrudates) on the day of baking (Fig. 3).

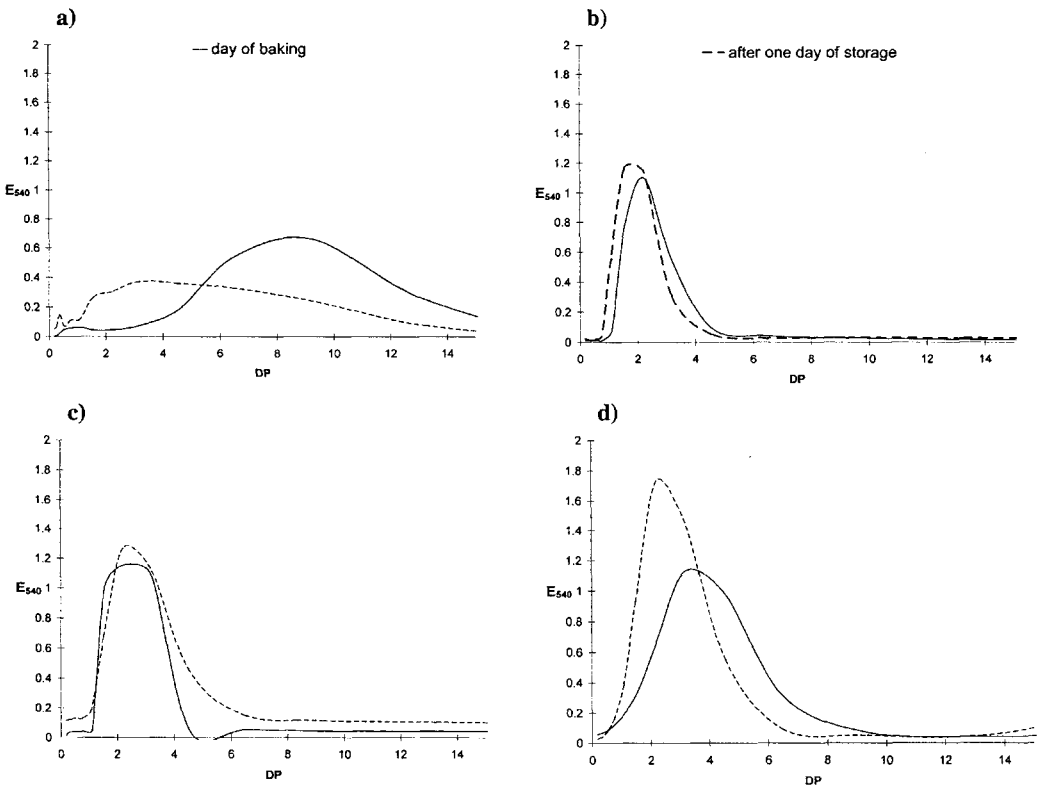


Fig. 4. Changes in fraction of low molecular mass dextrins present in the water extracts of crumb prepared from standard (a) and supplemented with corn (b), wheat (c) and potato starch extrudates (d) pup loaves.

The results show that bread staling corresponds to low mass dextrins content which could be acquired by addition not only of the enzymes but also starch degradation products such as extrudates.

Conclusions

Starch extruded at lower moisture content was more decomposed.

Addition of wheat, potato or corn starch extrudates doesn't affect or even improves organoleptic properties of model bread so they can be used as its component.

All pup loaves with supplement of extrudates were characterized by softer and more elastic crumb on the 3 day after baking than standard.

The reduction of crumb hardening seems to be less affected by changes in retrogradation process and more influenced by low mass dextrins presence.

The authors thank dr hab. A. Golachowski from Agricultural Academy of Wrocław for his help.

REFERENCES

[1] Cai W., Diosady L.L., Rubin L.J.: Degradation of wheat starch in a twin-screw extruder, J. Food Eng., **26**, 1995, 289-300.

[2] Cai W., Diosady L.L.: Model for gelatinization of wheat starch in a twin-screw extruder, J. Food Sci., **58** (4), 1993, 872-875.

[3] Chinnaswamy R., Hanna, M.A.: Macromolecular and functional properties of native and extrusion-cooked corn starch, Cereal Chem, **67** (5), 1990, 490-499.

[4] D'Appolonia B.L., Morad M.M.: Bread staling, Cereal Chem., **58**, 1981, 186-190.

[5] Davidson V.J., Paton D., Diosady L.L., Larocque G.: Degradation of wheat starch in a single-screw extruder: characteristics of extruded starch polymers, J. Food Science, **49** (2), 1984, 453-459.

[6] Diosady L.L., Paton D., Rosen N., Rubin L.J., Athanassoulias C.: Degradation of wheat starch in a single-screw extruder: mechano-kinetic breakdown of cooked starch, J. Food Sci., **50**, 1985, 1697-1699.

[7] Gambuś H.: Wpływ fizyczno-chemicznych właściwości skrobi na jakość i starzenie się pieczywa, Zesz. Nauk. AR, Kraków, 1997, Rozprawy nr 226.

[8] Kim S.K., D'Appolonia B.L.: The role of wheat flour constituents in bread staling. The Bakers Digest, **51**, 1977, 38-44.

[9] Krog N., Olesen S.K., Toernaes H., Joensson T.: Retrogradation of the starch fraction in wheat bread. Cereal Foods World, **34**, 1989, 282-285.

[10] Lin W., Lineback D.R.: Changes in carbohydrate fractions in enzyme-supplemented bread and the potential relationschip to staling, Starch/Stärke, **42**, 10, 1990, 385-394.

[11] Martin M. L., Hoseney R.C.: A mechanism of bread firming II. Role of starch hydrolyzing enzymes. Cereal Chem, **68**, 1991, 503-507.

[12] Morrison W.R., Laignelet B.: An improved colorimetric procedure for determining apparent and total amylose in cereal and other starches. J. Cereal Sci., **1**, 1983, 9-20.

[13] Neukom H., Rutz W.: Observation on starch retrogradation and bread staling, Lebensm. Wiss. u. Technol., **14**, 1981, 292-295.

[14] Owusu-Ansah J., van de Voort F.R., Stanley D.W.: Physicochemical changes in cornstarch as a function of extrusion variables, Cereal Chem, **60**, 4, 1983, 319-324.

[15] Qu D., Wang S.S.: Kinetics of the formations of gelatinized and melted starch at extrusion cooking conditions, Starch/Stärke, **46**, 6, 1994, 225-229.
[16] Yamada T., Suzuki K., Katuzaki H., Hisamatsu M., Komiya T.: GPC profile change of potato starch with extrusion processing, Starch/Stärke, **42**, 6, 1990, 217-223.
[17] Zheng X., Chiang W.C., Wang S.S.: Effect of shear energy on size reduction of starch granules in extrusion, Starch/Stärke, **47**, 4, 1995, 146-151.
[18] Ziobro R., Gambuś H., Nowotna A., Sabat R.: Starch extrudates in baking, Production and Uses of Starch International Conference, 6-8 April 1998, Edinburgh.

EKSTUDATY SKROBIOWE JAKO ŹRÓDŁO NISKOCZĄSTECZKOWYCH DEKSTRYN SPOWALNIAJĄCYCH STARZENIE SIĘ CHLEBA

Streszczenie

Zaobserwowano, że niskocząsteczkowe desktryny mogą zapobiegać starzeniu się pieczywa wpływając na interakcje pomiędzy glutenem i skrobią. Skrobia ekstrudowana jest w znacznym stopniu rozłożona, może więc być ona użyta do podniesienia zawartości niskocząsteczkowych dekstryn w chlebie. Dlatego w pracy przebadano wypływ 3% dodatku ekstrudatów skrobi pszennej, kukurydzianej i ziemniaczanej na jakość i starzenie się modelowych chlebków. Ekstrudaty skrobiowe sporządzano w jednoślimakowym ekstruderze laboratoryjnym Brabender 20 DN. Stwierdzono, że udział skrobi ekstrudowanych w cieście modelowych chlebków spowodował poprawę ich oceny organoleptycznej oraz zahamował twardnienie miękiszu, co na podstawie analizy chromatograficznej (GPC) ekstraktu z miękiszu przypisano roli niskocząsteczkowych dekstryn pochodzących z ekstrudatów.

Adresy Zarządu Głównego, Oddziałów i Sekcji Polskiego Towarzystwa Technologów Żywności

PREZES/ODDZIAŁ	ADRES
Prof. dr hab. Nina Baryłko-Pikielna Zarząd Główny	Polskie Towarzystwo Technologów Żywności ul. Rakowiecka 36, 02-532 WARSZAWA Tel/Fax (+22) 646 68 72
Prof. dr hab. Piotr Bykowski Oddział Gdański	ul. Kołłątaja 1 (MIR), 81-332 GDYNIA Tel.: (+58) 620 511; Fax.: (+58 620 28 31
Prof. dr hab. Zdzisław Targoński Oddział Lubelski	ul. Skromna 8 (AR), 20-704 LUBLIN Tel.: (+81) 52 54 770; Fax.: (+81) 533 57 33
Prof. dr hab. Jadwiga Wilska-Jeszka Oddział Łódzki	ul. Stefanowskiego 9/10 (PŁ) 90-942 ŁÓDŹ Tel.: (+42) 313 433/313 435; Fax. (+42) 313 402
Prof. dr hab. Tadeusz Sikora Oddział Małopolski	ul.Sienkiewicza 5 (AE), 30-033 KRAKÓW Tel.: (+12) 633 08 21; Fax.: (+12) 633 57 33
Dr hab. Andrzej Kuncewicz Oddział Olsztyński	Plac Cieszyński 1 (ART.), 10-957 OLSZTYN Tel.: (+89) 523 37 03; Tel./Fax (+89) 523 35 54
Prof. dr Kazimierz Lachowicz Oddział Szczeciński	ul.Kazimierza Królewicza 3 (AR), 71-550 SZCZECIN Tel.: (+01) 423 10 61
Dr Danuta Kołożyn - Krajewska Oddział Warszawski	ul. Nowoursynowska 166 (SGGW), 02-787 WARSZAWA Tel.: (+22) 843 90 41 w 1200
Prof. dr hab. Edward Pospiech Oddział Wielkopolski	ul. Wojska Polskiego 31 (AR), 60-624 POZNAŃ Tel.: (+61) 848 72 60, Fax.: (+61) 848 71 46
Prof. dr hab. Teresa Smolińska Oddział Wrocławski	ul. Norwida 25/27 (AR), 50-375 WROCŁAW Tel.: (+71) 205 284; Fax.: (+71) 205 273
SEKCJE	
Prof. dr hab. Barbara Szteke Analizy i Oceny Żywności	ul. Rakowiecka 36 (IBiPR-S), 02-532 WARSZAWA Tel. (+22) 499 167
Ekonomiczna dr Karol Krajewski	ul. Nowoursynowska 166 (SGGW), 02-787 WARSZAWA Tel.: (+22) 843 90 41
Technologii Mięsa prof. dr hab. Jan Mroczek	ul. Grochowska 272 (SGGW), 03-849 WARSZAWA (+22) 810 01 63
Technologii Tłuszczów prof. dr hab. Bronisław Drozdowski	ul. Narutowicza 11/12 (PG), 80-952 GDAŃSK Tel.: (+58) 347 15 37
Młodej Kadry mgr Iwona Jabłonowska	ul. Kazimierza Królewicza 4 (AR), 71-450 SZCZECIN Tel.: (+91) 423 10 61; Fax.: (+91) 423 13 47

Informacja dla Autorów

Pragniemy przekazać Państwu podstawowe informacje, które powinny ułatwić pracę redakcji i ujednolicić wymagania wobec nadsyłanych materiałów.

1. Będziemy na naszych łamach zamieszczać zarówno oryginalne prace naukowe, jak i artykuły przeglądowe, które będą miały ścisły związek z problematyką żywności.
2. Planujemy również zamieszczać recenzje podręczników i monografii naukowych, omówienia z naukowych czasopism zagranicznych, sprawozdania z konferencji naukowych itp.
3. Prace prosimy nadsyłać na dyskietce wraz z wydrukowanymi 2 egzemplarzami (format A4, maksymalnie 30 wierszy na stronie, 60 znaków w wierszu).
4. Objętość prac oryginalnych, łącznie z tabelami, rysunkami i wykazem piśmiennictwa nie powinna przekraczać 12 stron.
5. Na pierwszej stronie nadesłanej pracy (1/3 od góry pierwszej strony należy zostawić wolną, co jest potrzebne na uwagi wydawniczo-techniczne) należy podać: pełne imię i nazwisko Autora(ów), tytuł pracy, nazwę i adres instytucji zatrudniającej Autora(ów), tytuł naukowy.
6. Publikacja winna stanowić zwięzłą, dobrze zdefiniowaną pracę badawczą, a wyniki należy przedstawić w sposób możliwie syntetyczny (dotyczy oryginalnych prac naukowych).
7. Do pracy należy dołączyć streszczenia w języku polskim i w języku angielskim. Streszczenia powinny zawierać: imię i nazwisko Autora(ów), tytuł pracy i treść – maksymalnie 10 wierszy.
8. Nadsyłane oryginalne prace naukowe powinny zawierać następujące rozdziały: Wstęp, Materiał i metody, Wyniki i dyskusja, Wnioski (Podsumowanie), Literatura.
9. Literatura powinna być cytowana ze źródeł oryginalnych. Spis literatury winien być ułożony w porządku alfabetycznym nazwisk autorów. Każda pozycja powinna zawierać kolejno: liczbę porządkową, nazwisko i pierwszą literę imienia autora(ów), tytuł pracy, tytuł czasopisma, tom, rok, strona początkowa. Pozycje książkowe powinny zawierać: nazwisko i pierwszą literę imienia autora(ów), miejsce i rok wydania, tom. Informacje zamieszczone w alfabecie niełacińskim należy podawać w transliteracji polskiej. Spis literatury nie powinien zawierać więcej niż 30 najważniejszych pozycji.
10. Tabele i rysunki winny być umieszczone na oddzielnych stronach. Rysunki powinny być wykonane na kalce tuszem lub wydrukowane na drukarce laserowej. Każdy rysunek powinien być numerowany kolejno na odwrocie ołówkiem, należy również podawać nazwisko Autora i tytuł pracy, w celu łatwiejszej identyfikacji. Podpisy rysunków i tytuły tabel oraz objaśnienia w tabelach należy podać **w językach polskim i angielskim.**
    Rysunki wykonane za pomocą komputera prosimy dołączyć na dyskietce w formacie TIF lub WMF.
11. Materiałem ilustracyjnym mogą być również fotografie, wyłącznie czarno–białe.
12. Korektę prac wykonuje na ogół redakcja na podstawie maszynopisu pracy zakwalifikowanej do druku, uwzględniając uwagi recenzenta i wymagania redakcji. W przypadku daleko idących zmian, prace będą przesyłane Autorom.
13. Za prace ogłoszone w naszym kwartalniku Autorzy nie otrzymują honorarium, natomiast otrzymują egzemplarz autorski.
14. Materiały przesłane do redakcji nie będą zwracane Autorom.

ISSN 1425-6959

Wydanie czasopisma dofinansowane jest ze składek członków wspierających Polskiego Towarzystwa Technologów Żywności: **Agro Food Technology Sp. z o.o.** Czeladź; **Agros Holding SA** Warszawa; **Akwawit** Leszno; **Alima-Gerber SA** Rzeszów; **Animex SA** Warszawa; **Bielmar** Bielsko-Biała, **Biolacta Texel-Rhodia** Olsztyn, **Bolmar** Bodaczów, **Celiko SA** Poznań; **Coca-Cola Poland Services Ltd** Warszawa; **Hortimex Sp. z o.o.** Konin; **Krajowe Stowarzyszenie Mleczarzy** Warszawa, **Nadodrzańskie Zakłady Przemysłu Tłuszczowego** Brzeg; **Pekpol SA** Warszawa; **Pepsico Trading Sp. z o.o.** Warszawa; **Piast Browary** Wrocław; **Pozmeat SA** Poznań, **Poll Ltd Sp. z o.o.** Warszawa; **Regis Sp. z o.o.** Wieliczka; **Rolimpex SA** Warszawa; **PHU SIC** Gościnno, **Spółdzielnia Produkcji Piekarskiej i Ciastkarskiej** Kraków; **Van den Bergh-Foods Polska SA** Szopienice; **E.Wedel SA**, Warszawa, **Winiary SA** Kalisz, **Zakłady Przemysłu Tłuszczowego** Warszawa; **Źródło Pniewy Sp. z o.o.** Pniewy.

Warunki prenumeraty

Szanowni Państwo,
uprzejmie informujemy, że przyjmujemy zamówienia na prenumeratę naszego kwartalnika, zarówno Czytelników indywidualnych, jak i od instytucji, co powinno Państwu zapewnić bieżące otrzymywanie kolejnych wydawanych przez nas numerów.
Cena jednego egzemplarza wynosi 10 zł.

Zamówienia na prenumeratę, jak i na poszczególne numery prosimy kierować na adres Redakcji:

PTTŻ Oddział Małopolski

Redakcja Kwartalnika

„ŻYWNOŚĆ TECHNOLOGIA JAKOŚĆ"

31-425 Kraków, Al. 29 Listopada 46

Nr konta: PKO I O/Kraków 10202892-164353-270-1-111