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TECHNOLOGÓW ŻYWNOŚCI

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Suplement

Nr 2(23) Kraków 2000 Rok 7

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otrzymujecie Suplement nr 2(23) kwartalnika „Żywność", w którym publikujemy artykuły będące pokłosiem IX International Starch Convention (IX Międzynarodowej Konferencji Skrobiowej).

Teksty artykułów są wydrukowane w języku angielskim, ze streszczeniami w języku polskim, ponieważ Konferencja miała charakter międzynarodowy.

Wyrażamy nadzieję, że zamieszczone w tym Suplemencie artykuły przybliżą Państwu aktualny stan badań nad skrobią i zostaną przyjęte z zainteresowaniem.

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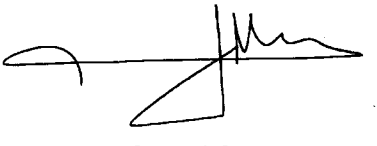

Tadeusz Sikora

This collection of the papers is a regular issue of the "Żywność" ("Food") journal, and at the same time. This volume plays a role of the Proceedings of the IX International Starch Convention held in Cracow on 13–16 June 2000.

The Proceedings can be regarded as a document that provides ample information on a number of topics with respect to structural properties of starch granules and starch gels.

This publication is made possible by the authors, the participants of the IX ISC, and the efforts of the reviewers. On behalf of IX ISC Organizing Committee I acknowledge their contribution with appreciation.

Prof. Dr. Piotr Tomasik, D.Sc.
(Head of IX ISC Organizing Committee)

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W. BERGTHALLER, H.-J. KERSTING

WHEAT – A CHALLENGING SUBSTRATE FOR STARCH PRODUCTION[#]

Abstract

In contrast to the world-wide given situation for maize as the main substrate in starch isolation, wheat gains an advantageous position in the European starch industry although technical starch yield cannot compete fully material prices. Its remarkable position profits also from wheat gluten as valuable by-product. Further indication for rising preference can be seen in the installation of new processing capacities in Europe. However, the economic situation of wheat starch production follows unavoidable fluctuations of wheat gluten markets. Political decisions play an important role, too.

The challenging situation connected with wheat as substrate for starch extraction is result of developments in equipment and remodelling of technology. The most important contribution consisted in an obvious shift of the relation of water to flour used for flour/water mixture preparation, starch and gluten extraction, and refining. This was initialised mainly by the introduction of separation techniques using centrifugal principles. With respect to limited availability of water and increasing costs for waste water treatment reduction of water supply is a steady target.

In close connection to developments in separation technology wheat and wheat flour should gain extended attraction. Published standards are limited and reveal at most characteristics oriented to the Martin process. With respect to recent developments in technology, alternative testing procedures have been proposed. Results demonstrate the suitability and specificity of the „Mixer method", a procedure adapted to flour/water relations in centrifugal separation. But, the time consuming procedure restricts general application. With respect to characteristics describing substrate properties, parameters of conventional wheat quality evaluation systems are measured additionally and assigned to quantities of the mixer method. An extended data base is expected to provide with measures to select the most suitable system for classification of wheat grain and wheat flour.

After all, the outlook should not omit to mention developments in conventional breeding and genetic engineering that will allow to affect starch granule characteristics, molecular structure and composition of wheat starch offering promising prospects in functionality and application of wheat starch.

W. Bergthaller, H.-J. Kersting, Federal Centre for Cereal, Potato and Lipid Research, Schuetzenberg 12, D-32756 Detmold, Germany.

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Introduction – policy and the market

In a recently published review [1] starch policy in the European Community was sketched by its measures and institutional framework. Export refunds (to compete at the world market) and import duties (to repel starch produced in third world countries) were indicated as main measures for the Communities successful activity. These conventional instruments in foreign trade are completed by production refunds given to industrial producers utilising internally manufactured starch in particular in the non-food sector. This system allowed the European starch industry to develop a very strong position on the world market.

A world wide actual collection and evaluation of starch production is extremely difficult to establish. In particular reliable data concerning regions and specific starch substrates are scarcely available. Starch production itself is still characterised by a dominating role of maize which stands on Community level for approximately 50% of the produced starch; a position that is not any longer invincible. Wheat and potatoes are going on to change relevant shares. Since EU potato starch production has been limited by introduction of fixed quotas for all member countries further development is expected to contribute exclusively to increases in the corresponding wheat starch portion. The variability in wheat starch production given within member states can be documented well by their position in an estimate for 1999 [2]. During the last decade the French wheat starch industry grew extraordinarily and leads indubitably with approximately $760 \cdot 10^3$ t, while Germany and Netherlands lie equalised at second and third position with approximately $360 \cdot 10^3$ t. The aforesaid countries dominate European starch production with a share of 75% (Tab. 1). In contrast, the Eastern European countries including Russia are indicated to produce just $113 \cdot 10^3$ t. In comparison to the position of the main wheat starch producers within the EU the generally known production capacity within these countries is very small and potential changes depend highly on economic and to some extent also on political developments (Tab. 2). A comparison of production figures within different regions of the world's production ($3270 \cdot 10^3$ t) documents again the role of the European Community (60%) in having the control over the world market (Tab. 3).

Which factors did promote the increased utilisation of wheat in starch production? Although according to Wintzer et al. [3] net production costs for wheat starch are higher (430 €·t^{-1}) than for maize starch (approx. 380 €·t^{-1}) drastic reductions in substrate prices evaluated on basis of net costs [4] as well as close neighbourhood of wheat production and processing were beneficial factors. Together with a slightly higher processing margin for wheat starch and a lower procurement price for wheat produced in the European Community also Entwistle et al. [5] found small advantages for utilisation of this crop in the UK.

Table 1

Estimates for wheat starch production in the European Community in 1999.

Country	Production [x 10^3 t]
France	761.2
Germany	357.0
Netherlands	357.0
United Kingdom	199.5
Belgium	178.5
Italy	52.5
Finland	31.5
Sweden	24.2
Total	1963.4

Table 2

Estimates for wheat starch production in Eastern Europe in 1999.

Country	Production [x 10^3 t]
Yugoslavia	31.5
Poland	26.2
Czech Republic	16.8
Hungary	13.1
Russia	10.5
Slovak Republic	7.9
Romania	5.2
Slovenia	2.1
Total	113.3

Table 3

Estimates for world-wide wheat starch production in 1999 (x 10^3 t).

Production in total	3270
European Community (8 of 15 countries)	1963
Eastern Europe	113
North and South America (USA, Canada, Mexico, Argentina)	596
ASEAN and Pacific Area	570

To deal with wheat starch without mentioning the role of wheat gluten is politically and economically short-minded since gluten was always an important co-product in the economical evaluation of wheat starch production. However, with regard to relationships between US administration and the European Commission the controversy on restriction of gluten imports to the US (a quota frozen to $25 \cdot 10^3$ t annually since 30 May 1998) just now produces further unpleasant conditions in mutual relationships. According to newspapers it is planned to divide the existing quota into quarterly parts. Authorities of the European Commission, in contrast, insist on removal of existing restrictions [6].

Developments in equipment and technology

Equipment

A comprehensive overview of recent technological developments in the production of wheat starch has been presented in 1997 by Meuser [7] when reporting the extraordinary progress in starch separation from wheat flour by the application of centrifugal forces. In the early stage of separation of wheat flour components 3-phase decanters represented the principle machines which delivered an overflow (light phase) containing high shares of pentosans, a nozzle phase (middle phase) consisting of few pentosans, high portions of B starch, gluten and fibre and a concentrate containing nearly the whole share of A starch and some B starch and fibres. The early and very effective separation of flour into its components was prerequisite for economically improved recovery of starch and vital gluten, and divers by-products. Centrifugal forces were used successfully, too, in replacing static separation procedures of starch refining. Finally, nozzle separators of the 3-phase type were the respective machines that allowed also remarkable progress in formation of economically enhanced process operations, in particular in reduced needs of process water for A starch washing and a high-yielded recovery of co- and by-products [8].

Further focus was given meanwhile to drainage of refined wheat starch by means of pressure filtration. Since its first reference [7] interesting solutions were reported [9-11] that are basis for the following presentation.

Pressure filtration

Vacuum drum filters or discontinuously working peeler centrifuges allow to reach a minimum water content of 36 to 40% in drainage of starch suspensions [12]. It's state of art that free water of this amounts results in thixotropic flow behaviour of starch cakes and impedes their transportation [7]. Based on successful drainage of mineral suspensions [13] pressure filtration has been applied also to starch de-watering, in particular with such starch suspensions where water removal remains critical. With wheat

starch that contains in general a serious amount of fine granules and that tends to produce difficulties in this respect cake formation and drainage can be drastically accelerated [7]. For native wheat A starch as well as for native corn starch the range of available water reduction was reported to reach 33 to 35% with systems of different design [9-11]. Relevant systems installed in starch plants are pressure drum filters [9, 10] and membrane filter presses [11].

In case of pressure drum filters the system consists of a filter drum covered with a specific filter cloth inside a pressure vessel and a discharge for release of drained starch (Fig. 1). Reported pressure levels can range from 1 to 6 bar. Practical working conditions are pressure levels of approximately 3 to 3.5 bar, drum rotation speeds from 1.2 to 1.6 min^{-1} and a specific starch throughput in the range of 450 to 800 $kg \cdot m^{-2} \cdot h^{-1}$. Installed filtration surfaces are given with 10 to 17 m^2 [9, 10].

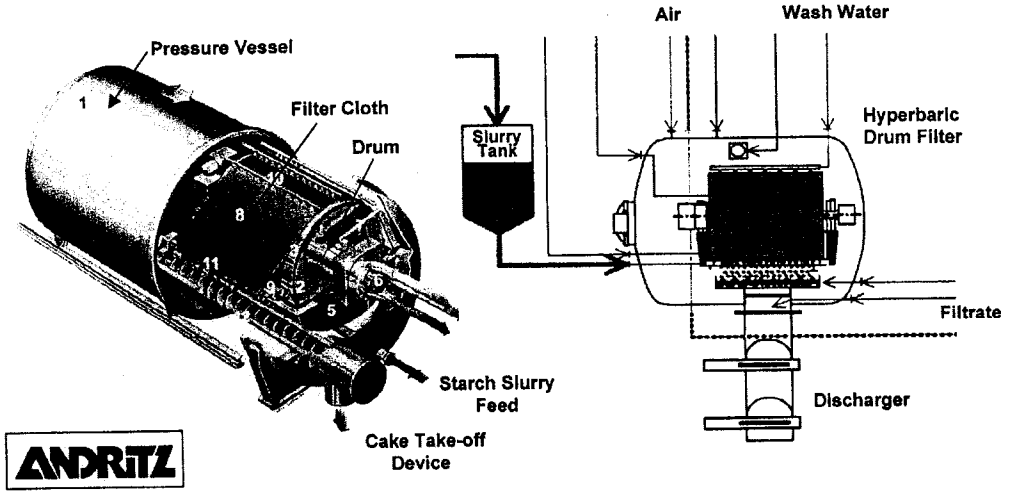


Fig. 1. Pressure drum filter system for starch slurry drainage.

A membrane filter press was presented as so-called diaphragm pressing system (Fig. 2). The filter consists of multiple filter chambers each of 45 to 60 mm height. A continuous filter cloth supported by propylene latticing is running through all filter chambers equipped with 4 to 5 mm thick rubber diaphragms by which the flow of starch suspension, filtrate, wash water and air are directed alternately. Pressure that can rise up to 16 bar is applied via the diaphragm by water. Additional drainage can be induced by blowing air through the pressed filter cake. Final cake water contents can be reduced to 33% for native wheat starch. The specific filtration rate is reported to reach 240 $kg \cdot m^{-2} \cdot h^{-1}$ and the maximum capacity to be 3.4 t. An almost continuous process is reached by a fully automated process operation.

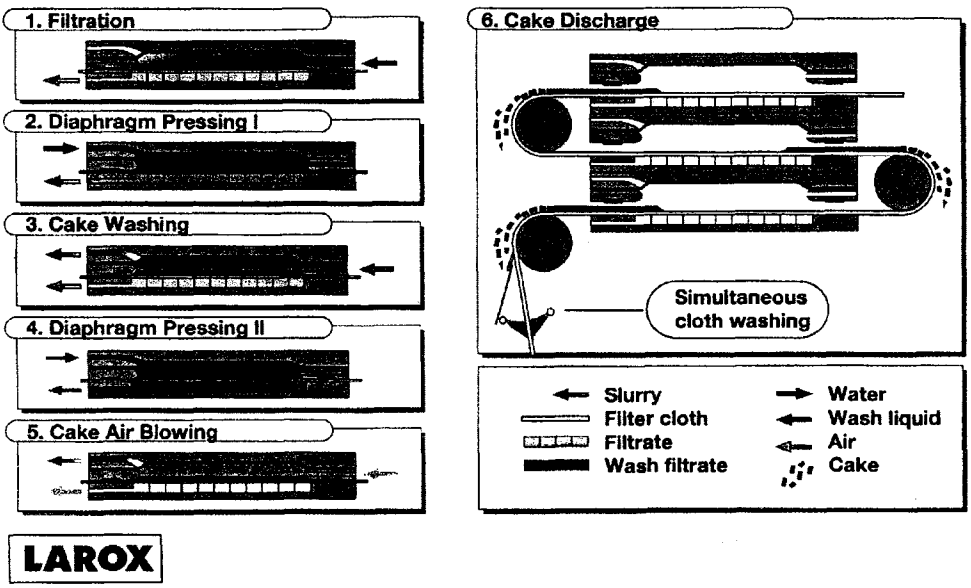


Fig. 2. Membrane pressure filtration system for starch slurry drainage with cake washing.

Technology

In 1999 Maningat and Bassi [2] presented an excellent overview about the state of art in wheat starch production. They reviewed in particular the situation in North America, where the Modified Martin Process, the Hydrocyclone Process and the Alfa-Laval/Raisio Process are standard procedures in industry. In contrast, more recent technology based on the HD (high pressure disintegration) or the Tricanter® Process were indicated as predominant in European industry. Driving force in process development was the reduction of fresh water use in relation to wheat flour utilised for preparation of a dough or batter and following extraction/separation of starch, gluten, fibres and further flour components (Tab. 4). While for the Martin process in its original form as well as for the batter process a water/flour relation of 15:1 was characteristic, technological improvements in both processes concerning in particular recycling of process water led finally to a ratio of 6:1. A concentrated flour-water system similar a baker's bread dough consisting of 1 part flour and 0.6 parts water was prepared initially for extraction. In other process proposals which used hydrocyclones or decanters as primary separation systems further reductions in the flour/water ratio to 4.5:1 and 4:1 were reported. More recent developments like the HD process which stands for mechanical disintegration of suspended flour particles under application of high pressure in a specific valve and/or decanter processes allowed ratios of 3:1 or 2.5 to 2:1.

Maningat and Bassi [2] described the main important starch extraction processes. Their principles should be recalled in the following.

Table 4

Water/flour ratios applied in wheat starch production.

Process	Ratio
Martin Process	15 : 1
Modified Martin Process	6 : 1
Batter Process	5-7 : 1
Hydrocyclone Process	4-5 : 1
Decanter Process	4 : 1
HD Process	3-2 : 1

Modified Martin Process

In order to satisfy requirements of reduced fresh water consumption and to adapt equipment to recent standards in efficient starch/gluten separation the traditional Martin Process underwent improvements resulting finally in a process represented by a scheme (Fig. 3) of the central part of the Modified Martin Process [2]. Conventionally

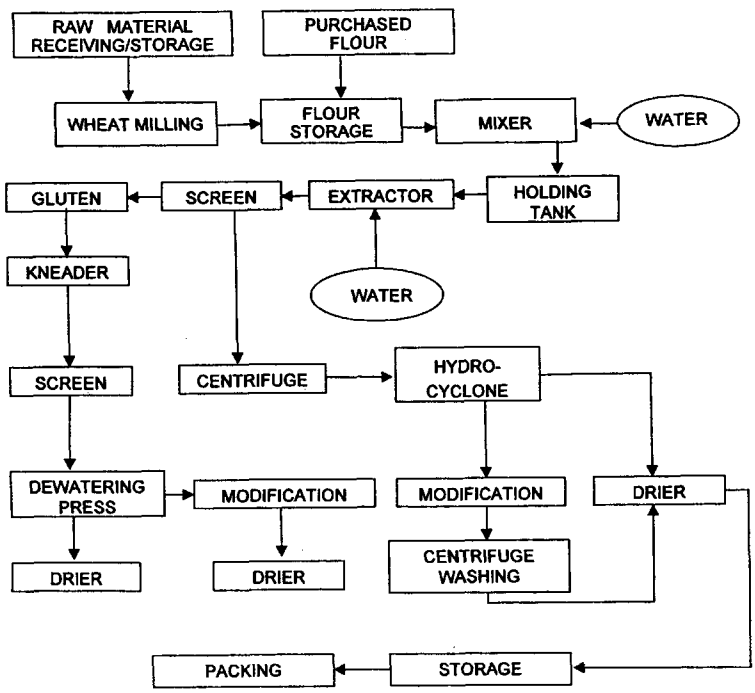


Fig. 3. Reduced schematic flow diagram of the modified Martin process [2].

or short milled flour is mixed with water (temperature = 32°C) in a continuous dough mixer in typical relations of 1.2:1 representing a dry substance content of approximately 47%. For complete hydration the produced cohesive dough undergoes a "rest". Then it is vigorously mixed and treated with turbulent agitation for quick gluten and starch separation which finally occurs in a long, slanted, hollow, rotating cylinder equipped with a 40 mesh stainless steel screen. Gluten is processed further in conventional manner to vital gluten or gluten products while the starch slurry is purified by sieving, centrifugation for B starch separation and hydrocyclone processing.

Hydrocyclone Process

Based on proposals of Verberne and Zwitserloot [15, 16] traditional processes (i.e. the Modified Martin Process) were modernised again by the use of hydrocyclones for gluten/starch separation (Fig. 4). After the continuous dough mixer the dough is allowed to mature for 10 to 20 min and then diluted into a homogenous suspension. Via a multistage hydrocyclone system where spontaneous gluten agglomeration occurs starch and gluten are separated according to their density difference. B starch and fibres are removed from gluten prior to flash drying. The starch slurry goes through a multistage hydrocyclone system for refinement and concentration and is finally dried after passing screens (75 and 50 μm for fine fibre removal).

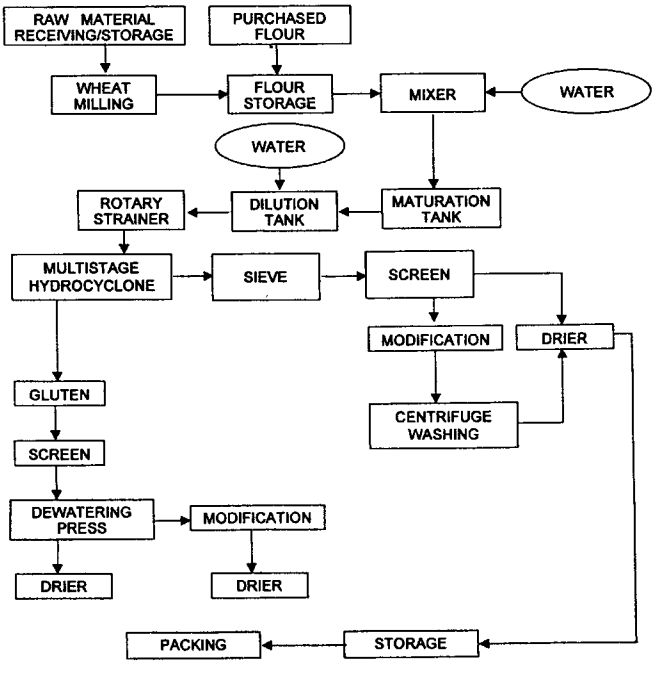


Fig. 4. Reduced schematic flow diagram of the hydrocyclone process [2].

Alfa-Laval/Raisio Process

In contrast to preceding described procedures the Alfa-Laval/Raisio Process (Fig. 5) uses a thick batter that passes a special disc type disintegrator for homogenisation. Starch and protein are then separated in a decanter type centrifuge. The resulting starch fraction that contains about 1% protein is further purified with screens for fine fibre removal and in two stages washed and concentrated in decanters. In the protein containing fraction (about 40% protein) gluten is developed by low-speed agitation via maturation. Aggregation of gluten particles into lumps is initialised in a disc type disintegrator which allows then separation of starch and fibre material by screening and succeeding drying to achieve vital gluten. From the filtrate of gluten screening all B starch as well as residues of A starch being present are recovered by decanting. A starch is further refined and B starch is dried after concentration [16].

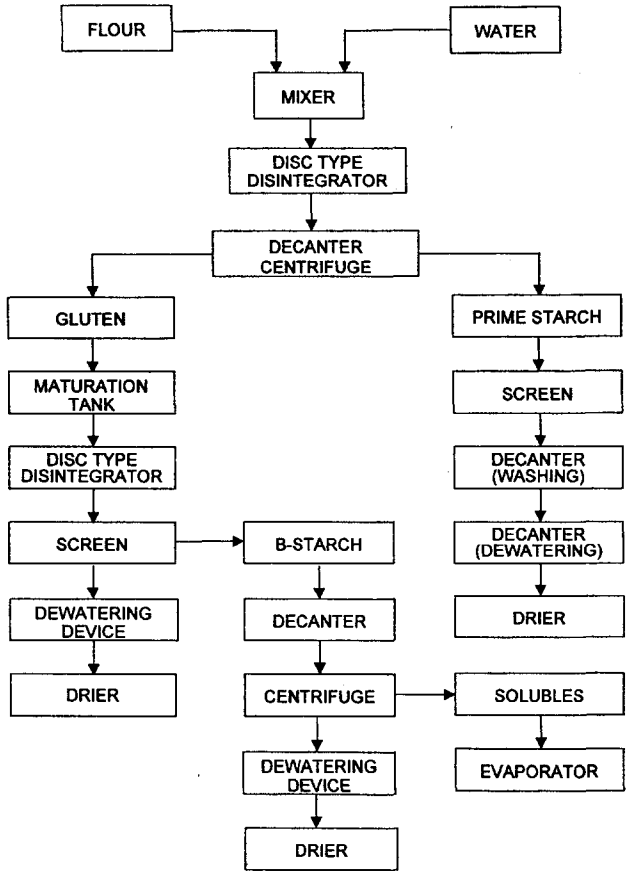


Fig. 5. Schematic flow diagram of the Alfa-Laval/Raisio process [2].

Westfalia Centrifuge/HD or Tricanter® Process

Decisive in modern process technology, however, was not only a very effective water regime during purification steps for wheat components but also a substantial improvement in initial flour hydration and subsequent separation of components from each other in early stages of selected process unit operations. In recent processes [7, 8] centrifugal techniques followed segregation of flour into starch, gluten, fibres and pentosans. A method described as very effective in splitting flour components was high pressure disintegration where shearing forces, friction and cavitation produced the disruption of the tissue within the specific valve of the homogenizer. This technique was successfully taken over from studies of maize starch extraction after pre-treatment of ground, de-germinated maize [17]. Extrication of starch granules, soluble proteins and pentosans from the hydrated flour protein matrix produces the prerequisite for aggregation of protein bodies to voluminous lumps. Because of their still lower density (approx. 1.1) compared to starch (approx. 1.5) they leave decanters with the middle phase of 3-phase decanters when transportation to the concentrate site is successfully impeded. A decanter screw equipped with a sluice disc forms while transporting settled starch to the concentrate outlet a dynamically changing ring-like sediment that separates the feeding and separation zone from the concentrate (drainage) zone [8, 18].

The principal process exists in varied designs, i.e. Westfalia Centrifuge/HD Process, Flottweg Tricanter Process, Barr & Murphy Process, Decanter-Based Weipro Process [2, 15, 19]. At the very beginning a batter/dough similar to other processes is produced and then disintegrated under high shear of a homogenizer. This homogenate is then separated in the aforesaid 3-phase decanter into three destinct phases. The concentrate consists almost entirely of A starch (less than 1% protein) which is refined to commercial quality wheat starch in multistage hydrocyclone units or in multistage 3-phase nozzle separators. The middle phase that consists of gluten, B starch and some fibre as well as the light phase having mainly pentosans and solubles are further separated, purified, concentrated and dried as described by Maningat and Bassi [2] or presented by Witt and Seiler [8].

Gluten drying

The effect of drying conditions on proteins in general is well known. Proteins undergo undesirable structural changes and loose their functional properties when treated under unfavourable conditions, for instance high drying temperatures. Wet wheat gluten is difficult to handle because of the visco-elastic properties and stickiness of this material. On the other hand, the preservation of these properties, in general indicated as vitality, makes the specific quality and value of carefully dried wheat gluten.

Leaving the refining step wet gluten exists in large sticky clots. To overcome the stickiness and to provide an adequate surface area for quick drying it is mixed with dry

product to be chopped into small lumps; for instance, into particles with a diameter less than 1 cm. Coating the wet material with the dry one balances furthermore heat transfer from hot air as drying medium and enhances water evaporation. Thermal stresses on particles transported in the hot air stream of the dryer are thus reduced in an order that avoids for the most part product temperatures detrimental to the proteins. Nevertheless, as a matter of size differences and of initial water content particles reach final moisture content at different times and thus after passing the drying zone several times. On average, gluten particles pass through a dryer more than three times. Using an industrial dryer mill, some particles were found to pass the drying zone up to seven times [20].

Industrial gluten drying occurs in flash dryers. Mainly ring dryers but also dryer mills are used. In both systems wet gluten undergoes changes in its visco-elastic properties as a result of the hot air temperature, the mixing ratio of wet to dry material (i.e. dry matter content of the feed) and the average number of add-back cycles. According to recently presented investigations [20] the visco-elasticity of commercial gluten samples differed significantly between production plants as a result of applied drying procedures. In gluten samples dried under test conditions the effect of hot air temperature was pronounced as soon as product temperature surpassed 60°C. Temperatures above this level are regarded as responsible for denaturation of gluten proteins. Surpassing of this temperature limit could be distinctly observed in simulation of a second and third add-back cycle. Reduced dry matter content in the feed had so far a similar effect.

Developments concerning substrates and testing methods

For substrate selection two sets of specifications representing either grain or flour are dominating [21]. With respect to the Martin process which was for a long time the predominantly utilised technology good dough formation was of prime importance. To meet this requirement minimum wheat grain protein content (N•5.7) was fixed for 14.1 to 14.7% in substance. Specifications indicate furthermore a soft grain type, high starch quantity and limited enzyme activity (Tab. 5). Flour characteristics require an equivalent quality level but specify additionally upper limits for mineral content (Tab. 6). The ongoing demand on wheat gluten as valuable co-product affect still substrate selection in particular with protein content.

Together with capacity extension and modernising of equipment and technology suitability evaluation of wheat for starch production is under investigation again since several years. In particular the expanded use of centrifugal separation techniques suggested the utilisation of wheat varieties showing different grain characteristics. Effects of these developments on ongoing investigations have been reported previously with regard to flour preparation, laboratory scale evaluation of starch extraction and relevant small scale processes as well [21, 22]. Even though the various investigations did con-

sider the practised new procedures they could not yet create a new criteria system for better suitable wheat varieties as grain or flour.

Derived from the fact that concentrated water/flour systems are more relevant in modern starch manufacture than dough systems and the importance of high mechanical energy input in gluten development prior to agglomeration two laboratory methods have been proposed as suitable in evaluation systems. These principles were regarded in both methods.

Table 5

Specifications for wheat grain and flour intended for starch production [22]

Grain Characteristics	
Minimum protein content [N.5,7](%)	12.0 to 12.5
Endosperm hardness	low
Falling number	medium to high
Amylograph consistency	medium to high

Table 6

Specifications used in wheat flour selection for starch production [22]

Flour Characteristics	
Maximum moisture content [%]	14.5
Maximum mineral content [%/% d.s.]	0.62/0.80
Minimum protein content [N·6.25]/% d.s.	12.0 to 12.5
Minimum falling number (s)	280
Minimum amylograph consistency (AU)	500
Damaged starch	low

In the gluten agglomeration test formation of the complex gluten structure follows water uptake and intensive extraction of soluble flour components including soluble proteins under the high mechanical strain of mixing the concentrated system. The applied active power is recorded time dependent. Derived estimates of the time necessary until significant power increase are used to characterise agglomeration.

In the mixer test the mechanical energy input is not only used in the concentrated but also in a diluted system comparable to decanting. This allows to evaluate the separability of the interesting flour components (i.e. A starch, B starch, gluten, and fibres) and in a further time-consuming procedure to determine their yield and purity.

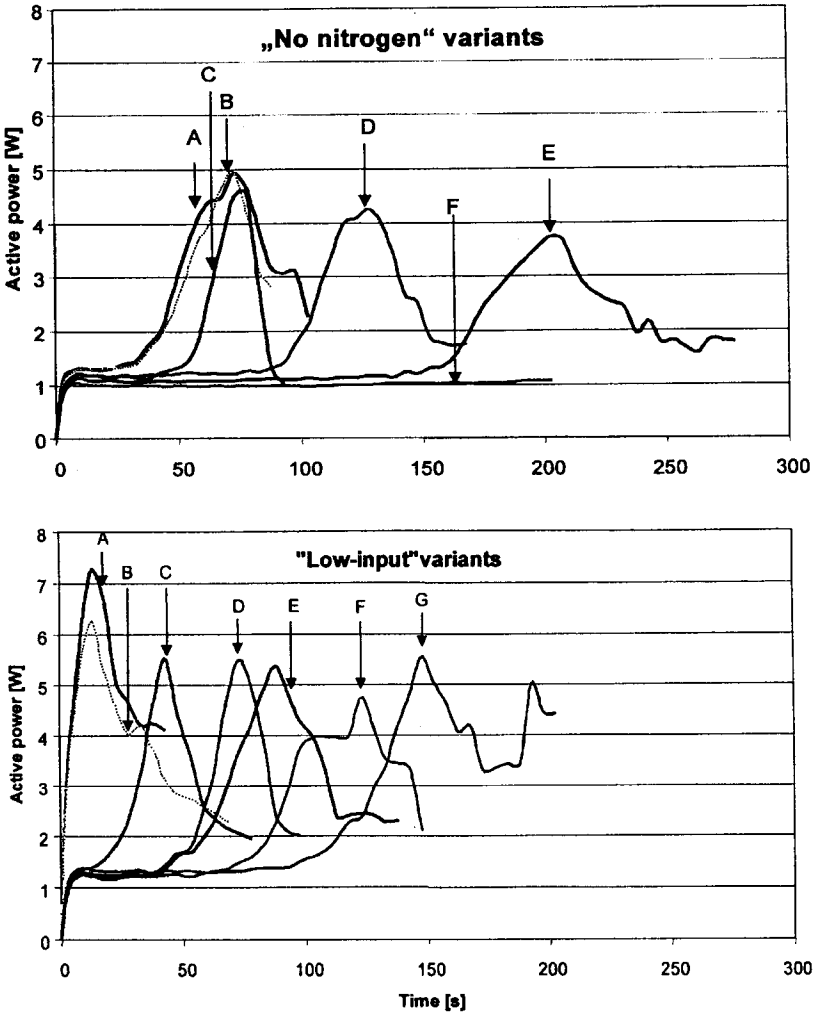


Fig. 6. Time-dependent formation of gluten of flour samples (cultivars: A = Residence, B = Ritmo, C = Atlantis, D = Contra, E = Contur, F = Crousty, G = Soissons) from nitrogen fertilisation trials in gluten agglomeration tests).

By applying these methods to divers sets of samples in particular such coming from fertilisation trials, it could be demonstrated that depending on the genetic potential of nitrogen acquisition of varieties grain produced under conditions of lacking or low-input nitrogen fertilisation will partially not allow satisfying processing [23]. Missing or inadequate gluten agglomeration becomes evident in long lasting or immeasurable agglomeration times (Fig. 6) or small gluten yields (Tab. 7). With some

exceptions in all "no nitrogen" and "low-input" variants dry gluten yield was smaller than flour protein content. Calculated reductions laying in between 0.3 and 0.9% were expected as normal. Within the "no nitrogen" variants, for cv. Contur dry gluten yield was reduced by 1.6%. An explanation for the observed loss might be found in an increased protein content of the respective B starch fraction. A comparable, but less pronounced situation was given for cv. Crousty, too. Along the "low-input" variants differently oriented variations could be found with cvs. Residence, Crousty and Soissons. The small unexpected increases in dry gluten yield in case of cv. Residence and Crousty cannot be explained, yet. For the gluten yield loss with cv. Soissons the phenomenon seems to bee similar to the "no nitrogen" variant of Cv Contur. As result B starch yields and/or fibre yields rise exceptionally (figures in bold) while A starch yield is smaller than anticipated. These results are of particular relevance when grain produced exclusively under organic fertilisation ("extensive farming") is intended for utilisation in starch production. Only varieties having high potential in nitrogen acquisition will thus be able to fulfil requirements.

Table 7

Yield characteristics of wheat varieties produced in a nitrogen fertilisation trial*.

Characteristics	Residence	Ritmo	Atlantis	Contra	Contur	Crousty	Soissons
"No nitrogen" variants							
Flour starch content (% d.b.)	83.7	83.7	83.3	82.5	83.0	83.0	---
Total starch yield (% d.b.)	82.1	83.9	83.6	83.5	85.2	**84.2**	---
A starch (%)	**71.9**	75.7	76.2	**73.1**	76.0	**72.5**	---
B starch (%)	**10.2**	8.2	7.4	**10.4**	9.3	**11.6**	---
Protein content (% d.b.)	2.6	2.5	3.2	2.9	**5.7**	4.6	---
Fibres yield (% d.b.)	1.1	1.1	1.3	1.3	**2.0**	1.3	---
Flour protein content (% d.b.)	9.1	8.4	9.2	8.7	8.5	8.5	---
Wet gluten (g)	23.6	20.0	22.8	21.8	17.5	21.7	---
Dry gluten yield (% d.b.)	8.6	7.6	8.5	8.1	**6.9**	7.6	---
"Low-input" variants							
Flour starch content	7g,g	80.4	80.4	80.5	81.8	80.9	**80.4**
(% d.b.)							
Total starch yield (% d.b.)	76.g	7g.2	80.5	82.3	82.2	80.7	81.0
A starch %	**66.6**	68.8	73.5	73.5	75.1	73.0	71.1
B starch (%)	**10.3**	10.4	7.1	8.8	7.1	7.7	9.9
Protein content (% d.b.)	2.3	2.2	2.5	3.3	**4.5**	3.1	**5.8**
Fibres yield (% d.b.)	0.9	1.2	1.1	1.3	1.3	**1.0**	2.0
Flour protein content (% d.b.)	13.3	11.7	12.3	10.6	10.9	10.9	11.9
Wet gluten (g)	39.5	31.6	32.3	29.2	29.8	**32.4**	28.9
Dry gluten yield (% d.b.)	**13.5**	11.0	11.8	10.3	10.3	**11.2**	10.6

*bold printed figures indicate divergences from expected levels

Developments in conventional breeding and genetic engineering

In contrast to maize extractability of starch was not defined being an interesting breeding task for wheat and played therefore never a important role in forming a new variety. Besides, in Europe wheat starch industry which was far of reaching processing capacities of maize starch production for a long time made in general use of baking flour quality available on the market. This situation was partly changed with the expansion of wheat markets in Asia and shifts in consumer demand to divergent suitability, for example for noodle and flat bread production. Following these demands and the fact that starch characteristics, in particular more waxy character, are connected with these applications, molecular strategies and plant breeding techniques were combined to alter expression levels of starch biosynthesis genes. By generation of mutant wheat lines with null alleles for GBSSI wheat lines could be formed with reduced amylose content or waxy wheat with more than 95% amylopectin [24]. Resulting starches showed higher peak viscosity and gelatinization temperatures, increased crystallinity and lower lipid content and thus a modified functionality in food products. Further presentations demonstrate availability of tools necessary for successful genetic modification of starch content, granule size distribution and shape, lipid content and different other aspects of starch functionality [24-26], but, with regard to the European Community the legal and the political situation as well are actually disadvantageous for their beneficial utilisation in modern biotechnology [27].

Recently presented preliminary results give an example that conventional breeding still may allow to affect the portion of small sized starch granules. Their quantity, in general, contributes significantly to the share of B starch separated in industrial processes. Crosses within *Triticum turgidum* and *Triticum aestivum* were basis of ongoing promising investigations [28].

Starch functionality and application

General considerations

Property profiles of starches are generated primarily by the botanical source and its genetic background [29]. It is well known that decisive contributions are coming from the ratio and structure of amylose and amylopectin. Applications in the food and non-food sector are, however, also determined by the morphology of starch granules (size distribution, form) and by the chemical composition of complex accompanying substances, as they are proteins, minerals, and lipids. With cereal starches serious technological relevance is ascribed to the lipid content (0.6–1.0%) since a certain part of lipids ("starch surface lipids") are hydrophobic surface compounds which affect starch characteristics decisively. As such they contribute to swelling and gelatinization prop-

erties, but also chemical reactivity and selectivity of reactions in granular state are expected to depend highly on the load of lipids [30, 31].

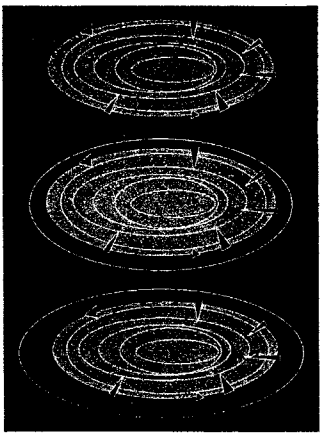
Empirical measurements performed under specific time and temperature procedures are in general successfully used to characterise standardised starch/water systems for application under practical conditions. Such measurements with wheat starches of different kind are well documented [12]. Since measuring conditions are often far from describing the real situation, these measurements provide just limited knowledge. More valuable information can be expected for example from rheometric measurements using well defined conditions of a horizontal cone/plate geometry in a relevant stress range [32].

Flow behaviour of starch suspensions

In starch technology the behaviour of aqueous suspensions plays an important role in process design. Description of the flow behaviour is a basic prerequisite in finding solutions for production processes. The multi-phase organisation of product systems impedes often an unmistakable characterisation of existing conditions. The typical temperature depending swelling behaviour of starch complicates such a description. It is well known, that the system starch/water forms suspensions of varied character in the temperature range from +5°C to 65°C. At higher temperatures (>65°C) the suspensions are transformed into pastes and then gels are formed by cooling. These transitions are influenced by a high number of different parameters which primarily affect packing within the given system [32].

With respect to aqueous suspensions of starch flow curves depend on characteristic parameters like concentration of dry substance, suspension density, suspension viscosity, sedimentation behaviour and stability. These parameters have to be considered in controlling measuring conditions. Nevertheless, such suspensions undergo steadily local and time dependent property changes which provide the flow curves a relative character. However, when measuring conditions are carefully adapted to practical conditions one can derive useful information even from measurements of quickly sedimenting suspensions.

Since surface compounds of starches are regarded as important for property profiles of starch/water systems starch products were used for measurement having very divergent surface properties as result of applied separation method. These products (commercial wheat starch; starch separated by a laboratory procedure: Glutomatic starch; starch recovered as insoluble fraction after Osborne fractionation: HMW starch) differed significantly in protein content (Fig. 7), but to a certain extent also in particle size distribution. In some way, they can be used to represent starches in different steps of industrial processing.



	Protein Content
Commercial Starch	0.3 %
Isolated Starch (Glutomatic)	0.8 -1 %
Starch Residue (Osborne) „HMWStarch"	4 -10 %

Fig. 7. Wheat starch products.

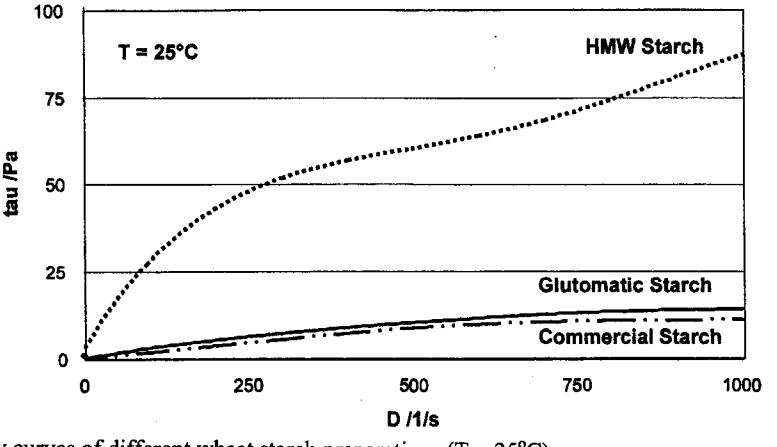


Fig. 8. Flow curves of different wheat starch preparations (T = 25°C).

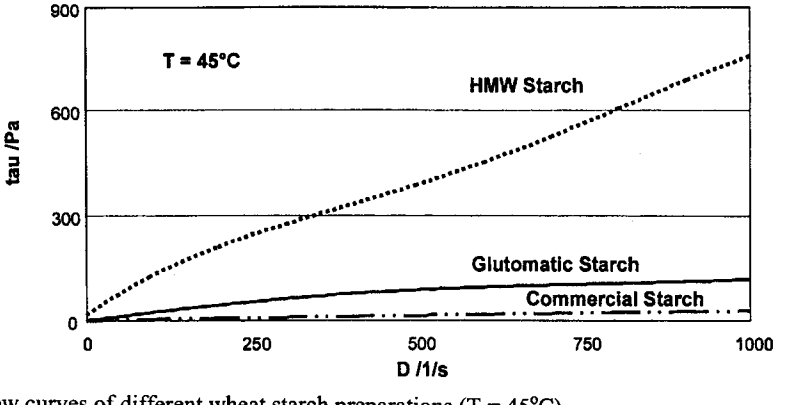


Fig. 9. Flow curves of different wheat starch preparations (T = 45°C).

Table 8

Specification of commercially available small-granule starches in comparison to the B-starch standard.

Quality characteristics	"HAMSTARCH ultrafine"	"Puramyl SP"	B-starch Standard
Colour	white	white	white/gray
Odour & taste	neutral	neutral	neutral
Moisture content (%)	10 - 13	15.0	max. 14
Starch content (% d.b.)	min. 97.0	-	-
Protein content (% d.v., Nx6.25)	0.3 - 0.6	max. 0.6	max. 5.81
Lipid content (% d.b.)	max. 0.1	-	max. 0.58
Mineral content (% d.b.)	max. 0.4	max. 0.6	max. 1.16
pH-value	5.5 - 6.5	5.3 - 6.7	-
Median particle size (μm)	3.4 - 4.2	-	-

Flow curves of aqueous suspensions of these starch products differed significantly when measured at T = 25°C (ambient room temperature) and T = 45°C (elevated temperature in processing, Fig. 9). At T = 25°C flow curves of commercial starch and Glutomatic starch were characterised by small but linear increases in slope while HMW starch demonstrated a higher, but not to drastic increase (Fig. 8). At 45°C the situation has changed. Now, even though the flow curve of the Glutomatic starch was separated from the one of commercial starch showing additional, even small contribution of structural changes within a dispersed phase of the suspension. The dispersed starch phase is considered of having changed its internal state of order. With regard to used scales a much greater contribution (nearly 10 times) to viscosity measurements, however, was observed with HMW starch. The temperature effects observed with the measured suspensions are expected to be different because of elucidated differences in type and amount of complex native accompanying substances. While in used commercial starch only lipoproteins and glycoproteins are present upon external layers and determine properties, amount and composition of HMW entities are decisive for the property profiles of Glutomatic starch, but to much greater extent for residues that remained on starch. This was in fact well reflected by the presented flow curves.

B Starch

A well known and until now unavoidable by-product of conventional processes in wheat starch production consists in an at most impure fraction of small sized (<10 μm) starch granules. Traditionally, this fraction is specified as B starch or, in Germany, also as "secunda" starch. Compared to regular A starch it contains much more impurities, e.g. proteins, lipids and minerals and resembles a product intermediate to Glutomatic starch and HMW starch. Because of utmost unknown functional properties, potential

application of this starch type is limited. Therefore, B starch is either pre-gelatinised and used as animal feed or transformed into saccharification products. However, some European starch producers recover small starch granules and try to turn this fraction into a high quality marketable product. Removal of impurities, in particular pentosans, is done by enzyme treatments having pentosanase activity followed by usual regimes of starch refinement which may allow to admix the purified fraction to regular grade A starch. Commercial small-granule starch products do not reach fully specifications of A starch but offer remarkable quality (Tab. 8). They are marketed by several wheat starch producers (Latenstein Zetmeel B.V., Nijmegen/The Netherlands, brand name: "Puramyl SP"; Jäckering Mühlen- u. Nährmittelwerke GmbH, Hamm/ Germany, brand name: "HAMSTARCH ultrafine") [33].

Conclusions

Starch production was always affected by agricultural policy of the European Community which produced now conditions (export and production refunds, import duties) that favour utilisation of wheat. It's therefore not astonishing, that EU wheat starch and gluten production dominates world wide this market segment. Eastern Europe's production figures, including Russia do not even pass 6% of EU production. Political implications, in particular between US and EC authorities, impede market relationships in case of wheat gluten. Similar to market activities also technological developments progress much faster in Europe, presumably because of environmental reasons. Driving force is the need for restricted use of process water.

On the other hand new separation techniques based on centrifugal principles are replacing older principles and lead to introduction of new processes. Some prospects of success can be seen in introduction of pressure filtration as result of technical improvements. Focussing on products, there is concern for functional properties of gluten. Improvements in gluten drying regimes, yet less studied, are expected to allow better quality. However, since process economy and product quality are based on substrates more attention should be given to better adapted wheat and flour quality. For testing suitability new methods are in question regarding centrifugal energy application; a surveying gluten agglomeration test and the mixer method, that allows detailed information. Concerning utilisation of better adapted and potentially transformed wheat molecular biological techniques together with conventional breeding are expected to provide material with prospects in starch content as well as starch composition and morphology. The acceptance of genetically modified wheat remains critical as soon as wheat components are intended for use in food products. With respect to expanded application of wheat starch a lack of information is seen in selected fields of functionality. Empirical methods provide limited knowledge. The state of art in fundamental rheological characteristics needs to be expanded together with new and criti-

cal consideration of functional impurities. Flow curves can be valuable tools. Finally, a still problematic co-product, the B starch fraction, is waiting for new solutions.

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PSZENICA - KONKURENCYJNY SUBSTRAT W PRODUKCJI SKROBI

Streszczenie

W przeciwieństwie do sytuacji ogólnoświatowej, gdzie kukurydza stanowi główne źródło skrobi, w europejskim przemyśle skrobiowym pszenica zajmuje istotną pozycję. Chociaż wydajność techniczna tej skrobi nie może konkurować cenowo z innymi skrobiami, to gluten pszenny jest wartościowym produktem ubocznym pozwalającym skrobi pszennej zająć ważną pozycję. Nowe instalacje o dużej wydajności ostatnio zainstalowane w krajach europejskich są dowodem wzrostu zainteresowania tym surowcem. Jednakże sytuacja ekonomiczna produkcji skrobi pszennej ulega nieuniknionym wahaniom na rynku glutenu pszennego. Ważną rolę odgrywają też decyzje polityczne.

Konkurencyjna sytuacja, związana z pszenicą jako substratem skrobi, wynika z unowocześnień aparaturowych i zmian w technologii. Najważniejszą jest tutaj zmiana ilości wody na daną ilość mąki potrzebna do wydzielenia glutenu i skrobi oraz ich oczyszczenia. Osiągnięto postęp przede wszystkim przez unowocześnienie sposobu oddzielania skrobi w wirówkach. Z uwagi na brak wody oraz konieczne ograniczenie objętości ścieków ten problem wciąż znajduje się w centrum uwagi. Opublikowane standardy ograniczają się do wytycznych zorientowanych na proces Martina. Lecz z powodu unowocześnień proponuje się obecnie nowe metody standaryzacji. Wśród tych metod należy zauważyć metodę mikserową, postępowanie stosowane do mieszanek wody z mąką poddawanych wirowaniu. Jednakże jest to metoda czasochłonna, co ogranicza jej powszechne stosowanie. Omawiane są też techniki mielenia w połączeniu z wybranymi właściwościami ziarna.

W końcu omówiono postępy w konwencjonalnej hodowli i inżynierii genetycznej, pozwalające uzyskać substraty o większej przydatności do produkcji skrobi.

W. BŁASZCZAK, J. FORNAL, A. RAMY, K. BORKOWSKI

THE ROLE OF STARCH GRANULES IN THE BALADY BREAD STRUCTURE FORMATION

Abstract

The microstructure of fresh and three days-stored Balady bread was studied using two different microscopic methods (LM and SEM). These both techniques revealed a great differences in the starch microstructure as well as protein distribution between two layers of fresh Balady bread. The lower layer of Balady bread is characterized by the greater extent of starch geletinization as compared to the upper one. The upper layer of Balady bread is formed by continuos protein matrix with embedded lenticular-shaped starch granules.

The microstructure of Balady bread after 3 days of baking differs from that of the fresh one mainly in the starch granules structure. The visible differences seem to be connected with different degree of starch gelatinization in each of the layers as well as free water (released from granules) redistribution between the layers. It was found that mainly these changes in stored bread allowed rapid retrogradation of the main soluble starch component – amylose.

Introduction

Dough ingredients combined with the old traditional Egyptian methods of the Balady bread production determine a unique and unusual form of this kind of bread. Balady flat bread differs significantly in appearance, microstructure, texture, nutritional value and taste from traditional European cereal baking products. The microstructure of bread and/or dough is highly variable and very often depends on the processing parameters and methods. Esspecially the methods of baking may vary widely from one type of bread to another [1]. Due to high temperatures (350–450°C), and short time of baking (2–4 min), the piece of dough of Balady bread raises in the oven and separates into two thin layers. The loaf is characterized by an open space between the top and bottom layer. The time and especially high temperature have also a significant influ-

W. Błaszczak, J. Fornal, Institute of Animal Reproduction and Food Research, PAS, Olsztyn, Poland; A. Ramy, Food Technology Department, National Research Centre, Cairo, Egypt; K. Borkowski, Olympus Optical Polska.

ence on deterioration of protein quality as well as the degree of starch gelatinization during baking [4, 5, 15]. The swollen and partially solubilized starch granules are the essential structural elements of bread. Cooling and staling of bread are responsible for transformation of both starch polymers (amylose, amylopectin), including gelatinization and crystallization.

The SEM and LM microscope techniques have been used to examine the distribution of the specific components within two layers of Balady bread. Moreover, much attention was paid to the studies on the role of starch granules in the structure formation of the fresh and stored bread.

Materials and methods

Balady bread was prepared using a commonly known baking procedure [4, 5, 8, 15]. The dough was fermented at 28°C for 40 min and divided into 180-g pieces. The dough pieces were placed on a wooden tray, which had been previously covered with a thin layer of bran. The dough pieces were placed in a fermentation cabinet for 15 min. After the first step of dough proofing, the pieces were flattened by rolling to a 20-cm diameter and 1.25-cm thickness, and left to a final fermentation for 50–60 min at 75–85% relative humidity [3]. The baking temperature reached 300–400°C for 2–4 min.

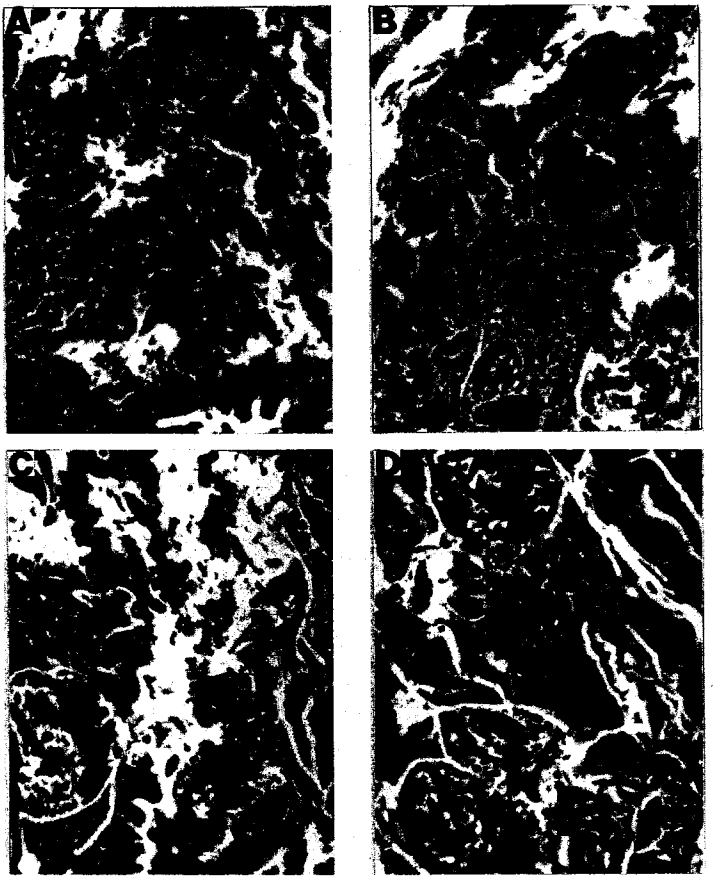
Moisture, ash and protein levels were determined by the ACC Methods: 44–15, 08–01, 46–10, respectively [16]. Fat content, crude fibres as well as sugars level were analyzed as recomended by the AOAC methods [1].

Small pieces of bread (lower and upper layer) have been prepared for the SEM studies by the freeze-drying method prior freezing in a liquid nitrogen. The dried samples were coated with gold and examined in a JSM 5200 microscope.

For the LM study, the small samples of bread were fixed in glutaraldehyde (dehydrated with ethanol series, polymerized and embeded in Historesin Kit medium according to producent data (Reichert-Jung, Germany). Sections, 2.5 μm, were cut with a microtome (Reichert-Jung, Germany) and stained with 0.1% Light Green and Lugol's solution. The sections were examinated and photographed with an Olympus BX 60 microscope.

Results and discution

The major structural change that takes place during baking process is starch gelatinization [2]. Light microscopy after iodine and light green staining provided considerable information about microstructure of both layers of Balady bread, which was different concerning the starch granules structure. In opposite to the lower layer of Balady bread, the upper one is reach in gluten proteins (Phot. 1a). The internal baking temperature reaches a point high enough to develop steam that 'puffs' the bread with



Phot. 1. LM pictures of Balady bread;
A/ upper layer of fresh bread; B/ lower layer of fresh bread; C/ upper layer after 48 hours of storage; D/ lower layer after 48 hours of storage.

almost explosive rapidity [6, 16]. Thus, the baking conditions as well as moisture content can affect significantly the changes in the protein structure resulting in a greater extent in protein redistribution mainly to the upper layer of bread. The structure of upper layer is created mostly by the continuos protein matrix with swollen starch granules embedded in it. The lower layer of bread (Phot. 1b) seems to be poorer in gluten proteins and consists mainly of gelatinized starch granules being in the higher stage of destruction. There is also observed an evident leakage of solubilized amylose through the equatorial groove to the intergranular space. The swollen starch granules together with solubilized amylose and amylopectin phase form a tightly-packed gel-like system. An interesting phenomenon, consisting in the different mechanism of starch gelatinization between the two bread layers could be also observed. The upper layer consists

of much less swollen starch granules at the first stage of swelling (elongated, lens shape A-type granules), while amylopectin-reach granules from the lower one are characterized by tangential deformation. These strongly pronounced microstructural changes in lower layer of Balady bread could be visible probably due to an easier access of starch to water in this part of bread as well as to a direct contact with the hot metal part in the oven. According to Faridi&Rubenthaler [6] the extent of starch gelatinization (under similar conditions) was generally higher for granules from centermost part of the loaf (85% for the lower crust), than that of granules from the exterior one (83.8% for the upper crust).

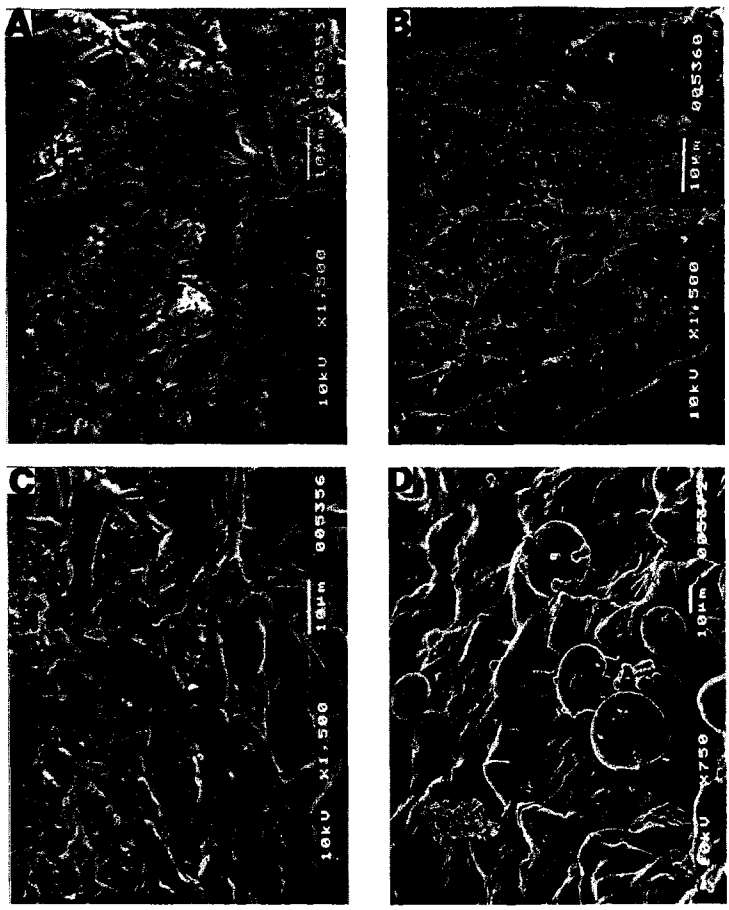
In order to obtain some additional details on starch and protein microstructure from layers of fresh Balady bread, the SEM technique was also performed. The continuos phase of the upper layer of Balady bread (Phot. 2a) seems to be composed of geletinized starch granules mounted in a gluten matrix.The characteristic filaments belonging to the solubilized and free amylose phase were visible in the lower layer of Balady bread (Phot. 2b).

Changes that occur during cooling and storage of starchy products are mainly related to retrogradation. Bread staling affects the starch microstructure as well as can influence gluten protein, what results from release of free water from retrograding starch granules. Redistribution, i.e. mobility of the free water in the whole crumb of bread, seems to be strongly related to the quality of gluten protein as well as starch properties [9, 18]. Water diffusion in staling bread was explained by Hoseney [10], as displacements of the molecule toward next-neighbouring binding sites, like -OH groups of the glucose units of polysaccharide molecules able to form hydrogen bonds. It is well known that during storage time water mobility decreases and the crumb structure becomes more firmer. In case of Balady bread the moisture content is not so high (Table 1). It is probably due to the moisture loss by evaporation during and immediately after oven baking as well as fast water redistribution between both layers.

Table 1

Chemical composition of fresh Balady bread.

	content % (dwb)
ash	1.01
fiber	1.16
lipids	1.32
protein	15.5
moisture	30.0
total carbohydrates	81.08



Phot. 2. SEM pictures of Balady bread;
A/ upper layer of fresh bread; B/ lower layer of fresh bread; C/ upper layer after 48 hours of storage; D/ lower layer after 48 hours of storage.

The microstructure of Balady bread after three days of storage differs from that of the fresh one mainly in the starch granules structure. The amylose, which was observed in fresh bread as a gel-like particles, now it seems to be strongly associated with amylopectin remnants and gluten protein matrix (Phot. 1c,d). The microstructural changes of amylose released (fresh bread) into insoluble crystallites are possible due to redistribution of free water as well as dehydratation of soluble starch during bread storage. The aggregated amylose can be expected to have a stabilizing effect on the gluten protein matrix (Phot. 1c) and probably it is responsible for the firmness of bread. [2, 12, 13, 19]. Photo 1d shows the field of the lower layer of stored Balady bread. The

changes in appearance of granule remnants (more shrunken, darker) seem to be a reason of rapid reorganization of the starch biopolymers within their structure [7]. Also the lack of solubilized amylose, observed in the lower layer of bread, can be connected with stronger dehydratation caused by ageing, as compared to the upper layer. According to these changes, it can be stated that the higher degree of granules gelatinization as well as presence of solubilized amylose strongly affected the starch retrogradation, during bread storage, especially in its lower layer.

In opposite to the above-mentioned observations, Faridi&Rubenthaler [6] reported that the main soluble starch material leached from crumb of freshly baked (415°C, 2 min) bread was predominantly amylopectin 1.62%. At the total amount of soluble starch material (2.42%), the amylose content was only 0.80%. They reported progressively decrease in amount of soluble starch (1,18%) as well as marked decrease in pasting properties after 48 hours of bread storage. Rapid and significant decrease in the amylose content (0.16%), obtained by these authors after 48 hours of storage, confirms our microscopic observations that amylose underwent retrogradation at a more rapid rate as compared to amylopectin (1.02%).

However, the mechanizm of retrogradation affects not only changes in the amylose structure [7]. Several authors suggested that during bread staling also some changes in the amylopectin structure, i.e. its recrystallization, take place, being the main cause of bread firming [6, 17]. Inagaki and Seib [11] clearly proved that the amylopectin recrystallization is stronger associated with crumb firming than amylose. The SEM photo 2c, seems to confirm this hypothesis, due to a bigger surface area of highly swollen and elongated amylopectin-reach granules they probably could easier interact with gluten protein matrix. In contrary, the presence of solubilized amylose (the gel-like phase) surrounding the swollen granules results in the formation of a strong gel on cooling as was shown on SEM photo 2d. These observations are in accordance with Martin et al., [14] suggestions about passive role of swollen starch granules in the crumb firming.

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STRUKTUROTWÓRCZA ROLA GAŁECZEK SKROBIOWYCH W CHLEBIE BALADY

Streszczenie

Składniki ciasta odgrywają niezwykle ważną rolę w produkcji tradycyjnego egipskiego chleba Balady. Są to płaskie chlebki wypiekane w temperaturze 350–450°C. Przy krótkim, 3–4 minut trwającym wypieku kawałki ciasta, rosną rozpoławiając się na dwie cienkie warstwy. W ten sposób w bochenku pomiędzy warstwą górną i dolną znajduje się pusta przestrzeń.

Za pomocą technik mikroskopowych SEM i LM zbadano rozkład specyficznych składników ciasta pomiędzy obie warstwy. Szczególną uwagę zwrócono na rolę gałeczek skrobiowych w tworzeniu struktury bochenków.

Okazało się, że w przeciwieństwie do dolnej warstwy, górna warstwa chleba Balady była bogata w białko, tworzące własną matrycę. Matryca glutenowa, zawierająca kleikowane gałeczki skrobiowe, tworzyła ciągłą fazę warstwy górnej. Warstwa dolna w ogóle nie zawierała fazy glutenowej i składała się głównie ze skleikowanych gałeczek skrobiowych.

Z powodu bardziej zaawansowanego kleikowania gałeczki skrobiowe w dolnej warstwie bochenka wykazywały istotne zmiany mikrostrukturalne. Gałeczki były bardzo zniszczone i wykazywały wyciek amylozy. Zdjęcia SEM wykazywały bardziej subtelne zmiany. W dolnej warstwie widać było charakterystyczne pasemka amylozy, natomiast w górnej warstwie widać było skleikowane gałeczki wbudowane w matrycę białkową. Wydaje się, że bezpośredni kontakt dolnej części bochenka z gorącymi elementami pieca ma istotne znaczenie dla stopnia skleikowania skrobi i zachowania się białka.

HYUN-JUNG CHUNG, EUN-HEE CHANG, SEUNG-TAIK LIM*

THERMAL TRANSITION CHARACTERISTICS AND GEL PROPERTIES OF HEAT-MOISTURE TREATED CORN AND POTATO STARCHES

Abstract

Normal corn starch containing 25 or 30% moisture and potato starch containing 20 or 25% moisture were heat-moisture treated at 120°C for 1 h and the changes in thermal transition characteristics and gel properties of the starches were examined. Granular crystallinity on X-ray diffractogram, especially for potato starch, was reduced by the heat-moisture treatment (HMT). At a limited moisture content (15% based on total weight), T_g measured in granular form of starch decreased by 2-6°C. At T_g, the change in heat capacity (ΔC_p) of the treated starch was substantially higher than of the corresponding native starch. Crystal melting of the heat-moisture treated starches, measured at 80% moisture, appeared to be biphasic on a DSC thermogram, in that the original endotherm became smaller while a new endotherm at higher temperature was enlarged by the HMT. However, the total melting enthalpy for starch decreased, indicating a partial loss of crystallinity. The degree of retrogradation under DSC was not significantly different between the native and treated starches. The HMT starches formed the gel with more opaqueness and brittleness. The gel stability from freeze-thawing treatment was slightly increased with corn starch, but decreased with potato by the HMT. Overall results on the paste viscosity and gel properties indicated that the HMT provided physical cross-linking effects on starch.

Introduction

The molecular arrangement in a starch granule can be altered by various physical treatments. Annealing and heat-moisture treatment (HMT) are two common physical means by which the treated starch can acquire modified properties without rupturing the overall granular shape. Annealing is generally carried out in the granular form of the starch with a large quantity of water at a temperature below the starch melting

Hyun-Jung Chung, Eun-Hee Chang, Seung-Taik Lim, Graduate School of Biotechnology, Korea University, 5-1 Anam-dong, Sungbuk-ku, Seoul 136-701, Korea.

* *Corresponding author. Tel.: 82-2-3290-3435; fax: 82-2-927-5201; e-mail address: limst@mail.korea.ac.kr*

point, whereas HMT is done with limited moisture content but at an elevated temperature [1].

The physical properties of a heat-moisture treated starch depend on the starch origin and treatment conditions used. Heat-moisture treated starch displays an increased paste stability and gelatinization temperature, regardless of its origin [2-8]. Collado and Corke [8] treated a sweet potato starch, and found that the starch paste became short and shear-stable and the starch gel exhibited marked increases in hardness and adhesiveness.

On X-ray diffraction patterns, root starches having a B-pattern showed a transition toward an A-pattern by HMT, whereas cereal starches remained in the inherent A-crystal pattern [3, 9]. Donovan et al. [6] reported that HMT made the starch melting endotherm on a DSC thermogram biphasic, and claimed that there was new crystal formation or crystalline rearrangement in the treated starch granules.

Hoover and coworkers [10-12] tested various starches of different origins, and claimed that HMT induced the changes not only in crystalline regions but also in amorphous regions in starch granules. They found that the amylose content and starch chain length were two important factors determining the physical properties of the final products.

Although a number of studies have been reported on heat-moisture treated starches, the structural transformation inside the starch granule is still not fully understood. In this study, the HMT effect on the thermal transition characteristics of crystalline and amorphous regions in starch granules was investigated with corn and potato starches, and the changes in physical properties of starch gel such as textural properties and freeze-thaw stability were also examined.

Materials and methods

Materials

Normal corn starch and potato starch were provided by Samyang Genex (Seoul, Korea) and Handuk Avebe (Seoul, Korea), respectively. Corn starch was adjusted to 25 or 30% moisture, whereas potato starch was adjusted to 20 or 25% because potato starch was more susceptible to heat and moisture than corn starch. The moisture-adjusted starch (200 g) in a pressure-resistant glass bottle (300 ml) was autoclaved at 120°C for 1 h. After cooling to room temperature, the starch was dried to approximately 10% moisture content in convection oven (40°C) overnight.

X-ray diffraction

The X-ray diffraction pattern of the native and heat-moisture treated starches was measured with a diffractometer (Rigaku Geigerflex G/max II-A, Tokyo, Japan) at 35

kV and 15 mA with Cu-K radiation and Ni filter. The scanning speed and diffraction range of 2θ were 1°/min and 5-50°, respectively.

Thermal transition analysis

Glass transition temperature (T_g), heat capacity change (ΔC_p), melting temperature (T_o, T_p, and T_c), and melting enthalpy (ΔH) were determined by using a differential scanning calorimeter (Seiko DSC 6100, Chiba, Japan). For the measurement of T_g, starch loaded in an open silver pan was equilibrated in a moisture chamber (>95% RH) until the moisture content reached 15%. The DSC measurement was carried out from 25 to 85°C at a heating rate of 2°C/min. Right after the initial heating, the sample was reheated to observe the glass transition only.

Pasting viscosity

Pasting viscosity profile was measured with a Rapid Viscoanalyzer (Newport Scientific, Warrenwater, Australia). Starch content in the aqueous dispersion was 7% (w/v) and the starch dispersion was heated from 25 to 95°C at a rate of 3.5°C /min, held for 10 min at 95°C, and then cooled to 50°C at 3.5 °C.

Gel preparation and texture analysis

Starch dispersion in water (10% w/v) was pasted in a boiling water-bath for 15 min while mechanically stirring. The paste was carefully poured into Petri-dish (50 mm diameter, 10 mm height) and stored with cover in a refrigerator (4°C) for 24 h. The gel was carefully removed from the petri-dish and tested by Texture Analyzer (TA-XT2, Stable Microsystems, Surrey, UK) with a cylinder plunger (12.8 mm diameter). The compression on the gel was done up to 60% of the height at a constant probe speed of 1.2 mm/sec, and initial modulus (surface firmness), fracture strain, and fracture strength (gel hardness) were measured.

Freeze-thaw stability

The starch gel was also frozen by storing at –20°C for 24 h and thawed at 25°C for 4 h. The gel was vacuum-filtered with an aspirator for 20 min. During the filtration, a weigh (600 g) was loaded on the gel to facilitate water release. The syneresis was calculated as the weight percent of the released water to the original water in the gel.

Results and discussion

X-ray diffraction

X-ray diffraction patterns of the native and heat-moisture treated corn and potato starches are given in Fig. 1. The treated corn starches retained the typical A-type dif-

fraction pattern of the original starch. At 30% moisture content for the treatment, however, a slight decrease in peak intensity on the diffractogram was found, whereas at 25% no significant change in peak intensity appeared. The heat-moisture treated potato starches also showed decreased peak intensity. It has been reported that excess heat or moisture for HMT results in reduced crystallinity [13]. Several researchers found that root starches had a transition tendency toward the A-pattern on X-ray diffractogram [3,9]. Under the HMT conditions used here, the small peak at 5° (marked by arrow) for native potato starch indicating the typical B-pattern disappeared, and the dual peaks at 22-25° seemed mixed together. However, due to the reduced peak intensity, identifying the transition was difficult.

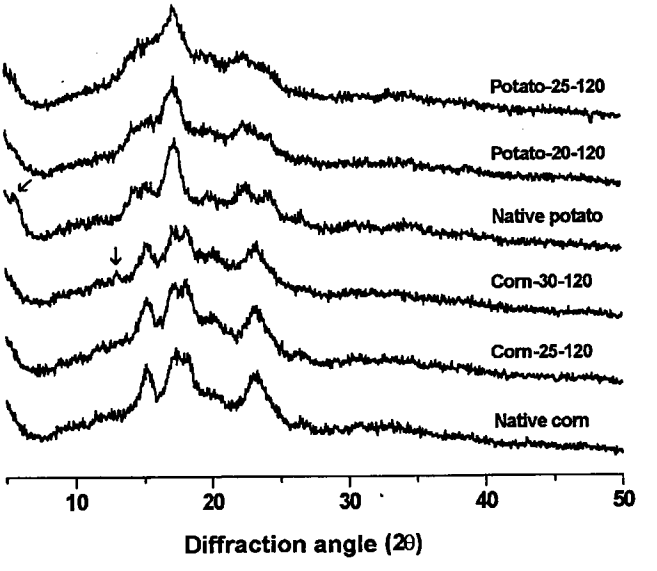

Fig. 1. X-ray diffractograms of native and heat-moisture treated corn and potato starches. The numbers indicate moisture content (20-30%) and temperature (120°C) for the treatment.

Kawabata et al. [9] also reported the amylose-lipid complex formation in cereal starch by pointing out a new peak presence at 13.1° (2θ) on X-ray diffractogram. The corn starch treated at 30% moisture showed a new small peak at approximately 13° (marked by arrow), which could be indicative of the amylose-lipid complex formation. The complex formation may result in the restriction of starch swelling, and a decrease in paste viscosity and clarity [10].

Glass transitions with limited moisture

The glass transition was determined in the native state of the starch so that the T_g values represented the transition of the inherent amorphous state of the starch. When the moisture in the starch was 15%, the T_g of native corn and potato starches appeared at 52.3 and 77.7°C, respectively (Table 1). This T_g difference (25.4°C) between the two starches remained not much changed during the HMT processes. The T_g difference suggests that there is a distinct difference in the amorphous chain conformation between the two starches. Several researchers have reported the differences in size, chain length and degree of branching between corn and potato amylose molecules [14-16]. Based on the literature, potato amylose was found to have higher degrees of polymerization and branching than corn amylose. Therefore, potato amorphous structure consisting of the bigger and more branched amylose chains has a higher thermal stability to resist the glass transition compared to corn amorphous structure at a limited moisture content.

Table 1

Glass transition and crystal melting of heat-moisture treated starches.

Starches	Glass transition				Starch crystal			
	T_1^* (°C)	T_g (°C)	T_2^{**} (°C)	ΔC_p (J/deg .g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
Native corn	40.5±4.2	52.3±2.9	62.0±2.1	0.018±0.005	62.6±0.1	66.9±0.1	75.5±0.3	18.7±0.2
Corn-25-120	40.2±2.7	47.7±0.8	55.1±2.1	0.058±0.014	62.8±0.1	74.2±0.3	79.6±0.5	14.4±0.2
Corn-30-120	39.5±0.8	46.8±0.6	53.2±1.7	0.082±0.005	63.4±0.6	76.7±0.1	80.3±0.6	8.2±2.5
Native potato	68.2±0.1	77.7±0.3	90.8±1.9	0.007±0.004	57.2±0.2	61.8±0.1	67.3±0.3	20.0±0.5
Potato-20-120	69.9±2.5	76.1±3.3	80.8±2.3	0.020±0.011	56.5±0.4	62.1±0.2	69.3±0.2	17.4±0.5
Potato-25-120	65.7±0.2	73.6±0.8	81.1±1.5	0.046±0.014	56.0±0.2	66.2±0.2	71.8±0.4	13.4±0.1

* onset of glass transition
** end of glass transition

As regards the HMT effect, the HMT decreased the T_g of both starches. For example, corn starch treated at 30% moisture displayed its T_g at 46.8°C, 5.5°C lower than that of native corn starch. Another important change detected on the thermogram was the degree of specific heat capacity change (ΔC_p) at the glass transition. The ΔC_p was increased substantially by HMT, and the temperature range for the transition became narrower (Fig. 2, Table 1). Especially the T_2 where the glass transition ended was significantly decreased. The crystalline regions in starch granules were interconnected by the continuous amorphous regions [17]. The crystal micelle presence thus reduced the

freedom of the neighboring amorphous starch chains. Billiaderis et al. [17] discussed the details of these crystalline effects and claimed the theoretical presence of inter-crystalline amorphous parts. This inhibitory effect by the intercrystalline parts on the mobility of amorphous chains caused the T_g of native starch to be higher than the value of the gelatinized starch [18].

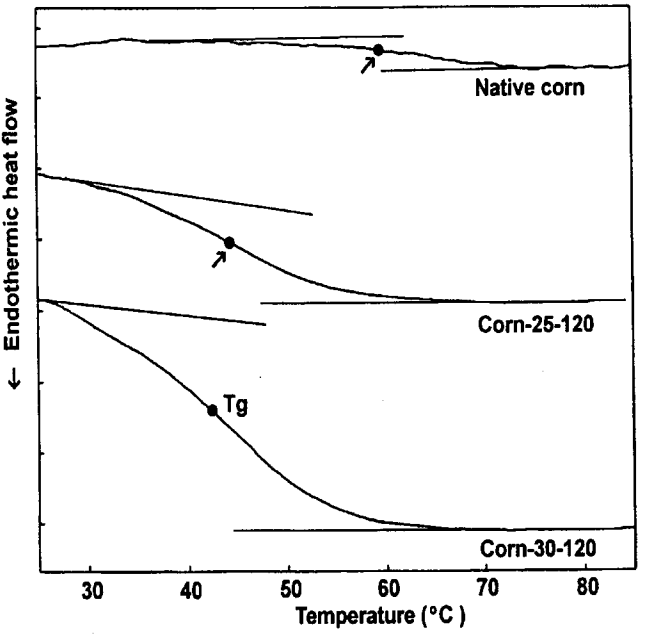

Fig. 2. The glass transition on DSC thermogram of native and heat-moisture treated corn and potato starches in the presence of limited moisture (15%). The numbers indicate moisture content (20-30%) and temperature (120°C) for the treatment.

We suppose that the increased ΔC_p of the heat-moisture treated starches was caused by the transformation of the intercrystalline parts into independent amorphous states during HMT. From this transformation, the amorphous portion in the starch granule could be raised. The reduced or removed cross-linking effect by the intercrystalline parts as well as the raised amorphous portions made the ΔC_p change increase and T_g decrease.

Melting and retrogradation of starch

By the HMT in this experiment, starch melting appeared to be biphasic and the transition temperature range was raised (Fig. 3, Table 1). There was no significant change in T_o, whereas T_p and T_c were substantially increased on the endotherm

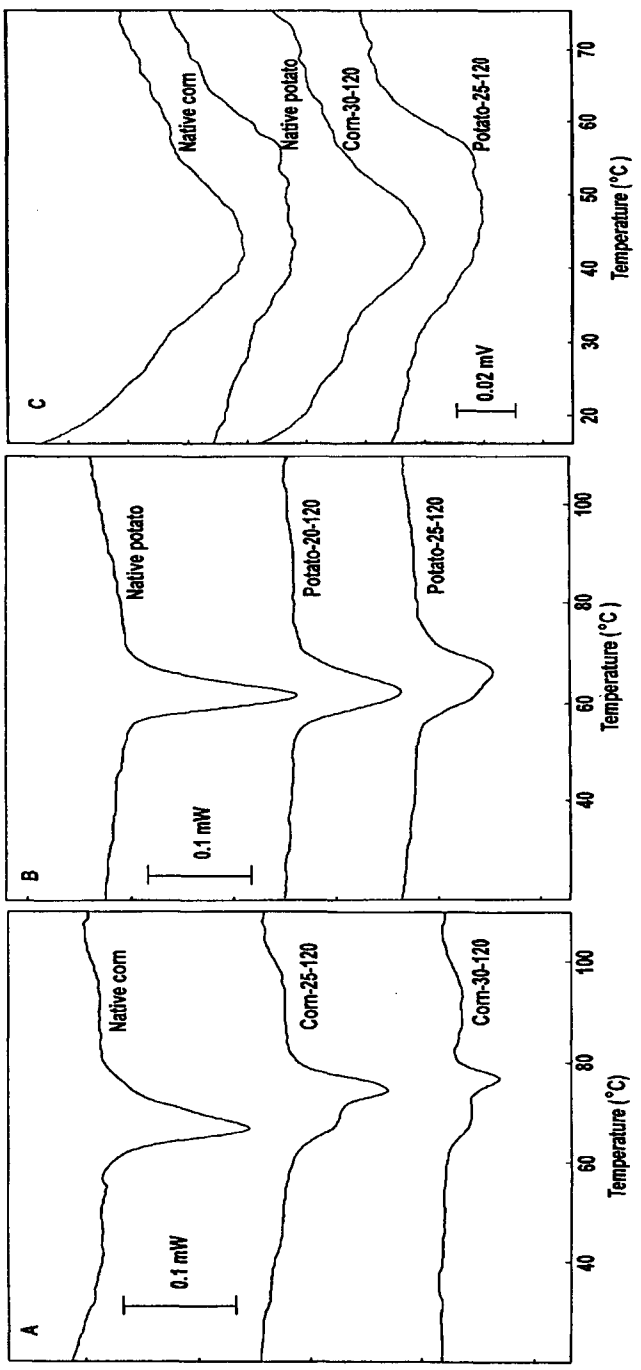

Fig. 3. DSC thermograms for the melting of native and heat-moisture treated corn (A) and potato (B) starches in the presence of excess water (4 x starch), and for the melting of recrystallized corn and potato starches (C). The numbers indicate moisture content (20-30%) and temperature (120°C) for the treatment.

(Fig. 3). The melting range increase resulted from the trend toward a smaller original endothermic peak in native starch while a newly formed high temperature peak appeared and became enlarged by the HMT. The melting range increase on thermogram by HMT has already been reported by several researchers. Donovan et al. [6] also found broad and biphasic transition with heat-moisture treated wheat and potato starches.

As discussed in the data of amorphous transitions, the transformation of the intercrystalline amorphous regions to amorphous phases may provide the short chains in the crystalline structure more freedom. Thus, the crystalline micelles undergo a structural transformation toward increased thermodynamic stability. This reformation in crystalline regions results in the newly-developed high temperature endotherm. The biphasic endotherm may indicate that this annealing undergoes heterogeneity, presumably in the location of the crystalline regions.

The thermal energy (ΔH) for melting the crystals in the treated starch was less than that of native starch (Table 1). The enthalpy reduction on DSC thermogram was substantial even though the crystallinity reduction based on the peak intensity on X-ray diffractogram was relatively small. Therefore, the HMT-induced transformation in this experiment seemed to be more significant in the short-range arrangements than in the long-range arrangements. The reduced enthalpy indicates that there was actual starch melting during the treatment. Some imperfect crystals in the native starch granules underwent HMT-induced melting resulting in the decreases in melting enthalpy.

Although the melting characteristics of heat-moisture treated starch were significantly different from that of the native starch, the retrogradation thermograms were identical (Fig. 3c).

Pasting viscosity

The pasting viscosity profile of potato and corn starches was significantly changed by the HMT (Fig. 4). As expected from the results in thermal transition and X-ray diffraction, the HMT at a higher moisture content caused more changes on the pasting profile. The HMT inhibited the swelling of starch granules, and pasting temperature increased and peak viscosity decreased, but the treated starch had improved stability during hot shearing.

The overall results in this experiment agreed with the initial findings by Kulp and Lorenz [2]. Hoover and Manuel [11] reported that the viscosity decrease by the heat-moisture treatments was caused by the interactions among the amylose chains and between amylose and residual lipids. The interactions induced by amylose chains might be concentrated in the amorphous regions under the treatment conditions where the moisture was not enough and the granular shape was unchanged. The amylose chain interactions in the amorphous regions formed the matrix having an increased rigidity of

the starch granules. Karahshi and Hizukuri [19] claimed that a small amount of monoglyceride increased the effect of HMT by producing a helical amylose-lipid complex. And the complex formation increased thermal stability to starch. The DSC data showing the raised melting range support this (Fig. 3, Table 1)

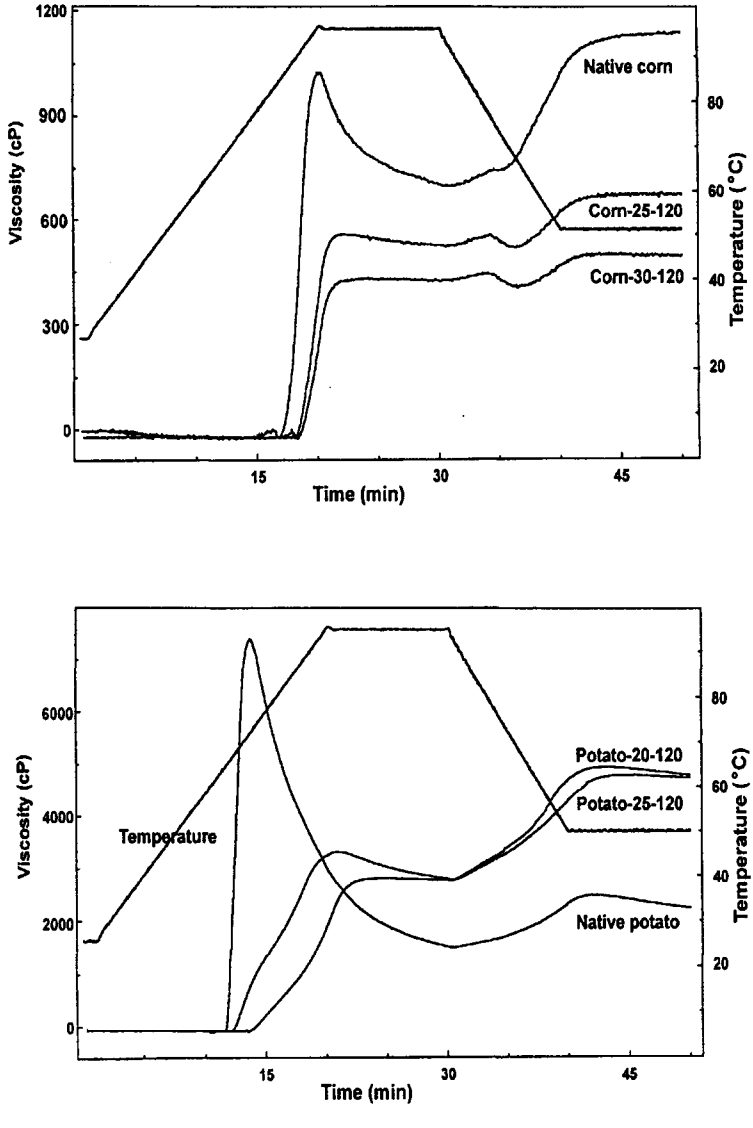

Fig. 4. Pasting viscograms of native and heat-moisture treated corn and potato starches. The numbers indicate moisture content (20–30%) and temperature (120°C) for the treatment.

Because potato starch was almost free of lipids, the interactions between amylose and lipids were not expected to occur. The absence of lipids might allow more amylose chains to form single helices or to interact each other, and this could be one reason for the greater changes in pasting profile of potato starch.

Gel texture

The textural properties of the gels of native and heat-moisture treated starches are given in Table 2. Both corn and potato starch gels showed reduced strain for gel fracture. Fracture strength, however, increased with the treated corn starch gels, whereas it decreased with potato starch gels. Native potato starch gel was very soft but not fractured up to the maximum strain used (60%). By the HMT, the potato starch gels also became brittle, resulting in the lower values for fracture strain (29 or 39%).

Initial modulus which represented the surface hardness increased by the HMT. Native corn and potato starches showed the initial moduli of 15.2 and $14.3 \cdot 10^7$ dyne/m^2, respectively. And these increased up to more than $27 \cdot 10^7$ dyne/m^2 by the HMT (Table 2). Therefore the HMT made the starch gel firmer but more brittle.

Table 2

Textural properties and syneresis by repeated freeze-thawing of native and heat-moisture treated starch gels.

Starches	Starch crystals			Syneresis	
	Fracture strain (%)	Fracture strength (g_f)	Initial modulus ($\times 10^7$ dyne/m^2)	1 cycle (%)	3 cycle (%)
Native corn	41.7^a	243.2^b	15.2^b	25.7^a	58.1^b
Corn-25-120	33.4^b	325.3^a	35.1^a	13.8^b	44.1^a
Corn-30-120	31.1^b	324.3^a	33.0^a	7.6^b	55.2^a
Native potato	NF	305.1^a	14.3^b	26.6^{ab}	45.0^a
Potato-20-120	38.6^b	251.5^a	27.7^a	19.6^b	51.1^a
Potato-25-120	28.9^b	218.4^a	29.6^a	50.0^a	67.6^a

Hoover and Vasanthan [10] also found the increased rigidity of starch gel by the HMT, and claimed that it was caused by the increased amount of amylose leaching by the treatment. The pastes and gels of the treated starches in this experiment appeared more opaque than those of the native starch. The opaqueness of starch pastes or gels may be caused by incomplete dispersion of starch granules, reassociation of the starch chains, and complexation of amylose with lipids. The restricted granular swelling as shown in the pasting curves and the amylose-induced interaction with other starch chains or lipids were attributed to the increased opaqueness.

Freeze-thaw stability

The syneresis of the starch gels while the freeze-thawing cycle was repeated are given in Table 2. With the first freeze-thawing, heat-moisture treated starch gels showed the reduced syneresis (13.8 and 7.6%, respectively at 25 and 30% moisture), compared to the value of native corn gel (25.7%). With three cycles of freeze-thawing, the gels of the heat-moisture treated corn starches remained still having better stability against the treatment than the native starch gel although the difference in syneresis was minor.

The intermolecular associations among the amylose chains that helped the gel formation for the heat-moisture treated starch affected the syneresis of the gel system. As shown in corn starch, the matrix formation between amylose chains in the amorphous regions and the restrict starch swelling as shown in pasting curves could raise the water stability in the gel matrix. But for potato starch these changes in the starch caused the opposite effect.

The pasting (Fig. 4) and gel characteristics (Table 2) showed that the HMT used in this experiment showed the physical changes which were commonly determined with chemically cross-linked starches. With minor cross-linking, the paste and gel stability of starch can be improved. But with excess level of cross-linking made the starch granules more intact and less swelling, and so gave the adverse effects. Therefore the HMT can be effectively used as the substitute of chemical cross-linking which is widely used for shear and acid stabilization of the potato or substituted starches.

Conclusions

The glass transition temperature measured at a limited moisture (T_g at 15% moisture) of corn or potato starch decreased, but the heat capacity change (ΔC_p) at the T_g was significantly raised by the HMT (20–30% moisture, 120°C for 1 h). Based on the DSC thermal transition data, the HMT may cause the intercrystalline amorphous parts to transform into an independent amorphous state, which would explain why the mutual effects between amorphous and crystalline regions were reduced. This transformation resulted in the reduced melting enthalpy of the starch and decreased T_g but increased ΔC_p. Annealing was facilitated during the treatment by the development of second endotherm at a higher temperature range than the original endotherm of native starch. The structural change by the HMT improved the paste and gel stability.

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TERMICZNE I KLEIKUJĄCE WŁAŚCIWOŚCI SKROBI OBRABIANEJ HYDROTERMALNIE

Streszczenie

Zwykłą skrobię kukurydzianą i skrobię ziemniaczaną obrabiano hydrotermalnie (HMT) (20–30% wilgoci, w 120°C, przez 1 godzinę, w zatopionej rurze szklanej), a następnie badano zmiany w temperaturze przejść fazowych i kleikowaniu. Obserwowana w widmie rentgenowskim krystaliczność skrobi ziemniaczanej obniżała się. W wyniku HMT T_g przy stosunku skrobia : woda jak 1 : 4 podnosiła się o 1–4°C. Jednakże przy ograniczonej wilgotności T_g spadało o 2–6°C. Skrobia ziemniaczana miała T_g o ok. 25°C wyższą niż skrobia kukurydziana bez względu na zastosowaną HMT. Zmiany pojemności cieplnej (OCp) w T_g, skrobi poddanych HMT były o wiele wyższe niż dla skrobi natywnych. Entalpia i temperatura początku przemiany także wzrastały dzięki HMT. Jak pokazały wykresy DSC topnienie krystalitów skrobi po HMT przy stosunku skrobia : woda = 1 : 4 było dwufazowe. Skrobia kukurydziana pokazała niewielki wzrost entalpii topnienia dla kompleksu amylazowo-lipidowego. HMT wywoływała wiekszą opalescencję żelu, który stawał się bardziej kruchy. Po HMT nieco wzrastała odporność żelu ze skrobi kukurydzianej na zamrażanie i rozmrażanie, natomiast żel ze skrobi ziemniaczanej zachowywał się odwrotnie. Dane o kleikowaniu wskazują, że HMT wywoływała takie zmiany, jak fizyczne sieciowanie.

I.M. DEMIATE*, N. DUPUY, J.P. HUVENNE, M.P. CEREDA, G. WOSIACKI

FTIR SPECTROSCOPY OF MODIFIED CASSAVA STARCHES PRESENTING EXPANSION PROPERTY#

Abstract

The property of expansion of cassava sour starch is very valued allowing the production of expanded gluten-free biscuits without need of extrusion, baking powder or yeast addition. Lactic fermentation and sun-drying are involved in the modification of cassava starch and are linked with the baking ability. Photo-oxidation was suggested as possibly responsible for chemical alterations of the starch macromolecules and in the present work cassava starch oxidation was considered. Potassium permanganate was employed for starch oxidation followed by lactic (LAC) or citric (CIT) acid treatment. Native cassava starch (NAT) as well as lactic acid treated and oven (LACOV) or sun-dried (LACSUN) samples were considered for comparison. One sample of commercial cassava sour starch (SOUR) was also analyzed. The results showed that both chemically (LAC and CIT) and photo-chemically (LACSUN and SOUR) modified samples presented baking property, but not NAT and LACOV. The carboxyl content was higher for the chemically oxidized samples indicating that they were more extensively modified. The FTIR spectroscopy data of these and some other samples resulted in a separation by their spectra, after being studied by principal component analysis (PCA). The presence of carboxylate groups (1600 cm^{-1}) was essential for differentiating the samples. By using partial least squares regression (PLS) on mean normalized data, it was possible to predict the expansion of the samples, that was positively related with carboxylate band (1600 cm^{-1}) and negatively related with another band at around 1060 cm^{-1}, that was assumed to be due to a degradative oxidation taking place at C-O bond (carbon 1 and oxygen 5) of the cyclic part of glucose.

I.M. Demiate, G. Wosiacki, Departamento de Zootecnia e Tecnologia de Alimentos, Universidade Estadual de Ponta Grossa, Praça Santos Andrade, s/n, 84001-970, Ponta Grossa PR Brasil. E-mail: demiate@interponta.com.br; N. Dupuy, J.P. Huvenne, Laboratoire de Spectroscopie Infrarouge et Raman (LASIR), CNRS, Bât C5, Université des Sciences et Technologies de Lille, 59655 Villeneuve d'Ascq Cedex France; M.P. Cereda, Centro de Raízes e Amidos Tropicais, Universidade Estadual Paulista, C.P. 237, Faz. Exp. Lageado, 18603-970, Botucatu SP Brasil.

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**corresponding author.*

Introduction

Cassava starch presents physico-chemical characteristics of interest both for native starch application as well as for using as raw-material for modifications. Cassava sour starch is an example of a different product obtained by fermentation followed by sun-drying in some South American countries. Mainly in Brazil and Colombia, known as *polvilho azedo* and *almidón agrio*, respectively, is very valued and unique for production of gluten-free biscuits and bread-like foods [1]. Studies indicated that only after sun-drying the fermented cassava starch presented expansion property. UV-irradiation of lactic acid treated cassava starch also promoted structural modifications on cassava starch that became expansible when baked [2]. There is no need of extrusion or addition of baking powder or yeast to the dough.

As cassava starch seems to be very susceptible to oxidation, and there were some indications that cassava sour starch could be partially degraded by photo-oxidation [3, 4], oxidizing chemical treatments were tested for starch modification [5]. Potassium permanganate associated with lactic acid was employed for treating cassava native starch and some properties of the modified samples were evaluated. FTIR spectroscopy was used for detecting structural changes on the samples in comparison with native and other modified ones [5, 6]. Sodium hypochlorite as well as hydrogen peroxide/Fe_2SO_4 associated with lactic acid were also considered as modifying agents and the samples tested for some characteristics [5].

The FTIR spectral study was selected due to previous results described in the literature [7-13]. Considering mid-infrared techniques for food analysis, Wilson & Tapp [13] cite qualitative and quantitative applications as advances that caused impact on the food sector; they predict that MIR spectroscopy is likely to continue to increase and develop in the near future.

The spectral data, when exploited by multivariate analysis (chemometrics), allow best interpretation of the information, that in many cases is not evident on the raw spectra. It is possible to relate latent variables with structural differences that appear in the spectra when they are conveniently explored. Both qualitative and quantitative methods can be used for data analysis and will make possible to classify the samples and to associate structural information with some physico-chemical or functional properties. Principal component analysis (PCA) is a qualitative method that when employed on FTIR spectral data allows classification of foods to be undertaken without any other chemical analysis [14].

Partial least squares regression (PLS) allows multicomponent quantitative analysis in mixtures, being able to choose the best spectral components for the regression with regard to the variation of the concentration [15]. PLS regression uses all spectral data to determine analyte concentration by factoring all wavelengths into the resulting

equation on any selected area of a spectrum. In the PLS approach, the concentration matrix is used at the same time that the spectral data matrix and, by balancing the spectral information and the related concentrations the method reduces the impact of large but irrelevant variation in the spectra [16].

Material and methods

Cassava native starch (NAT) was a gift of a Brazilian factory and was employed for producing the modified samples and also analyzed for comparison. Cassava sour starch samples (SOUR and SOUR2) were bought in Brazil on supermarkets and analyzed directly. For modifying the starch, reagent grade chemicals were used. Detailed description is found on Demiate [5] and Demiate et al. [6].

Starch modification

Cassava starch was suspended on 0.1 N potassium permanganate solution for 15 min under mild agitation at room temperature (*ca.* 20°C). After this period it was recovered by paper filtration with aid of a Büchner funnel and a vacuum pump. The moist starch was washed with de-ionized water and re-suspended in 1% (w/w) lactic (LAC) or citric (CIT) acid solution. This suspension was kept at room temperature for 30 min and then oven dried at 40°C. A part of the sample was washed with de-ionized water for complete elimination of excess acid (LACW and CITW).

Some other samples were considered, including another cassava native starch (NAT2) and modified ones (LAC2, LACW2, SHLAC, SHLAC2, OXLAC, OXLAC2, LACSUN, LACSUNW, LACOV, LACOVW, LACOVW2). The description of how these samples were produced is presented in Table 1, that also shows their carboxyl content, pH and expansion on baking.

pH and carboxyl content

The pH was measured in starch suspension after a 30 min stabilization period by direct electrode immersion. Carboxyl content was determined as described by Smith [17], by NaOH titration of the hot starch paste.

Expansion property

The expansion of the samples was determined as described on Demiate [5] and Demiate et al. [6]. The expanded biscuits were weighed and, after been made impermeable with paraffin, their volumes were determined by water displacement on graduated cylinders. Expansion values were expressed as specific volumes (SV) in mL/g.

Table 1

Source of modified cassava starch samples, their carboxyl content, pH and expansion on baking (SV, mL/g)#

Sample	Description[1]	COOH %	*p*H	SV (ml/g)
NAT	Native commercial cassava starch	0.113	6.0	3.2
NATW*	Washed NAT	0.090	5.8	3.2
NAT2*	Another sample of native commercial cassava starch	n.d.	6.0	3.2
SOUR*	Commercial cassava sour starch	0.349	3.5	10.0
SOUR2	Another sample of commercial cassava sour starch	n.d.	3.7	10.0
LACOV*	NAT immersed in 1% (w/w) lactic acid solution for 4 hours, drained and oven dried	0.698	3.0	3.2
LACOVW*	Washed LACOV	0.135	4.7	3.2
LACOVW2	Same as LACOVW but produced with NAT2	n.d.	4.9	3.2
LACSUN*	Produced with the same treatment as LACOV, but sun-dried for 8 hours instead of oven drying	0.563	3.0	11.5
LACSUNW	Washed LACSUN	0.135	4.7	10.6
LAC	NAT sample suspended in 0.1 N $KMnO_4$ solution for 15 min, drained and immersed in 1% (w/w) lactic acid solution for 30 min	0.405	4.1	18.0
LACW*	Washed LAC	0.203/***0.360***[2]	4.5/ ***4.2***[2]	17.3
LAC2*	NAT immersed in 0.06 N $KMnO_4$ solution for 15 minutes, drained and immersed in 0.79% lactic acid solution for 30 min	n.d.	6.0	17.4
LACW2	Washed LAC2	n.d.	5.8	16.0
CIT	Produced.by the same procedure as LAC sample but immersed in 1% (w/w) citric acid solution	0.495	3.5	14.6
CITW*	Washed CIT	0.225/***0.315***[2]	3..9/ ***4.0***[2]	12.9
OXLAC	NAT sample suspended on 0.05% $Fe_2SO_4.7H_2O$ solution for 15 min, drained and immersed on 0.86% lactic acid solution to which 2ml of 30% H_2O_2 were added. After 30 min starch was recovered and dried	n.d.	4.1	10.0
SHLAC*	NAT sample suspended in 2.4% NaClO solution for 15 min, drained and immersed in 0.86% lactic acid solution for 30 min	n.d.	3.7	8.0
OXLAC2*	Sample produced by the same procedure as OXLAC but with NAT2	n.d.	n.d.	10.8
SHLAC2	Sample produced by the same procedure as SHLAC but with NAT2	n.d.	n.d.	8.3

[1] Reactions were always carried out at room temperature (ca. 20°C) and oven drying was always made at 40°C.

[2] The values correspond to de-ashed samples

n.d.: not determined

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Infrared spectra

The mid-infrared spectra were collected employing an IFS48 Bruker spectrophotometer. The absorbance spectra were computed between 4000 and 700 cm^{-1} at 4 cm^{-1} resolution with the apodization function of the standard Bruker software. The 2000–700 cm^{-1} region was preferred for multivariate analysis due to the presence of well described absorption peaks. Starch samples were diluted into KBr pellets (1.5% w/w) and the transmission was the chosen technique for getting the spectra. Detailed description of the methodology is discussed on Demiate et al. [6].

Multivariate analysis

Principal component analysis (PCA) was employed for extracting systematic variation of the spectra. It models the variance/covariance structure of the data matrix into a model that considers noise as an error. The objective of applying PCA was to search for a separation among the samples based only on their FTIR spectral differences. Due to the characteristics of the mid-infrared absorption bands, it would be possible to find out the structural changes responsible for that separation.

The quantitative analysis was made by using the partial least squares regression (PLS). By employing this method it is possible to correlate mathematically the spectra matrix and other experimental data. In the present paper the expansion values were considered in order to find out structural changes that might be associated with this functional property. Representative samples were selected and a calibration model developed (*samples of Table 1). The expansion ability of the remaining samples was predicted.

Both qualitative and quantitative chemometric applications were performed by "The Unscrambler" software, version 6, from CAMO – Computer Aided Modeling (Trondheim, Norway).

Results and discussion

Cassava modified starches were produced and presented expansion property. It was possible to evaluate the samples in relation to their pH values as well as carboxyl contents. The FTIR spectral study was also done and, when evaluated by chemometric techniques, made evident some structural differences among the samples.

All samples that were exposed to oxidizing treatments (sun-drying or chemicals) presented expansion property. Native cassava starch samples and that only exposed to lactic acid and oven-dried did not have expansion when baked (Table 1). Even after elimination of soluble compounds by washings, the oxidized samples presented expansion, what suggests structural modification.

FTIR spectra

When the raw FTIR spectra were evaluated it was very difficult to discover any difference among the samples. As all samples were chemically very similar, their spectra should not evidence important changes (Figure 1).

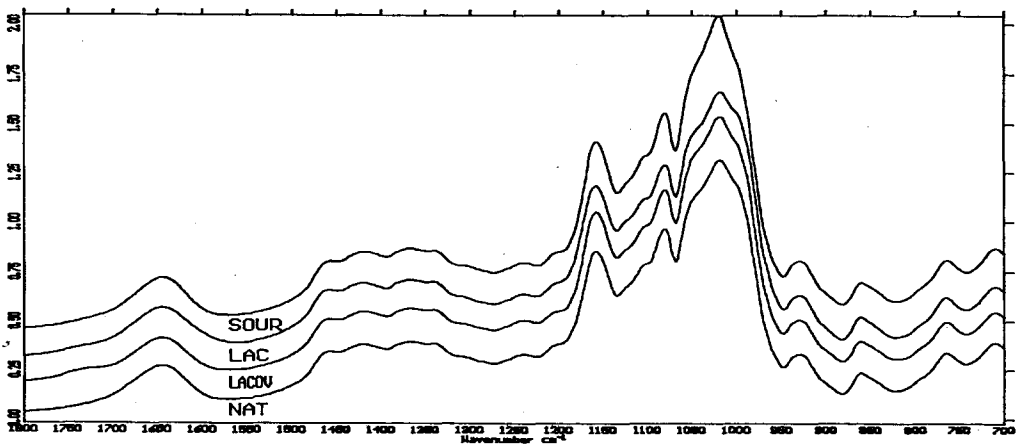

Fig. 1. Infrared spectra of some samples in the 1800-700 cm^{-1} spectral region
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Multivariate analysis

The spectral data were initially considered as a broad region (4000–700 cm^{-1}) for PCA analysis. In order to eliminate undesirable variations, the first derivative of the spectra was considered and the region 1800–1540 cm^{-1} was selected as the most important for sample separation. This selection was done after a long period of data exploitation without any important result. Also, the previous knowledge about chemical characteristics of the samples was important in the definition of the spectral region. Acid carboxyl groups have a high absorption at around 1730 cm^{-1} whereas the carboxylate form absorb at around 1600 cm^{-1}.

In Figure 2 it is possible to observe the sample separation and the structural information related to that. The principal components called "loadings" are presented as a distribution of the variance with respect to the wavenumbers. For each sample the original data may be reconstructed by the sum of the loadings multiplied by coefficients called "scores". The other figure presents, for each principal component, the scores with respect to the sample numbers. The common characteristics are modeled in one or several principal components for which the scores are not significantly different

according to the species. On the other hand, the information which differentiates the species contributes to principal components whose scores were significant. The classification of the samples was done by the scores since the characteristics of each species was established by the interpretation of the specific loading [18].

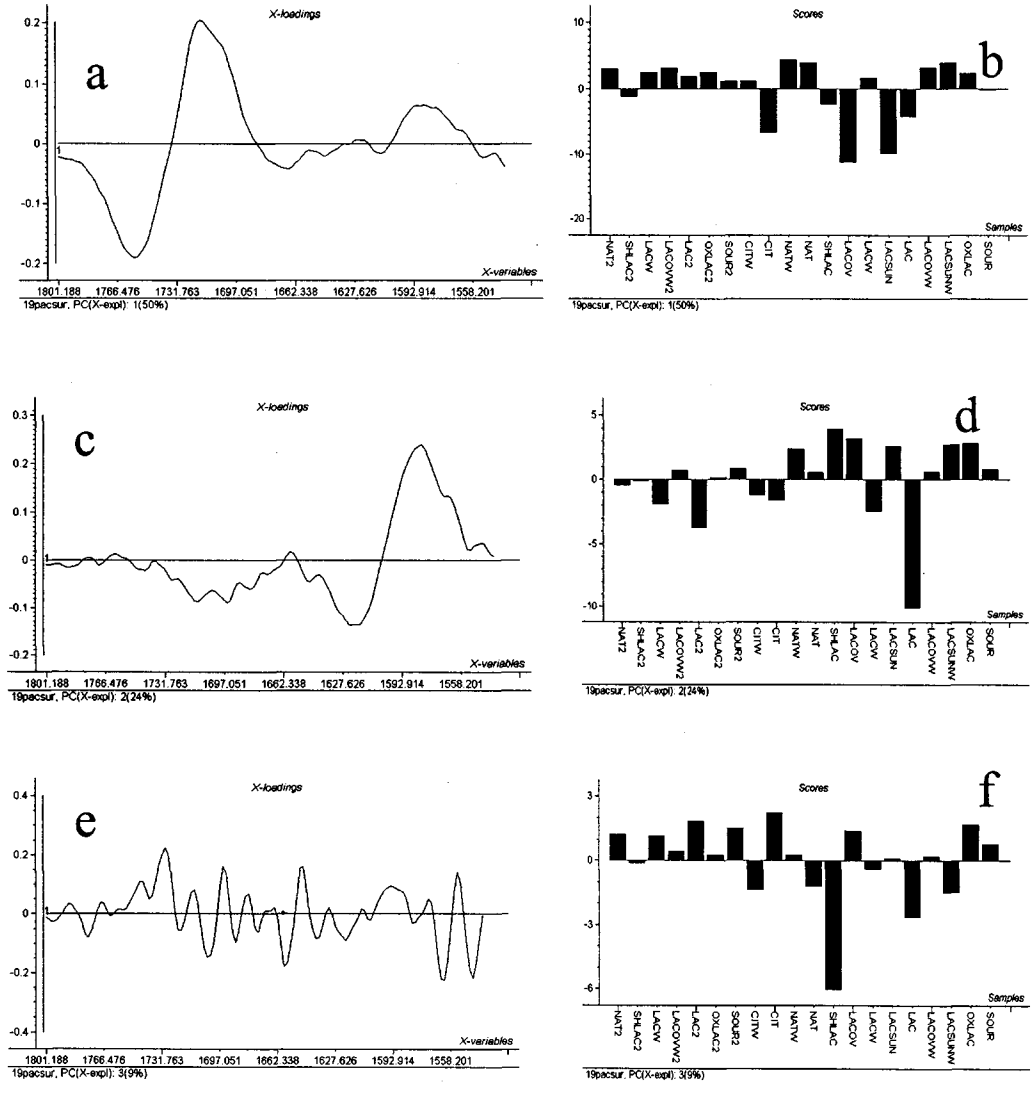

Fig. 2[#]. The three principal components (a, c, e) and the associated scores (b, d, f).
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The first and the second principal components showed the sample classification based on their acid and ionized carboxyl contents, respectively, and explained 74% of total variance. The third principal component did not present important sample separation.

The use of PLS regression allowed the prediction of sample baking behavior based on their FTIR spectra indicating a structural relationship. The calibration was done by using the PLS software in two spectral regions: [1800-1540] cm^{-1} and [1800-1525] and [1360-1030] cm^{-1} on the absorbance spectra obtained after mean normalization of data. The results are shown in Table 2.

Table 2

Predicted values of expansion by PLS regression, compared with observed values#.

Sample	Reference values	Predicted values in [1800 – 1540] cm^{-1} region	Predicted values in [1800 – 1525] - [1360 – 1030] cm^{-1} region
SHLAC2	8.3	9.5	11.8
LACW2	16.0	13.0	14.1
LACOVW2	3.2	6.1	2.2
SOUR2	10.0	9.3	7.5
CIT	14.6	18.4	16.2
NAT	3.2	11.3	2.6
LAC	18.0	44.8	23.6
LACSUNW	10.6	-6.9	7.2
OXLAC	10.0	-29.6	8.5
SEC		0.5	0.26
SEP		17.8	2.9

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The second spectral region predicted the expansion values with a lower standard error (SEP) if compared with the first one. There was positive interference of the 1600 cm^{-1} band and a negative contribution of a band at *ca.* 1060 cm^{-1}, as shown in figure 3. The first band is due to carboxylate presence in the starch molecules. The second band (1060 cm^{-1}) is not very well described as the first but may be attributed to C-O vibration on carbon 1 and oxygen 5 of the cyclic part of glucose (19).

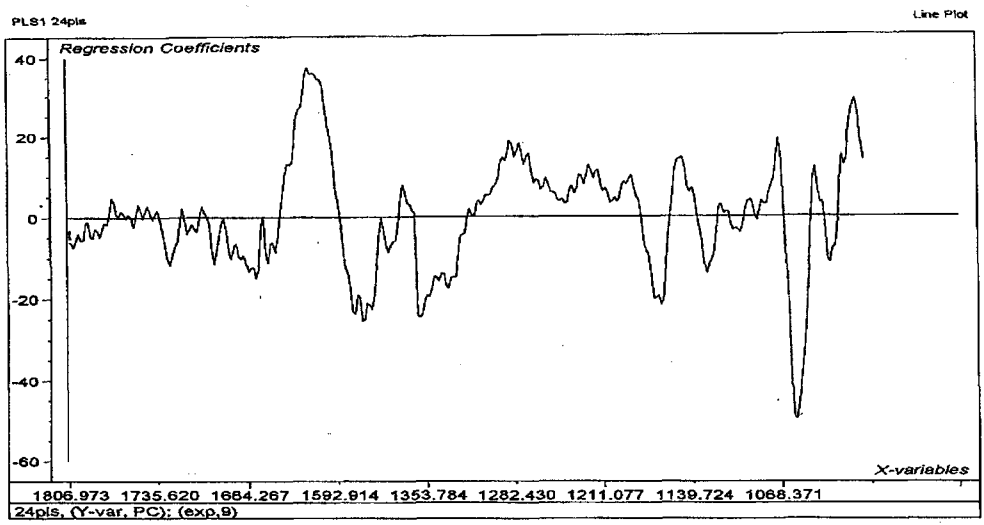

Fig. 3[#]. The regression coefficients obtained in the second spectral region.
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SPEKTROSKOPIA FTIR MODYFIKOWANYCH SKROBI TAPIOKOWYCH O WŁAŚCIWOŚCIACH EKSPANDUJĄCYCH

Streszczenie

Ekspandowanie kwaśnej skrobi tapiokowej pozwala otrzymywać bezglutenowe ciasto biszkoptowe bez potrzeby stosowania ekstruzji, proszku do pieczenia lub drożdży. Efekt ten osiąga się prowadząc fermentację mlekową skrobi i suszenie sfermentowanego produktu w słońcu.

Obserwowane zmiany właściwości chemicznych, zachodzące w trakcie tego procesu, przypisuje się fotoutlenianiu. W celu potwierdzenia tego poglądu porównano właściwości skrobi tapiokowej utlenionej $KMnO_4$, a następnie zakwaszanej kwasem mlekowym (LAC) lub cytrynowym (CIT), ze skrobią natywną (NAT), skrobią traktowaną kwasem mlekowym, a następnie suszoną w suszarce (LACOV) i w słońcu (LACSLIN) oraz handlową skrobią fermentowaną (SOUR).

Okazało się, że w przeciwieństwie do skrobi NAT i LACOV, skrobie LAC i CIT oraz LACSLTN i SOUR nadają się do wypieków.

Skrobie utleniane posiadały większą liczbę grup karboksylowych (1600 cm^{-1}). Na podstawie zmian intensywności tego pasma można było przeprowadzić interpretacje skutków utlenienia, posługując się analizą głównego składnika (PCA). Zmiany zachodzące w intensywności pasma przy 1600 i 1060 cm^{-1} zależały liniowo od stopnia eskpandowania.

Na podstawie nachylenia korelacji przypuszcza się, że degradacyjne utlenianie zachodzi pomiędzy C1 i O5 jednostek glukozowych.

JÓZEF FORNAL

STRUCTURAL PROPERTIES OF STARCH IN FOOD SYSTEMS

Abstract

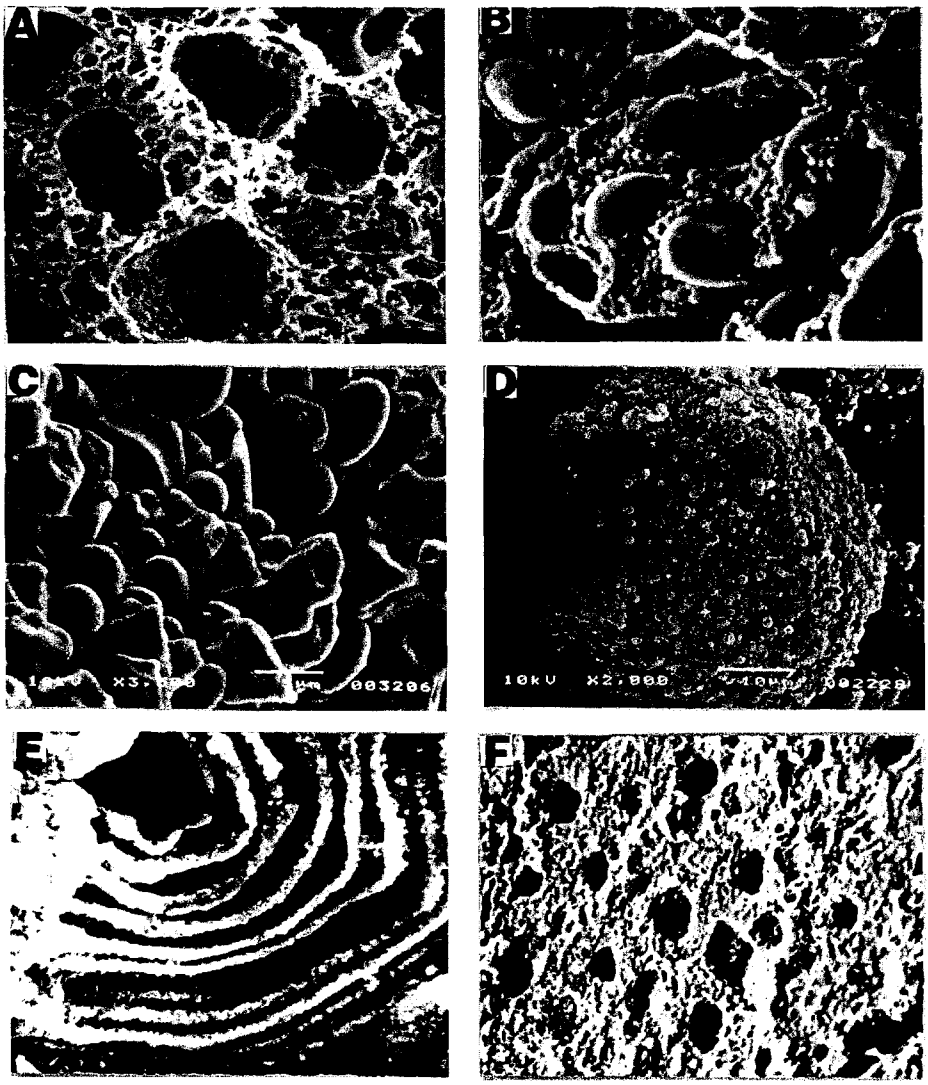
In the present work, structural changes of starch granules as seen by LM, SEM, TEM, CLSEM under different processing were shown, in relation to a function they were playing in the ready products. Special emphasis was also paid to starch change during different modification processes of the isolated starches for food and non-food uses. The structure of irradiated starches, resistant starch obtained by different methods, starches as encapsulating materials, high pressure treated starches as well as packaging materials were presented.

Starch, the most important storage component in many of the plant materials, is not only a source of energy for developing seed but also an important component of human diet. Its properties, depending on botanical source as well as processing are crucial for many functional properties of food.

Starch granules formed in amyloplasts differed in shape, size, localization within the cells as well as in proportions of granules fraction. Their appearance in cells is closely related to other cell components, mainly protein, being different in cereals (Phot. 1a), where protein matrix surrounds starch granules and in legume seeds (Phot. 1b), where additionally protein bodies envelope the granules [7]. Cereal starches are characterized by the presence of at least two fractions: large (10–45 μm) and small (1–10 μm). The first ones lenticular in shape are 70–90% by weight but only 30–10% by number, of the whole granule population, while the latest are spherical and more resistant to technological parameters. This is one of the reasons why in the starch industry fractionation of potato or wheat starch granules is being increasingly popular, or why the sources of starch with uniform and smallest size are searched for. Such starchy materials are oat (Phot. 1c) and amaranth (Phot. 1d) or quinoa [1, 8, 12, 28, 36]. Internal, lamellar structure and organization of starch granules is shown in Phot.1e where concentric rings and crystalline and amorphous parts of the granules are clearly dem-

J. Fornal, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland.

onstrated. Such picture can be obtained only after an enzymatic attack in controlled conditions (α-amylase treated starch in laboratory conditions germinated -as in the case of barley, or sprouted in non-controlled conditions).



Phot. 1. Microscope pictures of different starches;
A/ protein matrix of wheat grain in which starch granules are entrapped (amylase treated); B/ starch and protein in legume cotyledon cell; C/ oat starch granules of different shape and size; D/ amaranth starch agglomerate; E/ lammellar structure of barley starch; F/ cross-sectioned starch granules in corn grits.

The changes in starch (e.g. among others: swelling, solubilization, gelatinization and granule breakdown) occurring during technological processing, their interactions with other food components result in the physico-chemical properties of raw materials and finally creation of the new texture of the final products. The processes dominating in food technology are of thermal or hydrothermal nature [7, 8, 10, 11, 13-17, 20, 27, 31]. The example of moisture-heat or heat tratment is production of corn flakes and popcorn [7, 11, 15]. The technological process of the former is based on corn grit cooking in sugar-salt solution (2 hr at direct steaming at $1.47 \cdot 10^5$Pa), followed by drying (two stage -90 and 80°C for 40 min) and next flaking and roasting (390°C for 5 min). The structure of corn grit is composed of many polygonal cells in which starch granules stick closely to each other being surronuded by tiny residues of protein matrix. Starch granules of the control, untreated grit reveal "intra grain cavities" with an average diameter of about 2.5 μm. Pictures taken from grits subjected to longer cooking in liquor e.g. 35, 65 and 120 min ilustrated an increase in the dimension of pits observed to 5.5, 9.5 and 16 μm, respectively [7, 12]. It seems to confirm the presence of amylose concentrated in the central part of the granules and the amylopectin in the external one. Phot.1f shows additionally the elements of starch lamellar structure indicating its hydrolysis and considerable amount of minute globular structures, most probably dextrin-like ones. While hydrolysis takes place after 1 hr of the process and probably then shortening of starch granules chain length, division of their componental parts and a high increase in branching could be dominating. An increase in gelatinization degree (from 0 to 100%), drop in molecular weght (58.000–20.000) and viscosity of pastes of isolated starches (910 to 30 BU) confirmed these statements [12]. Drying and flaking of the hydrothermally treated grit and consequent roasting of flakes, due to a very high process temperature and crushing forces, are responsible for the completely different structure of final flakes. Instead ordered cellular structure, porous, spongy-like one, with air bubbles and characteristic air cell walls composed mainly of completely gelatinized starch, is formed. Microscope structure of flakes is to a great extent similar to that of buckwheat extrudate. In the latter, additional structural elements "pipe in pipe" of air bubles surface are visible. They are created during expansion of starch granules in the flower "bud mode" [12].

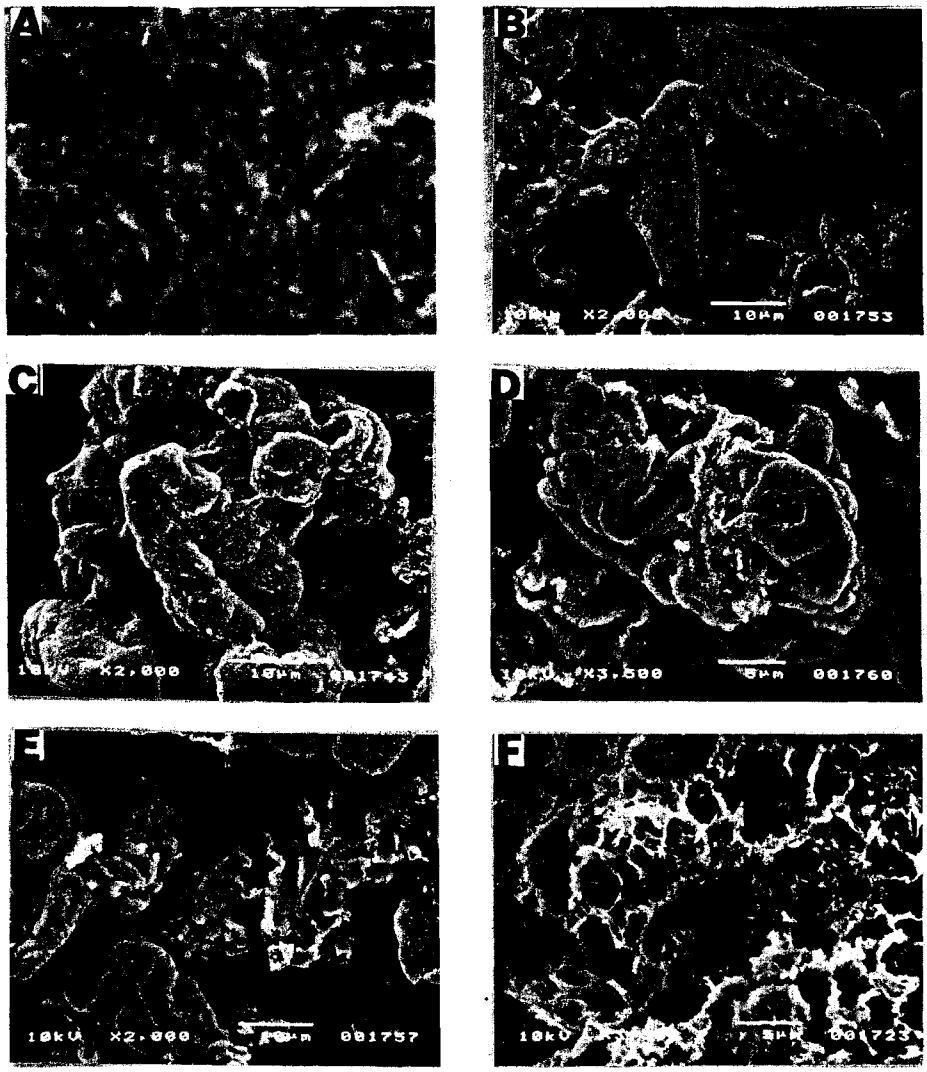
An interesting structure influencing specific texture of the product is developed during popcorn popping (heating of grains at 180°C / 6 min) where pressure inside the kernel reached 135 psi. The quality of the product strongly depends on the structure of material, mainly endosperm and pericarp. Translucent endosperm is regarded as closely associated with popping expansion being responsible for forming delicate network whereas the opaque one does not expand to such extent [7, 15].

Starch granules are also of great importance in the dough and bread structure formation [19, 24, 26]. A continuous, bimodal starch-protein structure of the dough is

based on the phenomenon of free water on starch granule surface which is responsible for creation of continuous starch phase with spaces between granules filled by gluten gel. Such a model of dough proposed by Eliasson and Larsson was confirmed also by Hug-Item et al. [17]. The LM picture of dough stained with iodine and Light Green is presented in Phot 2a. It shows small – round and large – lenticular starch granules embedded in protein part unequally distributed within preparation. The protein fraction is predominant in the dough structure, what was counted for about 60% of bread volume. Baking process resulting in creation of porous crumb structure of bread where swollen elongated and highly ordered at the pore surface granules are clearly visible. These granules are characterized by concentration of amylose zone along the length axis whereas outside the granules and arround protein matrix free leached amylose is seen (Phot. 2a). The bread crumb structure is highly porous, thus the pores diameter and volume as well as the pores wall structure (starch gelatinization degree) are responsible for mechanical / sensory (elasticity, hardness, cohesiveness, gumminess) properties.

Other cereal product where starch is the most important functional factor is pasta produced mainly from durum wheat [29]. Freeze fracture preparation and TEM examination illustrates unmodified starch granules and very minute globular components of gluten. After cooking starch, granules became swollen and gelatinized. Disintegration of starch granules is also clearly visible. Apearing starch subunits are separated or packed together into small clusters whereas protein matrix creates fibrillar network. Although the protein and in particular its subunits network is most responsible for the quality of pasta, such a product can be obtained without protein. Basing on the fact that starches of different origin have diverse gelatinization temperature and as such during technological pasta processing undergo different changes, pasta from rice, maize and potato starch was obtained. Crucial for good quality of such pasta is the phenomenon of starch repolymerization after cooking and creation of repolymerized starch network [29].

Except starches being produced on the large scale as maize (3.6 mln t in EU), wheat (2 mln t), potato (1.8 mln t) and tapioca in Asia, also other sources of starch are taken into consideration [6]. Among them legume starch [9, 10, 16, 18, 32] and starch from so called pseudo cereals (buckwheat, and amaranth) are worth noticing. Among legume seeds most popular are pea starches of different properties, which can be used as food ingredients. Some of them posesses the gelatinization capacity and viscosity profile comparable to these of cross-linked starches showing very good stability at high temperatures, shearing and pH levels, giving covering films or sliceable gels. When products are dried they markedly improve their crispness. Pregelatinized pea starch can be used in cold processes having high gelling capacity what makes possible, at a proper



Phot. 2. Starch granules deformation after heat and moisture treatment;
A/ starch granules in bread crumb structure (LM); B-F/ different stages deformation of pea starch granules during heating in water.

concentration and strong agitation, a rapid development of a firm gel. Therefore legume starches can be used in:

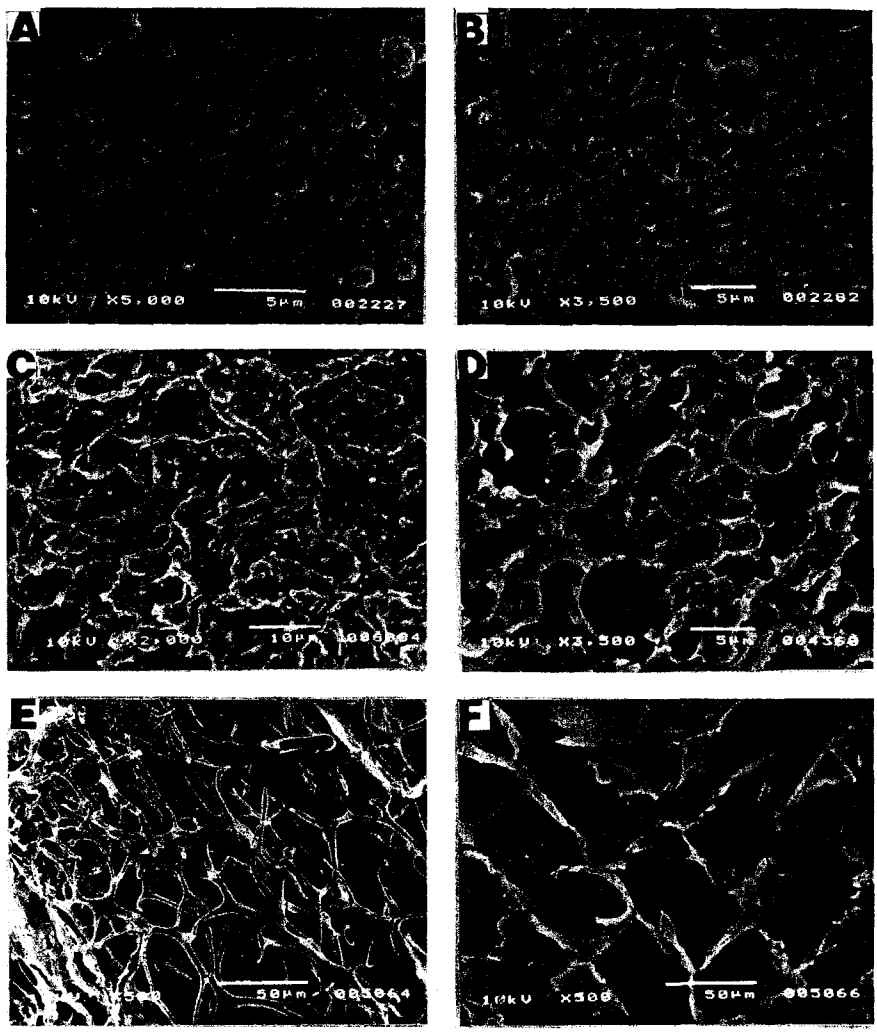
- preparation of gels (puddings) where it is possible to use up to 50% starch less than in comparable products obtained with corn starch,
- production of fruit and vegetable flakes with high cooking stability as well as pulpy texture after rehydration,

- production of instant desserts with desired texture.

The structure of legume (pea, faba bean) starch granules and their changes during heating in water suspensions are widely discussed in literature [7, 16, 20, 32]. Using the smear method, or embedding techniques for light microscope it is possible to illustrate leakage of amylose phase from the granule structure at 75°C and total disruption of granule structure at 98°C with the granule remnants of amylopectin nature embedded in the amylose phase [20]. To improve pasting properties of legume starches as well as their freeze-thaw stability, physical or/and chemical modification is used. Hindering retrogradation is visible by comparison of LM pictures taken from pea starch acetate with those of the native ones. Swelling and subsequent deformations leading at the end of heating to granule disintegration is clearly visible. The results of an additional study on extend of structural deformations that occur during heating of pea starch granules are shown in the Photos 2b-2f. The course of deformatiom and granule disintegration is in high accordance with the Bowler's theory which was developed only for lenticular wheat granules [4]. The initial swelling, which has a radial chracter, leads to flattenning of the granule shape. The amylose concentration took place in the middle part along the lenght axis of the granule folding at this particulary plane (Phot. 2b). It is a reason of folding the granule at this plane. At the next stages of deformation clear visible halves of the granule becomes closer to each other with visible preliminary division into smaller subunits. It is also evident that surface of the granules become intensively pitted and covered by amylose phase (Phot. 2c, 2d). At elevated temperatures, it is possible to observe that tendency to pucker is even higher (Phot. 2d, 2e). It means that some differences at the molecular level between granule axes are present. Probably, as in wheat lenticular starch granules, pea starch granule in xy (lenght-width) plane is composed mainly of molecules bound by covalent bonds in radial direction and in tangential direction by much weaker non-covalent ones. Higher temperatures disrupted non-covalent bonds resulting in the preferential swelling in tangential direction. It is the reason of visible deformation – puckerig of the granule out of xy plane. The last step is the disruption of granular structure of starch (Phot. 2f).

In the last decade, due to consumers demand, substantial reduction of caloric value of food is of great interest in the food manufacture. Lowering of such food ingredients as sugar, salt, cholesterol and fat in the human diet was achieved by special substitutes. Among them, fat replacers or fat substitutes are of starch origin. Native starch granules or modified ones are often used in the production of salat dressings, mayonnaises or processed cheese [21, 22, 34, 35, 36]. Due to special properties, some of native starches isolated from pseudocereals like amaranth are very useful in their manufacturing. The amaranth starch granules are extremely small and uniform in diameter 0.75–1.2 μm being much smaller than the smallest granules of the industrially produced rice starch. The content of amylose can vary, depending on species, from 4.8

to 22%, although, waxy species without amylose are also known. The shape and size of amaranth seed cells resemble that of pseudocereal buckwheat rather than of cereal grain. Starch granules are dominating structural elements whereas adherent to starch protein as well as cell walls were weakly marked. When starch is isolated from the seeds, unusually uniformed starch granules are assembled in greater agglomerates consisted of several hundreds of single granules (Phot. 3a). When amaranth starch is



Phot. 3. Pictures of different starch granules and their products;
A/ native amaranth starch garnules; B/ amaranth starch granules heated 15 min at 85°C; C/ yoghurt with starch as structurizing agent; D/ processed cheese with starch addition; E, F/ different kinds of starch gels.

heated in water suspensions at 55°C, the swelling of individual granules is visible. At elevated temperatures of 70 and 85°C respectively, the significant breaking of individual granules and their agglomeration initiating the network structure followed to form very delicate fibrous network structure after complete gelatinization (Phot. 3b) [36].

The properties of amaranth starch (low pasting temperature associated with a high rheological stability) were the base of its use in low-fat (less than 50%) mayonnaises [36]. If thickening power of amaranth starch was compared to that of potato and, it was found a close relationship between the mayonnaise viscosity and thickening agent addition, and despite of a slightly lower thickening power of amaranth starch as compared to the potato one, the concentration of 1.75% ensured thickening effect acceptable in the traditional product. It is worth stressing that low-fat mayonnaise produced with amaranth starch showed excellent sensory properties, better than these of potato starch. Most probably it resulted from the fine granularity of starch used. The only problem during long storage was the lack of rheological stability for product made from amaranth and potato starch as compared to corn starch. This can be improved by starch modification, using for example standard cross-linking and some stabilizing agents [36].

Another example of calorie-reduced products can be yoghurt and processed cheese (Phot. 3c, 3d). Fat substitutes used in both products can be based, among others, on microparticulate whey protein or starch. Modified starch preparations used under processing conditions are fused with the protein particles giving uniform matrix in which fat droplets or fat agglomerates are placed. Such a structure is responsible for desired texture properties (graininess, stickiness, mouth coating, and greasiness) as well as rheological properties (spreadability, stickiness or cohesiveness) not differing too much from the product without any fat substitute [5, 35].

Another important role that starch can play as a food component or as a pure preparation obtained by starch modification is so called resistant starch (RS) [23, 30, 34]. Resistant starch is a component of raw potatoes and green bananas or can be generated in food due to the action of heat and water. Its final amount to be present in foods is dependent on such parameters as starch concentrations, amylose/amylopectin content, starch/water ratio and energy supplied to the system. Retrograded starch is the most common RS starch in the diet and from the technological point of view, it is the most important type of resistant starch because it is formed as a result of food processing.

Resistant starch can be also produced from isolated starches by retrogradation, spray drying or by enzymatic modification. Depending on the process applied, RS (never being pure resistant starch but the mixture of its different forms) is characterized by different microscope structure and properties (water holding capacity -2.0-3.5 in

comparison to 668–732 g/g dmb wheat and potato native respectively, and fat absorption 1.2–1.6 g oil) [22, 23, 34].

The special properties of starch in food systems – gelling properties, are shown in Phot. 3e and 3f. Structure of such gels is strongly dependent on the proportion between both polymer of starch: amylose and amylopectin, as well as processing parameters [2, 13]. For microscope determinations of the structure of such gels, the factors of great importance are also methods of specimens preparation for analysis. The most important is the step of freezing of water-containing samples which can introduce ice crystal artefacts. The extent of structure damages is dependent mostly on the size of crystals formed. Therefore, to avoid this undesired phenomenon, the special intermediate velocity of freezing (1000°C/sec) is recommended [3]. The newly introduced methods of sample preparation for microscopy as well as new microscope methods will be mentioned latter.

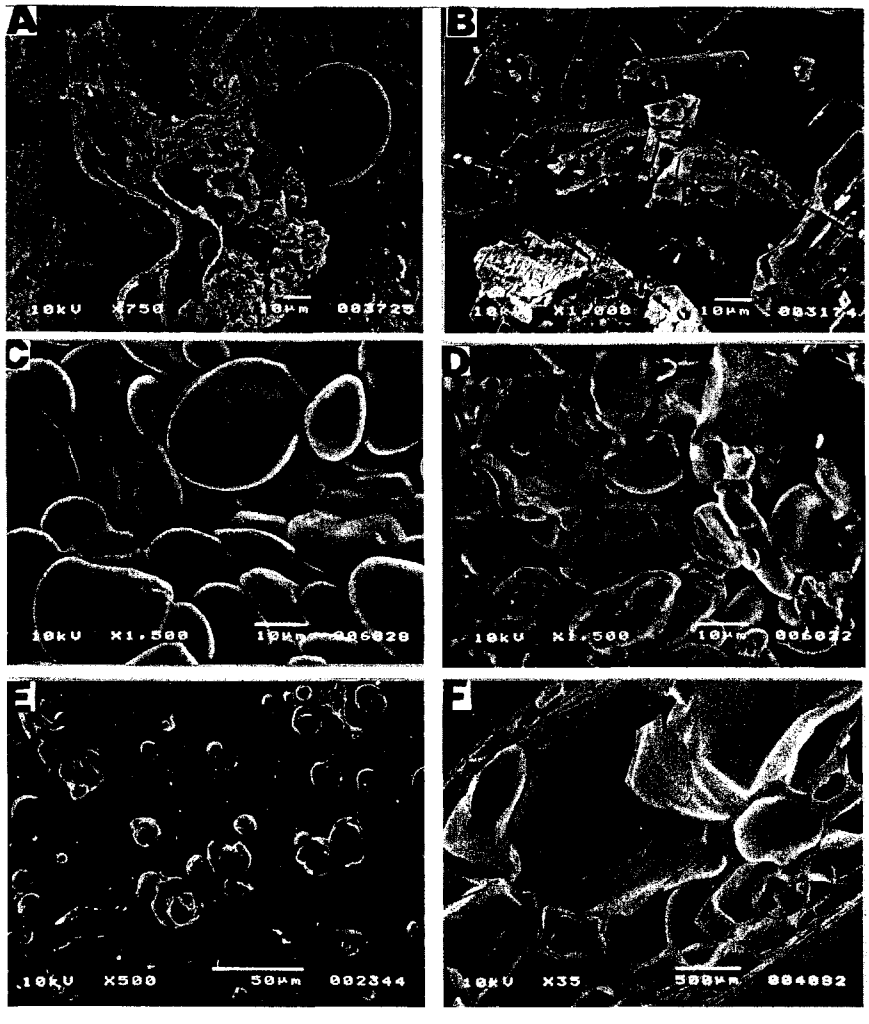
High water binding properties of modified starches as well as their enhanced gelling properties are very usefull for keeping quality of meat products to be portioned and long term stored in the supermarkets. The kind of modification strongly influences mentioned properties of different starches used for this purpose. This is especially visible after heating to the temperature which is reached inside the product during pasteurization [Phot. 4a].

Other popular starch derivatives used as food ingredients are such preparations as: cyclodextrins, porous starches and starch coacervates used as a vehicles for aromas, vitamins and food pigments/colourants or other substances (Phot. 4b) [38].

Except the mentioned above processing and products, very promissing ones in respect to starch properties, among others, are microvawe treatment and high pressure tratment. The changes induced in the granule structure are clearly visible for the former in LM, showing different pattern of breakdown in comparison to the structure of native granules. Spherical single structures or their agglomerates separated or perhaps tight together with brown amylopectin material are dominating in the smeared preparation. Thus, they can result in a rise of gelatinization temperature, drop in solubility of starch granules or viscosity. The extent of those changes was dependent on initial moisture of starch [21, 22]. Also high pressure can markedly influence structure and properties of starch. Depeding on processing parameters, we can obtain the products with different susceptibility to alpha-amylase and different rheological properties (Phot. 4c, 4d).

Starch granules are also used alone or in composition with polymers such as polystyrene to form structure and properties in packaging materials (foils, foams, alkogels) (Phot. 4e, 4f). The biodegradability of foils with starch addition is depending on the properties of starch in concentrates for foil obtaining, their percentage in the mixture and susceptibility to amylase attack. Being degraded first, starch is making place to other hydrolytical enzmyes slowly degrading another part of the foil. Different struc-

ture is formed in aerated products as for example plates, which become porous, and are due to many air cells of very low density [37].



Phot. 4. Examples of different use of starches;
A/ meat product; B/ cyclodextrins; C, D/ high pressure treated starch gels (3500 atm at 15 and 60 min); E/ starch in the structure of biodegradable foil; F/ structure forming properties of starch in foams.

Structural analysis of all foods, also these containing starch, in transmission and especially in giving three-dimensional structure impression, scanning electron microscopes are often difficult to interpret because of the artefacts appearing in thespecimen preparation procedure. As it was mentioned above the freezing velocity is one of the

most important factors influencing the extent of damaging by ice crystals formation in foods containing water [3]. To avoid this new method of freezing the sample – High pressure freezing (HPF) was developed, which can replace such methods like, for example, jet freezing or mirror slaming. In this technique, the sample is exposed to a very high pressure (2000 atm) and immediately frozen by jet of nitrogen. In 100 μm thick sample of the gel gelatine/water -5/5vv after HPF internal structure even on the depth of 50 μm is very clearly marked, whereas in the sample prepared by the traditional method structural changes caused by ice crystals formation are present on the depth of 5 μm and are even more pronounced on the depth of 15 μm. Also in milk gels such method of preparation, in comparison with the traditional one, reveals much more details of the structure. Preservation of protein structure in the casein network and fat globules is clearly visible. Also after use of immunization and gold labelling the localisation of β-lactoglobulin is much better visualised [3].

The development of new microscope techniques also creates new possibilities for more detailed structural analysis.

Among these methods some are very promissing. Field Emission Scanning Electrone Microscopy combined with cryo preparation allows observations of, for example, different materials with lamellar structure, which until now were possible only by the replication method in TEM. High resolution and no damages occurring at 2 kV is a great advantage of this method [3, 33].

Scanning Tunneling Microscopy is able to ilustrate in real time the surface relief with resolution of 2 A. The very small structures like for example the globular protein –vicyline with molecule length of about 100 A can be investigated. This microscopy can work also in water and low electrone energy (few V) what does not distroy the sample [3, 33].

The special potential for starch structure investigations represents Atomic Force Microscopy where the action of amylolytic enzymes on individual granule as well as the geometry of resulting pits and lamelles can be calculated [33].

An interesting and modest tool for starch containig foods can be Raman Microspectroscopy. This particularly method can give not only the structural images but it is a potential tool for measurements of forces acting between food components for example starch and protein [25].

Concluding the presented paper it can be stated that undoubtely an important role of starch granules in the formation of food structure and properties can be also visualized by microscope methods. The development in microscope technology can even better support the knowledge about starch itselfs and in food systems and also on the base of the new findings expected to create new desired properties of food.

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WŁAŚCIWOŚCI STRUKTURALNE SKROBI W ŻYWNOŚCI

Streszczenie

Przedstawiono możliwości obserwowania roli skrobi w kształtowaniu struktury żywności i jej przemian w trakcie przygotowywania żywności posługując się różymi technikami mikroskopowymi, a to: mikroskopem optycznym, transmisyjnym mikroskopem elektronowym (TEM), scanningowym mikroskopem elektronowym emisji polowej, scanningowym mikroskopem tunelowym, mikroskopem sił atomowych i mikrospektroskopią ramanowską.

H. GAMBUŚ, D. GUMUL, T. TUSZYŃSKI, M. WALCZYCKA

STARCH FROM IMMATURE CEREAL KERNELS AS AN IMPROVER OF BREAD

Abstract

The big starch grains of type A >10 μm in diameter are synthesised in cereal kernel until it reaches the early-waxy phase of maturity. In matured kernels 80% of total starch grains make up small grains of type B. It is known from earlier research that for creating the bread crumb structure big starch grains are needed because they swell and react with denatured gluten.

To improve the above mentioned proportions 5 and 10% of wheat, rye and barley starches were added to the baking of wheat breads from flour type 550. The starches were separated from kernels harvested in early and late-waxy phases. All breads were baked by direct method according to the same recipe. The volume of baked breads was measured, the sensory evaluation done and the texture profile of bread crumb was performed by TA-XT2 analyser. During three day storage hardness, springiness, cohesivness, gumminess, chewiness and resillence of bread crumb were estimated.

Starch additives did not affect the organoleptic value and the largest volume displayed breads with 3% starch additive from kernels in early-waxy phase of maturity irrespective of cereal species.

The origin and amount of added starch did not influence the texture parameters of bread with the exception of crumb hardness. All breads with starch additives were characterized by lower crumb hardness on the day baking and during three day storage in comparison with the standard bread. The most advantageous was an addition of 3 % of wheat and rye starches originating from kernels reaped in early-waxy phase of maturity. High resistance of such starch grains to swelling and pasting may have been responsible for that result (confirmed by DSC examination). Similar results can be obtained, for starch from mature kernels, only with the usage of certain inhibiting starch swelling substances in dough making. Usage of starches from wheat and rye kernels reaped in early-waxy phase of maturity eliminates addition of starch swelling inhibitors. The starch originating from grains of the early-waxy phase of maturity can be used as natural bread improver.

H. Gambuś, D. Gumul, Carbohydrates Technology Department, Food Technology Faculty, University of Agriculture, Cracow; T. Tuszyński, Department of Fermentation Technology and Technical Microbiology, Food Technology Faculty, University of Agriculture, Cracow; M. Walczycka Animal Product Processing Department, Food Technology Faculty, University of Agriculture, Cracow.

Introduction

It was already stated a long time ago that the differences in physico-chemical properties of starch of various sorts of flour have an influence on their baking properties [5, 11, 12]. Since starch granules take part in the formation of dough structure, because they arrange themselves with long axes towards the mixing direction and form a large surface, on which glutenous proteins can be absorbed [23, 24].

Smaller starch granules have a relatively bigger surface of the contact with the gluten, however, during a process of baking some interactions between denatured gluten and big swollen granules have an impact on crumb structure . It has been proven by Pomeranz and his co-workers [23] in their work using a scanning electron microscope (SEM).

Soulaka and Morrison [24] suggested that the optimal number of small granules of type B (less than 10 μm in diameter) of starch contained in the flour should be not more than 35%, because over the limit the volume of bread decreases.

However, in their works Lelievre and his co-workers [16] proved that the optimal granulation of starch depends on the amount of protein in the flour. According to these authors, the less amount of protein is contained in the flour, there should be more small granules of starch in order to get the best quality of crumb.

In the starch contained in mature kernels of cereal small granules of type B form 80% of the total number of starch granules [10]. However, till 30 days after flowering, that is in the early waxy phase of kernel maturity, almost only large starch granules of type A (more than 10 μm in diameter) are being synthesized. Therefore, in order to improve starch granulation an additive of starch isolated from immature kernels to bread dough seemed to be justified.

The aim of the conducted experiment was to check whether starch isolated from the cereal kernels harvested from the field in the early and late waxy phases of kernel maturity can be used as an improver of bread.

Material and research methods

The research material was commerial wheat flour of type 550 and wheat breads, in which 3 and 5% of the mass of the flour was replaced with wheat, rye and barley starches isolated from immature kernels harvested from the field both in the early and late waxy phases of kernel maturity.

The technological value of the wheat flour of type 550 was evaluated taking the following into consideration:

- a number of sedimentations with SDS (dodecylo-sodium sulphate (VI)) by the micro method [2],

- a falling number (LO) by Hagberg-Perten's method in the apparatus *Falling Number – 1800* (Norm ICC- Standard 107) [13],
- an amount of gluten in the apparatus *Glutomatic 2200* (Norm ICC-Standard No 137) [13] and a gluten index in a special centrifugal machine according to the instruction of the Parten Company,
- physical properties of dough in a farinograph and resistograph of the Brabender Company and according to the norm ICC – Standard no 115 [13].

A laboratory baking of the breads of dough consistency 350 j.B. was conducted using a direct method [6]. After one and a half hour cooling the breads were weighted and a baking loss and bread making performance were calculated [14]. The volume of the obtained bread was measured in a loose material using seeds of rape. The sensory evaluation of the breads was performed on the day of baking according to PN-89/A-74108 [22]. The quality of the bread was determined according to the number of the collected points.

In order to study the process of staling the breads were being stored in foil bags at the temperature of 20–24°C. Starting from the day of baking on, for the whole period of storing, that is four days, we marked:

- moisture of the crumb by the drying method according to PN-89/A-74108 [22],
- texture profile of the crumb using a texture analyzer TA XT2 with a programme XTR-1. The bread was sliced in halves and from each half one 3 cm thick slice was cut off. In both slices a texture profile was marked measuring the following parameters: hardness, springiness, cohesivness, gumminess, chewiness and resillence.

Results and discussion

The wheat flour of type 550 used for baking the breads was characterized by good baking quality (Table 1). The opinion is supported by a favourable falling number, big water absorption, a long period of dough consistency, as well as a high content of gluten of good quality [2].

The wheat bread baked from this flour was treated as a standard. The influence of starch additives on the quality of the obtained breads was presented in Table 2. As one can conclude from the data contained in the table, starch additives did not worsen the organoleptic evaluation. The breads with 3% of starch isolated from the kernel harvested in the early waxy phase of maturity were characterised by the biggest volume, even bigger than the standard bread, regardless of species of cereal. The bread of bigger volume had a worse performance, because its mass was the lowest what proved that the loss of water in this type of bread was the largest during baking. However, it had no influence on moisture of the crumb, which in all the breads was similar, but it increased their baking loss.

Table 1

Evaluation of technological value of wheat flour type 550.

Kind of indicator	Flour type 550
Sedimentation number (cm^3)	28
Falling number (s)	286
Water absorption (%)	56,0
Time of dough development (min)	2,1
Time of dough stability (min)	3,8
Dough softening (B.U.)	100
Quality number	47
Wet gluten content (%)	27,2
Gluten index (%)	79,0

No increase in bread volume was noticed along with the addition of 5% of the starch isolated from the kernel harvested in the early waxy phase of maturity, because probably a certain optimum of the size of starch granules in flour was disturbed. The optimal starch granulation in flour depends on the content of protein in it [16]. It seems that with the presence of 27% of gluten, as was marked in the used flour, a 3% addition of "immature" starch, which maintained a given number of big granules, was the most favourable for bread volume.

No increase in bread volume was observed while adding the starch isolated from the more mature kernel harvested in the late waxy phase of maturity to dough, because such starch already contains a certain number of small starch granules of type B (Table 2).

Analyzing the changes of moisture of the crumb in the tested breads (Table 3-5) one can come to the conclusion that the loss of water from the crumb during the period of storing was not big, both in the standard breads and those ones with the starch additives. The results confirm the well-known theory that bread does not always need to lose water while staling, because an old and hard crumb often contains the same amount of water as the fresh one [1, 5].

Both the origin and the size of the applied additives had no visible impact on the parametres of crumb texture with the exception of its hardness (Tables 3-5). All the breads with the starch additives were characterized by a lower hardness of crumb in comparison to the standard bread, both on the day of baking and during the period of storage. However, for the sake of this characteristic a 3% additive of wheat and rye starch, which were harvested in the early waxy phase of maturity, appeared to be the most favourable (Fig. 1, 2).

Table 2

Selected quality parameters of wheat breads supplemented with starch isolated from immature wheat, rye and barley.

Kind of bread	Weight of cold bread (g)	Total volume of bread (cm^3)	Bread volume from 100g flour (cm^3)	Yield of baking (%)	Total baking loss (%)	Moisture of crumb (%)	Sensoric evaluation	
							Scores	Grade
Wheat flour 100% standard	223,5	7 63	491	143,9	10,6	43,2	40	I
Early-waxy phase of maturity								
Standard + 3% wheat starch	222	**775**	**499**	143,0	11,2	43,7	40	I
Standard + 5% wheat starch	226	705	454	145,5	9,6	42,6	40	I
Standard + 3% rye starch	226	**780**	**502**	145,5	9,6	43,6	40	I
Standard + 5% rye starch	221	725	467	142,2	11,7	43,4	40	I
Standard+ 3% barley starch	222	**780**	**502**	143,0	11,2	43,1	40	I
Standard + 5% barley starch	225	685	441	145,1	9,9	43,0	40	I
Late-waxy phase of maturity								
Standard + 3% wheat starch	223	755	486	143,6	10,8	43,4	40	I
Standard + 5% wheat starch	226	740	477	145,7	9,5	42,7	40	I
Standard + 3% rye starch	224	720	464	144,1	10,5	43,5	40	I
Standard + 5% rye starch	227	750	483	146,2	9,2	43,5	40	I

Table 3

Effect of addition of starch derived from immature wheat on moisture and parameters profil texture of wheat bread crumb during storage.

Kind of bread	Storage days	Moisture of crumb (%)	Hardness	Springiness	Cohesivness	Gumminess	Chewiness	Resillence
Wheat flour 100% - Standard	0*	43,88	0,621	1,029	0,639	0,395	0,390	0,462
	1	43,71	0,775	1,013	0,471	0,365	0,383	0,207
	2	43,55	0,943	0,999	0,375	0,363	0,362	0,141
	3	43,21	1,082	0,981	0,335	0,355	0,340	0,122
Early-waxy phase of maturity								
Standard + 3% wheat starch	0	44,55	0,393	1,002	0,834	0,328	0,360	0,542
	1	44,41	0,714	1,001	0,451	0,361	0,345	0,198
	2	43,99	0,724	1,000	0,408	0,323	0,321	0,168
	3	43,66	0,862	0,958	0,333	0,238	0,239	0,130
Standard + 5% wheat starch	0	43,06	0,521	0,998	0,768	0,400	0,399	0,4675
	1`	43,05	0,861	0,975	0,441	0,382	0,366	0,1815
	2	42,84	0,911	0,962	0,360	0,332	0,323	0,131
	3	42,64	0,955	0,946	0,309	0,295	0,279	0,108
Late-waxy phase of maturity								
Standard + 3% wheat starch	0	43,81	0,490	1,163	0,782	0,383	0,440	0,483
	1	43,45	0,826	1,056	0,422	0,350	0,367	0, 181
	2	43,42	0,961	0,947	0,361	0,347	0,323	0,134
	3	43,39	1,020	0,923	0,324	0,328	0,236	0,115
Standard + 5% wheat starch	0	42,95	0,461	1,160	0,645	0,384	0,421	0,474
	1	42,91	0,923	1,051	0,416	0,372	0,399	0,175
	2	42,82	1,133	1,030	0,333	0,365	0,358	0,111
	3	42,70	1,166	0,984	0,305	0,345	0,341	0,109

* 0-day of baking, 1-first day after baking, 2-second day after baking, 3-third day after baking

Table 4

Effect of addition of starch derived from immature rye on moisture and parameters profil texture of wheat bread crumb during storage.

Kind of bread	Storage days	Moisture of crumb (%)	Hardness	Springiness	Cohesivness	Gumminess	Chewiness	Resillence
Wheat flour 100% - Standard	0*	43,88	0,621	1,029	0,639	0,395	0,390	0,462
	1	43,71	0,775	1,013	0,471	0,365	0,383	0,207
	2	43,55	0,943	0,999	0,375	0,363	0,362	0,141
	3	43,21	1,082	0,981	0,335	0,355	0,340	0,122
Early-waxy phase of maturity								
Standard + 3% rye starch	0	43,81	0,453	1,005	0,794	0,359	0,354	0,496
	1	43,77	0,732	1,003	0,472	0,347	0,317	0,222
	2	43,59	0,790	1,000	0,375	0,289	0,293	0,145
	3	43,20	0,848	0,998	0,325	0,261	0,255	0,120
Standard + 5% rye starch	0	43,52	0,400	1,053	0,807	0,346	0,330	0,488
	1	43,48	0,666	1,005	0,480	0,322	0,325	0,215
	2	43,39	0,865	0,989	0,354	0,319	0,308	0,135
	3	43,03	1,000	0,984	0,328	0,282	0,270	0,111
Late-waxy phase of maturity								
Standard + 3% rye starch	0	43,83	0,392	0,992	0,793	0,369	0,379	0,486
	1	43,61	0,889	0,987	0,478	0,330	0,364	0,216
	2	43,56	1,007	0,963	0,352	0,310	0,327	0,121
	3	43,54	1,120	0,931	0,331	0,283	0,268	0,119
Standard + 5% rye starch	0	43,47	0,449	0,968	0,782	0,382	0,357	0,473
	1	43,05	1,007	0,965	0,428	0,360	0,336	0,175
	2	42,95	1,082	0,960	0,388	0,351	0,317	0,146
	3	42,77	1,110	0,958	0,298	0,314	0,301	0,104

* 0-day of baking, 1-first day after baking, 2-second day after baking, 3-third day after baking

Table 5

Effect of addition of starch derived from immature barley on moisture and parameters profil texture of wheat bread crumb during storage.

Kind of bread	Storage days	Moisture of crumb (%)	Hardness	Springiness	Cohesivness	Gumminess	Chewiness	Resillence
Wheat flour 100% - Standard	0*	`43,88	0,621	1,029	0,639	0,395	0,390	0,462
	1	43,71	0,775	1,013	0,471	0,365	0,383	0,207
	2	43,55	0,943	0,999	0,375	0,363	0,362	0,141
	3	43,21	1,082	0,981	0,335	0,355	0,340	0,122
Early-waxy phase of maturity								
Standard + 3% barley starch	0	43,64	0,568	1,005	0,788	0,448	0,443	0,485
	1	43,59	0,785	0,988	0,438	0,383	0,343	0,183
	2	43,18	0,959	0,980	0,326	0,344	0,330	0,126
	3	43,04	1,198	0,977	0,324	0,325	0,322	0,113
Standard + 5% barley starch	0	43,81	0,623	0,965	0,781	0,486	0,464	0,474
	1`	43,58	0,852	0,958	0,423	0,371	0,371	0,174
	2	43,32	0,969	0,949	0,332	0,361	0,350	0,125
	3	42,96	1,262	0,941	0,319	0,311	0,349	0,119

* 0-day of baking, 1-first day after baking, 2-second day after baking, 3-third day after baking

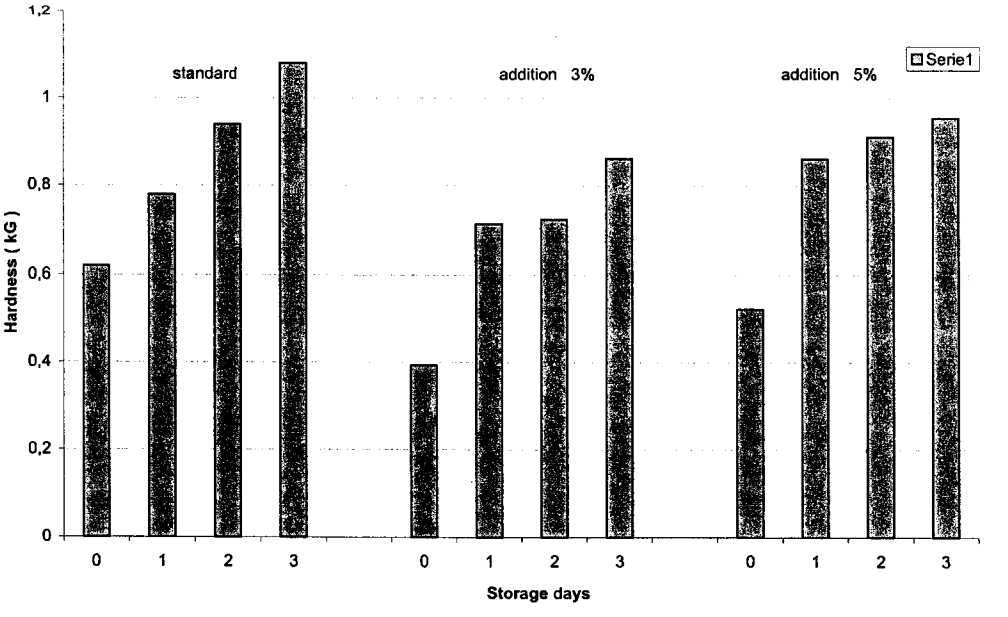

Fig. 1. Effect of addition of starch derived from wheat kernel in early-waxy stage of maturity on hardness of wheat bread crumb.

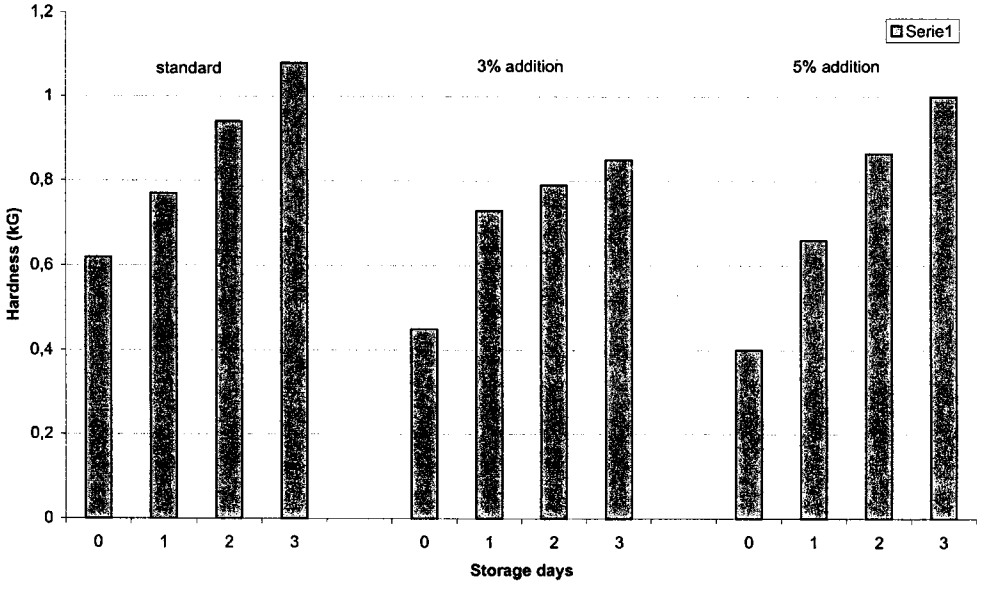

Fig. 2. Effect of addition of starch derived from rye kernel harvested in early-waxy stage of maturity on hardness of wheat bread crumb.

The inhibition of crumb hardening by a starch additive from the immature kernels was probably caused by a greater resistance of the granules of these sorts of starch to the process of swelling and pasting [8]. According to Martin and Hoseney [18, 19], the creators of the latest model of bread staling, this swelling of starch is the main factor defining a degree of hardening of bread crumb – the less swollen granules are and the smaller dilution of starch particles during the process of baking occurs, the smaller surface of contact of starch chains, which flowed out from the granules together with glutenous proteins and the weaker hydrogen bindings netting between protein and starch fractions during the period of storage, which causes the inhibition hardness of crumb.

As a result, the substances, which inhibit swelling of starch granules, also restrict the process of hardening of crumb. Using the starch from the kernel harvested from the field in the early waxy phase of maturity in dough , the addition of such substances seems to be unnecessary. Besides, the smaller swelling ability of the granules of the added starch could cause a greater plasticity of gluten, which according to many authors is the main factor of a longer preservation of freshness of crumb [1, 9, 17, 18].

A lower concentration of amylose gel of a worse sensor characteristic than starch gel could have an impact on a lower hardness of the crumb of the breads with the starch from the immature kernels on the day of baking [25, 26, 27]. As it is known, the starch granules collected from cereal kernels in the early waxy phase of maturity contain a bigger amount of amylopectin in comparison to the starch from mature kernels.

A favourable delay effect on crumb hardening is the least visible while using barley starch (Table 5), which in many scientists' opinion have the same baking values, as wheat and rye starch [3, 11, 12], but it does not derived from bread cereals.

Conclusion

1. The starch additives did not worsen an organoleptic evaluation and the bread of 3% starch isolated from the kernel harvested in the early waxy phase of maturity was characterized by the biggest volume regardless of species of cerals.
2. Both the origin of starch and the amount of the additives did not have any influence on the parameters of crumb texture with the exception of its hardness.
3. All the breads with the starch additives were characterized by a lesser hardness of crumb, both on the day of baking and during the 3 day period of storage compared to the standard bread. However, as far as this characteristic is concerned, the most favourable is a 3% addition of wheat and rye starch from the kernel harvested in the early waxy phase of maturity.
4. The favourable delay effect on crumb hardening was the least visible while using barley starch, which derives from no bread cereals.

5. The wheat and rye starch isolated from cereal kernels harvested in the early waxy phase of maturity should be considered as a natural improver of bread.

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SKROBIA Z NIEDOJRZAŁYCH ZBÓŻ JAKO NATURALNY POLEPSZACZ PIECZYWA

Streszczenie

Do momentu osiągnięcia przez ziarno zbożowe wczesno-woskowej fazy dojrzałości, syntezowane są w nim prawie wyłącznie duże ziarenka skrobiowe typu A o średnicy > 10 μm. W dojrzałych ziarnach zbóż 80 % ogólnej liczby ziarenek skrobiowych stanowią małe ziarenka typu B, a jak wiadomo z badań wcześniejszych, w tworzeniu struktury miękiszu chleba uczestniczą głównie ziarenka duże, które pęcznieją i wchodzą w reakcje ze zdenaturowanym glutenem.

W celu poprawienia tych proporcji, do wypieku chlebów pszennych z mąki typu 550 zastosowano 3 i 5 procentowe dodatki skrobi pszennej, żytniej i jęczmiennej, wyodrębnionej z ziarniaków zebranych z pola zarówno w stanie dojrzałości wczesno jak i późno-woskowej .

Wszystkie chleby wypiekano metodą bezpośrednią, według tej samej receptury. Oznaczono objętość uzyskanych chlebów, wykonano ocenę sensoryczną oraz oznaczono profil tekstury miękiszu chlebów analizatorem tekstury typu TX-XTA, z oprogramowaniem XTR1. Podczas 3 dni przechowywania oznaczono: twardość, spójność, elastyczność, sprężystość , adhezję, żujność i gumowatość miękiszu.

Dodatki skrobiowe nie pogorszyły oceny sensoryczną, a największą objętością charakteryzował się chleb z 3 procentowym udziałem skrobi wyodrębnionej z ziarna zebranego w fazie dojrzałości wczesno-woskowej, niezależnie od gatunku zboża.

Zarówno pochodzenie skrobi jak i wielkość stosowanych dodatków nie wywarły widocznego wpływu na parametry tekstury pieczywa z wyjątkiem twardości miękiszu.

Wszystkie chleby z dodatkami skrobiowymi charakteryzowały się mniejszą twardością miękiszu, zarówno w dniu wypieku jak i podczas 3 dniowego przechowywania, w porównaniu z chlebem standardowym, ale najbardziej korzystny ze względu na tę cechę okazał się 3 procentowy dodatek skrobi pszennej i żytniej pochodzącej z ziarna zebranego w fazie dojrzałości wczesnowoskowej. Efekt ten spowodowany był prawdopodobnie większą opornością ziarenek takiej skrobi na proces pęcznienia i kleikowania (co wykazały badania DSC), a co w skrobi ze zbóż dojrzałych uzyskuje się dopiero po zastosowaniu do ciasta odpowiednich substancji hamujących pęcznienie skrobi. Stosując do ciasta skrobie wyodrębnione z ziarna pszenicy i żyta, zebranego we wczesno-woskowej fazie dojrzałości, dodatek tych substancji wydaje się zbyteczny. Wobec tego skrobię taką zdecydowanie można uznać jako naturalny polepszacz pieczywa.

MAREK GIBIŃSKI

CHEMICAL COMPOSITION OF THE SELECTED VARIETIES AND STRAINS OF OAT

Abstract

Apart from many factors, quality of both plant and animal raw materials plays a crucial role in quality of food products as early as the beginning of the manufacturing process. It is clearly visible in plant raw materials in which quality at least at a certain stage can not be controlled by man. A sudden change in weather conditions can cause a big difference in technological parameters of the same variety of corn grown in different regions of the country during the harvest time. The criteria of purchase of the already mentioned corns are very simple and in case of oat very limited. It concerns some parameters pertaining to the science of commodities and that is all. Yet a grain does not only consist of shape, mass, density and colour. It mainly contains protein, starch, fat, cellulose and recently very popular β-glucans.

Mixing corns of different varieties, or the same variety, but of different parameters contributes to an average value of chemical properties, and consequently to obtaining commercial oat of general (poor) quality. A high health quality of oat due to its specific chemical composition should make technologists point out which varieties are to be used to either manufacture diet products, or fodder.

Aim of the work

The subject of this work is an analysis of chemical composition (protein, fat, starch, reducing sugars, β-glucans, cellulose and ash) of nineteen varieties and strains of oat coming from breeding work carried out at four stations in Choryń, Polanowice, Strzelce, Wielopole in 1999. Besides, it is important to stress the differences in certain values among the samples.

Experimental part

Research material and methodology

The research material of this work were bruised grains of the following varieties and strains of oats: Akt, Bajka, Cekin, CHD 1396, CHD 1441, CHD 1598, CHD 1698,

M. Gibiński, Department of Carbohydrate Technology, Academy of Agriculture, Kraków.

Chwat, Dragon, Dukat, Grajcar, Hetman, Jawor, Kasztan, Komes, Sam, Sławko, Skrzat, Szakal. The samples of the above-mentioned varieties and strains came from four stations of cultivation in Choryń, Wielopole, Strzelce, Polanowice. Before proceeding to the research work they were sifted through the sieves of 0,43 mm and ground in the laboratory grinder WŻ-1. The well sifted and mixed bruised grains were a research material.

In this work the following analyses were carried out:

- marking of dry substance according to Jermakow [10],
- marking of the content of protein according to Kjeldahl's method [4],
- marking of the content of fat [4],
- marking of the content of starch according to Clendenning [9],
- marking of the content of reducing sugars according Luffa-Schoorl's method [11],
- marking of the content of β-glucans according to ICC methods [2],
- marking of the content of cellulose according to AOAC methods [1],
- marking of the content of ash [15].

Results of reseach and discussion

The results of the conducted analyses have been put together in Table 1, which presents the chemical composition (protein, fat, reducing sugars, ash, β-glucans cellulose) of the nineteen varieties and strains of oats.

By evaluating the content of protein in each sample it is clearly visible that Komes is characterized by the maximum amount of protein (16, 49%) and Bajka is the minimum (11,41%) (Chart 1). Seven varieties and strains above the average (13,79%).

The obtained average content of protein – 13,8% is consistent with the data in professional literature. [6, 16], and does not differ from the world average, hovewer there are varieties in which the content of protein ranges from 20 to 23%, but they are grown for specific purpose (for instance, fodder).

The strain of CHD 1441 is characterized by the highest percentage of protein (15,45%) among the studied strains. The content of fat ranges from 10.56% (Kasztan) to 6,26% (Szakal) at the average of 8.07 for all the sample (Chart 2).

The marked content of fat (6,26% – 10,56%) is confirmed by the data in the works of Brown and Craddock [3, 6], who report that the content in defatted grain of oat ranges from 3% to 11,6%.

Kasztan grown in Polanowice is the variety with the highest content of fat as was marked during many years' research at the Department of Techonology of Carbohydrates of the Academy of Agriculture in Cracow. Other high-fat varieties are: Grajcar (9.35%) bred in Wielopole, Dragon (9,18%) bred in Choryń. A high content of fat is also characteristic for Akt, an unweeded variety, which contains 9.75% of fat. The

percentage of fat of the researched strains of oat belongs to the group of the lowest values (CHD 1698-7.00%; CDH 1598-7.06%; CHD 1441-7.06%).

Table 1

Chemical composition of nineteen varieties and strains of oats.

Oats	Station	s.m. [%]	Protein [%]	Fat [%]	Starch [%]	Red.sugar [%]	Ash [%]	β-glucan [%]	Cellulose [%]
Jawor	Choryń	90,19	11,79	7,74	49,80	0,40	1,91	3,70	14,33
Hetman	Choryń	90,07	16,27	8,18	52,69	0,42	2,12	4,06	15,65
Dragon	Choryń	90,00	13,71	9,18	47,99	0,39	2,07	3,68	16,71
Komes	Choryń	90,11	**16,49**	8,98	**43,64**	0,38	1,95	4,46	16,52
CHD 1598	Choryń	89,84	13,58	7,06	52,40	0,42	2,03	4,34	15,92
CHD 1698	Choryń	89,81	12,93	7,00	**58,98**	0,41	1,99	5,00	14,23
CHD 1441	Choryń	89,65	15,45	7,06	47,18	**0,52**	2,02	4,17	15,21
CHD 1396	Choryń	89,69	12,49	7,83	56,11	**0,31**	**1,78**	3,56	**12,71**
Cekin	Wielopole	90,13	13,39	8,42	52,64	0,32	1,91	**3,07**	14,03
Grajcar	Wielopole	**89,43**	13,91	9,35	50,41	0,36	1,82	3,54	13,95
Dukat	Wielopole	90,26	14,82	7,48	50,73	0,32	1,95	3,23	16,16
Akt	Strzelce	90,17	14,82	9,75	49,87	0,51	**2,42**	4,26	**17,25**
Bajka	Strzelce	90,04	**11,41**	7,46	51,86	0,47	2,02	4,56	16,62
Sam	Strzelce	89,96	12,73	7,21	54,38	0,52	2,00	4,47	15,70
Szakal	Strzelce	89,77	15,99	**6,26**	55,53	0,34	2,12	4,48	15,78
Chwat	Strzelce	90,01	14,48	7,39	53,36	0,51	1,96	**5,05**	15,86
Sławko	Strzelce	90,15	11,56	7,74	51,10	0,41	1,80	3,89	14,93
Kasztan	Polanowice	**90,49**	12,13	**10,56**	46,49	0,37	2,00	4,14	17,15
Skrzat	Polanowice	90,12	14,10	8,73	53,44	0,39	1,90	4,54	14,93

Sources: own research

Chart 1 Content of Protein %

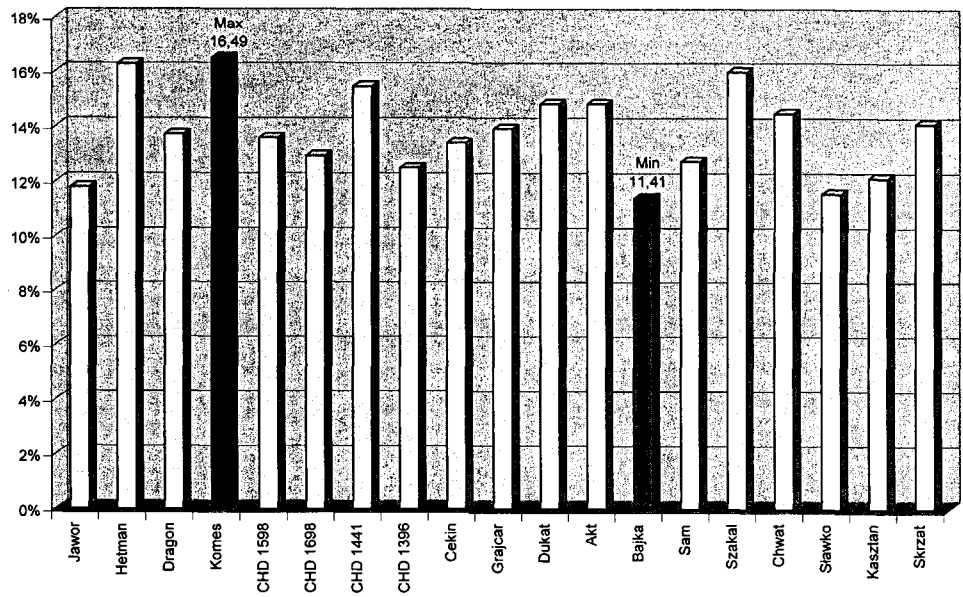
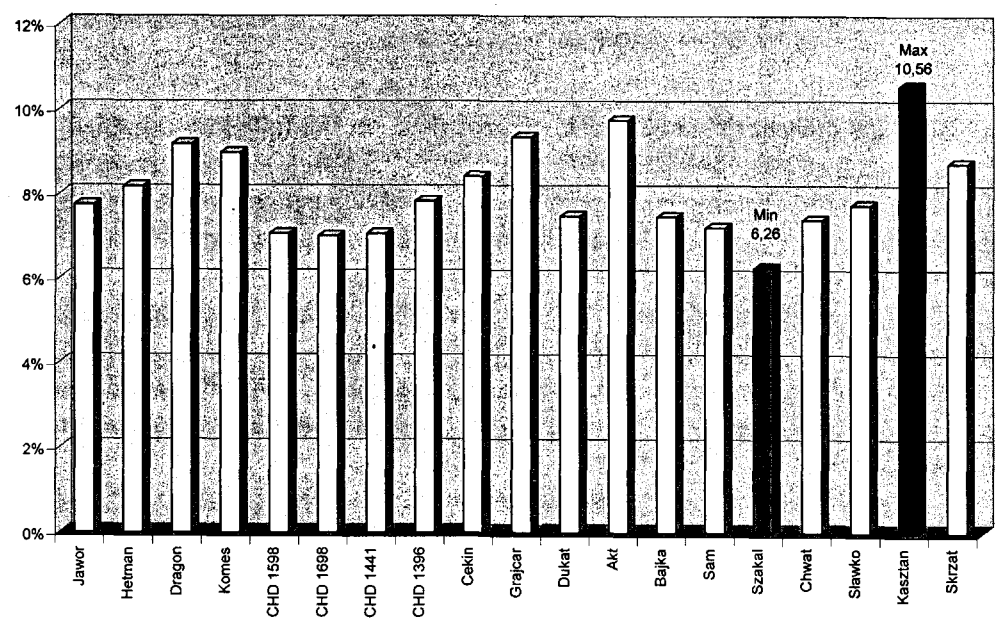
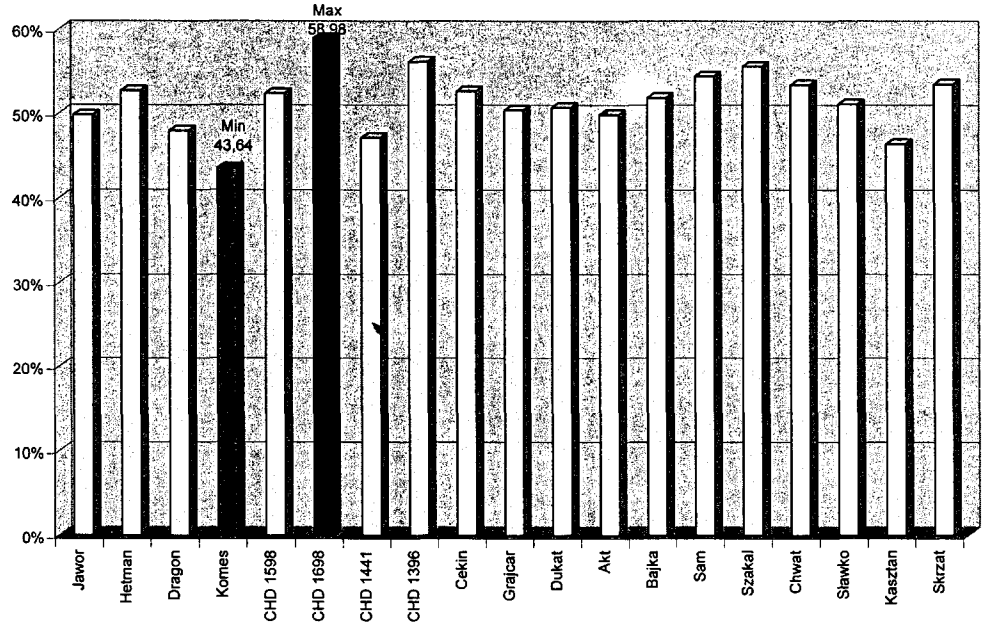


Chart 2 - Content of Fat %



While analyzing the amount of starch in the individual varieties, one can conclude that this value ranges from 58.98% (CHD 1698) to 43.63% (Komes). There are 10 samples above this value.

Chart 3 - Content of Starch (%)



The high content of starch is also characteristic for the following varieties: CHD 1396 (56.11%), CHD 1598 (52.40%) and Szakal (55.53%). Apart from Komes the following varieties belong to those containing a small amount of starch: Kasztan (46.49%), Dragon (47.99%). Among the strains CHD contains the lowest amount of starch (47.18%).

The marked average content of starch is not much different from the data in professional literature [6, 8, 13, 17], reporting that the content of starch in oat is 55%.

The samples of the varieties form Strzelce used for the analysis are characterized by a high content of starch. The average for the samples from this station has the highest value of 52.68%.

The content of reducing sugars ranges from 0.52% (CHD 1441) to 0.31% (CHD 1396). The average for all the measurements equals 0.41%. The difference in value results from a various state of maturity of corns during the harvest time [17].

The value of ash ranges from 2.42% (Akt) to 1.78% (CHD 1441) to 0.31% (CHD 1396) at the average value of 1.99%. The marked values of ash are similar to the data

from professional literature, in which the content of ash ranges from 1.65% to 2.00% [8]. Only the amount of ash for an unweeded sort of oat (Akt) exceeds this limit. The factors such as climatic and soil conditions, the year of harvest and fertilizing have an impact on the level of mineral components. Taking mechanization of harvest of oat, preparation of uniform mixtures for processing and a standarized way of processing in the factory into account, one can accept that a natural changeability in content of the basic components is not big and has no practical significance in men's feeding [6].

The varieties from Strzelce and Polanowice are characterized by a high content of β- glucans. The average values for these stations are 4.45% and 4.34% while the average for all the stations equals 4.12% for the range from 5.05% (Chwat) to 3.07% (Cekin). The marked values are consistent with the data from professional literature. It is generally accepted that the content of β-glucans varies from 3 to 7% [16].

In domestic oats the average value for many years is 4.6% [7]. Some authors [5, 17] think that the content of β-glucans is a characteristics for a given variety also depending on environment.

The amount of food cellulose varies from 17.25% (Akt) to 12.71% (CHD 1396) at an average value of 15.45%. The marked content of cellulose is confirmed by the data from professional literature [12, 13], which report that this value ranges from 10.7% to 19.4%.

Statistical interpretation

In order to emphasize the crucial correlations the obtained results of the chemical composition were subjected to a statistical analysis. With this end in view the importance of correlation coefficients was being verified by means of the test of essentiality using t-Student's statistics [18]. The obtained results are presented in Table 2.

Taking the data from Table 2 into consideration, one can conclude that there are directly and inversely proportional correlations of different strength. The most important correlations are in bold. There is no crucial correlation between protein and other components. The directly proportional are the folllowing components – ash and cellulose ($r = 0{,}6802$; $p = 0{,}001$), sugars and β-glucans ($r = 0.5997$; $p = 0.007$), sugars and ash ($r = 0.4606$; $p = 0.047$). The inversely proportional correlation is indeed between fat and starch ($r = -07127$; $p = 0{,}001$). The other correlations can not be considered as statistically important (a high or very high probability of no occuring a given correlation).

Table 2

Correlation coefficients for the analysed samples.

	Sugars	Fat	Ash	Starch	β-glucan	Cellulose
Protein	0,0221 p = 0,929	-0,0555 p = 0,821	0,4128 p = 0,079	0,012 p=0,961	0,1175 p = 0,632	0,4337 p = 0,064
Sugars		-0,1857 p = 0,447	0,4606 p = 0,047	0,0361 p=0,883	0,5997 p = 0,007	0,4707 p = 0,042
Fat			0,1354 p = 0,580	-0,7127 p = 0,001	-0,3109 p = 0,195	-0,0637 p = 0,795
Ash					0,303 p = 0,207	0,6802 p = 0,001
Starch					0,3543 p = 0,137	-0,2823 p = 0,242
β-glucan						0,3141 p = 0,190

Conclusions

1. Basing on the carried out analyses of the nineteen varieties and strains of oats one can affirm a high content of protein, fat and β-glucans, which decidedly inhances physiological and nutritious values of oats .
2. In the course of the past ten years we have been observing a significant rise in the content of protein, fat and starch in the grain of oats.
3. Taking the high nutritious values of oats fat and at the same time a big content of total fat (Kasztan) into consideration one can be tempted to isolate it on a industrial scale.
4. The varieties with a high rate of the content of cellulose and at the same time with a big number of β-glucans (Bajka, Chwat, Komes) can be used to produce cellulose preparations.
5. The unweeded variety of Akt due to its chemical composition is characterized by high rates of the content of protein, fat, starch, ash, β-glucans and cellulose can be a valuable raw material for producing diet food.
6. Taking a high biological value and a favourable composition of amino acids of oats protein and its big amount (Komes, Hetman) an attempt can be made to isolate protein from the oats on a commercial scale, or to use these varieties for fodder.
7. Basing on the carried out statistical analysis no inverse correlation between protein and starch has been confirmed, but one can notice positive correlations between ash and cellulose, sugars and β-glucans, sugars and cellulose, sugars and ash.

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SKŁAD CHEMICZNY WYBRANYCH ODMIAN I RODÓW OWSA

Streszczenie

Skład chemiczny owsa, zarówno pod względem ilościowym jak i jakościowym, decyduje o dużej przydatności tego zboża w żywieniu człowieka. Zawartość składników odżywczych w owsie w znacznym stopniu różni się od zawartości w pozostałych zbożach.

W niniejszej pracy przeprowadzono analizę składu chemicznego dziewiętnastu odmian i rodów owsa. Na podstawie otrzymanych wyników stwierdzono, iż niektóre odmiany mogą być cennym surowcem do produkcji żywności. Oznaczone wysokie zawartości białka, tłuszczu, skrobi, błonnika dla niektórych odmian i rodów owsa takich jak: Hetman, Akt, Chwat, Skrzat, mogą decydować o przydatności w przemyśle produkującym środki dietetyczne. Wysoka zawartość błonnika w przypadku: Aktu, Kasztana, Bajki, Komesa, Szakala, CHD 1598, Sama, mogą kwalifikować te odmiany i rody do produkcji preparatów błonnikowych. W przypadku Kasztana uzyskano najwyższy wskaźnik zawartości tłuszczu.

N. INOUCHI, Y. SUGIMOTO, H. FUWA

EFFECTS OF AMYLOPECTIN UNIT CHAINS ON THE STARCH PASTING CHARACTERISTICS

Abstract

Structures and properties of starches isolated from Japanese rice and different botanical sources were investigated. Amylopectin unit chain-length distributions were analyzed using HPLC and HPAEC-PAD for comparing the distributions of unit chains of isoamylase-debranched amylopectins. Thermal properties were measured by DSC. It was found that there were at least five groups of Japanese rice amylopectins having different unit-chain distributions. The four kinds of starches (Akenohoshi, Haiminori, Tashukei 431, and Kenkei 2064) had the amylopectins of long chain-length compared with the other rice starches. It was observed that the gelatinization temperature of their four native starches were higher and the enthalpy change (•H) of gelatinization of their four retrograded starches showed larger than the other rice starches. In the starches of different botanical sources, the ratios of unit chains of DP6-12 (Fr.A) of amylopectins of quinoa, barley, buckwheat, Japanese radish, and tulip were higher, and the gelatinization temperatures were lower than the values of other starches. The ratios of Fr.A of amylomaize V and VII were lower, and the gelatinization temperatures were higher than the respective values of other starches. There were highly negative relationships between the ratio of short unit chain-length in amylopectin and gelatinization temperature.

Introduction

The crystalline regions in starch granules are mainly composed of amylopectin. The X-ray diffraction patterns of starch granules change by the unit-chain length distribution of amylopectin [1]. Amylose has a close relation to starch retrogradation. Amylopectin also had the great influence with the phenomena of gelatinization and retrogradation of starch [2]. We found that the gelatinization temperature has a close relationship with the chain-length distribution of amylopectin of maize starches isolated from mature kernels of mutants with starch-modifying endosperm genes, and that the enthalpy change of gelatinization has a highly relation to the amylopectin content

Y. Sugimoto, Fukuyama University and Kansai Women's Junior College, Sanzo, Gakuen-cho, Fukuyama, Hirishima, Japan 729-0292; N. Inouchi, H. Fuwa, Asahigaoka, Kashihara, Osaka, Japan 582.

of them [3, 4]. This objective of this paper is to know the relationship between the chain-length distributions of amylopectins and the properties of gelatinization of starch by investigating various kinds of Japanese rice starches and starches of different botanical sources.

Materials and methods

Materials

The rice endosperm starches were prepared from milled rice obtained from the Ministry of Agriculture, Forestry and Fisheries of Japan by the cold and dilute alkali method of *Yamamoto* et al. [5, 6] in the laboratory. Normal and waxy maize and wheat starches were gifts of Sanwa Denpun Kogyo Co., Ltd. Amylomaize V and VII were gifts of Oji Corn Starch Co., Ltd. Potato starch was a gift of Tokachi Farmers' Cooperative. Sweet potato starch was a gift of Kagoshima Keizairengokai. The other starches of different botanical sources were prepared in the laboratory by a modification of *Schoch's* method [7]. The purification of amylopectin from rice starch were performed by the method of *Lansky* et al. [8].

Methods

Absorption curves of starch-iodine complexes were recorded with a Hitachi 3210-type recording spectrophotometer with mixtures containing 1 mg of starch, 2 mg of iodine and 20 mg of potassium iodide per 25 ml [9]. Measurement of gel filtration of isoamylase-debranched starch materials by Toyopearl HW55S-HW50S were described previously [10, 11]. Gel-permeation HPLC of isoamylase-debranched starch was performed by using the method of *Hizukuri* [12]. A high-performance anion-exchange chromatography with a pulsed amperometric detector (HPAEC-PAD) of isoamylase-debranched materials of starch was performed by using a Dionex model DX-300 system (Dionex Corp., Sunnyvale, CA, U.S.A.) according to the method described by *Koizumi* et al. [13, 14] with a minor modification. The thermograms of native and retrograded starches were recorded on a differential scanning calorimeter (DSC) (Setaram Micro DSC III, Caluire, France) by a modification of method of *Inouchi* et al.[3, 4]. X-ray diffractometry was performed by the method of *Hizukuri* et al. [15].

Results and discussion

The Tab. 1 shows that the λ max and blue value of iodine-starch absorption complex, and the amylose contents of starch determined by measurements of gel filtration, and the ratios of fractions of amylopectin determined by HPAEC-PAD. There are low amylose, middle content of amylose, high amylose, and waxy rice starches from the results of the λ max, blue value, and amylose contents. Fig. 1 demonstrates that

Tabela 1

Some structural characteristics of starches of Japanese rice and waxy maize.

Name of cultivar	Iodine complex spectra		GPC	HPAEC-PAD			
	λ max. (nm)	Blue value (at 680nm)	A.C. (9b)	Fr.A (96)	Fr.Bl (~)	Fr.B2 (9'0)	Group
Rice starch							
Saikai236	546	0.13	9.8	30.1	65.5	4.4	E
Saikai215	548	0.12	9.6	30.1	65.5	4.3	E
Milky queen	550	0.13	7.4	29.6	65.5	4.8	D
Kanto194	551	0.14	8.1	32.1	63.3	4.6	F
Hokuriku161	551	0.14	9.5	31.6	63.1	5.3	E
Ou344	555	0.15	10.8	31.6	64.0	4.4	E
Hokuriku180	561	0.16	11.0	33.8	63.0	3.2	F
Soft158	562	0.17	12.6	31.3	65.1	3.6	E
Haiminori	567	0.21	15.7	25.0	69.5	5.5	C
Hokkai280	568	0.17	14.0	33.0	62.2	4.8	F
Kanto195	571	0.23	20.5	31.1	64.6	4.3	E
Akenohoshi	572	0.21	15.7	24.9	70.4	4.7	C
Hinohikari	572	0.18	18.5	31.1	64.4	4.5	E
Koshihikari	572	0.22	18.5	29.2	65.8	5.0	D
Kanto186	572	0.23	17.8	31.2	64.6	4.2	E
Kanto188	572	0.23	17.5	31.0	64.1	4.9	E
Kanto198	573	0.23	19.6	30.9	64.8	4.3	E
Saikai231	574	0.23	18.1	29.7	65.6	4.7	D
Hokuriku184	574	0.26	20.3	31.1	63.9	5.0	E
Kenkei 2064	574	0.37	21.0	16.9	76.0	7.1	A
Hokkai287	576	0.22	18.9	32.2	63.9	3.9	F
Sasanishiki	578	0.22	21.2	31.2	66.0	2.8	E
Kirara397	578	0.22	21.2	31.1	65.2	3.7	E
Ou368	578	0.23	20.7	30.1	65.9	4.0	D
Hokuriku183	578	0.29	21.8	30.8	64.5	4.7	E
Nihonbare	582	0.27	23.0	30.8	65.0	4.2	E
Chugoku134	591	0.28	25.7	31.6	64.1	4.6	E
Hoshiyutaka	592	0.31	28.0	31.3	64.1	4.6	E
Kanto181	592	0.35	26.5	31.3	63.3	5.5	E
Yumetoiro	592	0.33	30.8	29.3	65.7	5.0	D
Waxy rice starch							
Hakuchomochi	523	0.05	0.0	30.6	64.9	4.5	E
Hakutomochi	532	0.06	0.0	30.7	64.3	5.0	E
Hiyokumochi	532	0.07	0.0	30.6	64.8	4.6	E
Hokkaimochi286	531	0.06	0.0	32.4	63.1	4.5	F
Asamurasaki	528	0.07	0.0	30.9	64.4	4.7	E
Hokurikumochi181	532	0.07	0.0	30.5	66.1	3.4	D
Tashukei431	535	0.09	0.0	24.7	70.8	4.5	C
Chugokumochi167	533	0.07	0.0	29.9	65.0	5.1	D

Saikaimochi223	532	0.07	0.0	29.4	64.2	6.4	D
Saikaimochi225	532	0.07	0.0	32.1	63.3	4.6	F
Saikaimochi227	530	0.07	0.0	30.7	65.2	4.1	E
Saikaimochi235	523	0.08	0.0	30.6	66.4	3.0	E
Waxy maize starch	535	0.09	0.0	23.9	72.2	3.9	B

A,C. : Amylose contents

Fr.A(96) : Ratio of tot al peak area of DP 6~12
Fr.Bl(96) : Ratio of total peak a rea of DP 13~36
Fr.B2(9ó) : Ratio of total peak a rea of DP >37

Group A : Fr.A 23.0%
Group B : 23.OsFr.A<24.5%
Group C : 24.5SFr.A<29.0%
Group D :29.OSFr.A<30.5%
Group E : 30.5SFr.A<32.1%
Group F : 32.1sFr.A

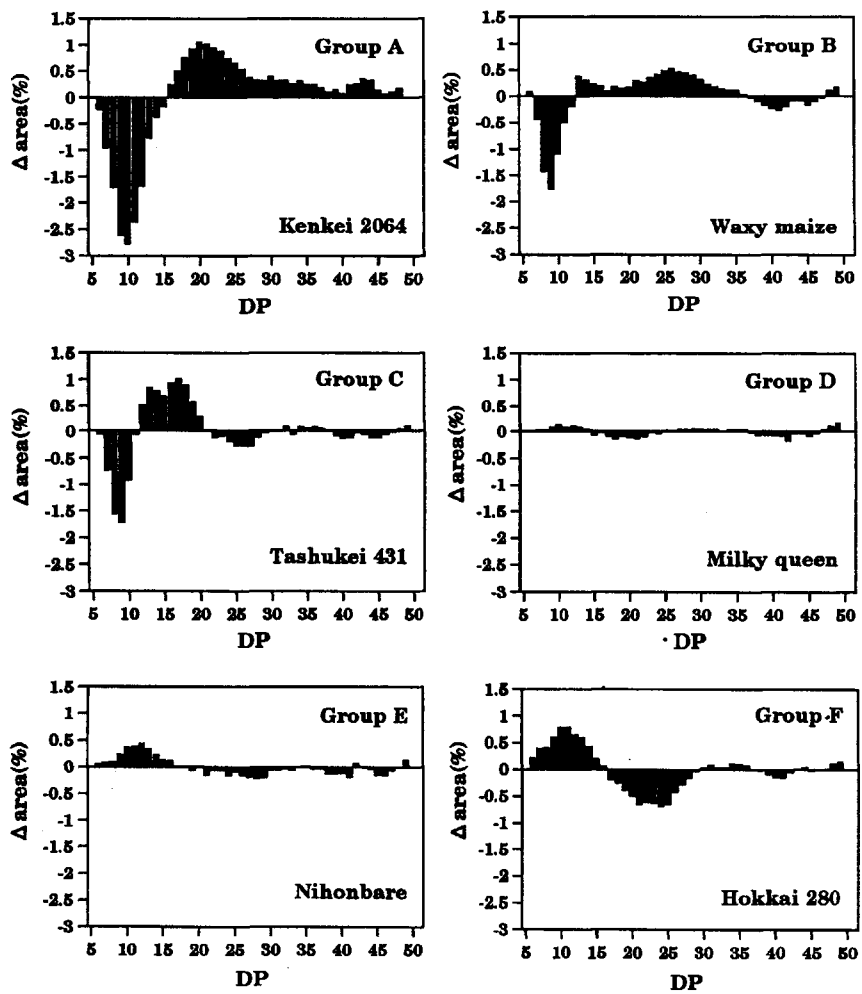

Fig. 1. Differences in chain-length distributions of debranched amylopectins of several rice varieties and waxy maize with comparison to Koshihikari rice amylopectin.

the differences in chain-length distributions of debranched amylopectins of several rice varieties and waxy maize with comparison to rice amylopectin of Koshihikari, the varieties accepted as the most delicious rice by Japanese people and had the best crop of rice in all varieties of Japanese rice, shown by the ratios of peak area of a PAD. It emerged that the chain-length distribution of rice amylopectin belonged to at least five groups (Group A, C, D, E, and F) determined by Fr.A contents (the ratio of total peak areas of DP 6-12). Most of Japanese rice starches belong to groups D, E, and F. The starches of Akenohoshi, Haiminori, Tashukei431 (Group C), and Kenkei2064 (Group A) had the amylopectins of longer chain-length compared with the other rice starches. Kenkei 2064 is an amylose-extender (*ae*) mutant rice.

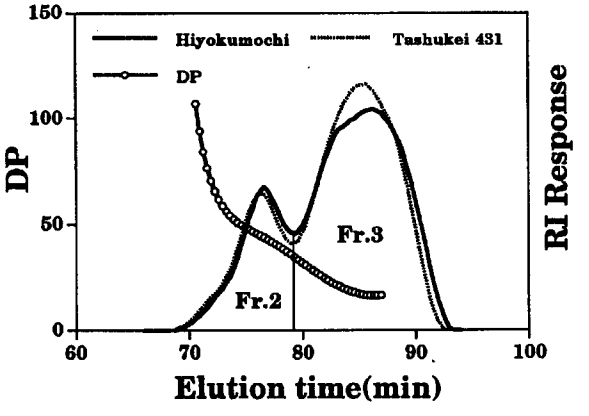

Fig. 2. Elution profiles of debranched waxy rice starches by HPLC-RI-LALLS.

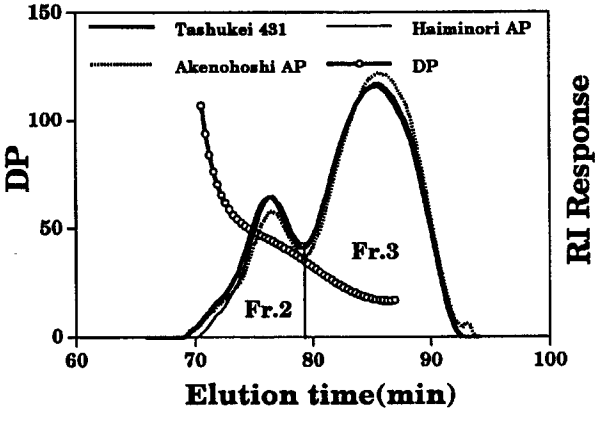

Fig. 3. Elution profiles of amylopectin (AP) of starches by HPLC-RI-LALLS.

Fig. 2 shows the elution profiles of debranched waxy rice starches of the most popular Japonica waxy rice (Hiyokumochi; Group E) and Tashukei431(Group C) by Gel-permeation HPLC. The average chain-length at vertex of Fr.3 of Hiyokumochi starch was about 15, and that of Tashukei431 was about 16-17. The elution profiles of debranced amylopectins purified from Haiminori and Akenohoshi starches which belong to the same group (Group C) were similar to that of Tashukei431. It could make sure that the unit chain lengths of amylopectins of these three rice starches (belong to Group C) were longer than the amylopectins of other starches except for Kenkei2064 from the results of Fig. 2 and Fig. 3.

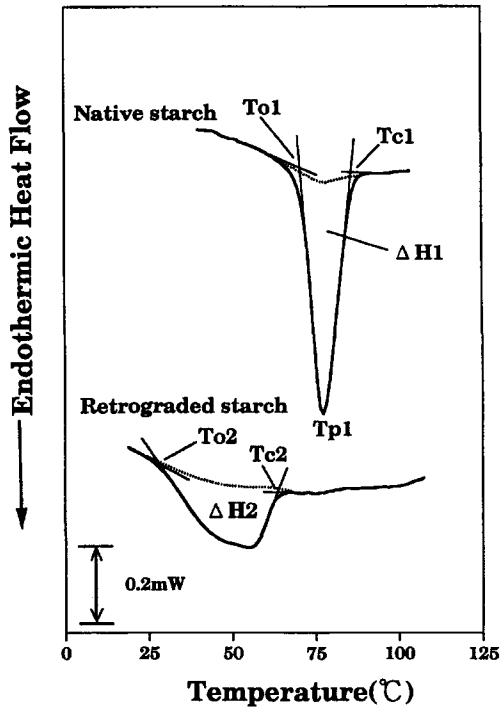

Fig. 4. Thermograms of native and retrograded rice starches of Tashukei 431 measured by micro DSC III (Retrograded starch was prepared by keeping the gelatinized starch in DSC pan in 5°C for 7 days).

Fig. 4 shows thermograms of native and retrograded starches of Tashukei431. The gelatinization temperatures and enthalpy changes of gelatinization of native and retrograded starches of rice and waxy maize are shown in Tab. 2. It was observed that the To1, Tp1, and Tc1 of native starches and Tc2 of retrograded starches of Akenohoshi, Haiminori, Tashukei431, and Kenkei2064 were higher and the •H2 of their retrograded starches were larger than the other rice starches. The To1, Tp1, and Tc1 of na-

tive starch of waxy maize were higher than common rice starches (belong to Group D, E, and F), and •H1 of native starch of waxy maize was larger than common rice starches, and the Tc2 of retrograded starch of waxy maize was higher and •H2 of retrograded starch of waxy maize was larger than common rice starches.

Tabela 2

Some thermal characteristics of native starches of Japanese rice and waxy maize and retrograded ones.

Name of cultivar	Native starch				Retrograded starch		
	To1 (°C)	Tp1 (°C)	Tc1 (°C)	ΔH1 (J/g)	To2 (°C)	Tc2 (°C)	ΔH2 (J/g)
Rice starch							
Saikai236	58.2	65.6	73.9	12.7	20.4	57.2	1.8
Saikai215	58.5	66.4	73.4	12.0	28.8	57.5	1.7
Milky queen	58.1	65.7	73.3	11.5	23.0	57.7	1.3
Kanto194	58.7	65.3	75.6	11.8	24.8	57.9	2.6
Hokuriku161	57.3	64.9	73.2	11.6	26.8	61.3	2.4
Ou344	54.5	62.1	70.9	10.9	26.9	56.3	1.5
Hokuriku180	54.2	60.7	69.0	10.4	21.6	57.1	1.2
Soft158	54.9	62.2	70.9	10.9	31.3	56.5	2.1
Haiminori	70.6	74.9	80.3	14.1	26.8	61.9	8.8
Hokkai280	54.2	60.7	69.0	10.4	21.6	57.1	1.2
Kanto195	56.4	62.1	70.0	11.1	22.4	59.1	3.2
Akenohoshi	71.6	75.3	80.0	12.9	27.4	62.5	9.7
Hinohikari	57.0	64.2	73.4	10.0	27.7	58.6	3.9
Koshihikari	58.8	64.3	72.2	11.9	32.0	56.6	2.9
Kanto186	56.6	62.8	70.5	11.4	33.4	57.5	2.5
Kanto188	58.8	63.5	69.9	10.4	31.6	57.5	1.8
Kanto198	58.0	63.6	70.9	10.9	27.6	57.1	1.8
Saikai231	58.7	64.1	71.3	11.1	31.6	56.8	2.3
Hokuriku184	58.6	64.0	71.4	10.7	29.7	57.2	2.1
Kenkei 2064	72.2	77.1	82.2	13.5	25.9	75.0	8.7
Hokkai287	53.8	59.8	67.0	9.7	21.9	57.5	4.4
Sasanishiki	56.4	62.7	71.1	10.0	28.1	57.1	2.8
Kirara397	54.6	60.1	67.3	10.1	27.0	57.1	1.8
Ou368	56.1	62.2	68.8	9.5	28.1	56.3	3.6
Hokuriku183	56.9	63.5	71.1	11.9	24.7	57.5	3.4
Nihonbare	56.8	63.5	69.2	9.5	25.1	55.9	2.9
Chugoku134	54.5	62.6	69.8	10.2	29.1	56.8	2.6
Hoshiyutaka	55.6	62.3	69.7	10.2	29.8	57.7	3.3
Kanto181	54.7	60.6	67.6	10.6	24.4	57.6	4.9
Yumetoiro	55.6	61.0	67.0	9.7	27.0	58.7	4.0
Waxy rice starch							
Hakuchomochi	50.8	59.7	75.6	11.5	26.0	55.9	1.1
Hakutomochi	56.4	65.4	79.3	10.2	29.9	57.8	0.8
Hiyokumochi	55.4	64.6	80.8	11.8	27.6	57.9	1.2

Hokkaimochi286	50.5	60.2	75.9	10.0	22.3	58.5	2,0
Asamurasaki	51.6	61.8	76.8	8.3	25.7	58.2	0.8
Hokurikumochi181	56.7	64.5	79.1	14.4	22.1	58.2	1,8
Tashukei431	71.0	77.1	86.0	15.9	27.9	63.3	10.9
Chugokumochi167	55.6	63.7	76.4	11.5	25.5	58.8	2,2
Saikaimochi223	57.5	65.0	79.6	11.5	-	-	-
Saikaimochi225	57.2	65.0	75.4	11.5	-	-	-
Saikaimochi227	56.9	64.6	75.8	12.3	30.1	58.1	0.9
Saikaimochi235	56.7	65.3	75.3	9.4	33.3	58.4	0.8
Waxy maize starch	62.5	68.2	78.6	14.3	26.0	62.6	6,4

To1 : Onset temperature (native starch)
Tp1 : Peak temperature (native starch)
Tc1 : Conclusion temperature (native starch)
ΔH1 : Enthalpy change (native starch)
To2 : Onset temperature (retrograded starch)
Tc2 : Con clusion temperature (retrograded starch)
ΔH2 : Enthalpy change (retrograded starch)

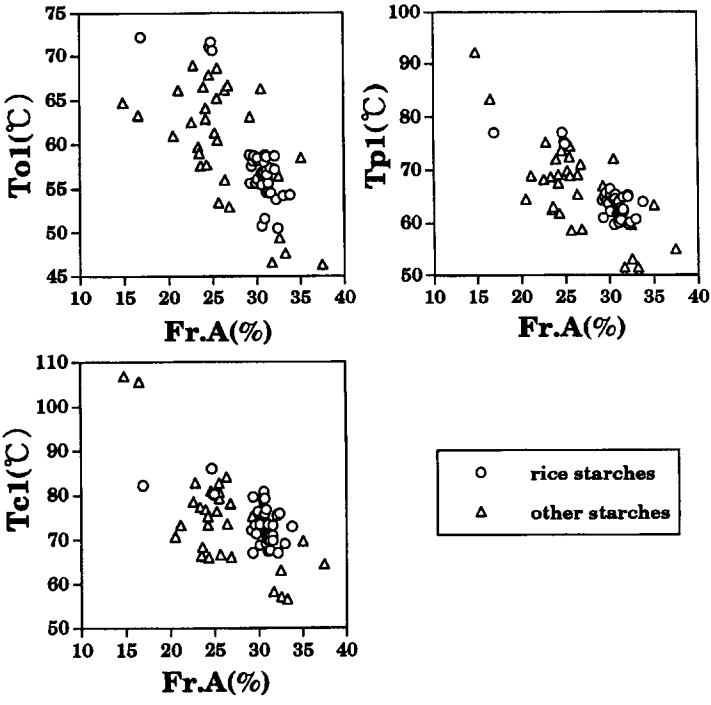

Fig. 5. Relationship between Fr.A and gelatinization temperatures.

Tab. 3 shows the λ max and blue value of iodine-starch absorption curves, the ratios of Fr.A, Fr.B1, and Fr.B2, the groups determined by Fr.A contents, and X-ray diffraction patterns of starches of different botanical sources. There were no relationship between X-ray diffraction patterns and the groups determined by Fr.A contents,

however, the chains with chain lengths longer than DP12 were mainly supposed to determine the X-ray diffraction patterns of starch granules.

Tabela 3

Some structural characteristics of starches of different botanical sources.

Sample	Iodine complex spectra		HPAEC-PAD				X-ray diffraction pattern
	λ max. (nm)	Blue value (at 680 nm)	Fr.A (%)	Fr.Bl (%)	Fr.B2 (%)	Group	
Job's tears	528	0.06	23.73	70.92	5.35	B	A
Proso millet	531	0.07	25.25	68.72	6.03	C	A
Waxy maize	535	0.09	23.90	72.20	3.90	B	A
Sorghum	536	0.08	22.83	72.53	4.64.	A	A
Amaranth	538	0.09	30.54	63.32	6.14	E	A
Kiwifruit	580	0.44	26.42	66.24	7.34	C	B
Taro	584	0.20	25.56	69.46	4.98	C	A
Potato	586	0.45	20.52	73.87	5.61	A	B
Quinoa	587	0.32	37.45	57.37	5.18	F	A
Sweet potato	589	0.40	24.61	70.79	4.60	C	C
Amylomaize V	590	0.67	16.55	77.69	5.76	A	B
Pumpkin	591	0.43	21.13	74.72	4.15	A	B
Normal maize	592	0.38	24.23	71.02	4.75	B	A
Wheat	593	0.41	26.87	66.76	6.37	C	A
Job's tears (nonwaxy)	593	0.38	24.00	71.16	4.84	B	A
Horse radish	593	0.46	25.66	65.70	8.64	C	B
Barley	594	0.39	32.51	65.06	2.43	F	A
Amylomaize VII	595	0.80	14.85	75.10	10.05	A	B
Indian lotus	596	0.42	23.50	70.45	6.05	B	B
Buckwheat	597	0.43	35.07	60.60	4.33	F	A
Dogtooth violet	597	0.49	31.69	61.82	6.49	F	B
Japanese radish	597	0.42	33.24	62.61	4.15	F	B
Foxtail millet	598	0.42	24.25	69.52	6.23	B	A
Lily bulb	601	0.58	24.32	67.44	8.24	B	B
Tulip	602	0.48	32.57	59.71	7.72	F	B
Tapioka	602	0.40	29.27	65.41	5.32	D	C
Konjak	603	0.42	25.52	70.28	4.20	C	A
Bamboo shoot	604	0.43	25.55	70.26	4.19	C	C
Kudzu arrowroot	604	0.41	26.38	68.73	4.89	C	C
Loquat (Seed)	613	0.50	23.63	71.15	5.22	B	C
Sago	616	0.47	26.79	67.80	5.41	C	C

Fr.A(96) : Ratio of total peak area of DP 6~12
Fr.Bl(96) : Ratio of to tal peak area of DP13~36
Fr.B2(96) : Ratio of total peak area of DP >37

Group A : Fr.A<23.0%
Group B : 23.OSFr.A<24.5%
Group C : 24.5sFr.A<29.0%
Group D : 29.OsFr.A<30.5%
Group E : 30.5sFr.A<32.1%
Group F : 32.1sFr.A

Tabela 4

Some thermal characteristics of starches of different botanical sources.

Sample	Native starch				Retrograded starch		
	To1 (°C)	Tp1 (°C)	Tc1 (°C)	ΔH1 (J/g)	To2 (°C)	Tc2 (°C)	ΔH2 (J/g)
Job's tears	59.7	68.7	77.3	12.9	27.1	60.9	4.9
Proso millet	61.3	69.8	76.4	14.6	28.0	59.5	5.6
Waxy maize	62.5	68.2	78.6	14.3	26.0	62.6	6.4
Sorghum	68.9	75.2	82.8	16.3	29.4	62.3	8.8
Amaranth	66.3	72.1	78.2	10.6	27.7	59.5	4.7
Kiwifruit	66.1	69.1	73.5	16.1	29.8	67.8	4.2
Taro	68.6	74.5	80.7	12.5	27.7	62.6	7.7
Potato	61.0	64.5	70.6	17.4	27.4	72.1	7.5
Quinoa	46.3	55.0	64.5	9.9	26.0	54.2	1.4
Sweet potato	67.8	73.7	81.0	13.3	29.2	66.8	7.1
Amylomaize V	63.3	83.3	105.5	15.3	-	-	-
Pumpkin	66.1	68.9	73.3	16.0	28.8	71.5	7.6
Normal maize	62.9	67.5	73.3	12.9	29.7	61.4	6.3
Wheat	52.9	58.8	66.1	9.2	28.0	59.6	3.8
Job's tears (nonwaxy)	66.5	72.0	76.8	13.6	28.1	62.4	7.3
Horse radish	53.4	58.6	66.7	14.7	28.5	67.2	4.9
Barley	56.4	59.6	63.1	11.2	28.7	57.5	2.7
Amylomaize VII	64.7	92.1	106.8	10.1	-	-	-
Indian lotus	59.0	62.6	66.4	13.7	28.2	65.2	7.3
Buckwheat	58.5	63.4	69.7	12.0	27.5	57.0	4.0
Dogtooth violet	46.6	51.5	58.3	13.7	29.5	64.6	4.1
Japanese radish	47.6	51.5	56.6	11.3	29.6	62.5	3.3
Foxtail millet	64.1	69.1	75.2	10.2	29.0	62.4	4.4
Lily bulb	57.7	61.8	66.0	13.9	28.3	68.4	5.6
Tulip	49.4	53.1	57.1	13.3	28.1	62.8	3.9
Tapioka	63.1	67.0	75.2	13.9	27.9	61.5	4.1
Konjak	65.2	72.4	82.7	14.2	28.3	65.0	6.9
Bamboo shoot	60.5	68.9	79.4	12.3	28.5	63.3	6.3
Kudzu arrowroot	56.0	65.4	84.1	14.6	29.5	65.8	7.6
Loquat (Seed)	57.6	63.1	68.3	11.6	30.1	64.9	7.2
Sago	66.6	71.0	78.2	16.1	28.6	63.8	7.2

To1 : Onset temperature (native starch)
Tp1 : Peak temperature (native starch)
Tc1 : Conclusion temperature (native starch)
ΔH1 : Enthalpy change (native starch)

To2 : Onset temperature (retrograded starch)
Tc2 : Conclusion temperature (retrograded starch)
ΔH2 : Enthalpy change (retrograded starch)

Tab. 4 shows the gelatinization temperatures and the enthalpy change of gelatinization of native and retrograded starches of different botanical sources. The amylomaize V and VII starches with low content of Fr.A had higher temperature for gelatinization. The ratios of Fr.A of amylopectins of quinoa, barley, buckwheat, Japanese radish,

dogtooth violet and tulip were higher, and the gelatinization temperatures were lower than the other starches. There were highly negative relationships between the Fr.A contents in amylopectins and the To1, Tp1, and Tc1 of starches of rice and the other botanical sources. This result shows that amylopectins in starch granules with shorter chain-length distributions have lower temperature of gelatinization.

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WPŁYW ŁAŃCUCHÓW AMYLOPEKTYNOWYCH NA CHARAKTERYSTYKĘ KLEIKOWANIA SKROBI

Streszczenie

Zbadano strukturę i właściwości skrobi wydzielonych z ryżu japońskiego i innych odmian botanicznych roślin. Pozorna zawartość amylozy w nich została określona przez pomiar intensywności widm absorpcyjnych kompleksów z jodem skrobi, przed i po amylolizie, pozbawiającej amylopektynę odgałęzień (izoamylaza).

Za pomocą HPLC ustalono rozkład długości łańcuchów bocznych amylopektyny a następnie, posługując się wysokorozdzielczą chromatografią anionowymienną z pulsacyjną detekcją amperometryczną (HPAEC-PAD), porównano ten rozkład z rozkładem w innych odmianach botanicznych skrobi przed i po amylolizie. Właściwości termiczne określono za pomocą skanningowej kalorymetrii różnicowej (DSC). Stwierdzono, że ze względu na rozkład długości łańcuchów amylopektynowych japońską skrobię ryżową można podzielić na 5 grup. Skrobie Akenohoshi, I-taiminori, Tashukei 431 i Kenkei 2064, miały dłuższe łańcuchy boczne w amylopektynie niż inne skrobie. Te cztery skrobie kleikowały przy wyższych temperaturach. Zmiany entalpii kleikowania tych czterech skrobi po zretrogradowaniu były wyższe niż dla

innych skrobi. Stosunek długości łańcuchów bocznych zawierających 6 i 12 jednostek glukozowych w odgałęzieniach w skrobiach, z chinoi, jęczmienia, gryki, rzodkwi japońskiej i tulipana był wyższy, a temperatury kleikowania niższe niż w przypadku innych skrobi. Stosunek liczby takich łańcuchów dla wysokoamylozowych skrobi kukurydzianych był niższy, a temperatury ich kleikowania, były wyższe niż te dla poprzednio wymienionej grupy skrobi.

V.N. KISLENKO, E.I. KURIATNIKOV

GRAFT COPOLYMERS OF UNSATURATED MONOMERS WITH STARCH

Abstract

The influence of reagent concentration on monomer conversion and grafting efficiency was investigated for the graft initiated by ammonium peroxidisulfate polymerization of methyl acrylate and natural oil onto starch.

The monomer conversion and grafting efficiency increased when the initiator concentration increased. An increase of the monomer concentration led to increase in the monomer conversion and a decrease in the grafting efficiency. The grafting efficiency of oils decreased in the order olive oil, linseed oil, sunflower seed oil. The conversion of natural oil decreased with increase in the oil concentration in the reaction mixtura.

The influence of reagent concentration on elongation at break, breaking strength, swelling capacity and water vapor permeability of films obtained from graft copolymer dispersions was investigated. The breaking strength was independent of the concentration of monomers and starch, but decreased when initiator concentration increased. The elongation increased with the increase in the methyl acrylate concentration. The water swelling capacity of films decreased with increase in the concentration of initiator and decrease in the starch concentration. It was independent of the oil concentration.

Introduction

The polymer solutions and dispersions can be used for obtaining glues, lacquers, paints in industry. Dispersions of acrylic polymers, solutions of poly(vinyl alcohol) [1-3], cellulose ethers [4] are used as film forming compounds. Homever, these polymers cannot be used in some cases, for example, in food or pharmaceutical industry, because dispersions of industrial polymers contain monomers, emulifiers and other additives. Therefore, further novel polymer dispersions based on natural substances are very interesting.

In this paper the results of the investigation of film forming dispersions based on the natural substances and physico-mechanical properties of film obtained from them are presented.

V.N. Kislenko, E.I. Kuriatnikov, Lviv Polytechnic State University, 12 bandera St., Lviv 79013, Ukraine.

Materials and methods

Industrial potato starch, linseed oil, olive oil and sunflower seed oil, purified from solid particles, distilled methyl acrylate and recrystallyzed ammonium peroxidisulfate were used. The solution of starch was obtained by heating of starch dispersion in distilled water at 97–98°C about 0,5 h. Graft copolymer was prepared of methyl acrylate and natural oil with starch obtained at 70°C by addition of ammonium peroxidisulfate as the initiator followed by addition of monomers, methyl acrylate and natural oil, into the starch solution. The reaction mixture was heated for 2 h at 70°C. The reaction mixture became milk in 5–10 min after blending reagents.

Oil conversion, S_o, was determined by centrifugation of dispersion at 8000 min^{-1} for 30 min. Oil separated from water dispersion. Oil conversion was calculated according to equation:

$$S_o = (m_o - m_k)100/m_o$$

Where m_o is the oil mass admixed into the reaction mixture, m_k is the oil mass separated after centrifugation.

It was found that under this condition the whole oil mass was separated from the solution. The experimental error was below 5%.

The methyl acrylate conversion, S_m, in dispersion was determined by the bromide – bromate method [5].

Grafting efficiency, GE, was detrmined by 80 h acetone extraction of methyl acrylate copolymer with oil from the film. The film contained the mixture of this copolymer with graft copolymer of these monomers to starch. The grafting efficiency was calculated according to equation:

$$GE = (m_g - m_c)100/m_g$$

Where m_g is the mass of polymer film without the mass of starch in it and m_c is the mass of copolymer of methyl acrylate and oil extracted from the film.

The sweeling capacity, δ, of the film in water was determined in the sample immersed for 24 h in distilled water at 20°C [6]. It was calculated with equation:

$$\delta = m_s\ 100/m_o$$

where m_s is the mass of swelling film, and m_o is the mass of dry film.

The water vapor permeability, P, of the film was detrmined as the relation of the mass of water evaporated from the pot covered with film to the mass of water, evaporated from the free water surface of this pot [7].

The breaking strength, δ, and the elongation at break, ε, of films was determined using "TIRA test 2200" instrument.

Results and discussion

Polymerization of natural oils, initiated by ammonium peroxidisulfate in starch solution practically does not occur. Oil conversion was below 5%. Conversion of natural oil was increased when methyl acrylate has been used as comonomer. Graft copolymer of methyl acrylate and natural oil starch was available in this process. A decrease in the reaction rate and monomer conversion was observed when reaction temperature decreased to 55–60°C. At temperature increased to 80–85°C, acrylate evaporated (boiling temperature 80.5°C), and the grafting efficiency significantly decreased. Therefore, the polymerization has been carried out at 70°C.

Dependence of conversion of natural oil, methyl acrylate and grafting efficiency on the reagent concentration in the mixture is given in Table 1. Increase in the linseed oil concentration from 0.7 to 4.2% led to decrease in the oil conversion. That can be rationalized in terms of the mechanism of the process. The equilibrium concentration of methyl acrylate in the water phase and oil droplets was established as a result of its diffusion from oil drops. Initiation of the polymerization proceeded in the water phase. Initiating radicals interacted with the methyl acrylate molecules and starch in the water solution. Then starch macroradicals, adding methyl acrylate molecules, initiated the graft polymerization. In the first stage, the oligomer radicals of methyl acrylate were formed in the water phase. These oligoradicals had surface-active properties and adsorbed on the surface of oil drops, where they initiated copolymerization of methyl acrylate to natural oil [8]. Therefore, the copolymerization of methyl acrylate with natural oil occurred at high concentration of methyl acrylate in oil. At its low concentration in droplets, the polymerization rate was very slow and in consequence the oil conversion was low. The polymerization of methyl acrylate proceeded in the water phase. It was suggested by increase in the monomer conversion when the concentration of methyl acrylate increased at the constant methyl acrylate-to-natural oil ratio. In this case, one could observe a decrease in the grafting efficiency of monomers onto starch. Evidently, copolymer of methyl acrylate with natural oil formed by the reaction of chain transfer from graft copolymer onto monomer molecules in the oil drops.

An increase in the starch concentration did not influence the natural oil conversion. However, that led to an increase in the methyl acrylate conversion. At the same time, conversion of methyl acrylate and natural oil increased, when the initiator concentration increased from 0.35 to 1.4% (Table 1). Evidently, the high viscosity of

starch solution led to an increase in the radical concentration in it, as a result of the gel-effect. In this case the grafting efficiency also increased.

Investigation of the influence of the oil nature on the grafting process showed (Table 2) that the oil conversion increased when the olive oil was used and decreased for sunflower seed oil. Evidently, it was associated with the number of the double bonds and the length of carbon chains in corresponding molecules. The methyl acrylate conversion and grafting efficiency were independent of the oil variety.

Table 1

Physico-chemical characteristics of graft copolymers of methyl acrylate and linseed oil with starch.

[MA], %	[St],%	[LO], %	[I],%	[S_o], %	[S_m], %	[GE], %
7,0	7,0	0,70	0,70	80	96	76
7,0	7,0	2,10	0,70	55	97	79
7,0	7,0	4,10	0,70	15	94	96
3,5	7,0	1,05	0,70	35	97	82
7,0	7,0	2,10	0,70	55	97	79
10,5	7,0	3,15	0,70	60	96	47
14,0	7,0	4,20	0,70	65	98	44
7,0	5,0	2,10	0,70	65	94	55
7,0	7,0	2,10	0,70	55	97	79
7,0	10,0	2,10	0,70	70	95	86
7,0	7,0	2,10	0,35	35	92	70
7,0	7,0	2,10	0,70	55	97	79
7,0	7,0	2,10	1,05	70	98	85
7,0	7,0	2,10	1,40	85	98	90

Table 2

Influence of the orgin of oil on physico-chemical characteristics of graft copolymers at the concentration of methyl acrylate 7.0%, starch 7.0%, oil 2.1% and initiator 1.4%.

Oil	S, %	S, %	GE, %	δ, %	P, %
Linseed	85	98	90	88	31
Olive	90	97	90	83	30
Sunflower seed	60	95	85	94	35

Physico-mechanical properties of the films were investigated involving the air dried films of graft copolymers of the 0.1 mm thickness. The film of initial starch could not be prepared because it was very friable.

Investigation of the elongation at the break of films showed, that it increases with the monomer concentration in the reaction mixture (Fig. 1, curve 1). At the same time, an increase in the starch concentration led to a decrease of elongation (Fig. 1, cuvre 2). Therefore, copolymer of methyl acrylate with natural oil plastified the starch containing film. An increase in the initiator concentration in the reaction mixture decreased the elongation at the film break although the monomer conversion and grafting efficiency increased (Fig. 1, curve 3). Evidently, it was due to cross-linking of polymers by a bimolecular termination of the macroradical chains. The swelling capacity of films in water decreased insignificantly when the initiator concentration in reaction mixture increased from 0.35 to 1.4%.

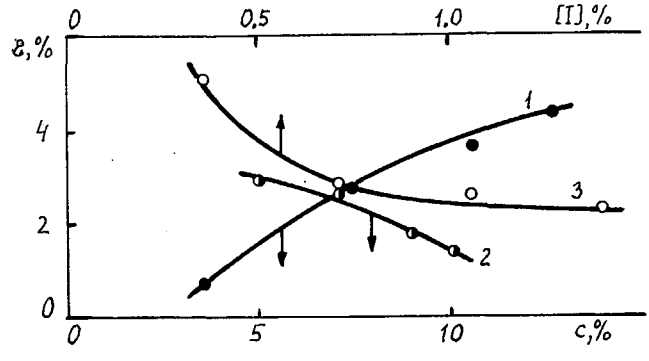

Fig. 1. Dependence of elongation at break of the graft copolymer of methyl acrylate and linseed oil with starch on reagent concentration in reaction mixture. Concentration of initiator was 0.7% (1,2), starch - 7% (1,3), methyl acrylate 7% (2,3), linseed oil 2.1% (2,3), mass ratio of linseed oil - methyl acrylate was 0.3 (1).

Decrease of the swelling capacity of film with the monomer concentration increase (Fig. 2, curve 2) and the starch concentration decreases (Fig. 2, curve 2) were observed. At the same time, an increase in the natural oil concentration in reaction mixture led only to insignificant swelling of the films (Fig. 2, curve 3). Evidently, that is the result of low conversion of oil.

Water vapor permeability of graft copolymers to starch increased with starch concentration (Fig. 3, curve 2). However, the water vapor permeability passed through the maximum, when the monomer concentration in reaction mixture increased at the constant ratio of methyl acrylate to natural oil (Fig. 3, curve 1). That could be explained as the result of decrease in the grafting efficiency.

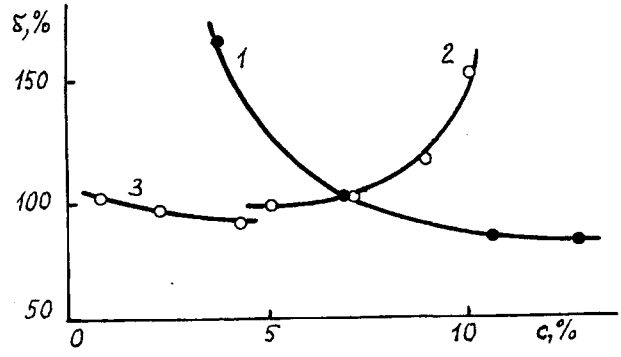

Fig. 2. Dependence of swelling capacity of the graft copolymer of methyl acrylate and linseed oil with starch on reagent concentration in the reaction mixture. Concentration of initiator was 0.7% (1,3), starch - 7% (1,3), methyl acrylate 7% (2,3), linseed oil 2,1% (2), mass ratio of linseed oil - methyl acrylate was 0.3 (1).

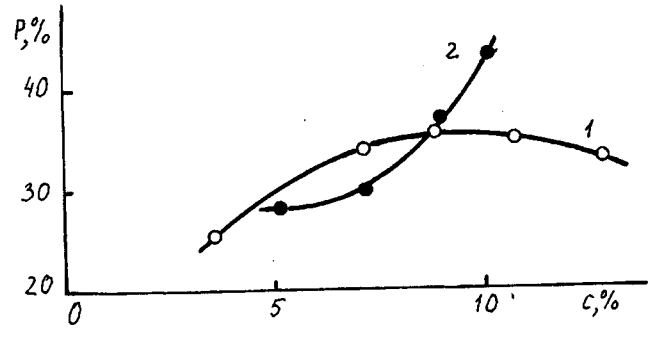

Fig. 3. Dependence of water vapor permeability of the graft copolymer of methyl acrylate and linseed oil with starch on reagent concentration in reaction mixture. Concentration of initiator was 0.7%, starch - 7% (1), methyl acrylate 7% (2), linseed oil 2.1 (2), mass ratio of linseed oil - methyl acrylate was 0.3 (1).

The breaking strength of the films insignificantly depended on the concentration of monomers and starch (Fig. 4, curves 1-3). An increase in the initiator concentration led to decrease in the breaking strength of the film (Fig. 4, curve 4). Oxidation of starch during graft polymerization could be involved leding to a change in the structure of the material.

The nature of oil had no influence on the physico-mechanical properties of the films of the graft copolymers of methyl acrylate and oil with starch.

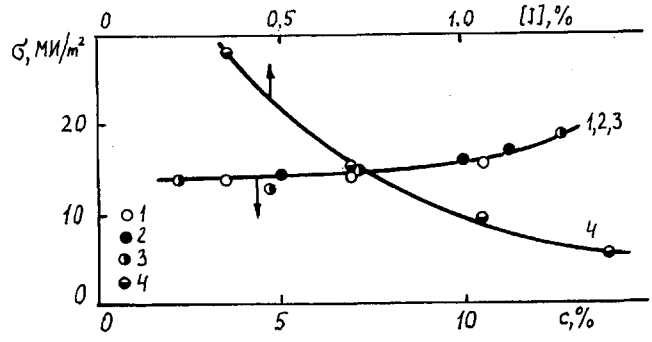

Fig. 4. Dependence of breaking strength of the graft copolymer of methyl acrylate and linseed oil with starch on reagent concentration in reaction mixture. Concentration of initiator was 0.7% (1-3), starch - 7% (1,2,3), methyl acrylate 7% (2-4), linseed oil 2.1% (2,4), mass ratio of linseed oil - methyl acrylate was 0.3 (1).

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SZCZEPIONE POLIMERY NIENASYCONYCH MONOMERÓW ZE SKROBIĄ

Streszczenie

Badano wpływ stężenia reagenta na konwersje monomeru i efektywność szczepiania w czasie inicjowanej peroksydisiarczanem amonu polimeryzacji akrylanu metylu i olejów roślinnych ze skrobią.

Konwersja i efektywność szczepiania wzrastała ze stężeniem inicjatora. Wzrost stężenia monomeru podnosi stopień jego konwersji, obniżając efektywność szczepiania. Efektywność szczepiania olejów roślinnych maleje w kolejności: olej oliwkowy > olej lniany > olej słonecznikowy. Stopień konwersji tych olejów maleje ze wzrostem ich stężenia w mieszaninie reakcyjnej.

Badano też wpływ stężenia reagenta na naprężenie wzdłużne w punkcie zerwania, wytrzymałość na zerwanie, zdolność pęcznienia i przepuszczalność dla pary wodnej filmów z tych materiałów. Wytrzymałość na zrywanie nie zależała od stężenia monomeru i skrobi i obniżała się ze wzrostem stężenia ini-

cjatora. Względne naprężenie wzdłużne wzrastało ze stężeniem metakrylanu metylu i malało ze wzrostem stężenia inicjatora. Pęcznienie filmu malało ze wzrostem stężenia inicjatora i malało ze wzrostem stężenia skrobi. Nie zależało ono od stężenia oleju roślinnego. Przepuszczalność pary wodnej wzrastała ze stężeniem skrobi i przechodziła przez maksimum zależności od stężenia monomeru.

J. KOROLCZUK, G. EL GARAWANY, J.M. MEMBRE, J.F. MAINGONNAT

TEXTURING CAPACITY OF VARIOUS STARCHES IN WHEY PROTEINS / POLYSACCHARIDES BASED MILK DESSERTS

Abstract

Experimental milk desserts were composed of reconstituted skimmed milk powder and whey protein isolate as a source of proteins, normal and waxy maize starch, potato starch and hydroxy propyl distarch phosphate (HPDP) as thickeners and ι-carrageenan (ι-C) as gelling agent. The protein and starch concentration varied between 0 and 6%, sugar between 0 and 20% and that of ι-C between 0 and 0.4%. The desserts were heat treated at 100, 110 or 120°C for 10, 20 or 30 minutes. The firmness of the desserts after 24 hours of storage at 20°C, was determined by a cone penetrometric method and expressed as stress in Pa.

The logarithm of the stress was a linear function of protein and starch content and of the logarithm of the ι-C concentration (g/100 g). To multiply the firmness of the experimental desserts by a factor of ten, it was necessary to increase the whey protein concentration by 11 to 15 g/100 g depending on the type of starch used. The same effect could be obtained with 5 to 9 g/100 g of starch. When the ι-C concentration increased tenfold the stress level was multiplied by a factor of 2.6 to 4. The desserts' firmness also depended on the heating time and temperature, even if this effect was small.

Introduction

Milk desserts are important industrial food products. A typical milk dessert is composed of 84 to 89% of liquid milk, 6.5 to 10% of sugar, 2 to 3.5% of starch 0.15 to 0.25% of carrageenan and 0 to 2% of chocolate in powder [7, 21]. Vanillin, carotenoids or xanthophylls are also added in small quantities <0.02% to improve the taste and modify the colour [21]. The gel-like texture of milk desserts is obtained by using starches and their derivatives as thickeners and some other polysaccharides as gelling agents. An important quality factor of milk desserts is their in-mouth perceived thickness. For many gel like products, including milk desserts, the relationship between the in-mouth perceived thickness and the objective viscosity follows a power law type

J. Korolczuk, G. El Garawany, J.M. Membre, J.F. Maingonnat, INRA-LGPTA, 369 rue Jules Guesde, B.P. 39, 59651 Villeneuve-d'Ascq, France.

equation [20, 29] i.e. the logarithm of the viscosity established by the sensory analysis is a linear function of the logarithm of the physically measured viscosity (Fig. 1).

For the starch pastes and gels, the viscosity and swelling capacity (Fig. 2 and 3) depend on the temperature, heating time and the shear rate [3].

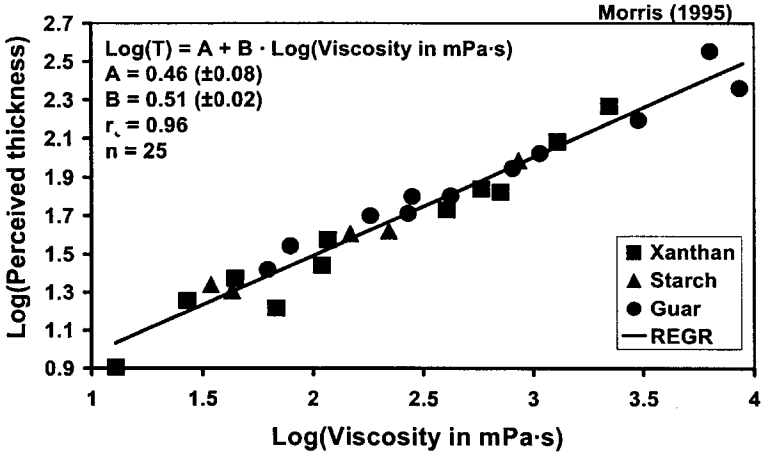

Fig. 1. Relationship between the logarithm of the in-mouth perceived thickness (T) and the logarithm of the apparent viscosity measured at shear rate = 50 s^{-1}, for the gels prepared from starch, xanthan and guar gums (based on the results published by Morris, 1995).

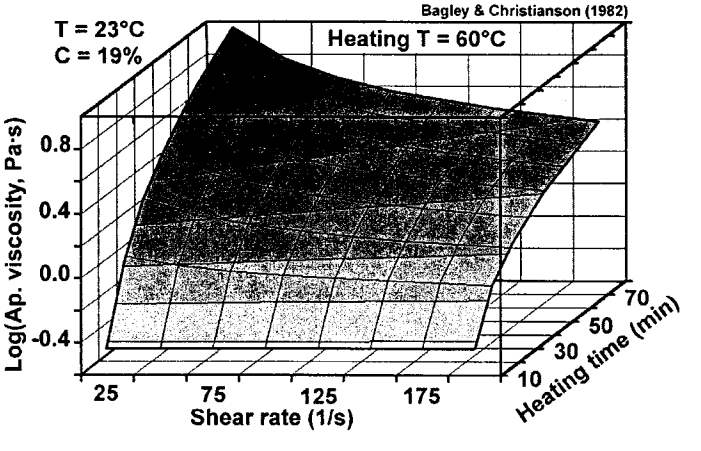

Fig. 2. Logarithm of the apparent viscosity of 19% wheat starch pastes at 23°C, as a function of the shear rate and the cooking time at 60°C (based on the results of Bagley and Christianson, 1982).

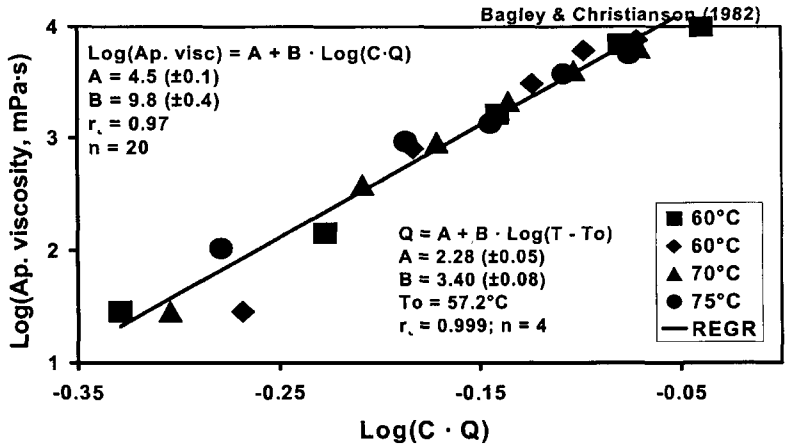

Fig. 3. Logarithm of the apparent viscosity of the wheat starch pastes at 60°C, as a function of the logarithm of the starch concentration (C in g/g) multiplied by the swelling capacity (Q in g/g), with Q being a function of the heating temperature (T in °C), for a constatnt heating time = 75 min (based on the results of Bagley and Christianson, 1982).

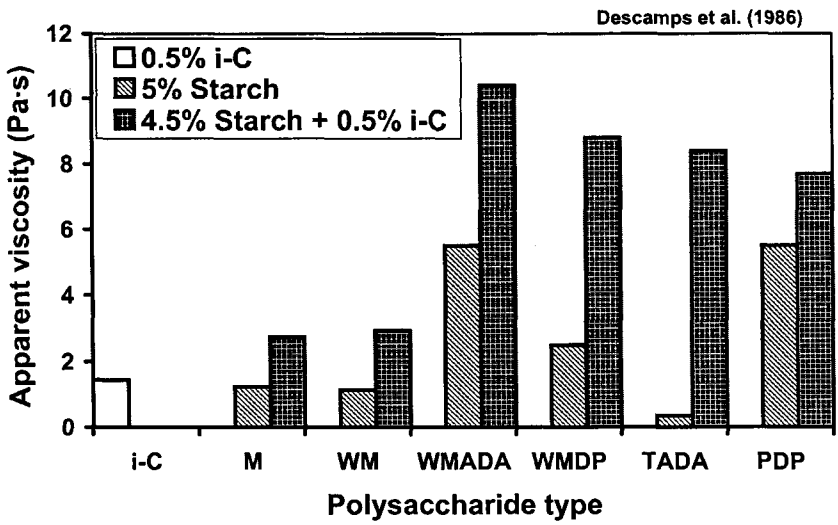

Fig. 4. Apparent viscosity at 20°C of gels prepared from different starches (4.5%) and 0.5% of i-carrageenan (ι-C). Starch type: M = maize, WM = waxy maize, WMADA = waxy maize acetylated distarch adipate, WMDP = waxy maize distarch phosphate, TADA = tapioca acetylated distarch adipate, PDP = potato distarch phosphate (based on the results of Descamps et al., 1986).

Due to the interactions between the starches and other polysaccharides, the observed viscosity of the mixtures is much higher (Fig. 4) than that which could be anticipated for non-interacting mixtures.

Owing to their high nutritional quality and gelling properties, whey proteins can be used as gelling agents in many food products including milk desserts [1, 2, 8, 9, 13-15, 17-19, 23, 31, 32]. The gel firmness of the whey proteins and starch gels increases exponentially (Fig. 5) with the dry matter content, i.e. the logarithm of the elastic modulus is a linear function of the protein or starch concentration [1].

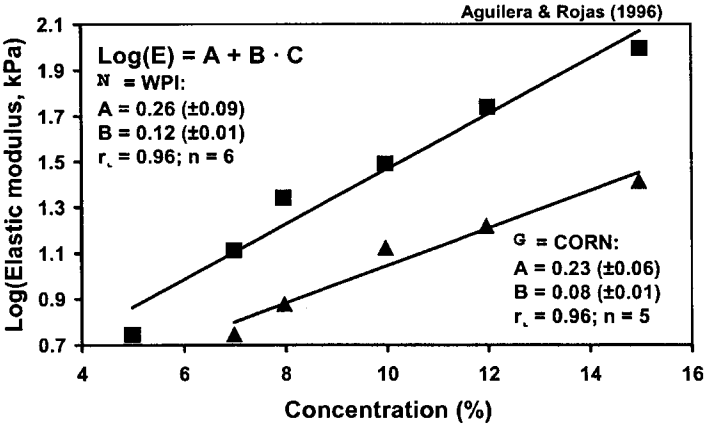

Fig. 5. Logarithm of the elastic modulus (E) of gels prepared by heating for 30 min at 85°C and cooling, as a function of the whey protein isolate (N) and corn starch (G) concentration (in % w/w) (based on the results of Aguilera and Rojas (1996).

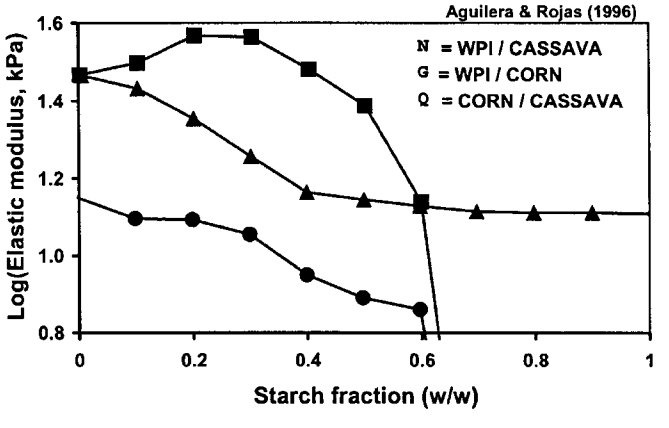

Fig. 6. Logarithm of the elastic modulus of mixed gels, prepared by heating for 30 min at 85°C and cooling, as a function of the starch fraction in the mixtures of: (N) = whey protein isolate (WPI) / cassava starch (CS), (G) = WPI / corn starch (CORN) and (Q) = CORN / CS. Total solids in all gels is 10% and the pH is 5.75 (based on the results of Aguilera and Rojas, 1996).

The firmness of mixed gels also depends, in some cases, on the protein to starch ratio (Fig. 6). Cassava starch pastes do not form gels below a 16% concentration, but when 2–3% cassava starch is mixed with 7–8% whey protein isolate, the resulting gels are stronger than either the pure protein or pure starch gels. No such interaction is observed for the corn starch mixed with whey proteins or with cassava starch.

Milk proteins interact and are precipitated by certain polysaccharides [6, 10, 11, 24, 30, 31]. Because of the interactions, the viscosity of milk protein / polysaccharide gels is multiplied by a factor of 2 to 10 (Fig. 7) in comparison with pure polysaccharides in water gels [26]. The viscosity multiplication factor depends not only on the polysaccharide and protein type but also on the heat treatment applied to the mixture. It is much higher for strongly acidic carrageenans than for neutral guar or slightly acidic xanthan gums.

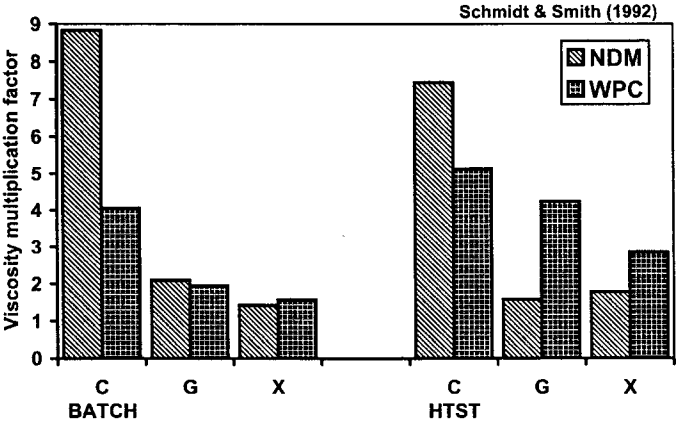

Fig. 7. Viscosity multiplication factor for the gels containing 0.1% C = carrageenan, G = guar, or X = xanthan gums and 11% (w/w) of (NDM) = non fat dry milk or (WPC) = whey protein concentrate, heated at 69°C for 30 min (BATCH) or at 80°C for 25 s (HTST) in comparison with the gels composed of 0.1% of C, G or X gums in water. Viscosity was measured at 4°C and at the shear rate 250 s^{-1} (based on the results of Schmidt and Smith, 1992).

The literature data suggest that the gel strength of milk desserts depends on several parameters: protein and starch type and their concentration, the presence of other polysaccharides and the heat treatment applied. Taking into account the existing power law type relationship between the sensory and the physical properties of milk desserts, we have chosen to use the logarithm of the gel stress measured by a penetrometric method as an indicator of the gel strength and of the texturing capacity of native and modified starches in the presence of ι-carrageenan and whey proteins, in relation to the heat treatment applied during the cooking of the desserts.

Materials and methods

The following raw materials were used in this study: normal and waxy maize starch, potato starch, hydroxy propyl waxy distarch phosphate (HPDP) from Roquette Frères, (Lestrem, France), ι-carrageenan from Sanofi Bio-Industries, (Carentan, France), wheat flour (WF) type 55%, sugar and chocolate powder (Van Houten) from the local supermarket, whey protein isolate 85% proteins in dry matter from Eurial, (Herbignac, France), low heat skimmed milk powder from NIZO, (Netherlands). Three samples of gel type market milk desserts, covering the large scale of gel firmness: Danette and Dany (Danone, France) and Crème Brulée (Triballat, France).

Two hundred grams of water suspensions containing: 0 to 6% (w/w) whey proteins, 0 to 4% milk proteins (low heat skimmed milk powder), 2 to 6% starch, modified starch or wheat flour, 0 to 0.3% ι-carrageenan and 0 to 20% sugar, were heated for 10, 20 or 30 minutes at 100, 110 or 120°C in a small (250 ml) reactor vessel with magnetic stirring. The pastes were then cooled to 90°C, poured into small (25 ml), screw sealed, plastic boxes and left overnight in a 20°C set water bath.

The texture of the desserts obtained was measured at 20°C, with a constant speed (2 mm/s), cone (20 degrees) penetrometric method [16] with a DY-30 (Adamel Lhomargy, France) traction - compression machine. The penetration depth was 20 mm and the full scale load of the force transducer was 1 N. The results of penetrometric tests are expressed as stress in Pa. The gel firmness of a part of experimental and market milk desserts was also measured by a dynamic rheological method with an AR1000 type rheometer (TA Instruments, England). A General Linear Models [25] method was used for the statistical analysis of the results.

Results and discussion

For market and experimental milk desserts gels, the logarithm of the stress level evaluated by the penetrometric method is linearly related to the logarithm of the complex modulus measured by the dynamic rheometric method (Fig. 8). It means that the simple to use, rapid and inexpensive penetrometric method gives results comparable to those obtained by a non-destructive, but time consuming rheometric method, and requiring expensive equipment. The gel firmness of milk desserts was evaluated by the viscometric method [3, 7, 14, 15, 17, 21, 26, 27, 31], by the compression method [1, 17] or by the rheometric method [12, 18, 22]. For rapid evaluation of the gel firmness of the large quantities of dessert samples, the penetrometric method could be a good choice.

For over 300 experimental desserts gels prepared and for all starches analysed in this work, the logarithm of the stress level was increasing linearly with the concentra-

tion of whey proteins and starch and with the logarithm of ι-carrageenan content (Fig. 9, 10 and 11):

$$\text{Log(S)} = A + WP / B + St / C + D \cdot \text{Log}(\iota\text{-C}) \quad (1)$$

where: S = stress in Pa measured by the penetrometric method, A = intercept or the hypothetical value of the logarithm of the stress for WP = 0, St = 0 and ι-C = 1. WP, St and ι-C are respectively the whey protein, starch and ι-carrageenan concentration in g/100g of solution. B and C are the coefficients indicating respectively the increase in protein and starch concentration (in g/100g) provoking ten fold increase in the stress. D is the increase in the log(S) for tenfold increase in ι-C concentration.

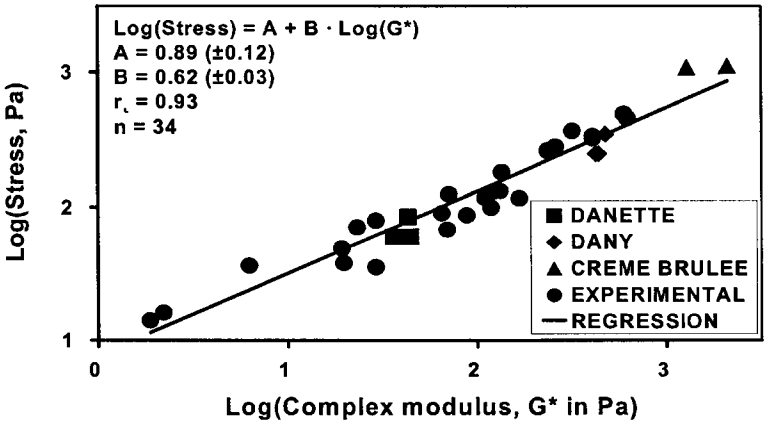

Fig. 8. Relationship between the logarithm of the stress evaluated by the penetrometric method and the logarithm of the complex modulus (G*) measured by the dynamic rheometric method.

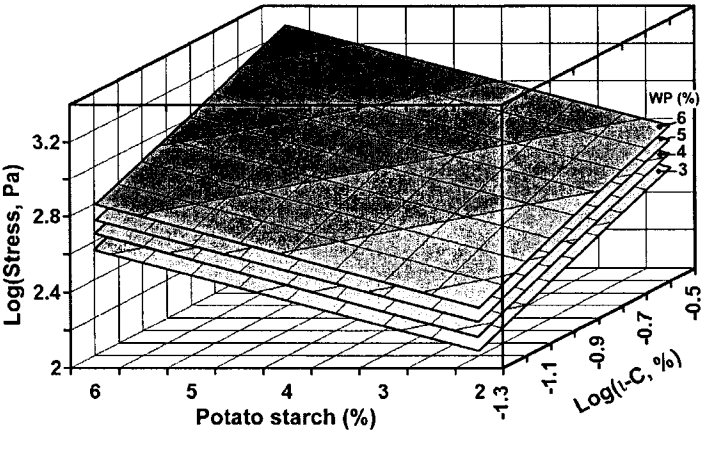

Fig. 9. Logarithm of the stress as a function of the potato starch, whey proteins (WP) and ι-carrageenan (i-C) concentration (in % w/w).

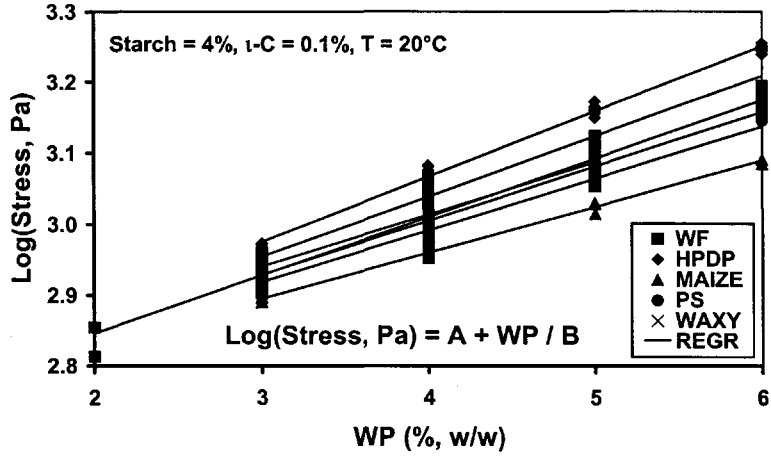

Fig. 10. Logarithm of the desserts' gels stress as a function of the whey proteins (WP) concentration for different starches: WF = wheat flour, HPDP = hydroxy propyl distarch phosphate, MAIZE = normal maize starch, PS = potato starch, WAXY = waxy maize starch, in presence of 0.1% of ι-carrageenan.

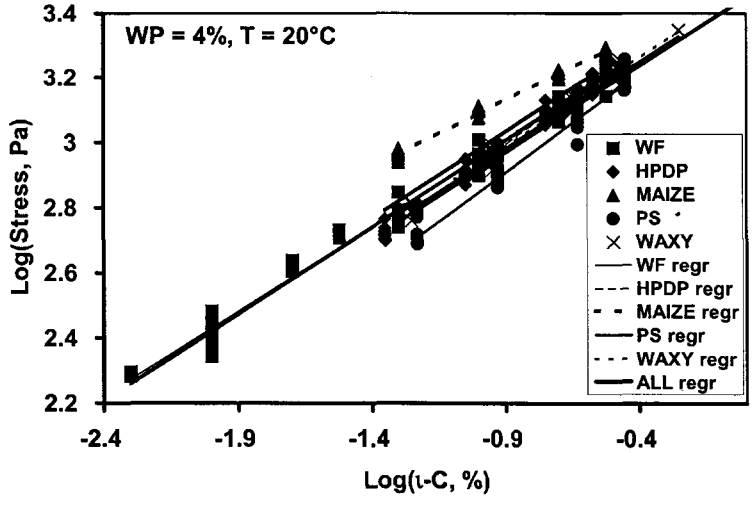

Fig. 11. Logarithm of the desserts' gels stress as a function of the logarithm of ι-carrageenan concentration for different starches: WF = wheat flour, HPDP = hydroxy propyl distarch phosphate, MAIZE = normal maize starch, PS = potato starch, WAXY = waxy maize starch, in presence of 4% whey proteins.

The coefficient A depends also on the starch, whey proteins and ι-carrageenan concentration. The value of coefficient B varied between 11 and 15 g/100 g for different starches (Tab. 1). Some of the differences were statistically significant. The highest B level (>15) was found for the maize starch, which gelatinises at a slightly higher temperature than whey proteins and on cooling, forms its own amylose and amylopectin gel structures independent of protein. This structure probably disturbs the weak protein gel that has already formed at high temperatures (>70°C) [1, 2, 14]. A similar effect was also found by Muhrbeck and Elliasson [22] for bovine serum albumin / starch gels. The lowest B value (<11) was found for the most heat resistant, and the most hydrophobic hydroxy propyl waxy distarch phosphate. This slightly acidic starch was, more hydrophobic than the others analysed and may interact with whey proteins as do other acidic polysaccharides such as carrageenans and CM-cellulose [4, 5, 10, 11, 24, 26, 28].

Table 1

Coefficients B, C and D from the equation (1) for different starches. SD = standard deviation.

Starch type	B	±SD	C	±SD	D	±SD	n
Wheat flour	12,20	0,45	5,40	0,81	0,52	0,03	76,00
HPDP	10,90	0,12	5,40	0,63	0,59	0,04	52,00
Maize	15,40	0,24	5,90	0,12	0,41	0,03	27,00
Potato	13,20	0,22	8,00	0,42	0,65	0,02	136,00
Waxy	13,60	0,55	8,90	0,88	0,55	0,02	42,00

Taking into account the coefficient C from the equation (1), two groups of starches could be distinguished (Tab. 1): one including hydroxy propyl distarch phosphate, maize starch and wheat flour which needed on average 5 to 6 % and the other group which includes potato and waxy maize starches, which needed 7 to 9% starch addition in order to multiply by ten the desserts' firmness expressed in stress units. Whey protein addition required to achieve the same effect was on average 13%. From the results of Aguilera and Rojas [1], presented in fig. 5 the coefficients B and C are 8.3% and 12.5%. respectively.

The value of the coefficient D varied between 0.4 and 0.6. Its lowest level (0.41±0.034) was observed for the maize starch gels. It was the only one of the starches analysed to form firm gels.

By using the parameters A to D from the equations (1), it is possible to calculate the stress level for a given dessert composition. Figure 12 shows the results of such a comparison for the milk desserts containing potato starch as thickeners and ι-carrageenan as gelling agent. The agreement between the experimental and calculated results is quite close for all the starches analysed in this work. Other models including

starch - protein, starch - ι-C, protein - ι-C and starch - protein - ι-C interaction coefficients could be applied to describe the texture dependence on the gel composition. We tried several of these more complicated models, but the results were not better than for the simple one described by the equation (1).

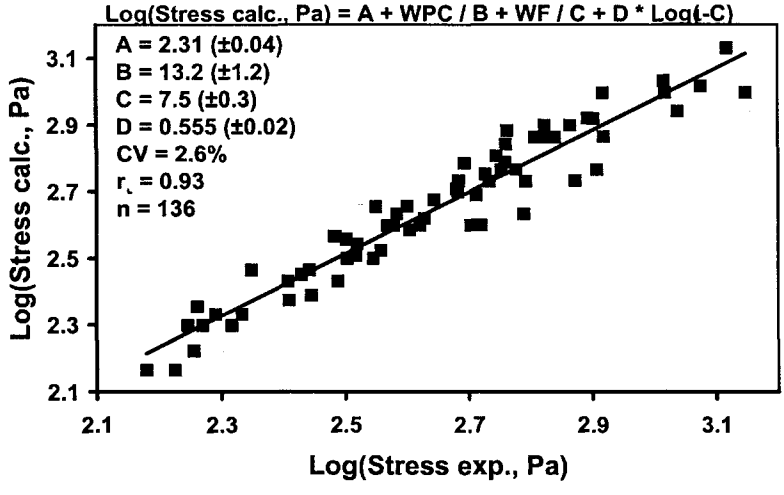

Fig. 12. Relationship between the logarithm of the stress calculated by the equation (1) and the experimental values, for the desserts gels containing whey proteins (2 - 6%), potato starch (2 - 6%), ι-carrageenan (0–0.3%), sugar 10% and chocolate powder 3%.

Cooking time and temperature are important technological parameters, influencing the firmness of pastes and gels of pure starch [3, 4, 15], of the mixtures of starch with proteins [14] and of other polysaccharides with proteins [26]. In this work we analysed the effect of the heating time (10–30 min) and temperature (100–130°C) on the desserts' gel firmness after 24 hours of storage at 20°C, for the desserts composed of whey proteins (0 or 4%), milk proteins (4 or 0%), wheat flour (4%), milk fat (3%), chocolate powder (3%), sugar (10%) and ι-carrageenan (0.1%). We used the same type of mathematical model as given in the equation (1) to evaluate the effect of the heating temperature (T in °C) and of the heating time (HT in minutes):

$$\text{Log}(S) = I + T / E + HT / F \qquad (2)$$

where: I = the point of interception or the hypothetical value of the stress (S) logarithm for T = 0 and HT = 0, E = the coefficient indicating the temperature increase provoking ten fold stress increase (if +) or decrease (if -), F = the coefficient indicating the heating time increase provoking ten fold stress increase (if +) or decrease (if -).

The results obtained show that the effect of the heating time and temperature are relatively small (fig. 13 and 14), and much smaller than those observed for the pure

starch pastes and gels [3, 4, 15] and for the milk proteins/starch mixtures [14]. This is probably because of multiple interactions between proteins, polysaccharides, fat and minerals which impair the formation of the three dimensional networks of whey proteins, amylose, amylopectin and ι-carrageenan.

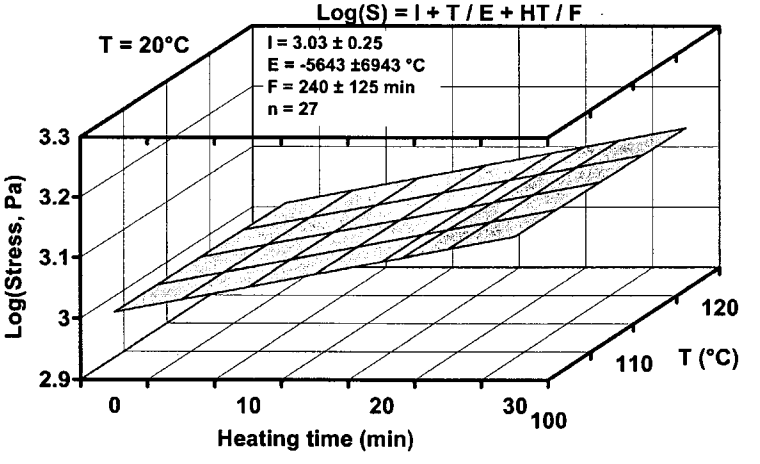

Fig. 13: Effect of the heating time (HT) and temperature (T°C) on the gel firmness at 20°C, of the dessert composed of the whey proteins (4%), wheat flour 4%, milk fat 3%, chocolate 3%, sugar 10% and ι-carrageenan 0.1%.

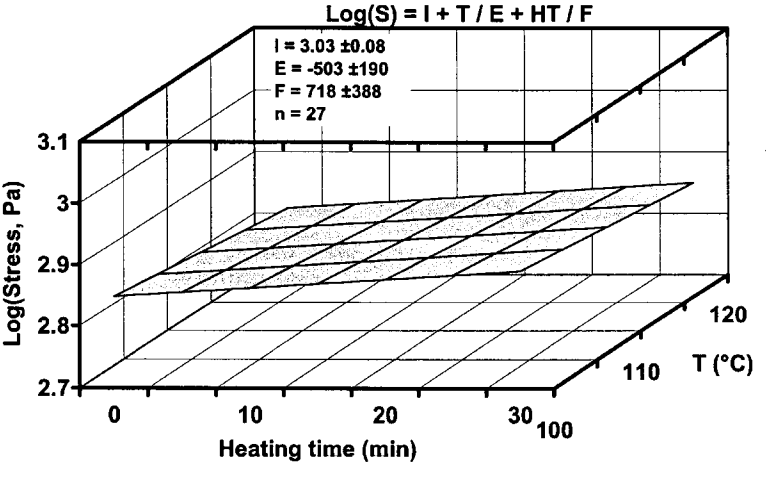

Fig. 14: Effect of the heating temperature (T°C) and the heating time on the gel firmness at 20°C, of the dessert composed of the milk proteins (4%), wheat flour 4%, milk fat 3%, chocolate 3%, sugar 10% and ι-carrageenan 0.1%.

Conclusions

1. The penetrometric method appears to be a useful tool for a rapid evaluation of the desserts' gel firmness.
2. Gel firmness expressed as the logarithm of the stress is a linear function of the whey proteins and starch and of the logarithm of the ι-carrageenan concentrations.
3. The effect of the cooking times within the analysed range (10–30 min) and temperatures (100–130°C) on the gel firmness was small.

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WŁASNOŚCI ŻELUJĄCE RÓŻNYCH SKROBI W DESERACH Z BIAŁEK SERWATKOWYCH I POLISACHARYDÓW

Streszczenie

Doświadczalne desery mleczne składały się z odtłuszczonego mleka w proszku i izolatu białek serwatkowych jako źródła białka, skrobi jako substancji zagęszczającej (normalnej i woskowej skrobi kukurydzianej, skrobi ziemniaczanej, hydroksypropylowanego fosforanu dwuskrobiowego) i ι-karagenu jako substancji żelującej. Stężenie białek i skrobi zmieniano w zakresie od 0 do 6%, cukru od 0 do 20%, i-karagenu od 0 do 0.4%. Desery były ogrzewane przy ciągłym mieszaniu w temperaturze 100, 110 lub 120°C w ciągu 10, 20 lub 30 min. Konsystencję deserów oznaczano metodą penetrometryczną po 24 godz. przechowywaniu w temperaturze 20°C.

Logarytm naprężeń ścinających był liniową funkcją stężenia białka i skrobi oraz logarytmu stężenia ι-karagenu. Aby naprężenia ścinające wzrosły 10 krotnie, stężenie białek serwatkowych powinno wzro-

snąć o 11–15% (g/100 g) zależnie od rodzaju stosowanej skrobi. Identyczny efekt uzyskuje się przy dodatku 5–9% skrobi. Kiedy stężenie ι-karagenu wzrastało 10 krotnie, naprężenia ścinające zwiększały się 2.6 do 4 krotnie.

Konsystencja deserów zależała też od czasu i temperatury ogrzewania, ale wpływ ten był stosunkowo mały.

G. LEWANDOWICZ, W. BŁASZCZAK, E. VOELKEL

IONIC STARCH DERIVATIVES OBTAINED IN MICROWAVE ASSISTED REACTIONS – STRUCTURE AND FUNCTIONALITY

Abstract

Commercial potato and wheat starches were substituted to DS = 0.04 by cationic and anionic groups separately (to obtain cationic and anionic derivatives, respectively) and jointly (to obtain amphoteric derivatives). Microwave assisted processes were carried out in oven emitting radiation of 2450 MHz frequency and 0.5 W/g energy. Reaction products were examined by rheological methods, light, and scanning electron microscopy, and X-ray diffractometry. Nitrogen and carboxymethyl group contents were also determined.

Incorporation of ionic substituents into the starch molecules significantly changes its physicochemical properties related to starch-water interactions, results in the decrease of the gelatinisation temperature, changes of the swelling characteristics, solubilisation and iodine complexation. The extent of these changes depends on type of processing (microwave or traditional) and starch species. Crystal and granular structures of investigated starches were only slightly damaged on derivatisation.

Introduction

Microwaves are known to be capable of generating heat deeply inside the penetrated medium. As quite competitive in cost to other methods of heating it has been used for thawing of frozen foods, drying, baking, rendering, pasteurisation and sterilisation. Microwave radiation seems applicable to starch processing, but thus far, it has not been used on commercial scale, despite of the opinion that solid state reactions are the most promising area of applying microwave ovens instead of traditional heating methods [1]. The problem of the influence of microwave radiation on the reactivity of different substances still remains unsolved. It is believed that microwave radiation (2450 MHz) does not activate any specific bonds in molecules, and consequently this forms of treatment will not lead to any kinetic differences compared to other form of

G. Lewandowicz, E. Voelkel, Starch and Potato Products Research Laboratory, ul. Zwierzyniecka 18, 60-814 Poznań, Poland; W. Błaszczak, Polish Academy of Sciences, ul. Tuwima 10, 10-817 Olsztyn, Poland.

heating [1]. In spite of this, there has been a growing interest in the use of microwave heating synthesis, so called „MORE chemistry" (microwave oven induced reaction enhancement), and in some processes catalytic effect is claimed [2-7]. Our previous work on the application of microwave ovens to obtain inorganic starch esters proved that microwaves shorten reaction times and do not affect molecular and submolecular structure of the derivatives as compared to conventional technology [8, 9]. Promising results of using microwave ovens to esterification, suggest applying them to other starch modification reaction, for example etherification. These type reactions is conventionally applied in starch industry to produce derivatives of ionic type i.e. cationic starches, carboxymethylated (anionic starches) and amphoteric starches. Ionic starch derivatives of different degree of substitution reveal unique physicochemical properties, useful in many branches of industry, especially in papermaking, building and mining. The aim of this work was to examine the influence of microwave processing on structure and functionality of ionic starch derivatives.

Materials and methods

Preparation of ionic starch derivatives

Microwave assisted reactions

Microwave assisted processes were carried out according to the Polish patent description [15].

Cationic derivatives: Commercial potato and wheat starches were sprayed with the solution of N,N,N-trimethyl-2-epoxypropylammonium chloride to incorporate 0.34% of nitrogen (basis of starch dry mass), alkalised with 1% calcium hydroxide and 1% sodium metasilicate (basis of starch dry mass) and carefully mixed to unification. The moisture contents of the prepared blends were adjusted to 35%. Then, the starch samples were dried in a Panasonic microwave oven emitting radiation of 2450 MHz frequency and 0.5 W/g energy, to reduce moisture content level below 20%.

Anionic derivatives: 100 g of commercial potato and wheat starches were sprayed with the solution containing 3.5 g of sodium mochloroacetate, mixed carefully with 12 g of sodium metasilicate, then the moisture contents of the prepared blends were adjusted to 35%. Microwave processing was performed as described above.

Amphoteric derivatives: commercial potato and wheat starches were sprayed with the chemicals necessary both to cationisation and anionisation. Blends adjusted to moisture content of 35% were processed in microwave ovens as described above.

Reference samples

Cationic derivatives (suspension reaction): commercial potato and wheat starches were slurried in the water solution of 3-chloro-2-hydroxy-N,N,N-trimethylpropylammonium chloride (0.34% of nitrogen basis of starch dry mass). The suspensions were alkalised with the solution of sodium hydroxide (2 moles per 1 mole of cationisation agent) and the reaction were carried out at the temperature of 35°C during 6 hours. Then the reaction mixtures were neutralised with diluted hydrochloric acid, filtered, washed twice with water and air dried.

Anionic derivatives (rotating roaster process): 100 g of commercial potato and wheat starches were sprayed with the solution containing 3.5 g of sodium mochloroacetate and mixed carefully with 12 g of sodium metasilicate. Then the starch samples were pre-dried at room temperature to moisture content below 20% and heated in rotating roaster at the temperature of 120°C for two hours.

Amphoteric derivatives (rotating roaster process): commercial potato and wheat starches were sprayed with the chemicals necessary both to cationisation and anionisation. Starch blends were processed in rotating roaster at the temperature of 120°C for two hours.

Analytical methods

Rheological properties

The course of gelatinization was monitored with a Brabender viscograph under the following conditions: measuring cartridge 0.07 Nm; heating/cooling rate 1.5°C/min; thermostating 30 min.

Nitrogen content

Cationic starches obtained in microwave process were previously purified by with hydrochloric acid. To this aim, starch samples were suspensed in 5% solution of hydrochloric acid, filtered, and washed with water to remove chloride ions and air dried. The nitrogen contents was determined according to EN ISO 3188 standard.

Carboxymethyl groups content

Carboxymethyl groups content was determined according to ISO 11216 standard.

Microscopic examinations

The starch samples to be examined by light microscopy were prepared by the smear method. To this end starch suspensions were heated at the initial gelatinization temperature (as measured acc. to Brabender), and at 95°C. A drop of the resulting paste

was applied to a microscope slide and, on cooling, the smear was stained with iodine acc. to Kaczyńska et al. [10], and observed with an Olympus BX60 light microscope.

The starch samples to be examined by scanning electron microscopy were prepared acc. to Fornal [11] and observed with a Jeol JSM 5200 microscope.

X-ray diffractometry

X-ray diffractometry was carried out with a TUR 62 Carl Zeiss 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

As it is shown in Table 1 microwave processing is a convenient way to obtain ionic starch derivatives of DS ≈ 0.04. Reference cationic starches obtained in a water suspension revealed lower degree of substitution in spite of relatively long reaction time (6 hours). Especially in case of wheat starch low reactivity was observed. Reference samples of anionic and amphoteric starches which were obtained in rotating roasters also revealed a lower degree of substitution as compare to microwave assisted product reactions.

Table 1

Degree of substitution of ionic starch derivatives obtained in microwave assisted and traditionally run reactions.

Starch origin	Reaction type	Nitrogen content [%]	Nitrogen degree of substitution	Carboxymethyl groups content [%]	Carboxymethyl groups degree of substitution
Potato	Microwave assisted	0.33	0.04	-	-
Potato	Suspension	0.30	0.03	-	-
Potato	Microwave assisted	-	-	1.47	0.04
Potato	Rotating roaster	-	-	1.39	0.04
Potato	Microwave assisted	0.33	0.04	1.49	0.04
Potato	Rotating roaster	0.27	0.03	1.08	0.03
Wheat	Microwave assisted	0.34	0.04	-	-
Wheat	Suspension	0.17	0.02	-	-
Wheat	Microwave assisted	-	-	1.46	0.04
Wheat	Rotating roaster	-	-	1.24	0.03
Wheat	Microwave assisted	0.34	0.04	1.42	0.03
Wheat	Rotating roaster	0.19	0.02	1.23	0.03

The incorporation of ionic substituents into the starch molecules significantly affected physicochemical properties related to the starch-water interactions. The substitution of cationic groups (DS = 0.02–0.04) resulted in a decrease of pasting temperature accompanied by a rapid increase in viscosity within a narrow temperature range and the occurrence of a sharp viscosity peak (Fig. 1, 2). The extent of these changes in microwave assisted ethers was more significant than that in products of suspension reaction, what could be a result of a higher degree of substitution [12].

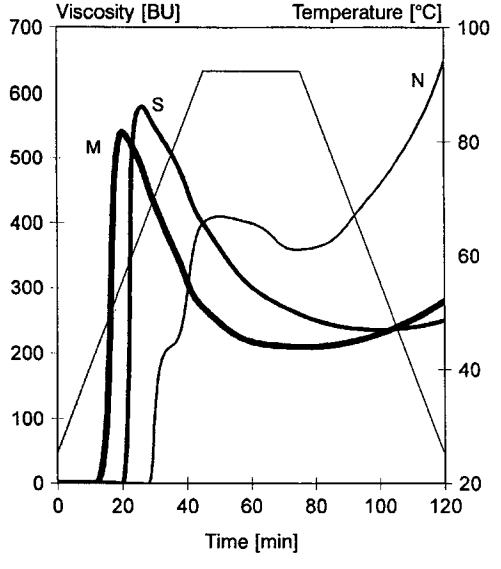

Fig. 1. Brabender viscosity curves (c = 3,3%) of potato starch cationic ethers as compared to native starch: N – native potato starch; S – suspension reaction product; M - microwave assisted reaction product.

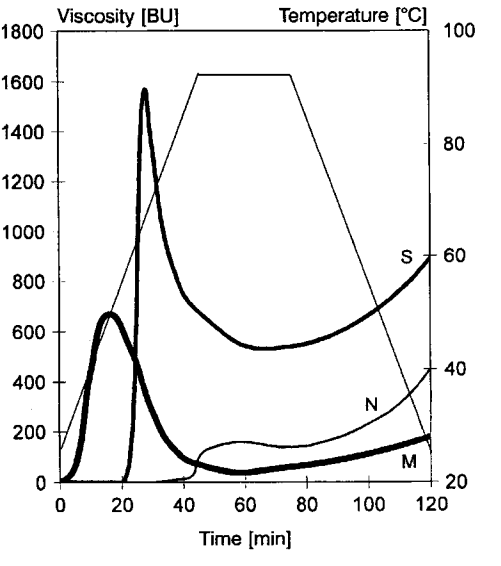

Fig. 2. Brabender viscosity curves (c=8%) of wheat starch cationic ethers as compared to native starch: N – native wheat starch; S – suspension reaction product; M – microwave assisted reaction product.

The substitution of anionic groups into the starch molecules also resulted in a decrease of pasting temperature. In case of potato starch it is accompanied by a rapid increase in viscosity within a narrow temperature range and the occurrence of a sharp viscosity peak, but extend of these changes were lower as compare to cationic derivatives (Fig. 3). In case of wheat starch, medium type of swelling characteristic was observed (Fig. 4). Rotating roaster reaction products revealed significantly lower viscosity than the derivatives from microwave assisted reactions. This observation could be explained by degradation phenomena accompanying the processing at relatively high reaction temperature (120°C).

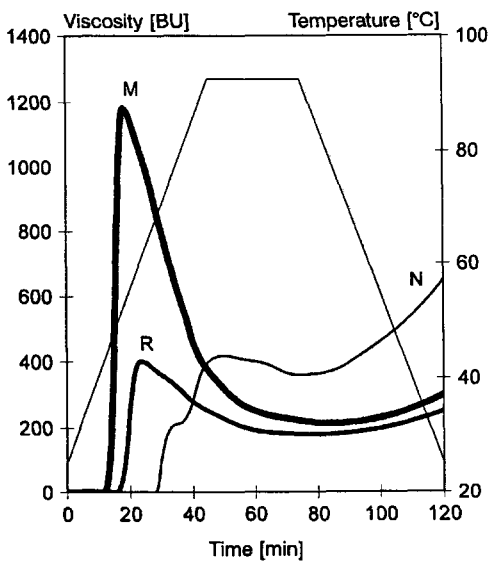

Fig. 3. Brabender viscosity curves (c = 3,3%) of potato starch anionic ethers as compared to native starch: N - native potato starch; R – rotating roaster reaction product; M – microwave assisted reaction product.

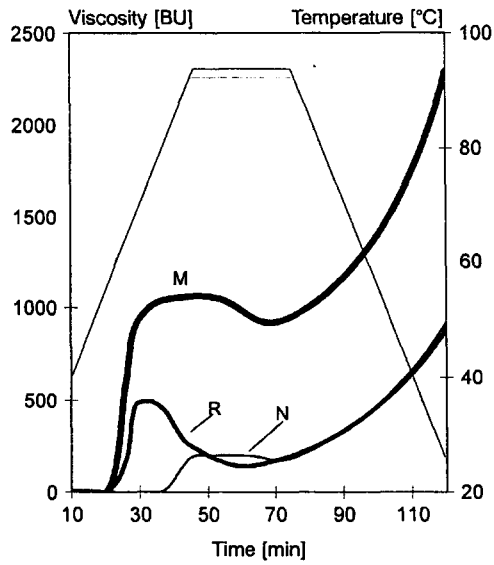

Fig. 4. Brabender viscosity curves (c = 8%) of wheat starch anionic ethers as compared to native starch: N – native wheat starch; R – rotating roaster reaction product; M – microwave assisted reaction product.

The incorporation of two types of ionic substituents into the starch molecules together, caused the decrease of pasting temperature, accompanied by different phenomena in case of potato than in case of wheat starch (Fig. 5, 6). In case of wheat starch an increase of the type of swelling characteristic was observed, whereas in case of potato starch a significant decrease in viscosity. As just as anionic, amphoteric derivatives obtained in rotating roaster, revealed lower viscosity than products of microwave reactions.

The above changes were confirmed by light microscopy. Native potato and wheat starches heated at pasting temperature give a characteristic behaviour – amylose leakage out of the starch granules [13, 14]. Cationic starches revealed similar solubilisation mechanism (pictures not shown) i.e. amylose leakage out of the starch granules. At the temperature of 90°C the solubilisation of native starches is advanced, amylose leaks completely out of granules, but amylopectin still forms aggregates which are the remnants of the granules [13, 14]. Cationic starches at the temperature of 90°C (pictures not shown) formed almost uniform mixture of soluble amylose and amylopectin. The colour of amylose and amylopectin – iodine complexes changed only a little as compared to native starches. Amyloses of different starch origin formed deep blue com-

plexes, whereas amylopectins formed red-brown complexes, (pictures not shown). The differences between solubilisation behaviour of cationic derivatives obtained using microwaves and in water suspension were not observed.

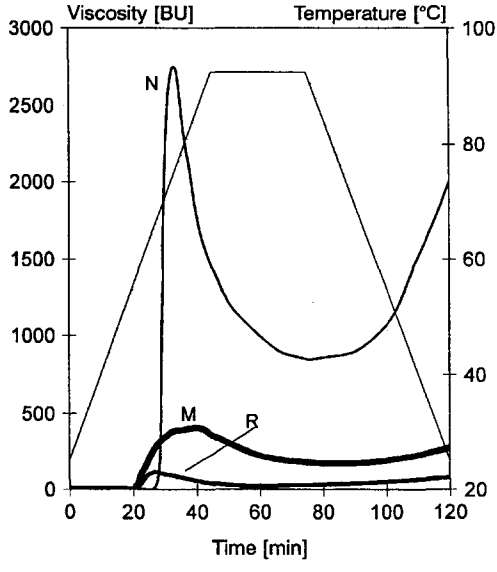

Fig. 5. Brabender viscosity curves (c = 8%) of potato starch amfoteric ethers as compared to native starch: N – native potato starch; R – rotating roaster reaction product; M – microwave assisted reaction product.

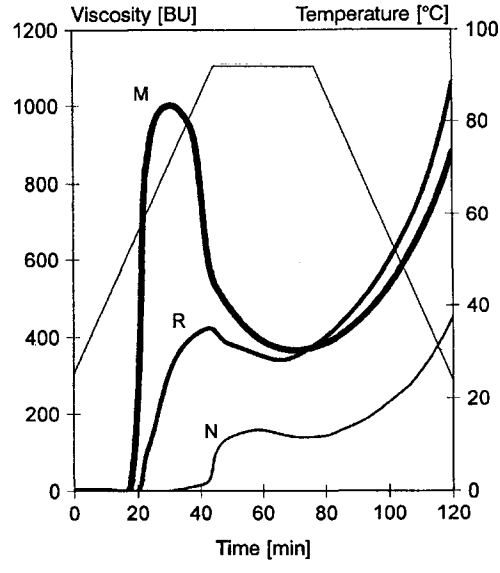

Fig. 6. Brabender viscosity curves (c = 8%) of wheat starch amfoteric ethers as compared to native starch: N – native wheat starch; R – rotating roaster reaction product; M – microwave assisted reaction product.

The substitution of anionic groups as well as together two types of ionic substituents into the starch molecules resulted in different phenomena in case of potato than wheat starches. Potato starch anionic derivatives, similarly to cationic ones, at the temperature of 90°C (pictures not shown) were almost completely soluble, what corresponded to their high type of swelling characteristics (Fig. 3). Amphoteric potato starch derivatives of restricted type of swelling characteristic (Fig. 5) at the temperature of 90°C (pictures not shown) revealed strongly limited solubilisation process – only part of amylose leached out of granules. The majority of the starch material remained insoluble. This observation pointed to the conclusion that cationic and anionic substituents probably formed amphions, which reduced solubility of starch.

Wheat starch anionic derivatives at the temperature of 90°C (pictures not shown) were only partially soluble, what corresponded to their medium type of swelling char-

acteristics (Fig. 4). Amphoteric derivatives of wheat starch at the temperature of 90°C (pictures not shown) were better soluble than anionic ones, what corresponded to higher type of swelling characteristic (Fig. 6). LM pictures of anionic and amphoteric derivatives obtained in rotating roaster (pictures not shown) were coloured more red-violet as compare to microwave equivalent samples what pointed to degradation phenomena occurring during processing at the temperature of 120°C.

Deep changes in type of swelling characteristics could suggest some changes in the structure of starch granules as an effect of derivatisation. This hypothesis was not confirmed by X-ray and SEM investigations however. The type of X-ray diffraction patterns of native starches maintained unchanged after substitution of all types ionic substituents (Fig. 7-9). The type of processing (microwave, suspension and rotating roaster) also only slightly reflected on relative crystallinity of investigated starches. Even heating in rotating roaster at the temperature of 120°C during 2 hours did not affect crystallinity of investigated starches.

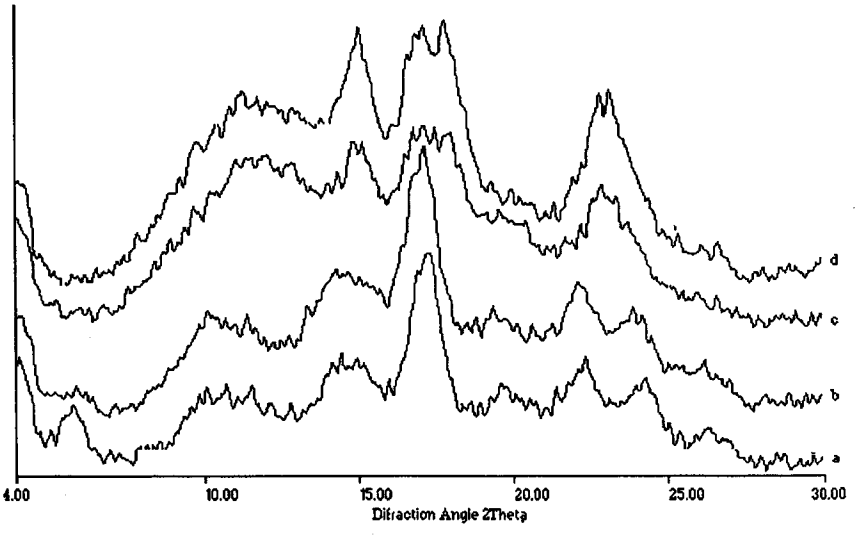

Fig. 7. X-ray diffraction patterns of the cationic starch ethers: potato starch microwave assisted reaction product (a); potato starch suspension reaction product (b); wheat starch microwave assisted reaction product (c); wheat starch suspension reaction product (d).

Scanning electron microphotographs of ionic starch derivatives proved that modification process induced only slight deterioration of starch granular structure (Fig. 10, 11). The strong alkaline conditions applied in all types of processing caused some gelatinization phenomena resulting in a leakage of starch material out of the granules. However, the extent of granules damages was the smallest in case of suspension reac-

tion products. Solid state reactions (microwave and rotating roaster) occurring by local action of alkalis on starch granules caused more extensive leakage of starch material out of the granules.

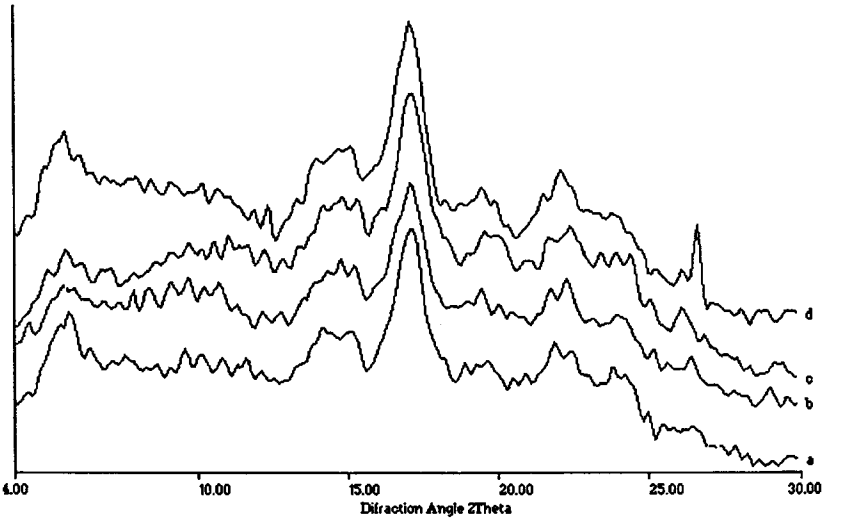

Fig. 8. X-ray diffraction patterns of potato starch ionic starch ethers: microwave assisted reaction amphoteric derivative (a); rotating roaster reaction amphoteric derivative (b); microwave assisted reaction anionic derivative (c); rotating roaster reaction anionic derivative (d).

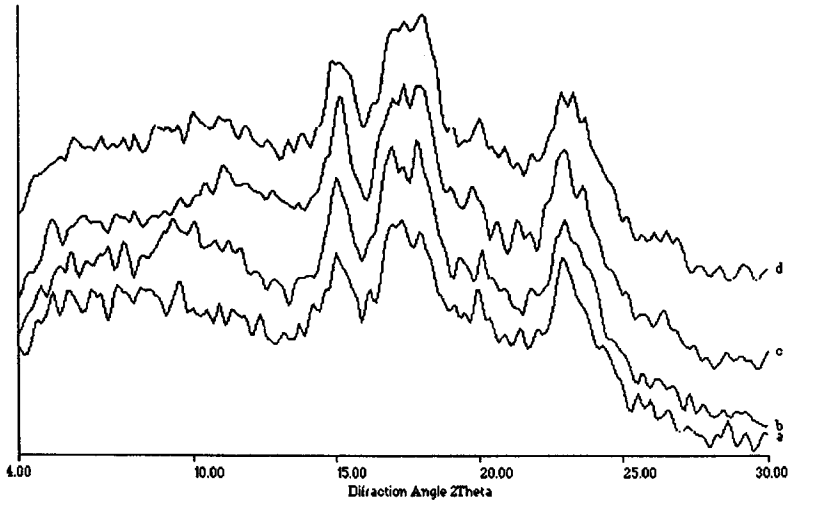

Fig. 9. X-ray diffraction patterns of wheat starch ionic starch ethers: microwave assisted reaction amphoteric derivative (a); rotating roaster reaction amphoteric derivative (b); microwave assisted reaction anionic derivative (c); rotating roaster reaction anionic derivative (d).

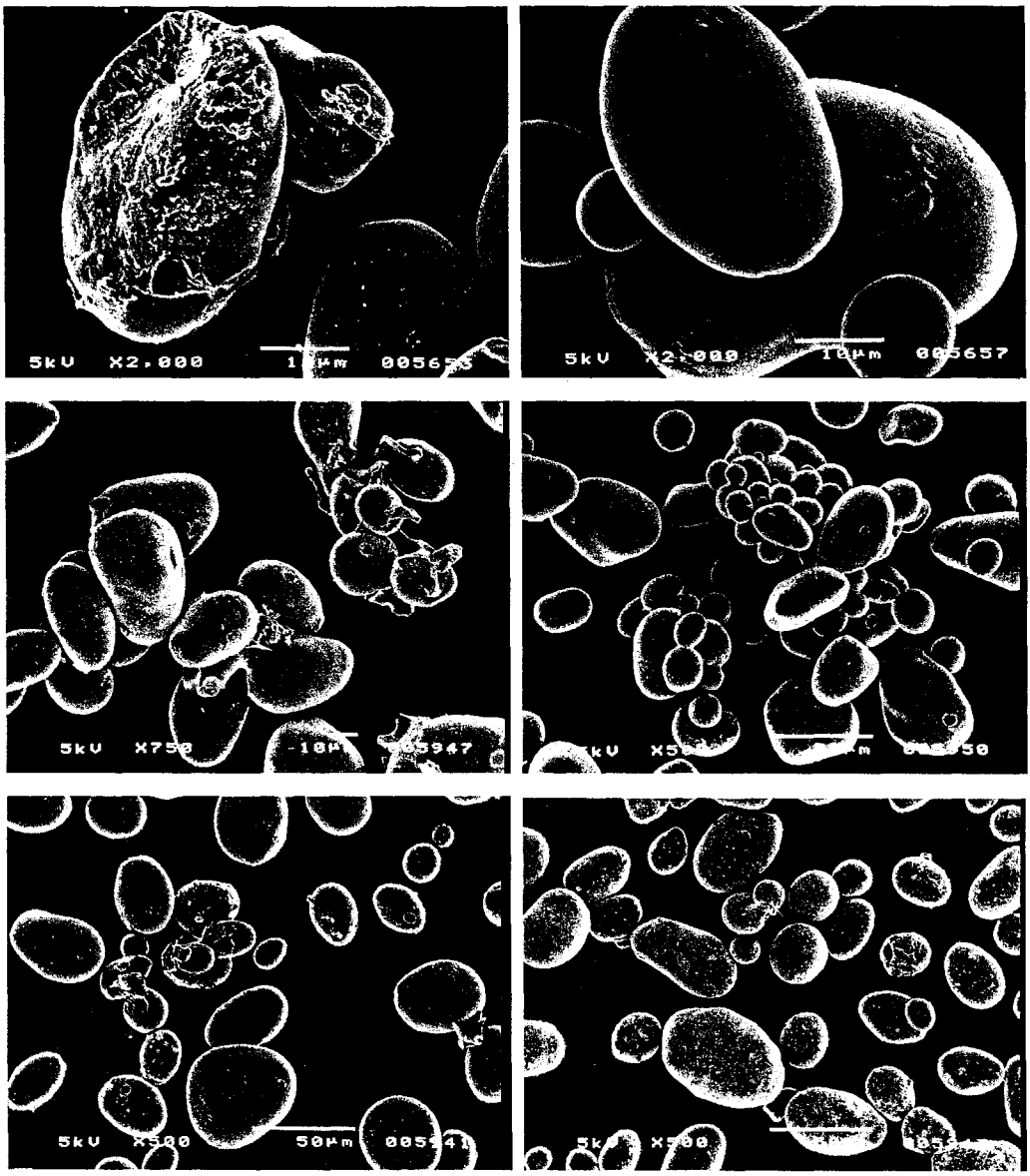

Fig. 10. SEM microphotographs of potato starch ethers: cationic derivative from microwave assisted reaction (A), cationic derivative from suspension reaction (B), anionic derivative from microwave assisted reaction (C), anionic derivative from rotating roaster reaction (D), amphoteric derivative from microwave assisted reaction (E), amphoteric derivative from rotating roaster reaction (F).

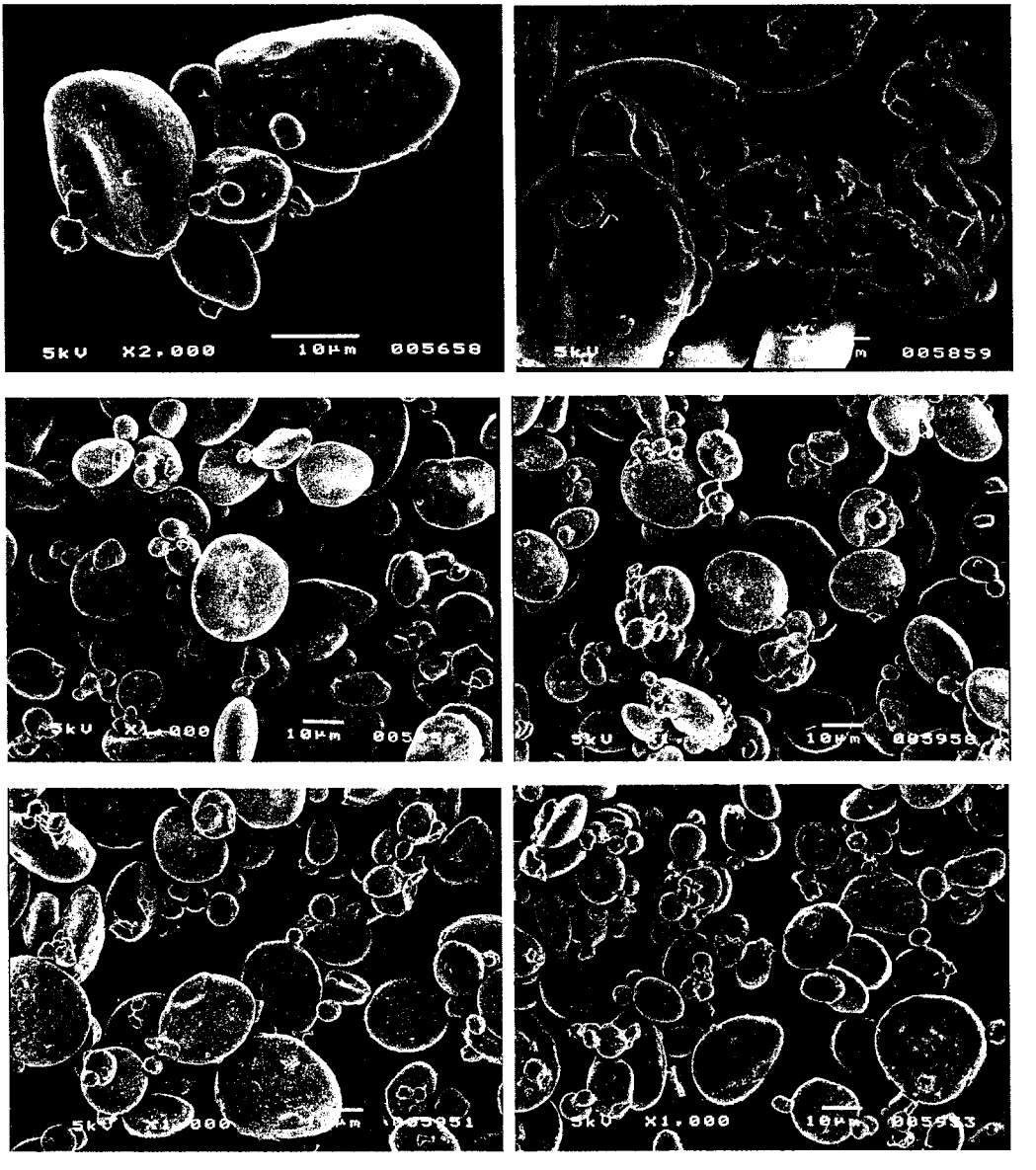

Fig. 11. SEM microphotographs of wheat starch ethers: cationic derivative from microwave assisted reaction (A), cationic derivative from suspension reaction (B), anionic derivative from microwave assisted reaction (C), anionic derivative from rotating roaster reaction (D), amphoteric derivative from microwave assisted reaction (E), amphoteric derivative from rotating roaster reaction (F).

Conclusions

1. Microwave processing is a convenient way to obtain starch ethers of DS ≈ 0.04.
2. The incorporation of ionic substituents into the starch molecules significantly affects their physicochemical properties related to starch-water interactions. These results in the decrease of the gelatinization temperature, changes of the swelling characteristics, solubilisation and iodine complexation.
3. The incorporation of ionic substituents into the starch molecules are not reflected in their crystal structure.
4. Microwave processing as well as suspenion reaction do not reveal degradation phenomena observed as changes in colour of starch-iodine complexes.
5. Rotating roaster processing causes some degradation phenomena observed as changes in colour of starch-iodine complexes.
6. Microwave processing caused deterioration in granular structure of investigated starches similar to those observed in rotating roaster produtcs.

Acknowledgement

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POCHODNE SKROBI JONOWYCH UZYSKANE W REAKCJACH W POLU MIKROFALOWYM. STRUKTURA I FUNKCJONALNOŚĆ

Streszczenie

Zbadano możliwości zastosowania energii pola mikrofalowego do otrzymywania skrobi kationowych (podstawionych grupami tetraalkiloamonio alkilowymi), anionowych (karboksymetyloskrobie) i amfoterycznych oraz wpływ takiego pola na strukturę i funkcjonalność produktów.

Natywne skrobie: ziemniaczaną i pszenną, podstawiono do DS = 0,04, stosując piecyk mikrofalowy 2450 MHz dający energię 0,5 W/g. Produkty badano za pomocą wiskografu Brabendera mikroskopowo (mikroskopy optyczny i SEM) oraz za pomocą dyfraktometrii proszkowej. Oznaczano też zawartość azotu w skrobiach kationowych według normy EN ISO 3188.

Stwierdzono, że zmiany spowodowane wprowadzeniem grup jonowych do skrobi zależą od sposobu dostarczania energii oraz od rodzaju skrobi.

DANUTA M. NAPIERAŁA

AMYLOSE AND GLUCOSE SOLUTIONS. A COMPARATIVE STUDY

Abstract

Structural parameters of retrograded amylose chains in aqueous solution were determined by means of a comparative analysis with glucose solutions stored under the same conditions and the same specific volume.

Viscosimetric and polarimetric measurements were carried out in glucose and amylose (from potato) solutions during storage, within the same concentration range of 0.005–0.02 (g/cm^3), and with a fixed low concentration of a polymer complexing agent ($5\ 10^{-5}\ g/cm^3$), added as a polymer chain stiffener.

From the ratio of limit viscosity numbers obtained for polysaccharide and monosaccharide solutions, the average asymmetric parameter of rigid amylose chain was calculated, value of which points to a significant length expansion of retrograded α-D-glucopyranose coil.

Measurements of the concentration dependence of the optical rotatory dispersion in retrograded amylose and its monomer unit solutions were used to determine the degree of coiling into helix of polysaccharide in aqueous solution.

Materials and methods

Studies were performed on aqueous solutions of soluble potato amylose (POCh, Gliwice, Poland) and anh. D-glucose of analytical grade (POCh, Gliwice, Poland). The glucose solutions were prepared at room temperature, while the amylose was digested for 30 min in boiling re-distilled water (200 cm^3). At the end of gelling, the same small amount of Rose Bengal sodium salt (Sigma, Poland) was added to all samples of amylose and glucose. The concentration range studied for both compounds was 0.005–0.02 g/cm^3 and the fixed low concentration of the dye was $2.5\ 10^{-5}\ g/cm^3$. The samples were stored in a darkness at 22.0 (± 0.5)°C. Viscosity measurements were carried out for samples stored for 3h, 24 h, 48 h and 100 h. The capillary method with the Ubbelhode viscometer was involved. Capillary diameter was 0.8 mm. Flow time for each sample was average of 5 to 8 runs.

D.M. Napierała, Department of Physics, Agricultural University of Poznań, 60-637 Poznań, Wojska Polskiego 38/42, Poland.

Optical rotatory dispersion (ORD) of the amylose and glucose solutions was measured at three wavelengths of 366 nm, 406 nm and 436 nm, respectively with a Polamat-A polarimeter (K. Zeiss, Jena, Germany) equipped with a 2 dm cell. Temperature was maintained constant.

Results and discussion

Viscosity measurements

Prolonged storage of amylose solutions resulted in significant structural and molecular changes in the system. The concentration dependence of the specific viscosity of amylose solution after 3 h, 24 h, two, and four days of ageing presented in Figure 1 relates to studies at low polymer concentration range, i. e. below 2%.

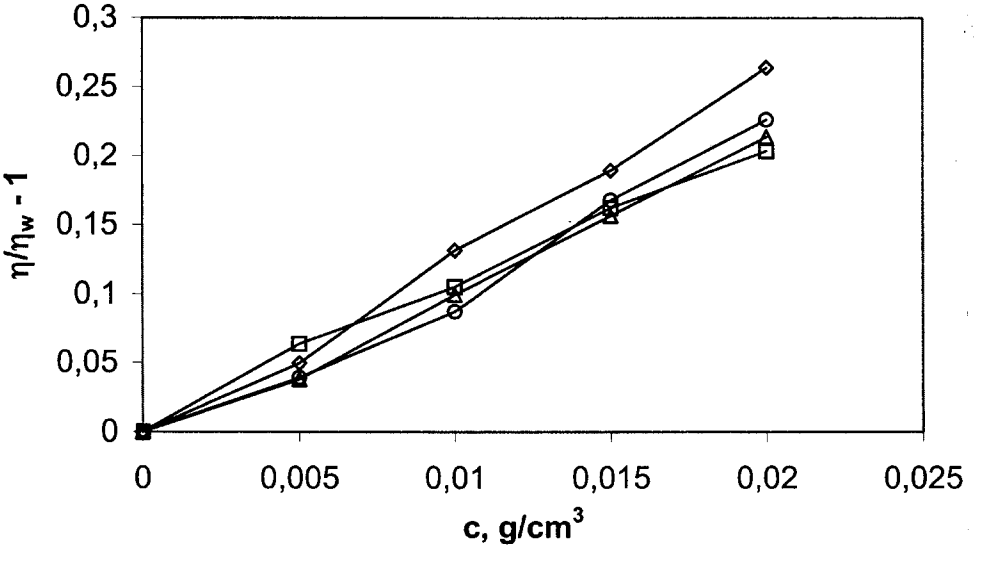

Fig. 1. The specific viscosity of a potato amylose solution in the presence of Rose Bengal (0.025 mM) as a function of polymer concentration after 3 h (□), 24 h (O), 48 h (Δ) and 100 h (◊) of storage at a constant temperature of 22°C.

In the first approximation the $[\eta/\eta_w - 1] = f(c)$ function for all samples is linear (Fig.1). Admixture of a small amount of Rose Bengal caused minor changes in the limit value of viscosity in comparison to pure water. The dye is known to complex to amylose [7] influencing in this manner the molecular motion of that polysaccharide

chains in solution. The dynamic viscosity of water, $\eta_{w,}$, is lower than that of Rose Bengal solution, η_{RB}, but the difference is negligible in the first approximation.

Assuming a linearity between specific viscosity and amylose concentration in the solution, the limit viscosity number $[\eta]$ was obtained. From the definition limit viscosity number $[\eta] = \lim \{[\eta/\eta_w - 1]/c\}$ when $c \rightarrow 0$. This number estimated for the amylose solution after 3, 24, and 48 hours of ageing is identical and reaches 10.6 ± 0.4 $(g/cm^3)^{-1}$, but it increases to 13.0 ± 0.4 on the 4-day amylose solution, where the effect of opacity in the 2% amylose concentration solution was observed.

The Flory's theory [3] of polymer solutions reveals that the limit viscosity number $[\eta]$ is related to the size of the macromolecule coil, precisely to the mean square of the inertial diameter of a coil, $\langle s^2 \rangle$:

$$[\eta] = \Phi \langle s^2 \rangle^{3/2} / M_p, \qquad (1)$$

where Φ is the universal constant, independent of the macromolecule character and solution. Its value of $4.2\ 10^{24}$ was calculated for the coil of a flexible polymer chain [6]; M_p – molecular weight of a polymer chain.

Based on Eq. (1), the ratio of $\langle s_r^2 \rangle / \langle s_f^2 \rangle$ was calculated for the mean square of the inertial diameter in amylose chain prior to retrogradation and in retrograded molecule. Resulting value of 1.36 indicates changes in the compactness of the random coil. Therefore, a significant increase in the inertial diameter of the amylose coil may lead to a break down in the phase continuity in the system. Independently of it, changes in the dynamics of the polymer motion in a liquid take place.

Let us compare the molecular dynamics of the amylose chain in water and its monomer unit, i.e. in glucose in solution of the same specific volume. We may consider the glucose solution as the limiting case of a non-branched α-D-glucan polymer solution. Thus, all differences between these two systems resulted from the polymerisation of small glucose molecules. The concentration dependence of the specific viscosity of the amylose and glucose solutions in the presence of Rose Bengal, after 100 h of storage is shown in Figure 2.

The limit viscosity number for the glucose - Rose Bengal solution is $[\eta] = 3.0$. Taking into account this value and that obtained for the α-D-glucopyranose chain, one may estimate an asymmetric parameter p for the amylose coil based on the relation between the limit viscosity number and the asymmetric parameter p in a suspension of ellipsoidal particles [1]:

$$[\eta] = v' 2.5 \left\{1 + \frac{0.4075}{2.5} (p - 1)^{1.508}\right\} \qquad (2)$$

where v' – is the partial specific volume of the particle, p – is the asymmetric parameter of the ellipsoidal particle, the ratio of its semi-axes.

In our case, the specific volume of amylose, v'_{AM} and of glucose, v'_{GLU} in solution is the same, so we obtained value p for the fresh amylose chain p = 7.2, and for retrograded amylose p = 8.4.

An increase in the asymmetric parameter of the amylose coil in solution stored for a long time speaks in favour of a tendency of the polymer chain to expansion by decoiling. Amylose chains in aqueous solutions stray from the ideal spherical shape. Due to complexation of dye to polymer, conformation of the amylose chain is affected. Effect of Rose Bengal on the viscosity of 2% amylose and glucose solutions is shown in Figure 2.

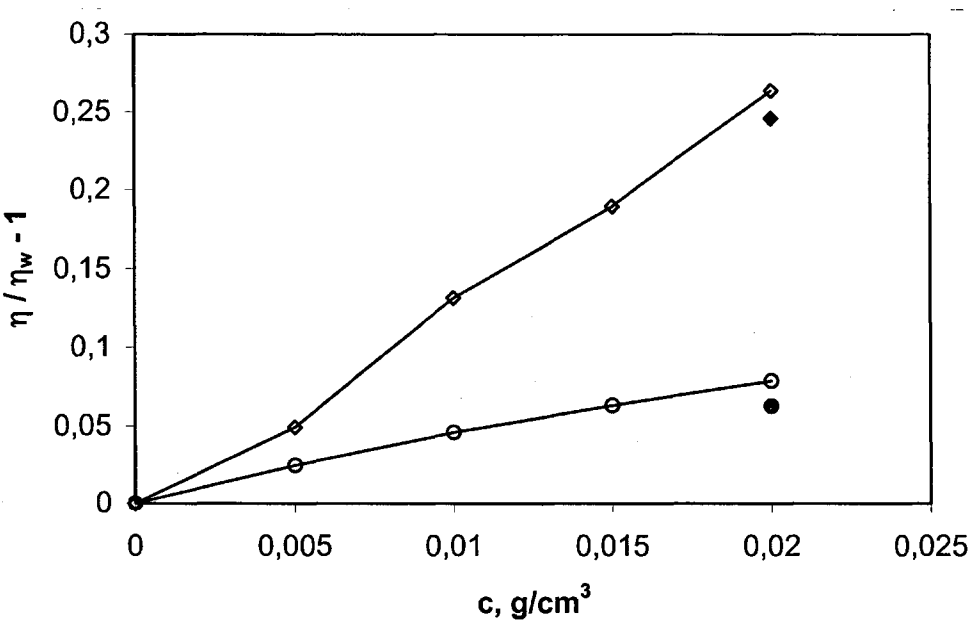

Fig. 2. Comparison of the concentration dependencies of relative viscosity of amylose (◊) and glucose (O) solution, stored for 100 h at the same conditions. (♦) and (•) - amylose and glucose solution without Rose Bengal, respectively.

Optical rotation measurements

The concentration dependence of the optical rotatory dispersion in glucose solutions, the monomer unit of the α-D-glucopyranose chain, and in amylose solutions of the same concentrations was measured during storage. The results of these measurements are presented in Table 1 and Figure 3.

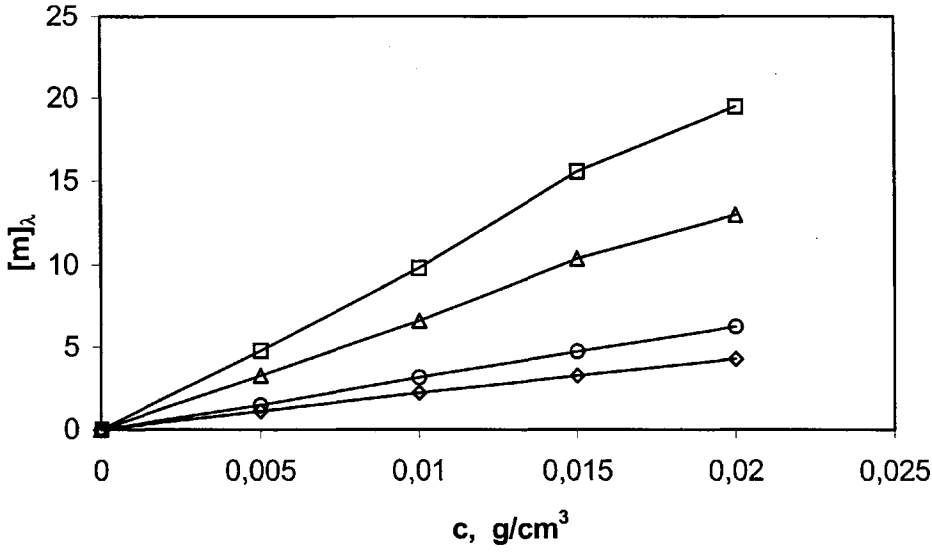

Fig. 3. Optical rotatory dispersion at two wavelengths of λ_1 = 366 nm and λ_2 = 436 nm, versus glucose (O, [366 nm], ◊ [436 nm]) and amylose (□, [366 nm], Δ [436 nm]) concentration.

Table 1

Optical rotatory (OR) dispersion vs concentration in glucose and amylose aqueous solution, measured at two wavelengths, λ_1 = 366 nm and λ_2 = 436 nm (after 48 h of storage).

No sample	Concentration, g/cm^3, 10^{-2}	D-Glucose		α-D-glucopyranose	
		$[m_{366}]$,deg	$[m_{436}]$,deg	$[m_{366}]$,deg	$[m_{436}]$,deg
1	0.5	1.12	0.83	4.79	3.27
2	1.0	2.78	2.18	9.82	6.60
3	1.5	3.63	2.96	15.55	10.38
4	2.0	6.06	4.04	19.47	13.00
5*	2.0	7.54	5.01	20.33	13.50

* without Rose Bengal

Analysis of the data from these sources and calculated values of specific rotation, provided the degree of amylose coiling in solution during ageing. The calculations were based on the Moffitt equation [2, 8]:

$$[m]_\lambda = A\, f(\lambda^2) + B\, f(\lambda^2) \qquad (3)$$

where: A, B – Moffitt's coefficients and $f(\lambda) = (\lambda_0{}^2 - 1)/\lambda^2$, λ_0 is a wavelength at maximum absorption.

Coefficient A depends on the contributions of the chiroptical centres of monomers in macromolecule to the optical rotation. Value of B depends on the contribution of the helices of the molecule to the optical rotation, and λ_0 is the wavelength corresponding to the maximum of the electronic transition of the molecule. Assuming for the amylose solution that $\lambda_0 = 153$ nm [5], both parameters of the Eq.(3) could be calculated. Due to the asymmetry of a helix, the helical structure is source of a some optical activity, which overlays the activity, induced by asymmetric carbon atoms in polymer chain. Comparing the optical rotation in the amylose and glucose solutions of the same number of asymmetric carbon atoms, one may assume, that the chain coiling in the polymer solution also contributes to optical activity. The average ratio of $[m]_\lambda$ for the amylose and glucose solutions, calculated from optical rotatory dispersion, equals to 3.1. This value is close to the ratio of the limit viscosity number for amylose and glucose stored in aqueous solution for less than 4 days. The degree of coiling, χ, can be obtained from Eq. (4);

$$\chi = ([m]_{AM} - [m]_{GLU})/[m]_{AM} \tag{4}$$

where $[m]_{AM}$ and $[m]_{GLU}$ – are optical rotations for amylose and glucose, respectively.

The value of $\chi = 0.68$ fit the data from the 1H-NMR method [5]. No changes in the degree of polymer coiling were observed in the amylose solution stored for 100 h.

Rose Bengal in amylose and glucose 2% solution decreased optical activity (Table 1).

The comparative analysis of behaviour of polymer and monomer in solution can be an important source of the information about structural changes and enables to determine some structural parameters of high-molecular systems.

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BADANIA REOLOGICZNE I POLARYMETRYCZNE (ORD) ROZTWORÓW AMYLOZY I GLUKOZY

Streszczenie

Ze stosunku względnej lepkości roztworów poli- i monosacharydu obliczono średni parametr asymetrii sztywnego łańcucha amylozowego. Jego wartość wskazuje na znaczne wydłużenie łańcucha amylozy w trakcie retrogradacji.

Pomiary zależności stężeniowej ORD retrogradowanej amylozy oraz α-D-glukozy posłużyły do wyznaczenia w roztworze wodnym stopnia skręcenia łańcucha amylozy w heliks.

ANNA NOWOTNA, HALINA GAMBUŚ, RAFAŁ ZIOBRO, WIKTOR BERSKI, GRAŻYNA LEWANDOWICZ, RENATA SABAT, ANDRZEJ CYGANKIEWICZ

STARCHES FROM WHEAT OF VARIOUS TECHNOLOGICAL VALUE

Summary

The research was carried out on the grain of wheat classified to different quality classes (A, B or C) grown in 1998-1999. Protein content, wet gluten were measured, and bread was baked from flour. When starch was extracted from the flour, the following analyses were performed: amylose and phosphorus content, and its swelling and pasting characteristics.

It was stated that investigated starches from the varieties of wheat belonging to different classes greatly vary in their properties.

It was stated with full confidence that a high pasting temperature and medium water binding capacity and solubility are favourable for baked bread volume.

Introduction

It was commonly believed that properties of proteins, mainly gluten, were responsible only for dough properties and a large volume of the obtained bread from wheat flour. But, for many years much attention has been devoted to the role of the main component of flour, that is starch. Sanstedt [16] and Hoseney et al. [8] defined the role of this component in dough and obtained in the process of baking bread. Starch is a substrate for amylases. It provides them with fermentable sugars. It is also a water system regulator, which brings gluten dilution to proper consistency. It has been scientifically proven that wheat starch has the best baking characteristics [1, 7, 8].

Many scientists were investigating the influence of the physico-chemical properties of the starch on dough and bread quality. Dennet et al. [2] claimed that a greater volume of bread was connected with the decrease in the amount of amylose. The stud-

A. Nowotna, H. Gambuś, R. Ziobro, W. Berski, , R. Sabat, Agriculture University of Cracow; G. Lewandowicz, Starch and Potato Research Laboratory in Poznań; A. Cygankiewicz, Institute of Plant Breeding and Aclimatization in Cracow.

ies of Werli and Pomeranz [14], followed by Gambuś [5] proved the important role of fatty substances in flour, especially glicolipids, but also phospholipids in the formation of dough structures.

The integrity of starch granules plays an important role in obtaining the optimal properties of dough. It depends on its swelling and pasting properties. Although Gambuś et al. [17] showed a negative correlation between baked model breads and starch solubility in water, the recent studies by Gambuś [4, 5] proved, that limited swelling and solubility positively influenced bread quality.

Apart from the starches of low viscosity, which have a negative impact on bread quality, the extent of viscosity of starch pastes plays no key role in the formation of bread structure [17].

Basing on a thermal analysis of wheat flour Eliasson et al. [3] claim that a high pasting temperature and a small paste enthalpy improves baking properties.

From the work of D'Appolonia and Gilles [1] one can conclude that baking properties of starch depends not only on a type of plant from which the starch was extracted, but also its variety. There is a lack of full information about the physicochemical properties of the starch extracted from wheat varieties belonging to different quality classes. Therefore, this problem is the subject of this article.

Materials and methods

Winter and spring wheat came from the two cultivation seasons of 1998 and 1999 [table1].

The above mentioned varieties were divided into classes according to the following quality parameters [9]: a falling number of grain, protein content in grain, sedimentation test, farinnographic analysis (water absorption, softening), bread volume, flour yield.

The starches were separated by the laboratory method [15], next they were analyzed to determine their total phosphorus content by Marsch [11], pasting characteristics of 8.5% water suspensions in a rotating viscometer Rheotest II, using spikes as a measuring device [6], water binding capacity (WBC) and solubility in water at 60^0 and 90^0C by modified the Leach's method [15] and apparent amylose content by Morrison and Laignalet [13].

Results and discussion

The starch was extracted from eight varieties of wheat (grown in the season of 1998–1999 (Table 1)), which were classified according to their quality [9] into A, B and C classes.

Table 1

Characteristics of the used for research material.

Kind of variety	Harvesting season	Variety of wheat	Quality class
Jara	1998	Torka	A
		Jasna	A
		Eta	A
Ozima	1998	Begra	A
		Almari	C
		Elena	C
Jara	1999	Torka	A
		Jasna	A
		Eta	B
		Helia	C
Ozima	1999	Begra	A
		Kobra	C
		Elena	C

The extent of changes of the physicochemical properties of the starches from the varieties of wheat of the same quality class is presented in table 2. From this table one can conclude that the value of the individual physicochemical properties do not considerably vary for the starches of different quality classes. On the contrary, the starches from the wheat of different quality classes were characterized by a similar range of changes of the individual properties.

Table 2

Properties of starch isolated from wheat of different quality classes.

Quality class	Starch properties							
	Amylose content	Total phosphorus	WBC [g/1g d.m.]		Starch solubilty in water [%]		Pasting characteristics	
	[%]	[mg%]	60°C	90°C	60°C	90°C	Pasting temp. [°C]	Maximum viscosity [B.u.]
A	17.9 - 22.1	47 - 60	5.1 - 6.9	9.1 - 10.5	0.5 - 5.0	7.7 - 10.7	63.5 - 79.5	46 - 101
B*	18.2	57	6.2	9.9	3.1	7.1	73	84
C	19.6 - 21.1	48 - 55	5.7 - 6.5	9.3 - 11.0	1.4 - 3.2	4.8 - 14.1	64.5 - 78.5	38 -58

*only one variety was analysed in this class.

Protein content, wet gluten and the physicochemical properties of the starch extracted from the flour of the specific variety were compared with the volume of baked bread. Pearson's linear correlation coefficient was computed between protein content and wet gluten and physicochemical properties of starch and bread volume.

It was proven there was no significant correlation between protein content, wet gluten and volume of bread, despite the well known role of gluten in the formation of baking value of wheat flour.

A highly significant (at importance level 0,01) positive correlation (r = 0,70) was obtained between pasting temperature of starch and bread volume. This correlation was presented in fig. 1 and was confirmed in Eliason's work [3]. The authors think that completing of starch pasting causes to finish the increase in bread volume in the oven, and if it occurs at a higher temperature, there is more time for bread volume to increase.

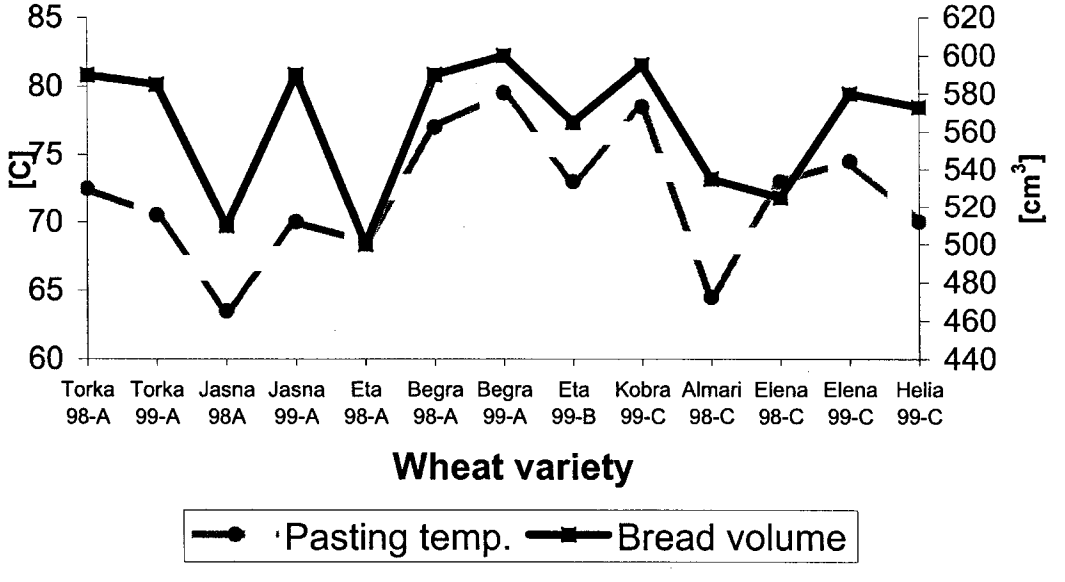

Fig. 1. Bread volume vs pasting temperature of starch.

In fig. 2 an inversely proportional correlation between bread volume and maximum starch pastes viscosity was presented. This observation is supported by a correlation coefficient between the mentioned properties which is r = -0,68 (at importance level 0,01). This correlation is contrary to the works of Gambuś et al. [17], where

model breads (40 g of dough) were made from gluten and starch of very different viscosity (27–474 mPa·s). These results were not confirmed in the later works by the above - mentioned author [4, 5], who baked model breads and came to the conclusion that functionality of starch in bakery goods did not depend on maximum paste viscosity, but on these physicochemical properties that influenced the size of swelling and pasting of starch during dough formation and baking.

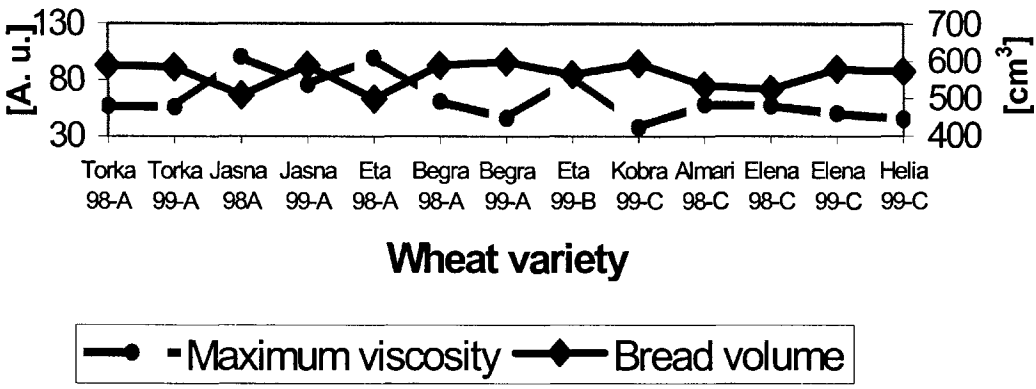

Fig. 2. Bread volume vs maximum viscosity of starch paste.

The content of starch lipids was measured by assessing the content of phosphorus [12]. Results obtained in the present work do not confirm the important role of starch lipids, mostly phospholipids, in the formation of dough structure, as was shown in the earlier works [5]. It is not surprising, because during the formation of dough from flour, glicolipids play the main important role. However, artificially created flour made from starch, dry vital gluten [5] was used for baking model breads and due to the lack of glicolipids a positive role of starch phospholipids was shown in stabilization of the gas pores of bread and in increasing of loaf volume.

The correlation between water binding capacity or solubility of starch and bread volume has not been confirmed. According to Sanstead's theory [16] the most suitable is such water binding capacity and solubility of starch granules, that ensures their proper contact with gluten with preservation of granule integrity. It leads to the conclusion that the most proper are medium values of these parameters.

Conclusion

The results obtained in the present work show that the properties of starch isolated from different varieties are not considerably different from various quality classes. It was only stated with full confidence that a high pasting temperature and medium water binding capacity and solubility in water of starch are favourable for obtaining optimal baking performance.

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SKROBIE Z PSZENICY O RÓŻNEJ WARTOŚCI TECHNOLOGICZNEJ

Streszczenie

Materiałem badawczym było ziarno pszenicy zaklasyfikowane do różnych klas jakości (A, B lub C), uprawiane w latach 1998-1999. Oznaczono w nim zawartość białka i glutenu mokrego, po czym z mąki wypieczono chleb. Po wyodrębnieniu skrobi z mąki, została ona przebadana pod względem: zawartości amylozy i fosforu oraz wyznaczono ich charakterystykę pęcznienia i kleikowania.

Stwierdzono, że pod względem przebadanych właściwości skrobie z odmian pszenic, należących do jednej klasy, znacznie różnią się właściwościami.

Stwierdzono z całą pewnością, że wysoka temperatura kleikowania skrobi wpływa korzystnie na objętość wypieczonego chleba.

DAVID LEE PHILLIPS, JIE XING, CHAN KONG CHONG, HAROLD CORKE

ANALYTICAL CHARACTERIZATION OF CHEMICALLY MODIFIED STARCHES BY FT-RAMAN SPECTROSCOPY

Abstract

We have recently developed an FT-Raman spectroscopic method for measuring the amount of chemical modification of starch samples from a diverse range of botanical sources and amylose contents. In this paper, we present results and FT-Raman spectroscopic calibration curves that can be used to measure the degree of chemical modification for starches that have been acetylated, succinylated, cationic modified, and maleic acid modified. The FT-Raman methodology we have developed is much faster than currently used wet chemistry techniques, is nondestructive of the sample, needs almost no sample preparation, does not require use of hazardous chemicals, and can be further developed for use as a quality control method for process control in manufacturing.

Introduction

Chemical modification of starches affects their physicochemical properties and their desirability and effective use in manufacturing processes and for particular products [1-4]. It is important to control the amount of chemical modification in order to optimize the starch physicochemical properties for a desired application or product. Usually wet chemistry techniques are still widely used to measure the level of chemical modification in modified starches [5-7]. However, these wet chemistry techniques require time-consuming periods of sample preparation, are destructive of the starch sample, involve chemical hazards and related disposal costs, and are not applicable for use as a quality control method for process control in manufacturing.

We have recently developed applications of Raman spectroscopy to determine the degree of chemical modification in modified starches. In this paper, we present results for the following chemically modified starches: acetylated, succinylated, 3-chloro-2-hydroxypropyltrimethyl ammonium chloride (CHPTAC) cationic modified, and male-

D.L. Phillips, C.K. Chong, Department of Chemistry; Department of Botany; J. Xing, Harold Corke, The University of Hong Kong, Pokfulam Road, Hong Kong.

inated. The different chemical modifications have characteristic Raman marker bands that increase in intensity (relative to the native starch Raman bands) as the amount of chemical modification increases. The characteristic Raman bands associated with the functional group of the chemical modification can be used as a convenient indicator of the degree of chemical modification in modified starches. The ratio of the intensity of the characteristic Raman chemical modification band to the intensity of an internal standard native starch Raman band can be used to prepare a Raman calibration curve for the degree of the chemical modification of the modified starch samples. These calibration curves can then be used to find the amount of chemical modification for unknown starch samples from their Raman spectra. The Raman spectroscopic method for measuring the degree of chemical modification in modified starches allows much faster determinations than currently used wet chemistry techniques, is non-destructive of the starch sample, and is less prone to interference from residual impurities than wet chemistry methods.

Because each chemical compound has a different vibrational spectrum, the different substances contributing to a sample spectrum can be distinguished and this helps to minimize interference from impurities on the Raman spectra of a sample. The intensity of Raman bands depend linearly on the amount of compound present in the sample [8, 9] and Raman spectroscopy has long been used as a quantitative analytical method in the pharmaceutical and polymer industries and has been more recently finding increasing applications in food science and industry [9-28]. The Raman band frequencies and relative intensities can sometimes vary significantly with the structure of the compound and the surrounding molecular environment and this may make it necessary to use different calibration curves for samples with different amylose contents and/or botanical source.

Materials and methods

Parent starch samples were purchased commercially and chemically modified to varying degrees as detailed in references 20, 21, 23-25, 27 and 28 to obtain the acetylated, succinylated, CHPTAC cationic modified and maleinated starch samples used to determine the Raman calibration curves. The degree of substitution of the chemically modified starches were measured using the standard wet chemistry methods as described in detail elswhere [20, 21, 23-25, 27, 28]. The FT-Raman spectra of the dry starch samples were obtained using an FT-Raman spectrometer (Bio-Rad, Cambridge, MA) that employed a backscattering geometry. Typical collection times for the Raman spectra were in the range of 5–10 min per sample with spectral resolution of 4 to 8 cm^{-1}. The ratio of the intensity of the appropriate Raman marker band for the chemical modification to the intensity of the Raman band of the parent starch used as an internal standard was determined from the Raman spectra [20, 21, 23-25, 27, 28]. This

ratio was plotted versus the degree of substitution of the chemical modification found from the wet chemistry methods to derive the Raman calibration curves.

Results and discussion

Figures 1–4 display typical FT-Raman spectra of chemically modified starches with varying levels of modification for acetylated, succinylated, CHPTAC cationic modified and maleinated starches. The Raman marker band for the chemical modification functional group (1732 cm^{-1} Raman band for acetylated starches, 1730 cm^{-1} Raman band for succinylated starches, 761 cm^{-1} Raman band for CHPTAC cationic modified starches and the 1600–1760 cm^{-1} region Raman bands for maleinated starches) typically varies strongly with the degree chemical modification. The ratios of the intensity of the chemical modification Raman marker band to the intensity of the parent starch Raman band chosen to be the internal standard were plotted versus the degree of chemical modification determined from the standard wet chemistry methods to obtain the Raman calibration curve (see plots reported in references 20, 21, 23-25, 27, 28). Least squares linear regression fits to the Raman calibration curves found linear correlation coefficients that had values of $r > 0.99$ which indicates a very high level of linearity of the Raman marker band intensity with the amount of chemical modification. This excellent linearity indicates that the Raman method calibration curves can be used with confidence to determine the degree of substitution for acetylated, succinylated, CHPTAC cationic modified and maleinated starches [20, 21, 23-25, 27, 28]. Table 1 presents typical results for the linear regression analysis of the Raman calibration curves derived from the FT-Raman spectra shown in Figures 1–4. The Raman calibration

Table 1

Linear Regression Parameters For Plots of The Ratios of the Raman Marker Band Intensities to the Intensity of the Internal Standard Raman Band (y) Versus the Degree of Chemical Modification Determined From the Standard Wet Chemistry Method (x). Note $y = m x + b$ where y is the ratio of the Raman marker band intensity to the intensity of the internal standard Raman band; x = the amount of chemical modification measured using the standard wet chemistry method; m = the slope; and b = the y-intercept. See references 20, 21, 23-25, and 28 for more details of the plots and linear regression parameters.

Sample and Modification	Slope (m)	y-intercept (b)	Correlation coefficient (r)
Wheat, Acetylation	0.02277± 0.00114	-0.00162± 0.00225	0.9963
Waxy Maize, Succinylation	0.69427± 0.03182	-0.00183± 0.00144	0.998
Waxy Maize, Cationic	38.81527± 1.135461	+1.8585± 0.0478	0.998
Waxy Maize, Maleinated	6.8741	-0.0006	0.9978

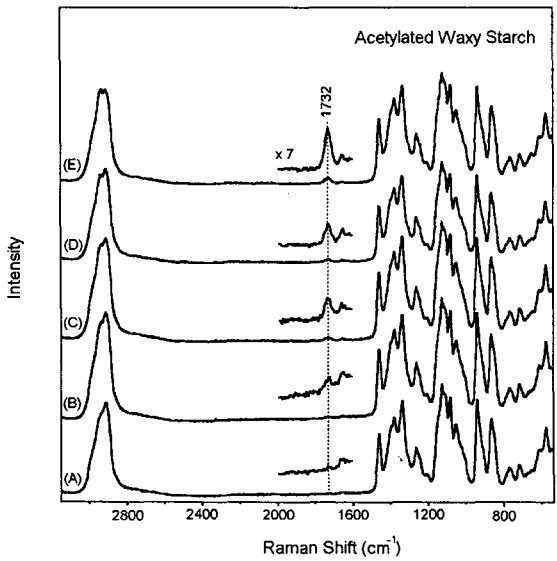

Fig. 1. FT-Raman spectra of a control non-acetylated wheat starch sample (A) and four acetylated wheat starch samples with differing amounts of acetylation (B-E). Noticeable changes occur in the 1732 cm^{-1} Raman band as the level of acetylation changes. The 1732 cm^{-1} band has been magnified by a factor of 7 so as to more easily observe its intensity changes.

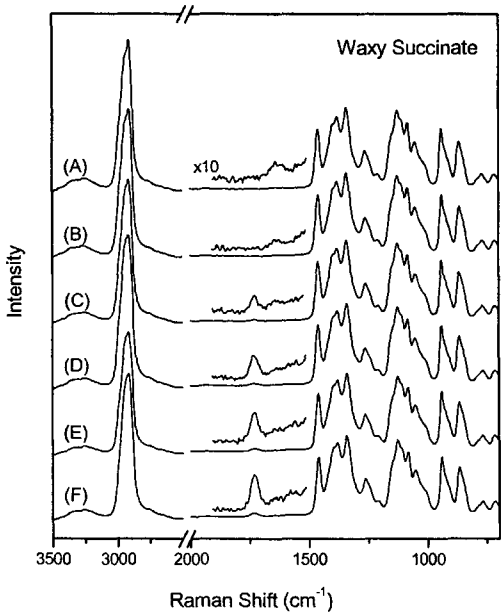

Fig. 2. FT-Raman spectra of a control non-succinylated waxy maize starch (A) and four succinylated waxy maize starch samples (B-E) with varying degrees of succinylation. The Raman band at 1730 cm^{-1} correlates with the degree of succinylation. The inset of each spectrum shows an expanded view (x 10) of the 1730 cm^{-1} C=O stretch Raman marker band.

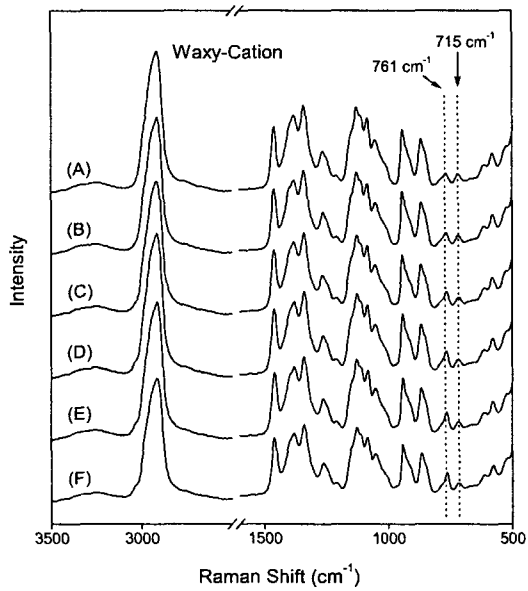

Fig. 3. FT-Raman spectra of a control non-cationic modified waxy maize starch (A) and five cationic modified waxy maize starch samples (B-F) with different degrees of cationic modification. The Raman band at 761 cm^{-1} increases in intensity as the degree of cationic modification increases.

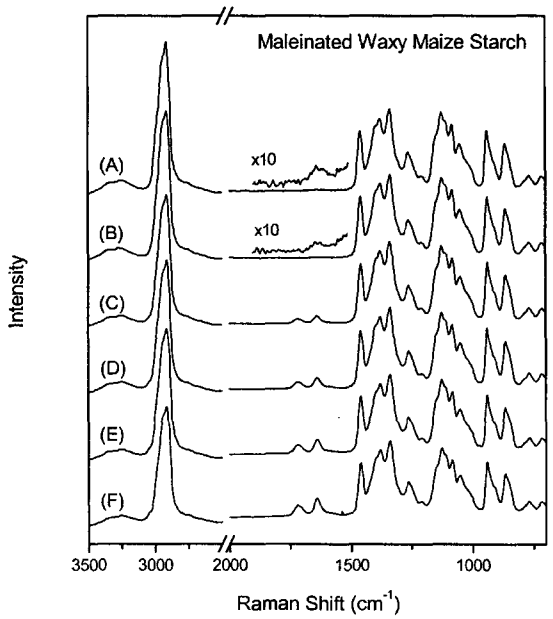

Fig. 4. FT-Raman spectra of a control non-maleinated modified waxy maize starch (A) and five maleinated modified waxy maize starch samples (B-F) with different amounts of maleate modification. The Raman bands in the 1600 to 1760 cm^{-1} region increases in intensity as the level of maleate modification increases.

curves show some variability due to the amylose content and the botantical source [20, 21, 23-25, 27, 28]. To obtain the best accuracy for the Raman method determination of the degree of chemical modification in modified starches, it is advisable to develop a separate Raman calibration curve for the particular type of starch for which one wants to make routine measurements. The developed Raman calibration curves can be used to obtain the degree of chemical modification for modified starch samples that have unknown levels of modification from their Raman spectra. This Raman technique needs almost no sample preparation, is non-destructive of the sample, is much faster than commonly used wet chemistry methods and is feasible to be further developed for quality control situations in manufacturing processes.

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ANALITYCZNA CHARAKTERYSTYKA CHEMICZNIE ZMODYFIKOWANYCH SKROBI ZA POMOCĄ SPEKTROSKOPII RAMANOWSKIEJ FT

Streszczenie

Opracowano metodę spektroskopową (widma ramanowskiego z transformacją Fouriera) nadającą się do pomiaru stopnia chemicznej modyfikacji próbek skrobi bardzo różniących się pochodzeniem botanicznym oraz zawartością amylozy.

W pracy przedstawiono krzywe kalibracyjne nadające się do tego rodzaju analizy w przypadku skrobi acetylowanych, sukcynylowanych, maleinowanych i kationizowanych.

Opracowana metoda jest o wiele szybsza od obecnie stosowanych, jest nieniszcząca, nie wymaga przygotowania próbek, stosowania toksycznych reagentów i z powodzeniem może zostać rozwinięta jako metoda kontroli jakości w produkcji i reżimu samej produkcji.

S. PIKUS, J. JAMROZ, E. KOBYLAS

SMALL ANGLE X-RAY SCATTERING (SAXS) INVESTIGATIONS ON POTATO STARCH IN SUSPENSIONS

Abstract

Small angle X-ray scattering (SAXS) is one of the methods used for the analysis of the starch structure. In this manner, involving changes in electron density structure of starch becomes available. By variation of the media in which starch is suspended wider differentiation of starch structure is possible. Usually such studies are carried out in water. In this paper the SAXS method was successfully used for investigation of starch structure in such media as water, methanol, 1 M solution of HCl, 1 M solution of NaCl, and 0.1 M solution of I_2 in KI. Analysis of SAXS curves for those suspensions proved some differences in curve pattern. Especially interesting data were obtained from a comparison of SAXS curves for starch suspensions in 1 M HCl and 1 M NaCl. Effective scattering of starch suspension in 1 M HCl in the initial part of the curve, as compared to the 1 M NaCl case, indicates that the diffusion of Cl^- ions to various regions of the starch structures depends, among other things, on the pH of the solution. These results demonstrated that the SAXS method could be used for identification of the starch structures to which specific media diffuse and, therefore, for investigating the impact of various factors on this diffusion.

Introduction

Analysis of the structure of starch is a subject of great interest. Linearly bound glucose residues may form a spiral structure – the, so called, helix in the particle of amylose. Stability of that structure is preserved by hydrogen bindings.

In the structure of amylopectin, part of the branches glucose residues chains are distributed parallelly producing a three-dimensional species. Therefore, starch demonstrates the presence of crystalline regions and gives diffraction peaks [1-3]. Figure 1 shows, a simplified structure of starch. In the beam model of the amylopectin particle,

S. Pikus, E. Kobylas, Department of Crystallography, Faculty of Chemistry, University of Marie Curie-Skłodowska, sq. Marii Curie Skłodowskiej 3, 20-031 Lublin, Poland; J. Jamroz, Department of Biological Elements of Food Technology and Feed, Agricultural University, Doświadczalna str 48, 20-236 Lublin, Poland.

one can distinguish dense parallel periodically arranged fragments considered as crystalline regions (Fig. 1a).

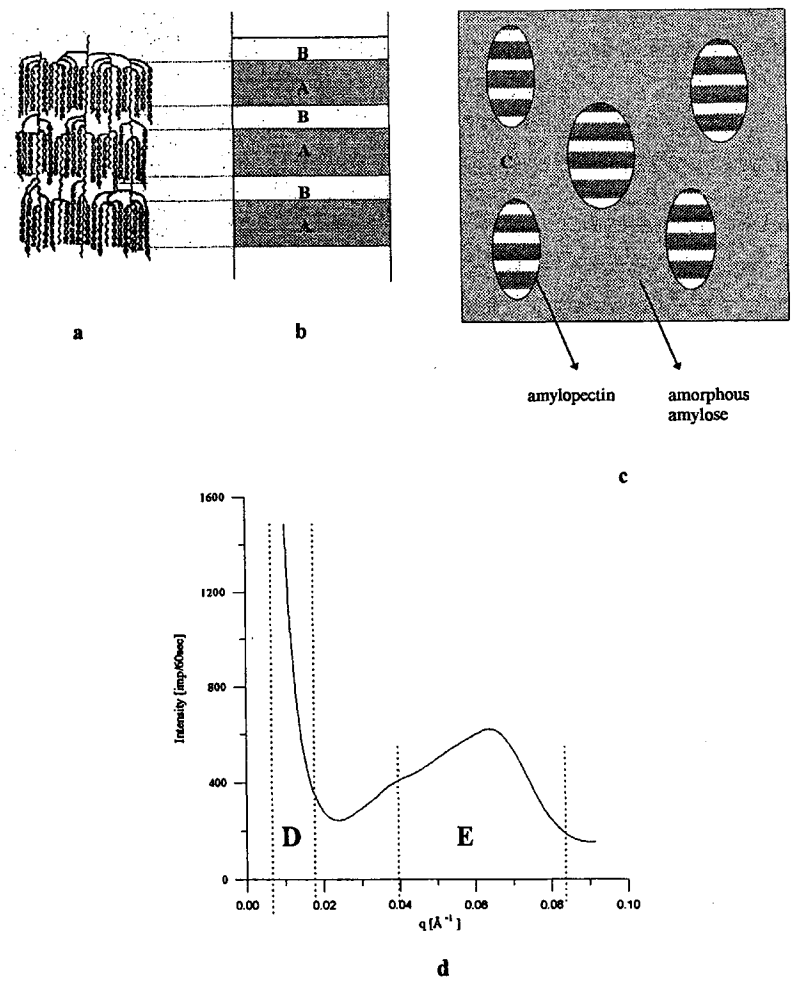

Fig. 1. Scheme of starch structure (1a, 1b, 1c) and an example curve of SAX scattering of the potato starch suspension in water (1d).

Also amorphous regions can be recognised, where few amylopectin chains are not ordered. Those regions are placed alternately, which derives from daily and nightly starch accretions. Thus the structure of amylopectin grain can be presented in a way shown in Fig. 1b. It is a lamellar, semi-crystalline structure. Figure 1c shows placement of amylopectin grains in relation to the amorphous amylose. The above scheme is greatly simplified, but sufficient in terms of possibilities of using the SAXS method for starch structure investigations [4-7]. Actually, crystalline and amorphous layers of

amylopectin are bedded in shape of mutually shifted growth rings, which gives a tetragonal superstructure.

Small angle X-ray scattering always occurs if in the investigated material there are areas (regions) that vary in terms of electron density from the environment. The greater the difference in electron density, the greater intensity of scattering. Also the size of those regions affects the shape of the scattering curve. The bigger the scattering objects, the smaller the angel of the scattering. The SAXS method can, therefore, be used for investigating objects (areas) of dimensions of 1–1000 nm.

Investigating the starch structure in terms of electron density distribution (within the limits of region size of 1–1000 nm, not on the atomic level) one may expect some differences between the amorphous part and the crystalline part of amylopectin (A-B) and between amylopectin and amylose (A-C, B-C). In dry starch, the differences in electron density between the mentioned regions are very small. Therefore, SAX scattering is so small that it is impossible to interpret it in terms of starch composition. The situation changes when electron density of one of the phases is changed using different methods. It can be obtained by preparing a water suspension of starch. It turns out that water diffuses to the areas (regions) of starch in a non-uniform way and therefore it differentiates its structure in terms of electron density. Figure 1d shows an SAXS curve for native potato starch suspended in water.

As it can be seen, the SAXS curve after its initial fast decrease, shows a peak at a value of the q vector equal to 0.063 $Å^{-1}$, which corresponds with the interplanar spacing d = 100 Å The initial section of the curve, marked in broken lines (D), is responsible for the presence of differences in electron density of great areas of amylopectin in relation to amorphous amylose (A, B-C). Presence of the peak (E) derives from the occurrence of alternate regions A and B in the structure of amylopectin and the d value approximately determines thickness of the layer A+B [4]. As mentioned above, such form of the SAXS curve was registered for water suspensions of starch, when water easily diffuses to regions B, and not to regions A, which increases the difference of electron density and causes the occurrence of the peak. Change in electron density of region C causes changes in the first part of the SAXS curve.

The points presented above lead to a conclusion that the SAXS method can be used to investigate the process of absorption of different media to different areas of the starch structure. Thus, the aim of this paper was to examine the SAX scattering of starch suspensions in various liquid media.

Material and methods

Because the aim of this paper was to prove that diffusion of different compounds to the structure of starch affects the small angle scattering, measurements of a number of suspensions of potato starch in water, HCl, NaCl, iodine, and methanol were taken

using the SAXS method. Table 1 presents the chemical composition of the investigated suspensions.

Samples were prepared directly prior to the measurements. Potato starch was mixed with an appropriated solvent and the suspension was placed in a cuvette (thickness 0.5 mm) with windows covered with plastic foil.

Table 1

Chemical composition of the investigated suspensions.

Sample	Starch concentration	Solvent
S1	45%	water
S2	45%	CH_3OH
S3	45%	1 M HCl in water
S4	45%	1 M NaCl in water
S5	45%	0.1 M I_2 in 1M KI in water

SAXS measurements

Measurements were performed on a slit-collimated Kratky camera using a Cu anode tube as the radiation source. A proportional counter with a nickel filter and a pulse-height analyser were used to measure the scattered intensity. The scattered intensity measurements were carried out for each of the investigated samples as well as for the empty cuvette (background scattering). The background scattering curve was each time subtracted from the scattering curve for an investigated sample. Absorption coefficient was also measured for each sample. Subsequently, the SAXS scattering curve were recalculated considering the differences in adsorption coefficient.

The measurements were carried out in the range 2θ from 0.076 to 6.52° in 0.0076 to 0.038° intervals and counting time of 100 sec. Scattering curves are presented in the intensity versus q

$$(q = \frac{4\pi \sin\theta}{\lambda}, \text{ where } \lambda \text{ - X-ray wavelength, } 2\theta \text{ - scattering angle}).$$

Geometry of SAXS camera and other conditions of the SAXS experiments allowed for the treatment of the scattering curves obtained as slit-smeared data for beam of infinite length.

Results and discussion

SAX scattering occurs when there are areas in the investigated sample which differ from the environment in terms of electron density. In a dry state, starch did not

show such a difference, but when water was added the situation changed. Water is absorbed faster by the amorphous part (especially in amylopectin), which increases the difference in electron density in the structure of starch. A clear peak then appears on the SAXS curve, which originates from the part of starch of a lamellar structure (Fig. 1b). It is obvious that the rate of water absorption by different parts of the starch structure depends on many factors (e.g. temperature) and thus the SAXS measurements must be taken at a certain time; for temperatures around 20°C this time cannot exceed several hours. It affects the accuracy of the results obtained and the SAXS curve might show numerous „wild" oscillations. Due to the fact that the phenomenon of specific fast diffusion of water to the amorphous part of amylopectin and the impact of this phenomenon on SAXS curves are known, further in this paper the SAXS curves obtained for various starch suspensions are compared with the SAXS results for the suspension of starch in water. Figures 2, 3, 4, and 5 display SAXS curves for samples S2, S3, S4, and S5, respectively. Furthermore, every figure contains a curve of SAXS of the starch suspension in water (S1). Analyzing the SAXS curves attention should be paid to two phenomena: presence or absence of the diffraction peak and the intensity of scattering at different ranges of the q vector.

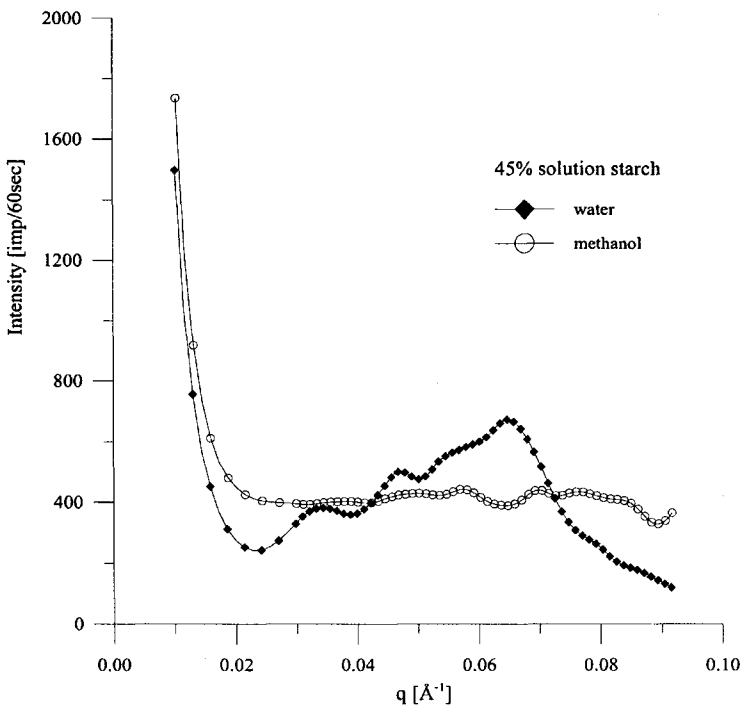

Fig. 2. Scattering curve of potato starch in water and methanol.

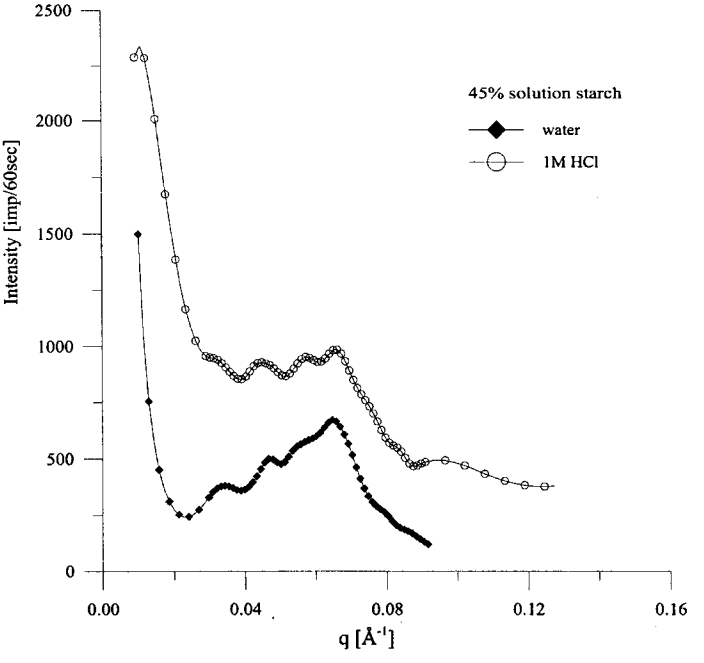

Fig. 3. Scattering curve of potato starch in water and 1 M HCl.

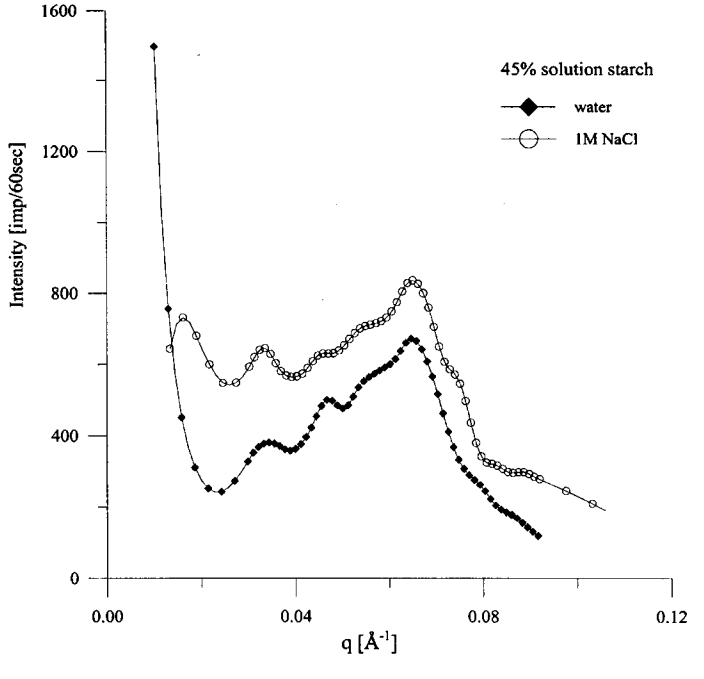

Fig. 4. Scattering curve of potato starch in water and 1 M NaCl.

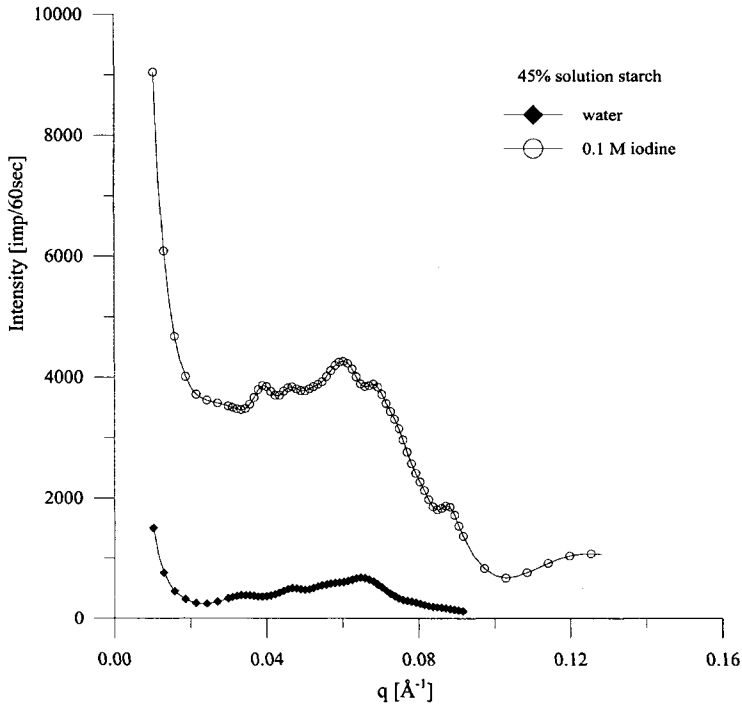

Fig. 5. Scattering curve of potato starch in water and 0.1 M I_2 in KI.

The curve of SAX scattering of the starch suspension in water presented in Figs. 2, 3, 4, and 5 shows a clear wide peak with a maximum at q = 0.063-0.065Å^{-1}. Analyzing the shape of this peak one should remember that the presented data are slit smeared type and they have not been mathematically smoothed. The curve of scattering of the starch suspension in methanol (waterless) does not show any peak. Its course is very similar to the curve of dry starch scattering. This proves the lack of the specific diffusion of methanol to the starch structure. The SAXS curve of the starch suspension in 1 M HCl in water has a very interesting course. Theoretically one may suspect that along with the diffusion of water, there will be a diffusion of Cl^- ions (they are the only ones that affect the change in electron density). Introducing Cl^- ions should increase SAXS. As shown on the figure, the intensity of SAX scattering is greater. The scope of the diffraction peak is also clear, although it is less distinct than in the case of starch suspension in pure water. However, interesting is an especially high growth of scattering in the initial part of the SAXS curve. It proves that Cl^- ion diffusion reached not only the amorphous part of amylopectin, but also the C region (Fig. 1), which caused scattering to greater structure objects and an increase of scattering in the initial part of the SAXS curve.

The course of SAXS curves of starch suspensions in 1 M NaCl and water (Fig. 4) is very similar: both curves show clear diffraction peaks. Therefore, it can be assumed that Na^+ and Cl^- ions diffuse only in places of water diffusion. The comparison of the course of SAXS curves of starch suspensions in HCl and NaCl shows the impact of pH on the way of water diffusion to the starch structures [8]. Data presented in Fig. 5 indicate that iodine ions diffuse together with water to some parts of starch. The visible diffraction peak proves it. Scattering is much more intense that in the suspension of starch in water. It is clear, however, that in the first part of the SAXS curve, scattering is greater than for water, which can be interpreted that the solution of I_2, KI, and water do not diffuse exactly in the same way as pure water. A significant growth of SAX scattering after the introduction of iodine ions to the starch structure suggests that those ions could play the role of indicators that would better exhibit the differences in starch structure. The results presented above clearly prove that the SAXS method can be used to investigate the process of various media diffusion to the starch structure. Through investigating the shape of SAXS curves, it allows us to identify those parts of the starch structure to which specific media diffuse. At the same time it allows us to investigate the impact of various factors (pH for example) on the diffusion. Use of intense radiation from a synchrotron for obtaining SAXS curves, which means shortening the time of measurement, will allow us to investigate the kinetics of this diffusion.

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BADANIA SKROBI W CIEKŁYM OŚRODKU METODĄ NISKOKĄTOWEGO ROZPRASZANIA PROMIENIOWANIA RENTGENOWSKIEGO (SAXS)

Streszczenie

Metoda niskokątowego rozpraszania promieniowania rentgenowskiego jest jedną z metod badania skrobi. Pozwala ona zobaczyć budowę skrobi, ujawniającą się jako zmiana rozkładu gęstości elektronowej. Gdy obserwacje prowadzi się w ciekłym ośrodku, zmiany rozkładu gęstości elektronowej są łatwiej-

sze do zaobserwowania. Dotychczas stosowanym do tego celu ośrodkiem była jedynie woda. W niniejszych badaniach użyto też innych ośrodków. Skrobie zawieszono w wodzie, metanolu, 1 M kwasie solnym, 1 M wodnym roztworze NaCI, i 0,1 M roztworze J_2 w KJ. Analiza krzywych SAXS pokazała, że różnią się one między sobą. Różnice te były szczególnie widoczne w przypadku zastosowania 1 M kwasu solnego i 1 M roztworu NaCl. Szczególnie duże rozproszenie promieniowania przez skrobię w kwasie solnym w początkowym przebiegu krzywej wskazuje na dyfuzję anionu Cl^- do różnych regionów gałeczki, przy czym dyfuzja ta zależy od pH roztworu. Metoda może być przydatna w badaniach strukturalnych skrobi.

K. PIYACHOMKWAN, A. JARERAT, C. DULSAMPHAN,
C.G. OATES, K. SRIROTH

EFFECT OF PROCESSING ON CASSAVA STARCH QUALITY: 1. DRYING

Abstract

The long-term outlook for cassava starch is uncertain, this is despite the economic advantage afforded to this product by recent decline in cassava price. The problems are due to a restricted portfolio of functional properties coupled with a final product that is variable in-terms of its quality. The quality of extracted cassava starch is dependent on many factors, especially processing. One key problem area is that of drying the dewatered cake. In this study, it was shown that the properties of dried starch were different to those of its non-dried counterpart (cake). After drying swelling power and solubility decreased, these changes were in-line with those exhibited by heat-moisture treated starch prepared by incubating 25% moistened starch at 100°C for 16 hr. Dried starch had higher peak temperature than its original cake but lower pasting temperature, which contrasted to the effect of heat-moisture treatment. Dried starch from moist cake had a broader endothermic peak indicated by a larger gelatinization temperature range and lower peak height index, similar to heat-moisture treated starch. Despite apparent changes in functional properties during drying of cassava starch, the cause of the change is not entirely known. Generally, changes reflect a hybrid of heat-moisture treatment and hydrothermal effect.

Introduction

Starch, unmodified as well as modified, has many properties that collectively contribute to its usefulness in a wide range of food and non-food products. The world market for starch destined for industrial use is continuously on the increase. This trend occurs despite the restricted range of crops from which starch is extracted on a commercial basis. The most important crops are potato, corn, wheat and tapioca. Corn is the main source of starch; of the total world starch production, about 83% is derived

K. Piyachomkwan, A. Jarerat, Cassava and Starch Technology Research Unit, National Center for Genetic Engineering and Biotechnology, Bangkok, Thailand; C. Dulsamphan, K. Sriroth, Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand; C.G. Oates, K. Sriroth, Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Kasetsart University, Bangkok, Thailand.

from corn, 7% from wheat, 6% from potato and 4% from cassava [6]. The dominant position of cornstarch is a reflection of the cost advantages of this crop. However, given the ready availability of cheap cornstarch, products are often adapted to facilitate incorporation of this starch in their formulation. The range of functional properties provided by cornstarch is on the increase as genetically modified corns are developed with specific functionality, and advances are made in starch modification technology. Cornstarch technology is therefore at state of technological sophistication whereby the starch is tailored to the needs of the product rather than the product to the starch. The cornstarch industry has also responded to the need by the user industries for a high, consistent quality product.

The cassava starch industry, in contrast, has not invested in variety development or modification for improved starch functionality. Globally, this industry is still struggling to deal with problems of starch quality variability. Given the chemical composition of cassava root, starch from this source should be more pure than cornstarch. Unfortunately, this is not necessarily the case. Further, the lack of by-products is an impediment to further income generation by the industry.

The portfolio of functional properties (quality) of cassava starch is highly variable between batches. Starch quality is influenced by many factors starting with the process of starch synthesis during plant and root development through to inconsistencies in the starch extraction process. In terms of starch properties, there is a substantial G by E effect. The main environmental effects being mediated by duration of plant growth are the rainfall immediately before harvest. Environmental effects are expressed by differences in the structural and the physicochemical properties of the starch granules deposited in roots [1, 12, 13, 16, 17]. Fluctuation in soil temperature also causes an alteration in starch properties [2, 7]. During the manufacturing process, mechanical grinding of fresh roots can lead to damage of starch granules and subsequent changes in water interactions and enzyme susceptibility. Extraction processes employing sulphur dioxide also lead to alteration in the granule stability [18].

Modification of the granular structure and property of starch can occur, accidentally or intentionally, during processing. In the starch extraction process the final stage is potentially problematic in terms of altering starch quality. The combination of high temperature and moisture can precipitate structural changes in the architecture of starch granules, known as "hydrothermal" treatment [5]. Starch granules in excess water, when subjected to sufficient heating, swell irreversibly becoming water-soluble. This process is associated with a loss of granule integrity and birefringence, a process known as gelatinization. Two further thermal treatments, heating an aqueous suspension of starch granules at a temperature below that at which gelatinization occurs ("annealing" treatment) or heating moistened starch (water content less than 30%) at a higher temperature than that at which gelatinization occurs ("heat-moisture" treat-

ment), do not result in complete loss of starch structure (starch gelatinization). Annealed and heat-moisture treated starch remains as discrete granules that are water-insoluble. Yet, modification of the granule structure and associated properties are evident. Annealed starch is characterized by an alleviate gelatinization temperature and reduced gelatinization temperature range [19]. Heat-moisture treatment also alters both structural and physicochemical properties of starch granules [4, 9, 11].

Extraction of cassava starch involves a dewatering stage consisting of a horizontal centrifugal basket running at a low speed of 800 to 900 rpm. Discharged starch cake is of high moisture content (35–40%; [16, 17]). Final moisture reduction of the moistened cake occurs in a flash dryer. Temperature fluctuation of the flash dryer occurs, often in the range 160 to 180°C. Given the profound influence of heat and moisture on the starch granule structure, inconsistencies at the drying stage could be a responsible for some of the quality variability in the final product. Strategies for eradicating this variability could involve either improvement in the dewatering process such that the final cake has lower moisture content or improvements to the heating system.

This study is part of a larger project that is investigating the influence of processing on starch quality. This paper reports on an investigation to probe the effect of commercial drying on cassava starch properties. Quality of starch cake, before and after factory drying and at different levels of cake moisture, was investigated. Comparison was also made with heat-moisture treated starch.

Materials and methods

Drying process

A cassava starch factory situated close to a cassava-producing region in the Northeastern part of Thailand was chosen for the study. The factory selected had a production capacity of 200 tons cassava starch per day. No sulphur dioxide was used in the extraction process. Eight sets of locally-made dewatering centrifuges were installed to reduce starch cake moisture. Each centrifuge had a discharge capacity of 239.0±23 kg cake/cycle (10 minutes). Feeding rate of the cake to dryer was 8 tons dry solid/hr. Starch cake was dried in a flash dryer at a temperature of 170°C. Sampling was as a pair of cake and its starch after drying. Time interval for sampling between cake in the feeder and dried starch from cyclone was determined by the dryer manufacturer to be about 10 second after feeding.

Heat-moisture treatment in laboratory

Cassava starch was extracted, in water, from fresh cassava roots and dried at 50°C. After sieving through a 90-μm screen, moisture was adjusted to 25% moisture content and samples were equilibrated overnight. One hundred grams of moistened

starch sealed in bottles was subjected to 100°C in hot air oven for 16 hours [5]. After treatment, samples were unsealed, dried at 50°C and kept in cool place.

Analytical methods

Moisture content of samples was determined by drying at 105°C to constant weight [3]. Starch content was determined by a polarimetry method [3]. Paste viscosity properties were investigated by a Rapid Visco Analyzer (RVA 4, Newport Scientific, Australia) according to Sriroth et al. [18]. Thermal analysis was determined by Differential Scanning Calorimeter (Perkin Elmer DSC 7, Norwalk, CT;). The peak height index (PHI) is reported as the ratio of enthalpy (ΔH) and the difference between peak and onset temperature (T_p-T_o) [10]. Swelling power and solubility at 85°C followed the method of Schoch [14]. Degree of hydrolysis of samples was measured using α-amylase and amyloglucosidase, following the method of Wang *et al.* [20]. Reducing sugar was analyzed using Somogyi-Nelson method [15] and total sugars by the method of Dubois et al. [8].

All data was statistically analyzed at 95% confidence level by Completely Randomized Design (STATGRAPHICS Plus Version 3.0, USA).

Results and discussion

Heat-moisture treatment is believed to cause changes to the physical order within starch granules. These changes do not affect the morphology of the granule visually but influence starch properties. Heat moisture treated samples, compared to native starch, have higher gelatinization temperature, lower peak viscosity but higher cold paste viscosity. Solubility and swelling power are lower [5, 9]. Alteration in the physical properties of cassava starch occurs when starch is moistened (25% moisture content) and kept under controlled heating conditions that are higher than the gelatinization temperature (>66°C). After subjecting cassava starch to heat-moisture treatment, solubility and swelling power were lower. This is similar to the response of wheat and potato starches, to similar treatment. Gelatinization endortherms of heat moisture treated cassava starch are broader; this is because of the final peak temperature is elevated. Despite extension of the final peak temperature enthalpy of gelatinization was lower and hence PHI (Table 1). Change in the RVA paste viscosity profile of treated cassava starch is also evident. On heat-moisture treatment, starch paste viscosity was significantly decreased; peak viscosity of untreated and treated starch were 368 and 304 RVU, respectively. Yet, paste stability during heating was increased, indicated by starch paste breakdown from 234 RVU for untreated starch to only 95 RVU for treated starch. Cold paste viscosity of treated starch was improved; final viscosity of untreated and treated starch was 205 and 330 RVU, respectively (Table 1). Change in paste vis-

cosity of heat-moisture treated cassava starch was similar to those reported in a previous study by Abraham (1993). The susceptibility of heat-moisture treated starch to enzymatic hydrolysis was also lower (Table 1).

Table 1

Change in starch property by heat-moisture treatment*.

Property**	Native starch	Heat-moisture treated starch
Swelling power at 85°C	26.33	21.99
% Solubilty at 85°C	48.71[a]	20.79[b]
Gelatinization		
- Onset temperature (°C)	65.85[b]	72.18[a]
- Peak temperature (°C)	70.95[b]	78.63[a]
- Gelatinization temperature range (°C)	10.19	12.91
- Peak height index (PHI)	2.94[a]	1.48[b]
- Enthalpy (J/g)	15.00[a]	9.52[b]
Paste viscosity		
- Pasting temperature (°C)	72.90[b]	81.33[a]
- Peak viscosity (RVU)	368[a]	304[b]
- Trough viscosity (RVU)	135[b]	209[a]
- Final viscosity (RVU)	205[b]	330[a]
- Breakdown (RVU)	234[a]	95[b]
- Setback (RVU)	71[b]	121[a]
Degree of hydrolysis (%)	41.65[a]	33.63[b]

*Moistened starch (25% moisture content) was kept with completely sealed at 100°C for 16 hr.
**Values in each row with different letters are significantly different at $p < 0.05$.

Table 2

Swelling power* and %solubility*, at 85°C, of cassava starch obtained from cakes with different moisture contents after flash drying in cassava starch factory.

Moisture content of cake** (%)	Swelling power		%Solubility	
	Cake	Starch	Cake	Starch
30.1-33.0	58.08[a]	44.75[b]	31.47[a]	28.04[b]
33.1-36.0	60.67[a]	44.79[b]	26.82[a]	23.59[b]
36.1-39.0	63.93[a]	45.03[b]	25.92[a]	21.21[b]
39.1-44.0	65.30[a]	46.12[b]	27.67[a]	24.23[b]

*Values in each row with different letters are significantly different at $p < 0.05$.
**$n = 31$.

In a cassava starch factory, hydrothermal induced changes may take place between the point at which moistened starch exits the dewatering centrifuge and the flash

dryer. The present study 73 sample pairs (cake and dry starch) were investigated for signs of heat-moisture treatment that may have occurred during the drying process. Care was taken to ensure that samples were only collected when the dryer temperature was around 172±2.0°C. The moisture of the cake varied from 30 to 44% and could be categorized into 4 levels including low moisture cake (30.1 to 33.0%), medium moisture cake (33.1 to 36.0%), high moisture cake (36.1 to 39.0%) and very high moisture cake (39.1 to 44.0%). Moisture of the dried starch samples was 10.90±0.96% and starch content was 97.97±0.82% for cake and dried starch. Changes in starch properties due to possible hydrothermal effects were evident (Table 2 to 4); these changes were expressed for certain starch properties and were dependent on cake moisture content. The changes were similar to those seen in heat-moisture treated starch produced in the laboratory. Dried starch from the cakes of different moisture content had significantly reduced swelling power (Table 2, Figure 1). Peak viscosity of dried starch was also significantly different comparable to its original cake (except the low moisture cake, Table 3). Surprisingly, viscosity change of dried starch from the factory was incompatible with the heat-moisture treated starch previously observed in the laboratory.

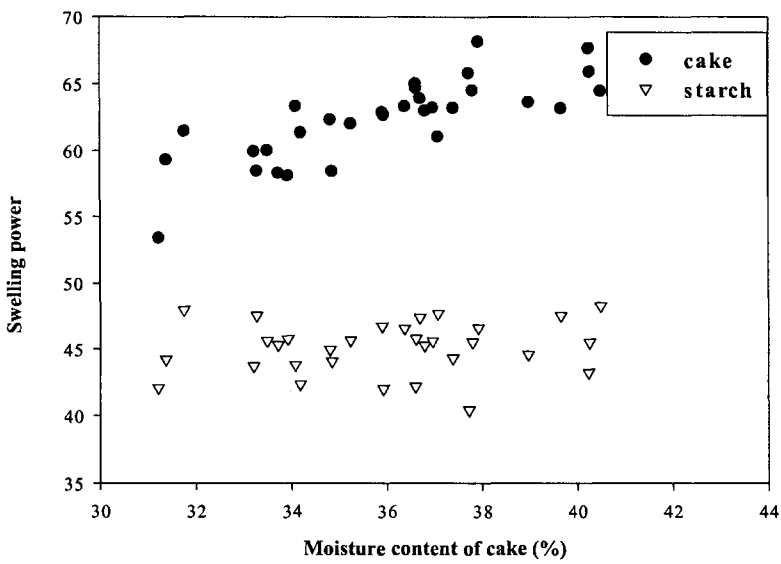

Fig. 1. Swelling power of starch cakes with different moisture contents and their equivalent dried starch samples collected from cassava starch factories.

Heat treatment of moistened starch cake in the factory produced dried starch with increased peak and cold paste viscosity; the changes are not as clear as those found for swelling power (Figure 2), but still significantly different. In contrast, to the laboratory

results, dried starch from all moisture cakes exhibited a significant reduction in pasting temperature (Table 3). Changes in gelatinization of the cake and its equivalent starch were also evident. In accordance with pasting temperature, heat treatment resulted in a lower gelatinization temperature of dried starch relative to its original cake (Figure 3). Dried starch collected from the factory also had lower enthalpy than its original cake (Figure 4). The endothermic peak of gelatinzation of dried starch was broader but of lower peak height than that of its original cake; the PHI of dried starch was thus lower than that of the cake (Table 4). Presumably, heat treatment during drying induces structural changes in starch granules thus affecting their gelatinization process.

Table 3

Paste viscosity* of cassava starch obtained from cakes with different moisture contents after flash drying in cassava starch factory.

Moisture content of cake** (%)	Peak viscosity (RVU)		Final viscosity (RVU)		Breakdown*** (RVU)		Pasting temperature (°C)	
	Cake	Starch	Cake	Starch	Cake	Starch	Cake	Starch
30.1-33.0	394	393	217	223	240	239	69.60^{a}	68.71^{b}
33.1-36.0	390^{b}	397^{a}	227	231	240	243	68.74^{a}	68.17^{b}
36.1-39.0	387^{b}	400^{a}	220^{b}	229^{a}	242	247	68.68^{a}	68.05^{b}
39.1-44.0	375^{b}	393^{a}	215	221	234	242	68.69^{a}	67.97^{b}

*Values in each row with different letters are significantly different at $p < 0.05$.
**n = 73.
***Breakdown = Peak viscosity – Trough viscosity

Table 4

Thermal analysis* of cassava starch obtained from cakes with different moisture contents after flash drying in cassava starch factoreis.

Moisture content of cake** (%)	Onset temperature (°C)		Temperature range*** (°C)		Enthalpy (J/g)		Peak height index***	
	Cake	Starch	Cake	Starch	Cake	Starch	Cake	Starch
30.1-33.0	61.00	60.39	11.03	12.19	14.58	12.21	2.66	2.01
33.1-36.0	60.97	60.29	10.24	10.67	13.42	12.66	2.70^{a}	2.39^{b}
36.1-39.0	60.92^{a}	60.43^{b}	9.24	10.42	14.30^{a}	12.45^{b}	3.13^{a}	2.40^{b}
39.1-44.0	60.72	60.48	9.87	9.45	13.91	12.15	2.84	2.59

*Values in each row with different letters are significantly different at $p < 0.05$.
**n = 21.
*** Temperature range is reported as the difference between the final and onset temperature; peak height index (PHI) is reported as the ratio of enthalpy (ΔH) and the difference between peak and onset temperature (T_p-T_o).

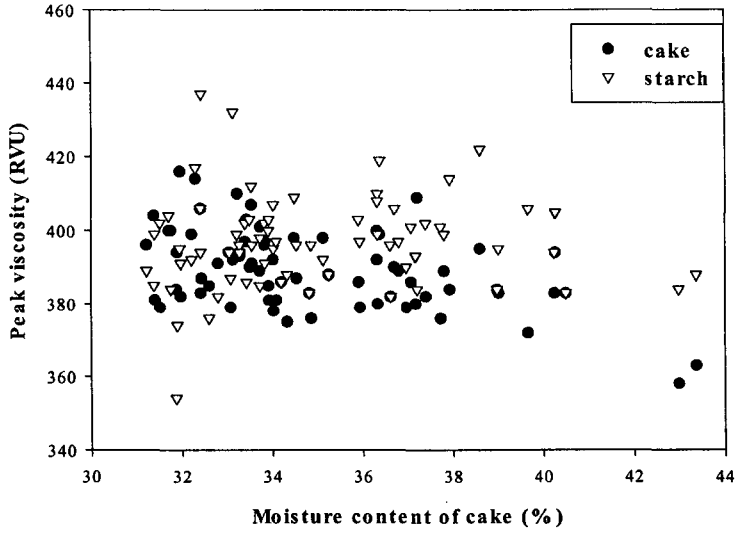

Fig. 2. Peak viscosity (RVU), as determined by a Rapid Visco Analyzer, of starch cakes with different moisture contents and their equivalent dried starch samples collected from cassava starch factories.

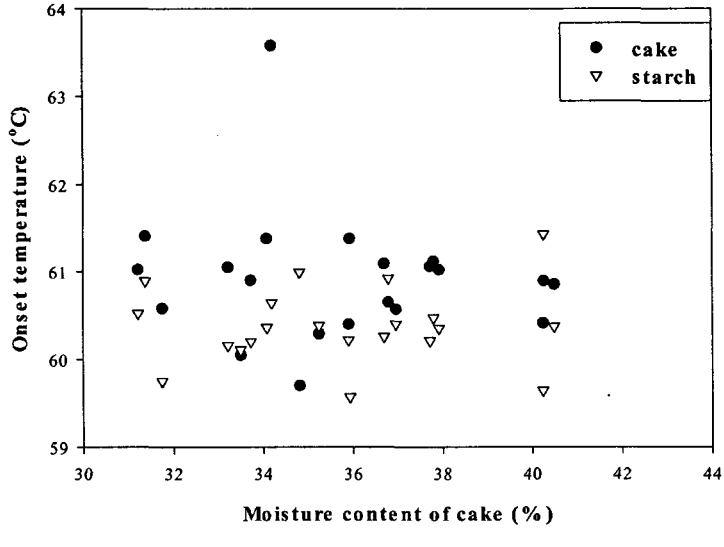

Fig. 3. Onset temperature (°C), as determined by Differential Scanning Calorimeter, of starch cakes with different moisture contents and their equivalent dried starch samples collected from cassava starch factories.

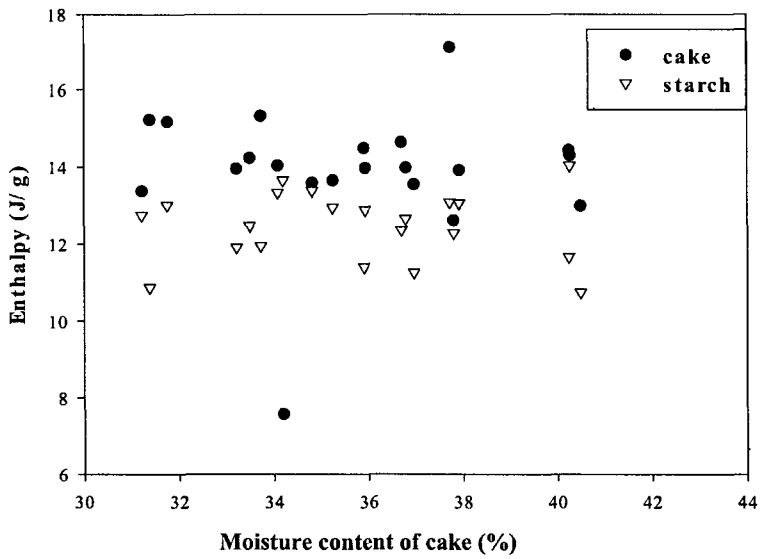

Fig. 4. Enthalpy (J/g), as determined by Differential Scanning Calorimeter, of starch cakes with different moisture contents and their equivalent dried starch samples collected from cassava starch factories.

Conclusion

Drying is a critical step in the starch extraction process and may account for final product quality inconsistency. In addition to the physical process of drying, when starch cake with 30-44% moisture content is subjected to heat treatment changes in some of the functional properties occurs. Yet, the apparent direction and magnitude of these changes, during drying, of cassava starch are not in agreement with the effects of heat-moisture treatment. It is suggested that cassava starch dried under factory conditions may undergo some form of hydrothermal treatment, which leads to alteration in the functional properties of the starch.

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WPŁYW OBRÓBKI NA JAKOŚĆ SKROBI TAPIOKOWEJ: 1. SUSZENIE

Streszczenie

Z powodu obniżenia ceny na skrobię tapiokową, długoterminowe prognozy dla tej skrobi są niepewne mimo wielu jej zalet. Wynika to z ograniczonej liczby istotnych właściwości funkcjonalnych tej skrobi i ich niekorzystnych zmian w trakcie przechowywania.

Jakość ekstrahowanej skrobi tapiokowej zależy od wielu czynników, przede wszystkim od sposobu jej wydzielania. Kluczowym problemem jest suszenie odwodnionego placka skrobiowego. W niniejszych badaniach pokazano, że właściwości skrobi suszonej i nie suszonej (placka) różniły się od siebie. Po suszeniu malała zdolność pęcznienia i rozpuszczalność. Zmiany te były liniowe względem zmian zachodzących w trakcie przechowywania skrobi zawierającej 25% wilgoci, w 100°C, przez 16 godzin. Odwrotnie niż w przypadku obróbki temperaturowej wilgotnej skrobi, skrobia suszona miała wyższą temperaturę w punkcie maksimum lepkości i równocześnie niższą temperaturę kleikowania, niż skrobia otrzymana z placka. Skrobia z placka wykazywała szerszy pik endotermiczny wskazujący na szerszy zakres temperaturowy kleikowania. Równocześnie pik ten był niższy, w czym skrobia ta przypominała produkt z termicznej obróbki wilgotnej skrobi. Nie wiadomo co jest przyczyną zaobserwowanych zmian.

WERNER PRAZNIK, ANTON HUBER

MOLECULAR STRUCTURE AND PHYSICO-CHEMICAL PROPERTIES OF PSEUDO CEREAL STARCHES

Abstract

Glucans of pseudo cereal starches with significant differences in their branching pattern – amaranth, quinoa and buckwheat – were investigated upon the correlation of their molecular characteristics with technological properties. Consistency of glucan conformation, in particular persistance against elevated temperature, acidic pH and mechanical stress was investigated with respect to consequences on molecular and supermolecular structures of starch/DMSO-solutions.

For analytical purposes starch glucans were separated by semi-preparative size-exclusion chromatography (SEC) and obtained fractions were tested upon their iodine-complexing potential. Amaranth was found to be short chain branched (scb ≡ amylopectin type); quinoa to be scb-type, but consisting of longer branches than amaranth; buckwheat was found to be a mixture of scb-glucans with approx. 24% of long-chain branched (lcb ≡ amylose-type) glucans.

Molecular weight (degree of polymerization) for DMSO-dissolved starches was determined absolutely by means of aqueous SEC. Weight average molecular weights (M_w) were found close to $12 \cdot 10^6$ g/M for the investigated samples. Dimensions of starch glucan coils were estimated from SEC-data combined with universal calibration: values between 2–40 nm were found without significant differences for the three starches. However, in spite of these minor differences, the investigated starches differ significantly in their inter- and intramolecular interaction potential. Thus, obviously interaction potentials are strongly controlled by branching patterns, glucan-coil packing densities and by the ability to form supermolecular structures.

Introduction

Although diversity of technological qualities of cereal starches is as widespread as the variety of basic cereals, this list continuously is expanded by newly breeded and technologically modified species.

W. Praznik, 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; A. Huber, Institut für Chemie / Polymer Characterization, Heinrichstrasse 28, A-8010 Graz / Austria.

As starch is strongly determined by the biological history of the starch containing system a systematic analysis of technological qualities needs information from different levels: history of biological conditions, macroscopic granula-level characteristics, information about molecular dimensions and about conformation on molecular and supermolecular level. Whereas granule-characteristics such as composition (percentage of glucans, proteins, lipids, salts), size and geometry of granules, degree of cristallinity and water content ever have been considered to be important, the influence of molecular-level characteristics such as degree of polymerization (mean values, mass and molar distributions), branching pattern and interactive potential of starch glucans often is ignored or, at least, underestimated [1, 2]. Nevertheless, it is well known that in particular kind and percentage of amylose-type non/long-chain branched (nb/lcb) and amylopectin-type short-chain branched (scb) glucan fractions strongly influence macroscopic starch qualities [3, 4]. Starches of pseudocereals amaranth, quinoa and buckwheath, which differ in these molecular qualities, are investigated upon the consequences of these differences on technological properties [5-12]. 5,,,,,,,12

Experimental

Material

Starch of buckwheat was isolated from *Fagopyrum esculentum* (Nestelberger/ Austria); starches of amaranth and quinoa were prepared from *Amaranthus cruentus* and from *Chenopodium quinoa* (Posch/Austria), respectively.

Molecular characteristics

Dissolution

DMSO/starch glucan solutions were prepared for semi-preparative low pressure SEC-system in the range between 0.5–2 mg/mL for high pressure SEC analysis. To ensure elimination of supermolecular associates, dissolution was extended up to 100 hours with permanent stirring at 80°C. To avoid interfering phenomena by exergonic mixing energies in SEC-matrix, DMSO/starch solutions were mixed 1:1 with aqueous eluent (0.005 M Na_2CO_3) before chromatographical analysis.

Molecular weight distribution and glucan dimensions

For absolute molecular weight analysis, estimation of glucan coil dimensions and packing densities within occupied volumina, the SEC system (TSK PWM, PW 6000G, PW 5000G, PW 4000G, PW 3000 G; each 300 mm, ID 7.5 mm, ToyoSoda / J) was connected to dual-detection of mass (interferometric refractometer: Optilab 903, Wyatt Technology/US) and scattering intensity (low angle laser light scattering instrument

(KMX-6, TSP/US; injected sample volume: 260 µL of approx. 0.5 mg/mL solutions; eluent: 0.005 M aqueous Na_2CO_3). Estimation of glucan coil dimensions was achieved by universal calibration of SEC-chromatograms. For branching analysis of separated components, 1 mL fractions were stained with iodine reagent.

For these experiments data acquisition with integrated data reduction was managed by software package CODAwin32 and data processing with software package CPCwin32 (both: a.h group/Austria).

Branching analysis by iodine staining

The percentage of amylose-type nb/lcb-glucans was obtained from iodine-complexing potential (stock solution: 125 mg suspended with 400 mg KJ in 1000 mL H_2O; before application an equivalent of the stock solution was diluted 1:1 with 0.1 M acidic acid to provide pH 4.6). 1 mL of this reagent was added to 1 mL starch solution (100 µg glucan/mL) either from the bulk or from SEC-obtained fractions. Non-complexed scb-glucans were monitored by extinction at 525 nm (E_{525}), iodine-complexed nb/lcb-glucans at 640 nm (E_{640}).

Amylose-content of investigated starch solutions (10 mg glucan in 1 mL DMSO, diluted with 0.005 M Na_2CO_3 to a final concentration of 100 µg glucan/mL) were determined by referring to amaranth (0% amylose) and phosphorylase-synthezised non-branched $\alpha(1\rightarrow4)$ glucan (100 % amylose).

Finally, a ratio of amylose-type non/long-chain branched and amylopectin-type short-chain branched glucans was obtained by computing the extinction-ratio E_{640}/E_{525}.

Branching analysis by enzymatic fragmentation and subsequent fragment analysis

Starch samples were dissolved in DMSO (31 h, 80°C) and mixed with acetatbuffer (0.5 mL starch suspension + 1.5 mL buffer; final pH 5.3); debranching was achieved by incubating 5 µL Isoamylase (Hayashibara, EN 102) and stirring for 17 h at 38°C. Enzyme was denaturated by heating (5 min 95°C) and the sample solution was desalted with DOWEX mixed bead ionexchanger.

Analysis of debranched glucans was done by means of SEC (Pharmacia Superose 12 (l = 300 mm, ID = 10 mm) + Merck Fractogel TSK-HW 40 (S) (l = 150 mm, ID=10 mm); eluent 0.06M sodium-acetate pH 5.5; injection volume: 300 µL; flowrate: 0.42 mL/min) and HPAEC-PAD system (Dionex: Bio LC Modell 4000i System, working electrode: gold, reference electrode: Ag/AgCl, column: Carbo-Pac PA 100; injected volume: 20 µL; flowrate: 1,2 mL/min; gradient profile: 0 min 90% A/10% B to 90 min 10% A/90% B: A = 150 mM NaOH, B = 150 mM NaOH + 500 mM $NaOOCCH_3$).

Preparation of ß-limit dextrin: starch samples (1% in DMSO) were mixed with sodium-acetat buffer (1 mL starch suspension + 3 mL buffer; final pH 5.3) and incubated with 4.55 µL ß-Amylase (SIGMA, A-7005) for 2 h at room temperature. Enzyme

was denaturated by heating (5 min 95°C) and the sample solution was desalted with DOWEX mixed bead ionexchanger.

Technological Qualities

Gelatinization behaviour and resistance against mechanical, chemical and thermal stress

Gelatinization behaviour of aqueous starch suspensions was investigated with a cone/plate-Rheometer (Physica/Germany; system MK 250; 5% (w/w), 50°C, shear rate of 100 s^{-1} and a heating rate of 2°C/min up to 95°C).

Paste viscosity was determined with a Brabender Viscoamylograph E (Brabender, Duisburg/Germany, 10% (w/w), temperature program 30–90°C with a heating rate of 1.5°C/min, 75 rpm, holding period: 30 min, cool to 30°C).

Shear stability was determined in a cylinder-geometry rheometer (Physica/ Germany; system cylinder Z3 DIN, smooth spindle; 5% (w/w) aqueous starch suspension; shear rates: 5 min at 100 s^{-1}, 5 min 1000 s^{-1} and again 5 min at 100 s^{-1}). Shear stability is computed as the similarity between viscosity at the end of first period (η_{before}) and viscosity after second period (η_{after}) of shear stress in terms of shear stability percentage.

Acid resistance was determined for aqueous starch suspensions (5% (w/w), pH 3.0, 2 M citric acid, viscosity at 95°C before and after acidification at 100 s^{-1}. The degree of resistance was determined as the ratio of viscosity before ($\eta_{initial}$) and after ($\eta_{pH\ 3}$) acidification.

Freeze/thaw stability was determined according a modified method of Schoch [13,14]. For a freeze/thaw cycle the resulting suspensions were stored overnight at -7°C and thawed the next day in a 30°C water bath. The amount of liberated water was determined after centrifugation. For a next freeze/thaw cylce the starch pastes were resuspended with the liberated water, homogenized and once again stored overnight at -7°C. Freeze/thaw-stability is computed as the percentage of liberated water in a series of six freeze/thaw-cycles. Each freeze/thaw-cycle is assumed to be equivalent to a three week storage at 4°C.

Results and discussion

Molecular characteristics

With dual detection of mass (DRI) and scattering intensity (LALLS) absolute information about molecular weight and degree of polymerization for SEC-separated starch glucan fractions, and thus, about degree of polymerization distribution for the investigated starches could be achieved (Fig. 1).

The maximum value of degree of polymerization was found for all of the investigated starches in the range of 150 000 ± 20 000 glucose units – a surprising fact, as no significant difference was found for scb-type starch glucans (amaranth, quinoa) and scb/lcb-mixed-type (buckwheat) starch.

Minimum dp-values however, differ significantly for the different starch: the applied dissolving-process obviously reduced supermolecular structures of amaranth, and quinoa but left supermolecular structures buckwheat. As mean values of degree of polymerization, for instance weight average degree of polymerization (dp_w), strongly depend on the width of degree of polymerization distribution, dp_w-values for the investigated starches differ significantly. Resulting molecular weight distributions in Fig. 1 are normalized to unity-area to enable fast identity-checks via matching/ mismatching areas.

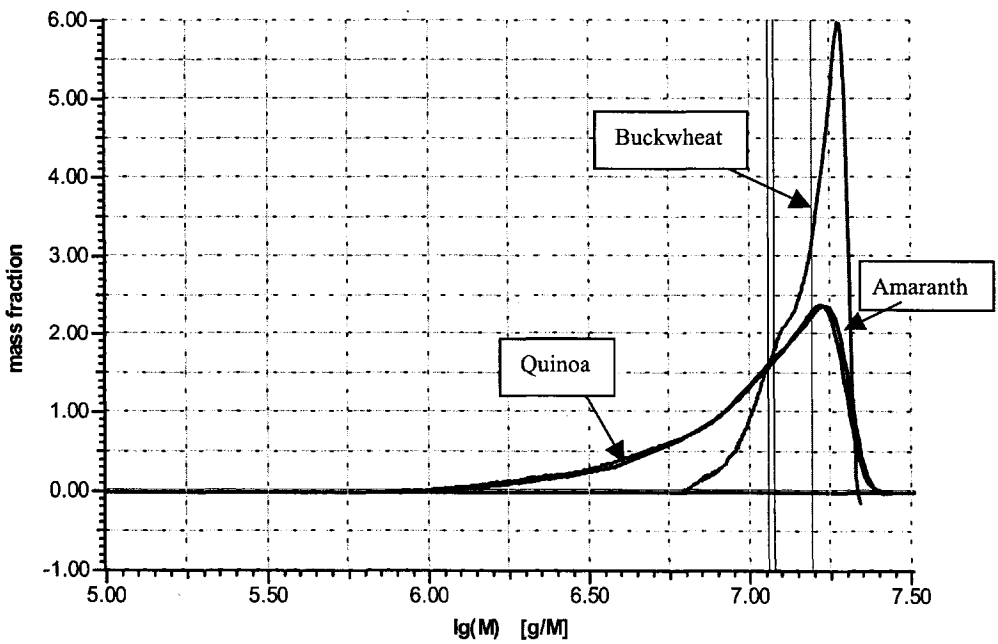

Fig. 1. Amaranth (M_w=11.8·10^6 g/mol ≡ dp_w=72500 Glc), Quinoa (11.3·10^6 g/mol ≡ dp_w=70000 Glc) and Buckwheat (15.4·10^6 g/mol ≡ dp_w=94900 Glc).

Tab. 1 lists the values for computed dimensions and packing densities of glucan-coils. All of the investigated starches consisted of glucan coils which occupied volumina with sphere-equivalent radii (R_e) between 2–40 nm. Different to the fluctuating dp_w-values, R_e-values for maximum-mass fraction (R_ep(Max)) were found very similar for all investigated samples.

Tabela 1

Mean values of degree of polymerization and dimensions of DMSO-dissolved glucan coils of investtigated starches from universal calibrated SEC-data.

Molecular characteristics	amaranth	quinoa	buckwheat
dp(Min) [Glc]	5 000	4 600	38 000
dp(Max) [Glc]	167 000	161 000	134 000
dp_w [Glc]	72 500	70 000	94 900
sphere equivalent radius of glucan coils			
R_e(Min) [nm]	2	2	2
R_e(Max) [nm]	40	40	40
R_ep(Max) [nm]	27	28	29
relative packing density of glucan coils			
molecules, corresponding to dp_w	ref = 1.0	0.9	1.3
R_e = 2-5 nm molecules	ref = 1.0	0.9	7.6
R_e = 35-40 nm molecules	ref = 1.0	0.9	0.8
average	ref = 1.0	0.9	3.2

ref: reference; Min: minimum value; Max: maximum value; dp: degree of polymerization; dp_w: weight average degree of polymerization; R_e: sphere equivalent radius of occupied volume by a dissolved glucan molecule; R_ep(Max): R_e for maximum mass glucan-fraction;

Referring to amaranth, relative packing densities for each glucan fraction was estimated with determined molecular weight (degree of polymerization) and corresponding occupied volumina: the fraction containing the largest molecules dp(Max), the fraction of smallest molecules dp(Min) and the fraction in the vicinity of weight average degree of polymerization (dp_w). Whereas no significant difference in the packing densities of the most voluminous molecules between the investigated starches could be found, differences were significant for the small molecules, in particular for buckwheat. Glucan coils of buckwheat were 7 times higher in packing density than amaranth and quinoa.

For investigation of branching characteristics the starch samples in a first approach were investigated upon their iodine-complexing potential in bulk solution. Minimum interaction was found for amaranth, resulting in a value of 0.46 for the E_{640}/E_{525}-ratio and thus, indicating that amaranth consists of scb-glucans only. Amylose-percentages for quinoa and buckwheat were determined from observed E_{640}/E_{525}-ratio-values referring to E_{640}/E_{525}-values obtained for mixtures of scb-glucan amaranth mixed with increasing percentages of synthetic nb-glucans (Tab. 2).

For more detailed information, the starches then were separated on SEC-system (Fig. 2). Similar to results of bulk-investigations, the E_{640}/E_{525}-profiles classify amaranth as scb-glucan starches with comparable uniform E_{640}/E_{525}-values close to 0.5. Likewise for quinoa a quite uniform E_{640}/E_{525}-elution profile was found, but with sig-

nificantly higher iodine-complexing characteristics. But although significantly higher in terms of E_{640}/E_{525}-values, quinoa glucans even were classified as scb-type but consisting of longer branches than amaranth. Buckwheat obviously contain two populations with respect to iodine-complexing potential: a scb-fraction of large molecules with minor iodine complexing potential and a fraction of midrange-size molecules with significantly higher iodine complexing potential and thus, with longer branches.

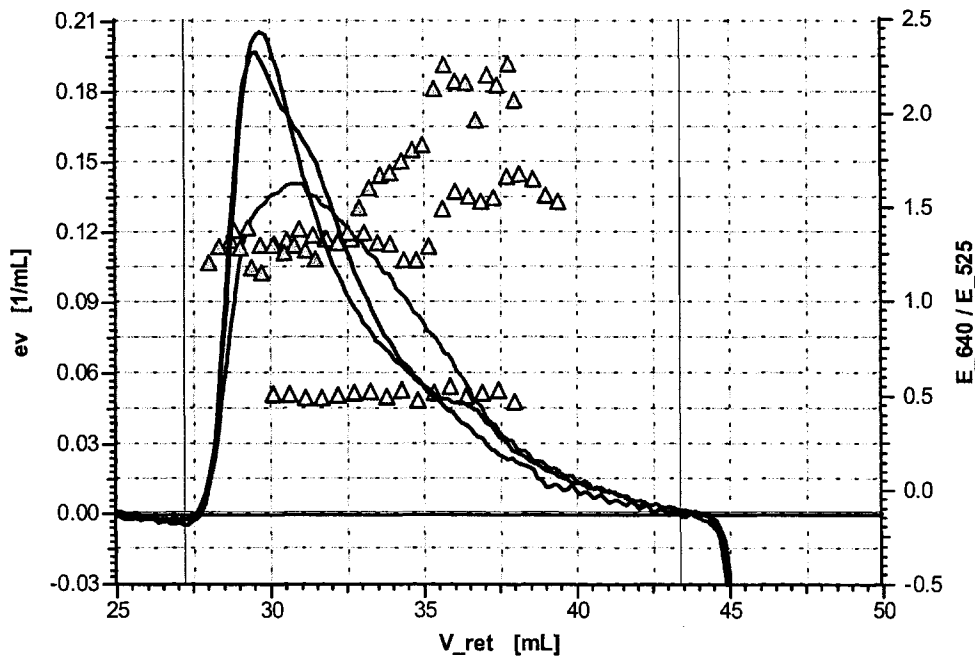

Fig. 2. Normalized elution profiles (area = 1.0) of SEC-separated starch samples (amaranth, quinoa, buckwheat) with superimposed iodine (E_{640}/E_{525}) ratio.

Supplementary to iodine staining, enzymatically catalyzed debranching and subsequent fragment analysis by means of chromatography was performed to investigate the mean branching patterns of the three starch glucans.

Tabela 2

Iodine/glucan-complexing potential E_{640}/E_{525} and correlated percentages of amylose-type nb/lcb glucans and amylopectin-type scb glucans and arithmetic population analysis of nb/lcb and scb-contributions.

Type of glucan	amaranth	quinoa	buckwheat
bulk solution:			
E_{640}/E_{525}	0.46	1.40	1.51
amylose-type nb/lcb-glucans [%]	ref.=0	20	25
SEC + E_{640}/E_{525}-fraction analysis:			
amylopectin-type scb-glucans: E_{640}/E_{525} 0-1.5 [%]	100	80	76
amylose-tpe nb/lcb-glucans: $E_{640}/E_{525} > 1.5$ [%]	ref.=0	20	24
SEC + population analysis:			
selective SEC-elution section (k_{av} 0.0-0.5): 1-4 [%]	72	68	56
selective SEC-elution section (k_{av} 0.5-1.0): 5-7 [%]	28	32	44
amylose-type nb/lcb-glucans (k_{av} 0.5-1.0): [%]	ref.=0	4	16

E_{640}: extinction at 640 nm; E_{525}: extinction at 525 nm; k_{av}: SEC-separation coefficient (selective separation range: 0.0-1.0);

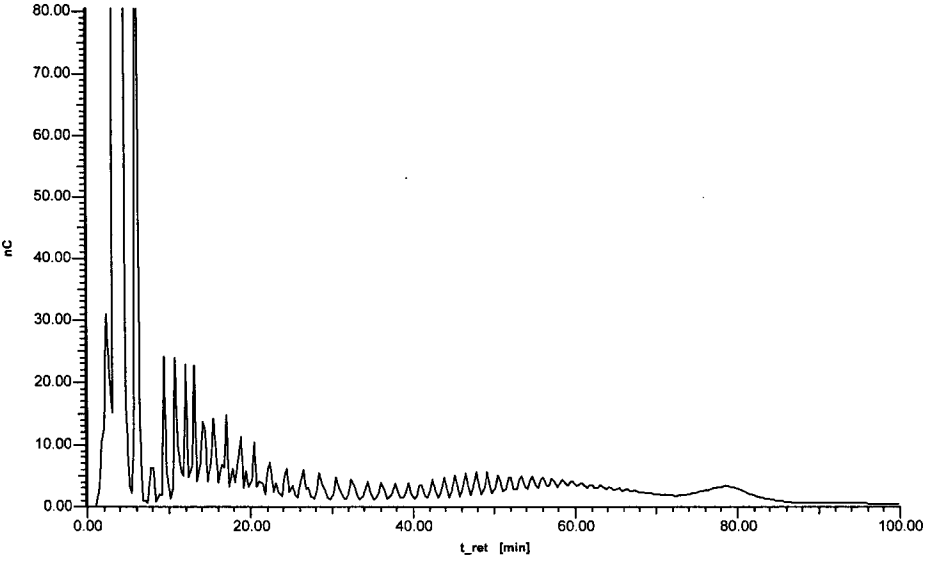

Fig. 3a. HPAEC-PAD analysis after application of ß-Amylase and Isoamylase for amaranth.

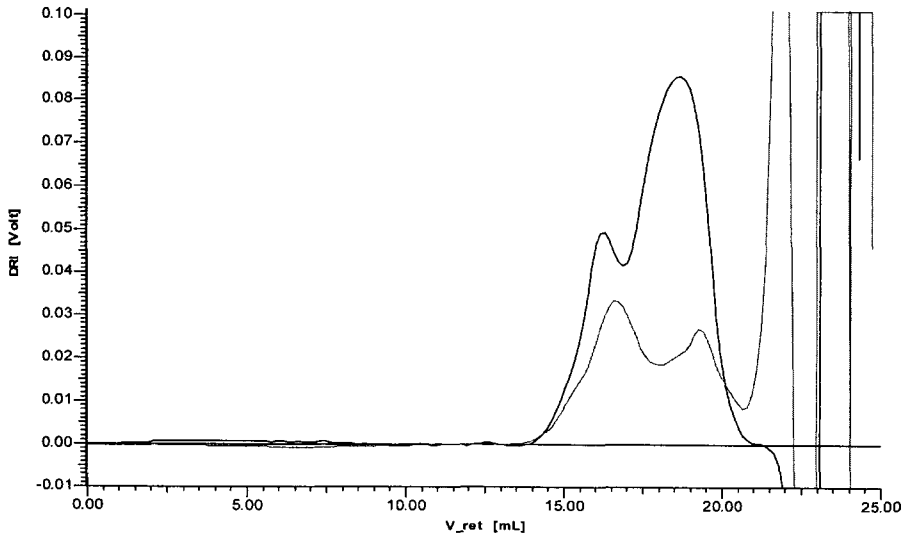

Fig. 3b. SEC-analysis of amaranth glucans: debranched native starch; debranched ß-limit dextrin.

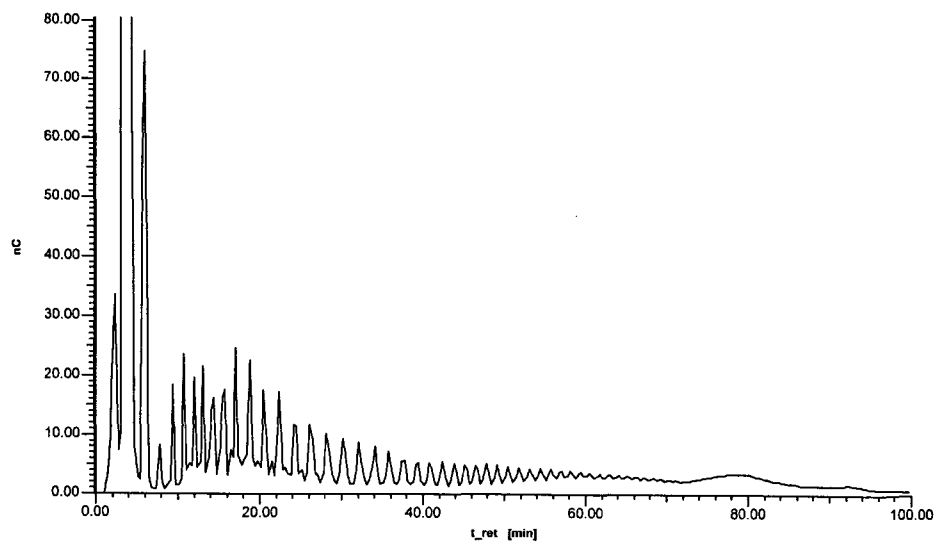

Fig. 4a. HPAEC-PAD analysis for debranched ß-limit dextrin of quinoa starch.

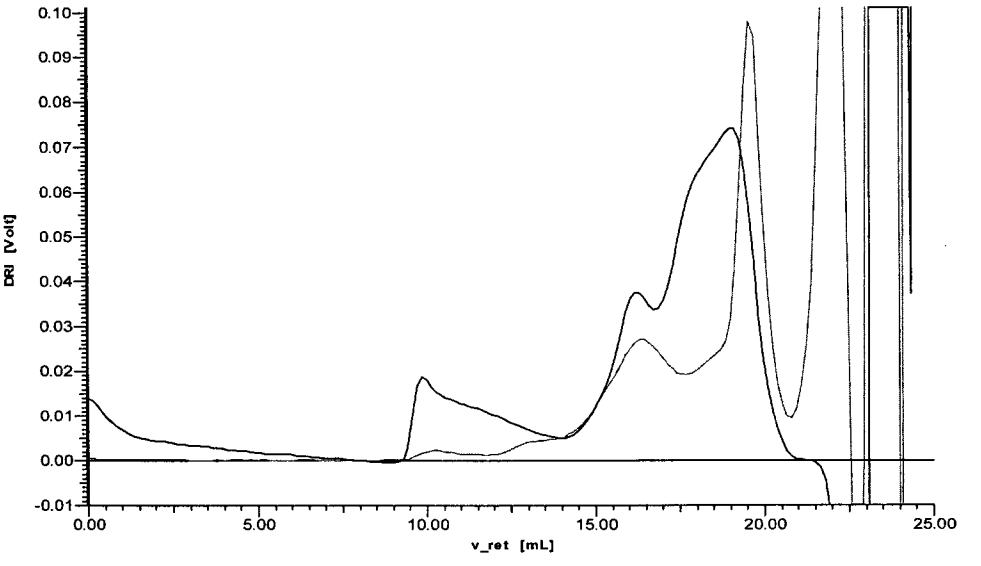

Fig. 4b. SEC-analysis of quinoa starch: debranched native starch; debranched ß-limit dextrin.

HPAEC-PAD-chromatograms of debranched ß-limit dextrin and SEC-elution profiles of debranched native starches and ß-limit dextrins for amaranth and quinoa are displayed in Fig. 3a-b and Fig. 4a-b, respectively. HPAEC-PAD-data of quinoa indicate the higher amount of glucan-dps exceeding 13 compared to amaranth. However, the SEC-elution profiles of debranched native starches show that quinoa but not amaranth consist of molecule fragments with dps exceeding 13 (approx. 20% of total mass of glucans elute between 9–15 mL). This again confirms, that quinoa is formed by longer glucan chain lenths than amaranth.

Technological qualities

Gelatinization / Temperature Dependence of Viscosity

Dependence of viscosity on temperature was determined for 5% (w/w) starch-suspensions in the range between 55–95°C (Fig. 5). Amaranth shows a maximum of disintegration at 75°C; quinoa, although uniform but with longer branches, starts disintegration at comparably lower temperatures and keeps this process without significant maximum over the observed temperature range till 95°C.

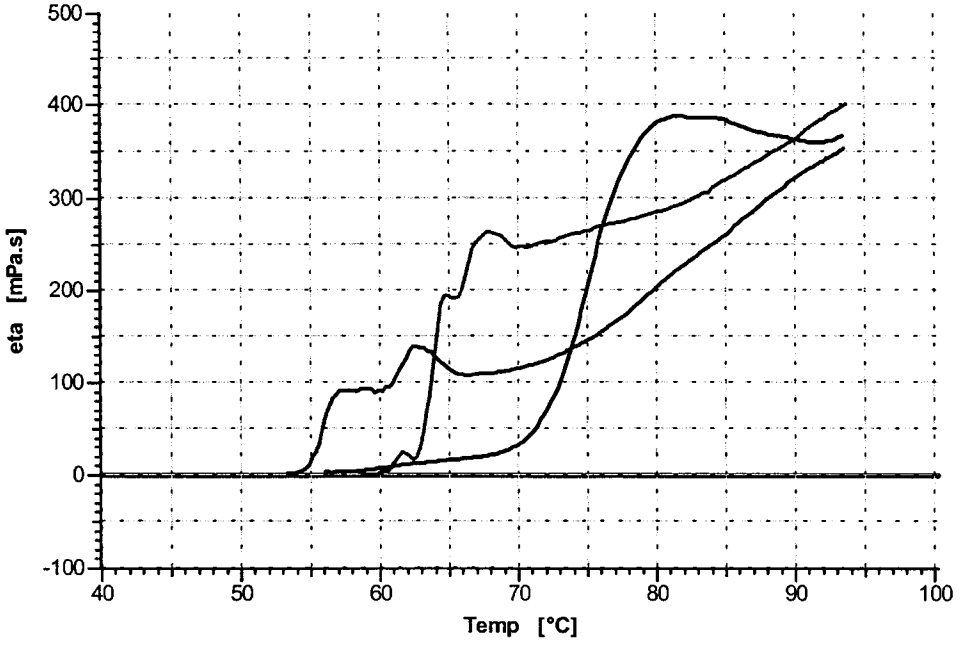

Fig. 5. Amaranth, Quinoa, Buckwheat - temperature dependence of viscosity.

Compared to quinoa glucans, which become continously disintegrated, the performance of amaranth rather is a phase-transition than a destruction phenomenon. Analysis buckwheat results in loose stabilized supermolecular glucan-structures which break up comparably easily. However, increasing viscosity with increasing temperature indicates an initial transition kind of cracking the glucan-packing at 65°C, followed by a continous disintegration of components beyond 65°C.

To monitor disintegration behaviour upon controlled energy input and accompanied tendencies to re-constitute supermolecular glucan structures, Brabender viscosity was determined for 10% (w/w) aqueous starch suspensions (Fig. 6). Although determination of Brabender viscosity for 10%-suspension is a-typical, such high concentrations were applied because for amaranth a minimum of such concentrations were required to obtain reasonable responses.

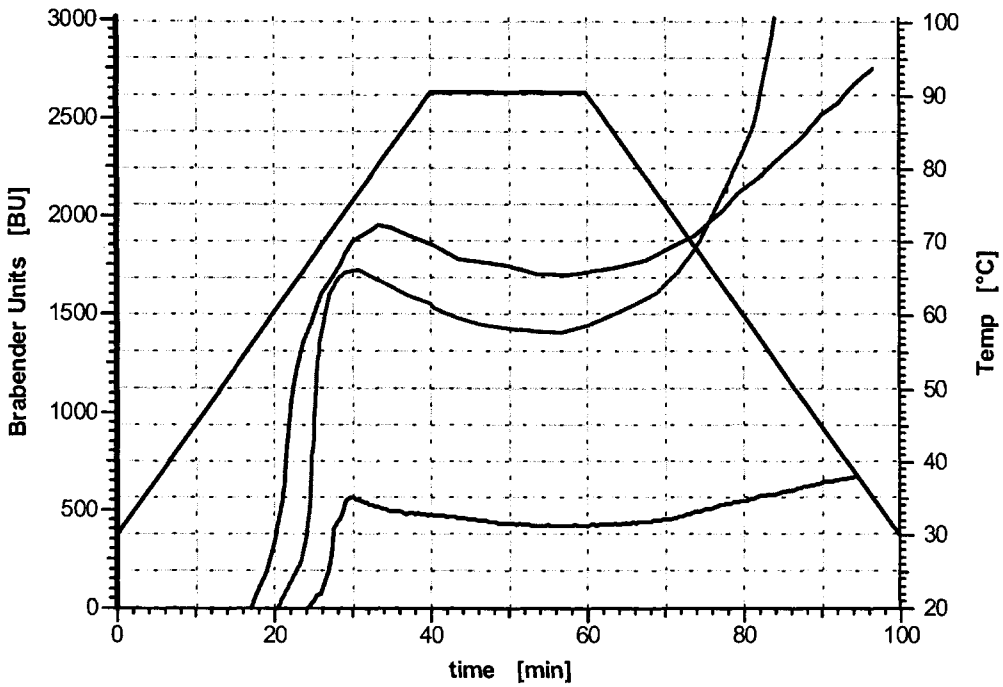

Fig. 6. Amaranth, Quinoa, Buckwheat - Brabender viscosity.

Amaranth forms the most stable structures which stand the applied temperature program significantly better than quinoa: after comparable minor disintegration in the initial temperature raising period, no further significant increase of viscosity was observed. Disintegration of quinoa glucans starts at lower temperatures than for amaranth, but is of similar magnitude. However, different to amaranth, disintegrated glucan/glucan interaction potential of quinoa glucans is high. Such a performance matches with results obtained from iodine-complexation: significantly longer branches for quinoa than for amaranth. The performance of buckwheat in the initial temperature rising period and the subsequent holding period is very similar to quinoa: supermolecular glucan structures of buckwheat are seriously disintegrated, but exhibit a high glucan/glucan-interaction potential. In the cooling period these interactions exceed the detectable scale, indicating a pronounced tendency to re-constitute supermolecular structures.

Resistance against mechanical, chemical and thermal stress

The three 5% (w/w) aqueous starch suspensions were found to be thixotropic. Viscosity decreases if shear stress is applied and regenerates or even exceeds the initial value after shearing is stopped.

In general, the applied shear stress of 1000 s^{-1} for 5 min didn't affect the investigated starches completely (Tab. 3): only loose-stabilized components within the supermolecular glucan-structures became disintegrated. An observed slight increase of viscosity for amaranth and quinoa should be due to a slightly increased reconstitution-tendency of supermolecular structures after shearing. The slightly decreased viscosity of buckwheat suspensions after shearing should be caused by destruction of loose-stabilized supermolecular structures.

Tabela 3

Molecular and technological characteristics of amaranth, quinoa and buckwheat.

	amaranth	**quinoa**	**buckwheat**
molecular characteristics			
iodine complexing potential of high molecular components: E_{640}/E_{525} E_{640}/E_{525} -correlated branching characteristics	0.4 - 0.55 scb	1.3 - 1.4 scb	1.2 - 1.4 scb
iodine complexing potential of low molecular components: E_{640}/E_{525} E_{640}/E_{525} -correlated branching characteristics lcb-glucans [%]	0.5 - 0.55 scb -	1.5 - 1.7 scb -	1.6 - 2.2 lcb 24
weight average molecular weight: M_w [g/M]	$11.8x10^6$	$11.3x10^6$	$15.4x10^6$
relative packing density of molecules	ref = 1.0	0.9	2.6
technological characteristics			
isolated from / provided by	*Amaranthus cruentus*	*Chenopodium quinoa*	*Fagopyrum esulentum*
moisture [%]	12.4	13.2	11.0
glucan content [% dry matter]	97.0	97.9	95.8
Brabender visc. 90→30°C: $[BE]_{30°C}/[BE]_{90°C}$	1.6	1.5	2.7
visc.of 5% aqu.suspension at 95°C [mPas]	122	187	230
stability against shear stress	high	high	medium
acid resistance	non	medium	low
status of starch-suspensions after the first freeze/thaw cycle	pasteous: thin-liquid	pasteous: gelous	gel: stiff
freeze/thaw stability	high	non	high

scb: amylopectin-type short-chain branched glucans; nb/lcb: amylose-type non-branched/long-chain branched glucans; E_{640}: extinction at 640 nm; E_{525}: extinction at 525 nm; M_w: weight average molecular weight;

Different to the applied shear stress, citric acid of pH 3 causes significant changes. Stability of scb-starches amaranth and for the mixed-type scb/lcb-glucans buckwheat was found to be bad. However, glucans of quinoa performed surprisingly well and kept more than half of the initial viscosity.

Consistency of aqueous starch suspensions after an initial freeze/thaw-cycle range from: scb-glucan amranth 'thin-liquid paste', quinoa 'gelly paste' and buckwheat 'stiff gel'. Quinoa liberates water already in the first freeze/thaw cycle of 20%. Quite different in their molecular characteristics, amaranth and buckwheat perform similarly well in a sequence of seven freeze/thaw cycles. These starches obviously form supermolecular structures, which are not collapsing at the applied conditions.

Acknowledgement

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STRUKTURA MOLEKULARNA I WŁAŚCIWOŚCI FIZYKOCHEMICZNE SKROBI PSEUDOZBOŻOWYCH

Streszczenie

Zbadano glukany skrobi pseudozbożowych o istotnych różnicach w ich rozgałęzieniach (amarantus, chinoa i gryka). Ich charakterystykę molekularną skorelowano z właściwościami technologicznymi. Niezmienność konformacji glukanów, zwłaszcza pod wpływem wzrastającej temperatury, odczyn kwaśny i naprężenia mechaniczne rozważano pod kątem wpływu tych parametrów na strukturę molekularną i supramolekularną roztworów skrobi w DMSO.

Skrobiowe glukany wydzielono przez półpreparatywną chromatografię na sefadeksach (SEC) a uzyskane frakcje charakteryzowano za pomocą reakcji z jodem. Amarantus miał krótkie rozgałęzienia (sbc = typ amylopektynowy), chinoa również należała do typu sbc, ale jej rozgałęzienia były dłuższe niż u amarantusa, a gryka była mieszaniną glukanów sbc z 24% domieszką długich rozgałęzień (lcb = typu amylozy).

Ciężar cząsteczkowy (stopień polimeryzacji) skrobi w DMSO został wyznaczony metodą absolutną za pomocą SEC. Badane próbki miały średni ciężar cząsteczkowy bliski 1,2·106 g/M. Rozmiar heliksów glukanów skrobiowych wyznaczony za pomocą SEC w połączeniu z uniwersalną kalibracją wynosił 2–40 nm bez istotnych różnic dla wszystkich trzech skrobi. Mimo tego badane skrobie różniły się istotnie ich miedzy- i wewnątrzcząsteczkowym potencjałem. Wynika stąd, że potencjał oddziaływań zależy w znacznym stopniu od rodzaju rozgałęzienia i upakowania w heliksach od czego zależy struktura supramolekularna.

R. REZLER, H. M. BARANOWSKA, S. SURMA, S. POLISZKO

INVESTIGATION OF STARCH GELS BY MEANS OF THE RELAXATION METHOD

Abstract

Analysis of structural changes of starch gels of the polymer content from 0.05 g/cm^3 to 0.10 g/cm^3 has been performed on the basis of results of a study by the DMTA and ^{1}H NMR methods. For gels of starch concentration below 0.08 g/cm^3, the changes of the rigidity modulus are insignificant; a small increase is observed only in the first three hours of measurements. Gels of a higher starch content are characterised by much higher initial value of the rigidity modulus, which significantly increases with the time of measurements. The values of the rigidity modulus are determined by the density of network segments. In the systems with starch concentration up to 0.08 g/cm^3, the initial and the final concentrations of the network segments show insignificant differences, while for the gels of a higher starch content these concentrations differ significantly.

As follows from results of NMR relaxometry experiment, in the gels of a polymer concentration up to 0.08 g/cm^3 no changes in water dynamics in time have occurred. In those of starch concentration above 0.08 g/cm^3, the relaxation rate of water has changed in time. The maximum in the water relaxation rate observed after 3–4 hours of measurements indicates the process of water fixing in hydrates. A further monotonic decrease of the relaxation rate suggests that the water initially involved in the gel structure formation is evacuated from the starch network. This process should be related to respiralisation of amylopectin chains, whose participation in the gel structure formation increases with increasing polymer concentration. The results suggest that for the starch gels of polymer content lower than 0.08 g/cm^3 the gel structure formed on cooling is saved and not subject to development. In the time range studied no changes in the parameters studied which could suggest the occurrence of starch retrogradation have been observed, which has been interpreted as a result of a small contribution of amylopectin chains in formation of the gel spatial network.

Introduction

In food products polysaccharides may play the structure building role as thickeners, stabilisers, regulators of functional properties etc. [4, 6]. From the physical and chemical point of view the majority of food products are gels. Therefore, recognition

R. Rezler, H. M. Baranowska, S. Surma, S. Poliszko, Department of Physics, Agricultural University of Poznań, 60-637 Poznań, Wojska Polskiego 38/42, Poland.

of the mechanisms of gelation is of great importance. Amongst the gelating products starch obtained from potatoes as well as from grains has the greatest use in food industry. The gelating properties of starch or its isolated components as well as products containing starch have been used in production of deserts, fruit mousse sauces and baking bread. All the products are systems of different water content, which is decisive for spatial structure formation in starch gels. The widespread methods of structural investigation such as X-ray radiography analysis [10, 11], differential thermal and calorimetric analysis [7, 8, 21] and nuclear magnetic resonance [5] provide indirect data which are not sufficient for interpretation of structural conditions of mechanical and rheological properties of biopolymer systems.

Much more information on molecular conditions of mechanical and rheological properties of the structures investigated can be obtained with the use of methods of dynamic-mechanical analysis [9, 13, 14], which can be also used to investigate starch systems [12, 15, 19]. Moreover, in non-reticulated systems the density and stability of links determines mechanical and rheological properties of systems. In order to recognise the molecular processes accompanying the formation of spatial structures of starch, the kinetics of the structuring processes was analysed as a function of the concentration of solutions.

Investigation of starch gels with the NMR relaxometry methods was mainly performed for concentrated systems. For the starch concentrations up to ca. 45% a fast exchange of water, manifested as a monoexponential recovery of magnetization [17, 18] was observed.

This study reports an analysis of the process of spatial structuring of starch in time on the basis of results obtained by the DMTA and NMR methods.

Materials and methods

The study was carried out for samples of starch hydrogels obtained from solutions of starch from the wheat species *Triticum durum* (Sigma) prepared as paste on heating for 3/4 hours under constant stirring. A constant concentration of the mixture was maintained by continuous addition of water to compensate for its loss. The study was performed on the systems containing starch in the concentrations from 0.05 g/cm^3 to 0.10 g/cm^3. Immediately after preparation the solutions were placed in the measuring chamber of the rheometer and in the measuring tubes of the NMR spectrometer. Measurements were begun after stabilisation of the temperature of the gels (1 hour after the end of heating) and performed every 20 min by DMTA and every hour by NMR for 10 hours, at about +20°C.

Measurements of spin-lattice relaxation times $T_1 = (1/R_1)$ were carried out using an NMR pulse spectrometer operating at 30 MHz. The standard inversion-recovery pulse sequence was applied, recording 29 free induction decay (FID) signals for each

preset parameter of the sequence and from 60 to 90 points on the FID signal. The distances between RF pulses were changed from 0.001 to 3 s. The repetition time was 50 s. The relaxation times were calculated using the program Cracspin [20]. For all systems studied a monoexponential recovery of magnetisation was recorded.

In the conditions of sinusoidal variable mechanical interactions of frequency ω, the viscoelasticity of the materials investigated is manifested as a phase shift of the angle δ between strain and stress. So, the stress σ is described by:

$$\sigma = \sigma_o \exp(i\omega t + \delta) \tag{1}$$

while the strain by:

$$\gamma = \gamma_o \exp(i\omega t) \tag{2}$$

where ω is the angular part while δ is the angle of the phase shift.

The ratio of stress to strain is described by the complex shear modulus:

$$G^* = \frac{\sigma}{\gamma} = \frac{\sigma_o}{\gamma_o} e^{i\delta} = \frac{\sigma_o}{\gamma_o}(\cos\delta + i\sin\delta) = G_1 + iG_2 \tag{3}$$

where G_1 and G_2 are the real and the imaginary parts of the modulus, respectively.

The real part G_1 is called the dynamic modulus and it is related to that part of the potential energy of strain, which is saved during periodic deformations. The imaginary part G_2 of the modulus G^* is called the modulus of loss and is related to the part of energy which is dissipated in the form of heat. The values of G_1 characterising a gel network made of flexible macromolecules depend on the number of moles per a unit volume of the macromolecule segments n_s effectively transferring stresses in the network:

$$G_1 = n_s RT \tag{4}$$

The values of the loss modulus G_2 are determined by the local coefficient of viscosity:

$$G_2 = \omega\eta_L \tag{5}$$

The method of dynamical and mechanical analysis (DMA) consists in determination of variability of the modulus in a wide range of sinusoidally variable mechanical interactions.

The results presented in the paper were obtained on the basis of measurements made with an instrument working on the principle of free vibration (the inverse torsional pendulum). For measurements of low strength systems such as hydrated starch (hydrogels), the measuring cells used were in the form of coaxial cylinders. The gel

studied was placed between the walls of cylinders. The outer cylinder was fixed to the base of the instrument, while the inner cylinder was connected to an inertial element with a rod. The inertial element was made of a brazen cylinder hanged on an elastic string fixed to the upper wall of the casing. In the middle part of the stiff rod there was a measuring transducer recording angular displacements of the rod and thus also displacements of the sample investigated. The whole system was fully computer controlled. The mechanical system of the pendulum was fixed to a granite base mounted on a polystyrene table ensuring elimination of external interactions, such as vibrations of the base and other mechanical interactions [16].

The following parameters were measured: frequency of free vibrations f, logarithmic decrement of the system damping with a sample Δ, and without a sample. The values are denoted as f_o and Δ_o. The components of the complex modulus of rigidity were calculated from the following relationships:

$$G_1 = 4\pi^2 I F_g (f^2 F_{dk} - f_o^2) \tag{6}$$

$$G_2 = 4\pi^2 I F_g (f^2 \Delta - f_o^2 \Delta_o) \tag{7}$$

where:

$$F_{dk} = 1 - \frac{\Delta^2}{4\pi^2} \tag{8}$$

and F_g is a coefficient depending on geometric size and shape of a sample:

$$F_g = \frac{R_w^{-2} - R_z^{-2}}{4\pi l} \tag{9}$$

where R_w is the radius of the mobile inner cylinder linked to the pendulum, R_z is the radius of the outer fixed cylinder, l is the height of the gel layer.

Results and discussion

In order to recognise the processes accompanying the formation of spatial structures in starch solutions, taking place from the moment of reaching room temperature, analysis of the structure forming processes was made as a function of the concentration of solutions. The most suitable parameters characterising the kinetics of structure formation were assumed to be the number of macromolecular segments forming the spatial network per a unit volume of the forming gel n_s and a local viscosity coefficient η_L determined by the interaction of the network segments with the solvent and the rest of the polymer.

An increase in the values of the rigidity modulus with the time of crosslinking has been observed for the starch systems of different concentrations of the component (Fig. 1).

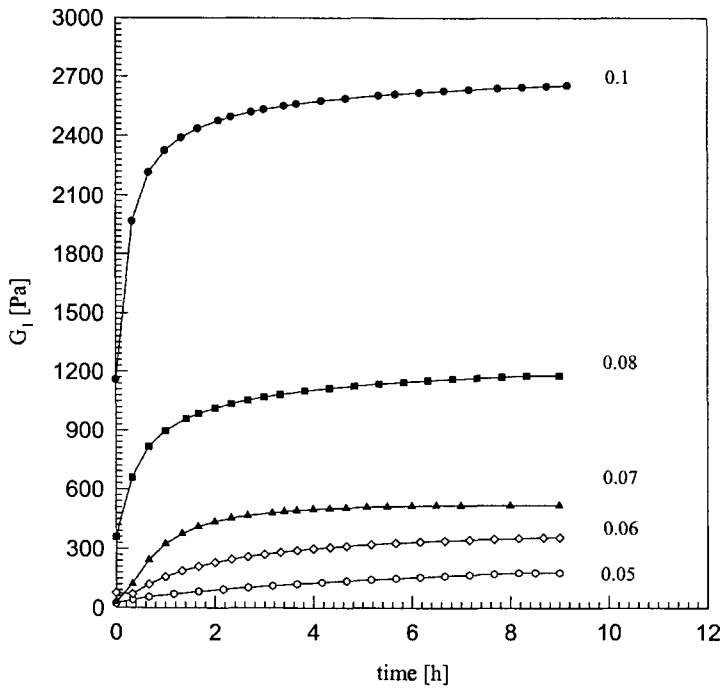

Fig. 1. Kinetics of changes of dynamic rigidity modulus during the process of starch gels formation at different starch concentrations.

As it can be observed in the range of concentrations from 0.07 g/cm^3 the initial values of the rigidity modulus vary very little with starch concentration. A small increase of its values was observed in the first 3 hours of the experiment. In the systems of the polymer concentration up to 0.08 g/cm^3, a significant increase in the initial values of the rigidity modulus was noted with increasing polymer concentration.

The relationship between the rigidity modulus of highly elastic polymer network and the concentration of segments in the network can be approximated by the expression (Ferry, 1970):

$$n_s \cong \frac{G_1}{RT} \tag{10}$$

An increase in the values of the rigidity modulus with the time of crosslinking has been observed for the starch systems. This fact indicates that the concentration of the effective segments in the network increases tending to the asymptotic values $n_{\infty s}(c)$. For the polymer systems the kinetics of crosslinking can be well described by the equation of the Avrami type [1, 2, 3], in which the time exponent is $m \cong 1$:

$$n_s(t) = n_{os} + [n_{\infty s} - n_{os}]\{1 - \exp[-(kt)^m]\} \qquad (11)$$

The value of this exponent means that recrystallisation in these systems runs at the initial stage of nucleation of the crystal forms that do not reach the critical size. Figure 2 presents the relationship between the initial and the final concentrations of segments in the network as a function of the square of the starch concentration.

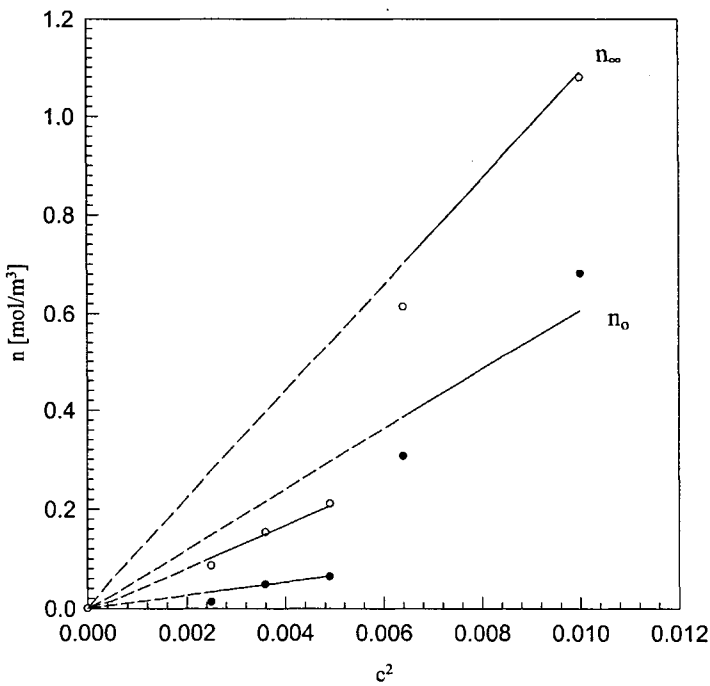

Fig. 2. The initial n_0 and the final n_∞ concentrations of the network segments as a function of the square of starch concentration in the system.

As follows from the plots presented in Fig. 2, the initial n_0 and the final n_∞ concentrations of the segments change linearly with c^2 (where c is a polymer content) and the slopes of the lines depend on the range of the starch concentration. The values of n_0 and n_∞ in starch gels of concentrations lower than 0.08 g/cm^3 show insignificant differ-

ences. For starch gels of higher concentrations the concentration of network segments increased in time. It means that with the ageing of gel, the fragments of macromolecules subsequently joining the network undergo adsorption on the nodes enhancing their functionality. Therefore, for the equilibrium network $n_\infty \sim c^2$, the inclination angle of anamorphoses, depending on the mean functionality of the network nodes $n_s = f(c^2)$, increases.

As follows from this dependence, at a given starch concentration in the system and at a constant temperature, an increase in the gel rigidity with time is a result of the development of the nodes realised by binding of new segments of macromolecules, which leads to an increase in the mean functionality of the nodes stabilising the network. The links between adjacent fragments of different macromolecules in a solution initiate a formation of the spatial network in the systems. The mechanism of the starch network formation involves spiralling of starch fragments and their association to bihelical forms characteristic of native and retrograded starch [22, 23].

For the systems of concentrations lower than 0.08 g/cm^3, long chains of amylose are responsible for formation of spatial structure of the network. With increasing concentration of the polymer in the system also short chains of amylopectin start to contribute in the formation of the spatial network, which is manifested as an increase of the initial concentration of segments in the network n_0. Therefore, the observed increase of the concentration of network segments in time and thus an increase in the rigidity modulus, are related to the formation of the network by chains of amylopectin.

DMTA measurements bring only the information on macroscopic changes in the biopolymer network dynamics. Analysis of the molecular dynamics of water in these systems was possible on the basis of NMR data.

The spin-lattice relaxation rate changes in time for the systems studied are shown in Fig. 3.

Changes of the spin lattice relaxation rates in time are only observed for the systems of the polymer concentration above 0.08 g/cm^3. The relaxation rates increased in the first 3–4 hours of the experiment and then monotonously decreased. The increase in the relaxation rates in the first hours recorded for starch gel suggests that water molecules are captured at the network nodes during the network formation. The later continuous decrease in the relaxation rate testifies to an increase in the water mobility in the systems studied as a result of evacuation of water from the network nodes. For systems with starch content below 0.09 g/cm^3 the relaxation rates are not time-dependent, which suggests that the dynamical state of the water does not change.

The results obtained by NMR and DMTA methods suggest that during the process of retrogradation, water molecules also participate in the initial formation of amylopectin network. The amylose network formed on cooling does not change in time, which explains no changes in the spin-lattice relaxation rate.

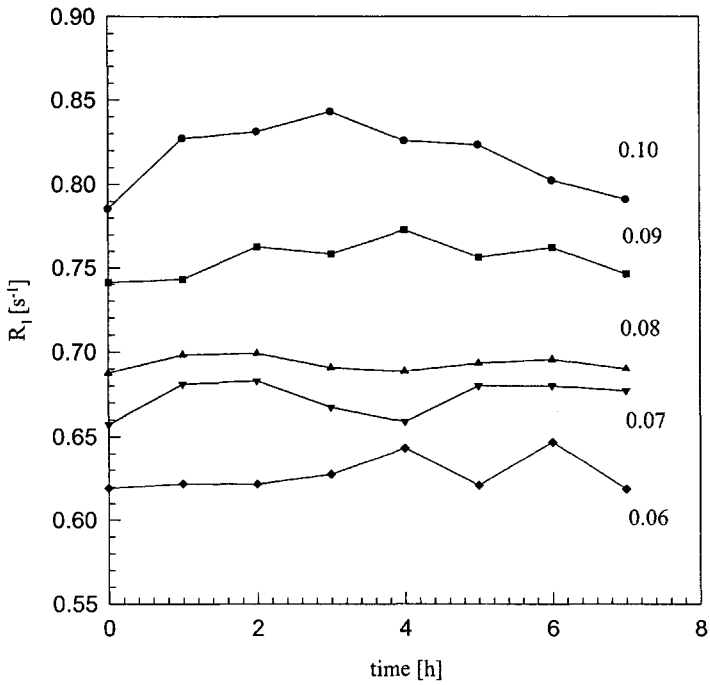

Fig. 3. Changes of the spin-lattice relaxation rates for starch gels in time.

Conclusions

1. Result of the relaxometric measurements of starch gels of the polymer content up to 0.10 g/cm^3 have shown that the process of retrogradation of the system leading to formation of a spatial network at a constant temperature can occur in the systems of a high polymer content (above 0,08 g/cm^3). The phenomenon involves association and spiralling of amylopectin chains into bihelical forms.
2. The process of retrogradation, leading to formation of the amylopectin network, takes place in the first 3–4 hours of the experiment.
3. In the systems studied, the process of amylose retrogradation on cooling leads to formation of a network whose structure does not change in time.

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BADANIE ŻELI SKROBIOWYCH METODAMI RELAKSACYJNYMI

Streszczenie

W prezentowanej pracy podjęto próbę analizy zmian strukturalnych żeli skrobiowych o niskiej zawartości polimeru (od 0,05 g/cm^3 do 0,10 g/cm^3). Badania prowadzono w czasie do 10 godzin po osiągnięciu przez układ temperatury otoczenia.

Technikę DMTA wykorzystano do określenia zmieniających się w czasie parametrów reologicznych, określających stopień usztywnienia struktury żelu.

Stan dynamiczny wody w układzie, w czasie formowania struktury, analizowano wykorzystując technikę ^{1}H NMR.

Uzyskane rezultaty pozwoliły stwierdzić, że dla żeli o koncentracji skrobi poniżej 0,08 g/cm^3 zmiany modułu sztywności są niewielkie, a wzrost jego wartości obserwuje się przez trzy pierwsze godziny trwania eksperymentu. Po upływie tego czasu nie stwierdzono dalszych zmian wartości tego parametru. Żele o wyższej zawartości skrobi (powyżej 0,08 g/cm^3) charakteryzują się dużo wyższą wartością początkową modułu sztywności i obserwuje się znaczny wzrost wartości tego parametru w czasie.

Wartość modułu sztywności determinowana jest gęstością segmentów sieci. Stwierdzono, że w układach o koncentracji skrobi do 0,08 g/cm^3 początkowa i końcowa koncentracja segmentów sieci zmienia

się w niewielkim stopniu. Zróżnicowanie ich wartości można zaobserwować w układach o wyższej zawartości skrobi.

Pomiary relaksacyjne wykonane techniką NMR wskazują, że w żelach o koncentracji polimeru do 0,08 g/cm^3 woda nie zmienia swojego stanu dynamicznego w czasie. Jedynie układy o zawartości skrobi powyżej 0,08 g/cm^3 charakteryzują się zmiennymi w czasie wartościami szybkości relaksacji wody. Obserwowane po trzech pierwszych godzinach trwania eksperymentu maksimum wartości szybkości relaksacji wskazuje na proces uwięzienia wody w hydratach. Dalszy monotoniczny spadek wartości szybkości relaksacji pozwala wnioskować, że woda początkowo biorąca udział w formowaniu struktury żelu jest ewakuowana z sieci skrobiowej.

Uzyskane w badaniach rezultaty sugerują, że w przypadku żeli skrobiowych o zawartości polimeru nie przekraczającej 0,08 g/cm^3 struktura żelu, jaka powstała w procesie ochładzania, jest zachowana i nie ulega rozbudowie. Rozbudowa węzłów sieci w czasie, z udziałem wody obserwowana jest jedynie w układach o koncentracji polimeru przekraczającej 0,08 g/cm^3.

MARIA SORAL-ŚMIETANA, MAŁGORZATA WRONKOWSKA

RESISTANT STARCH OF PEA ORIGIN

Abstract

Possibility of preparation of a pea resistant starch concentrate and its sorption of hydrophobic substances were studied. Pea starch appeared a good source of resistant starch concentrate. The use of thermostable alpha-amylase in technological process provided the preparation containing up to 70% of resistant starch. It contained the admixture of mineral and organic nitrogen compounds. Its crystallographic pattern belongs neither A- nor B-type. The pea-RS concentrate had the affinity to bile acid, deoxycholic, and also to cholesterol although the latter is not as efficient than that of native pea starch. Thus, pea resistant starch concentrate has potential regulatory properties and, therefore, it might be used as a food component in special diets or for preventive, prophylactic, and therapeutic purposes.

Introduction

Starches as the major reserve of polysaccharides in plants are an important and abundant food component. Native granules of starch are predominantly composed of two polysaccharide macromolecules, amylose and amylopectin and remarkably little of any other substances. The precise nature of both macromolecules varies in different sources. Starch as an organic polymer is subjected to physical, or/and thermal and hydrothermal processes in food production. During heating to 100°C the starch granules disrupt and form phase-separated mixtures of amylose and amylopectin. Under low or no shear conditions, the system is probably bicontinuous in amylose and amylopectin [4]. However, after a high shear, the amylopectin becomes continuous, with almost spherical amylose inclusion. This process changes the starch granules, their functional properties and susceptibility to endogenous enzymes and bioavailability. Actual WHO/FAO recommendation states that an optimum diet for humans of all age groups, adults with regular physical activity, except children under two years, should provide at least 55% of total energy from various sources of polysaccharides [9]. In many food-

M. Soral-Śmietana, M. Wronkowska, Polska Akademia Nauk, Instytut Rozrodu Zwierząt i Badań Żywności, Oddział Nauki o Żywności, ul. Tuwima 10, 10-747 Olsztyn.

stuffs about 10% of total starch remains undigested by pancreatic alpha-amylase in the human small intestine. This limited hydrolysis of starches is dependent on intrinsic factors such as type of resistant or indigestible starch. However, extrinsic factors such as viscosity of the environment (meal) are also important. The viscosity affects diffusion of enzymes, size of food particles upon chewing, and passing time through the colon [3, 4].

The origin of starch is of significance for macroscopic functional properties and of importance for biological and technological applications. Grain legumes, such as beans, peas or lentils, in raw and processed form, are characterised by low starch availability *in vitro* [6, 7, 13] and *in vivo* [10]. The pea starch has a limited use in the food production, but it can be used as a preferential source of resistant starch with it potential biological and therapeutical functions in the human gastrointestinal tract [11, 14].

In this paper, the preparation of resistant starch concentrate from isolated pea starch is described. The *in vitro* studies proved the ability of the concentrate to adsorb some hydrophobic substances as well as bile acids and cholesterol. This is an important property from the viewpoint of prophylaxis, prevention and/or therapy of certain diseases.

Material

Pea starch 'Nastar' was kindly gifted from Cosucra S.A., Belgium. It originated from native starch extracted from the kernels of yellow smooth pea. The following enzymes were used: liquid thermostable alpha-amylase from *Bacillus licheniformis* (Termamyl 120L, Novo Nordisk); solid amyloglucosidase (Fluka 10115, 70.7 U/mg, from *Aspergillus niger*); solid alpha-amylase (Sigma A-3176 [EC 3.2.1.1], 28.6 U/mg, from porcine pancreas). Other reagents used in the experiments were as follows: cholic acid (Sigma C-6445), deoxycholic acid (Sigma D-4297), taurocholic acid (Sigma T-9034), L-alpha-lecithin (Sigma P-5394), reagent kits for the enzymatic determination of cholesterol (P.O.CH., Gliwice cat. No 178132140). These were prepared in the solution of 0.05 M phosphate buffer, composed of monobasic sodium phosphate and dibasic sodium phosphate at various pH (from 6.0 to 7.6). Glucose was determined using glucose oxidase-peroxidase (GOPOD) and chromogen kits from Cormay following the manufacturer's instructions.

Experimental pea-RS concentrate. Resistant starch preparation was obtained from commercial pea starch. It was obtained in a laboratory scale in the course of physico-biochemical process using thermostable alpha-amylase (Termamyl 120L) according to the method described for wheat and potato starches [11]. Pea starch was suspended in distilled water (1:3.5), autoclaved (121°C/ 1h) and cooled (4°C/12 h). After 45 min of starch (1g) hydrolysis by thermostable alpha-amylase (0.4 ml Termamyl was diluted in 10 ml of 0.05 M phosphate buffer pH 6.0), the sample was autoclaved (120°C/20 min)

to inactivate the enzyme. After autoclaving the sample was washed several times with distilled water (sample:water, 1:5) to remove soluble α-glucans. The pea-RS concentrate was lyophilised and powdered to particles < 400 μm.

Methods

Chemical components: nitrogen was determined by Kjeldahl method and ash was determined after mineralisation in muflon oven at 700°C according to standard chemical methods [1].

The resistant starch was characterised according to Champ's procedure (the A-method) [2]. The sample (100 mg) was incubated with 500 U porcine pancreatic alpha-amylase at 37°C for 16 h. The products of hydrolysis were extracted with 80% ethanol and the extracts were discarded. Undigested material was dissolved in 3 ml of 2 M KOH and hydrolysed with amyloglucosidase (20 U) at 65°C for 90 min. Free glucose was finally analysed using the oxidase-peroxidase glucose test. Absorbance was measured spectrophotometrically at 500 nm in 1 cm cuvette.

The *in vitro* digestibility of starch preparations was determined using 200 U of porcine pancreatic alpha-amylase per 1 gram of sample. The enzyme solution was prepared in phosphate buffer pH 6.9 (0.05 M) with the addition of $CaCl_2$ (3 mM). The sample (200 mg) was suspended in phosphate buffer pH 6.9 (20 ml) and the alpha-amylolysis was carried out for: 1, 3, 6, 24 hours at 37°C. Prior to hydrolysis, isopropanol (100 μl) was added to the sample to inhibit the growth of microbes during incubation. At fixed time intervals, the sample (1 ml) in centrifuging capped tubes was mixed with 95% ethanol (4 ml) to inactivate the enzyme. The kinetics of hydrolysis was measured as an equivalent of maltose read from the maltose standard curve.

The sorption of bile acids (cholic, deoxycholic, taurocholic) was measured by the *in vitro* analysis. The sample (100 mg) was treated with solution of each bile acid (10 ml). The solutions were prepared in 0.1 M phosphate buffer pH 7.6 for each bile acid in 2 μM/ml concentration. The samples and parallel control samples were incubated at 37°C for 30 minutes.

Centrifugation was carried out at 2000xg for 5 min. The sample (50 μl) was treated on agitation with 70% sulfuric acid (5 ml) and freshly prepared 1 ml solution of furan-2-aldehyde (2.3 g/l). Absorbance was measured at 510 nm after 80 minutes. The results were expressed as per cent of bile acid sorption.

The cholesterol sorption was measured by the *in vitro* analysis. The sample (100 mg) was combined with emulsion composed of: 1% lecithin, 1.375% sodium salt of deoxycholic acid and 0.225% cholesterol prepared in 0.1 M phosphate buffer pH 6.8. (2 ml). The 1-h incubation was carried out on shaking at 37°C. The kinetics of cholesterol sorption by 20 μl emulsion was analysed for 10-minute intervals using reagent

kits. The results were expressed as per cent of cholesterol sorption by the sample at each time interval.

The scanning electron microscope (SEM) analysis was conducted for the native pea starch, pea-RS concentrate from this starch and the pea-RS concentrate after 24-h hydrolysis by pancreatic alpha-amylase. Samples were lyophilised, then mounted on aluminium stubs with double sided adhesive tape, held in nitrogen stream to remove loosely stuck particles, coated with gold in a JEE 400 vacuum evaporator and observed in JSM 5200 microscope at 10 kV.

Results and discussion

Comparison of the chemical content of pea starch and its preparation indicated that the latter was the concentrate of resistant starch with mineral and organic nitrogen compounds (Table 1). Higher levels of ash and nitrogen compounds may result not only from the concentration of starch components but also from the contribution of commercial enzymatic preparation Termamyl. However, the level of particular components in resistant starch concentration is dependent mostly on botanical source of starch granules. This is proved by comparing the pea-RS concentrate obtained from pea starch in this study with the preparations previously obtained from wheat and potato starches [12]. If we assume the resistant starch in the pea-, wheat- and potato-preparations as the major component, then at similar amounts of RS obtainable from respective starches the smallest amount of the accompanying was found for the pea starch preparation. The content of nitrogen and mineral compounds was twice as high in wheat RS-preparation and three times higher in the potato one [12]. The crystallographic pattern in the X-ray diffractograms was untypical. It was neither of the A- nor B-type [G. Lewandowicz, unpublished data]. Comparison of microelectronograms (SEM) of native pea starch granules (Fig. 1) with the pea resistant starch concentrate (Fig. 2a, 2b) allowed to observe fine granular subunits in the structure of the preparation (Fig. 2a) as well as crystalline shells with granular subunits on the edges of the surface (Fig. 2b). This picture was similar to that described by Gallant et al. [5], who studied alpha-amylolysis of granular starch of different origin. From 24-h hydrolysis of pea-RS concentrate with pancreatic alpha-amylase more compact structure resulted together with a decay of granularity as well as appearance of clear fragments of feather-like (Fig. 3a) and filament (Fig. 3b) characters. The kinetics of amylolysis (Fig. 4) shows that the availability of native pea starch for pancreatic alpha-amylase was similar to that of native wheat starch [12]. It was confirmed also by the way the enzyme attacked the granules of pea and wheat starches [11]. On the other hand, pancreatic alpha-amylolysis of pea-RS concentrate was similar to that of native potato starch considered a reference resistant II-type starch. *In vitro* enzymatic availability allows to

assume that the pea-RS concentrate closely resembles native potato starch having large amount of RS that is highly resistant to pancreatic alpha-amylase.

Table 1

Chemical composition of native pea starch and pea resistant starch concentrate[1].

Sample	Nitrogen [% d.m.]	Ash [% d.m.]	RS content in sample [% d.m.]
Native pea starch	0.2 ±0.02	0.1 ±0.01	42.6 ±1.2
Pea-RS concentrate	1.3 ±0.04	4.8 ±0.04	69.9 ±2.4

[1] Values given are means of four replications; ± standard deviation

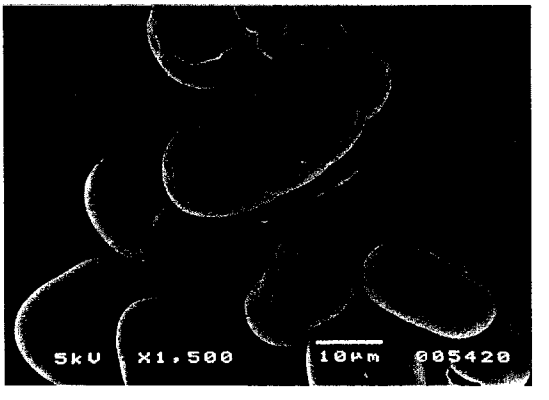

Fig. 1. SEM-microelectronogram of native pea starch.

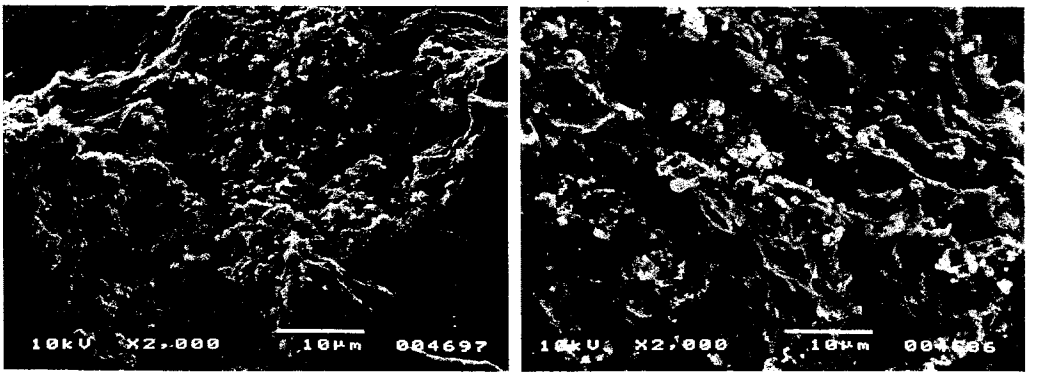

Fig. 2a; 2b SEM-microelectronograms of pea resistant starch concentrate.

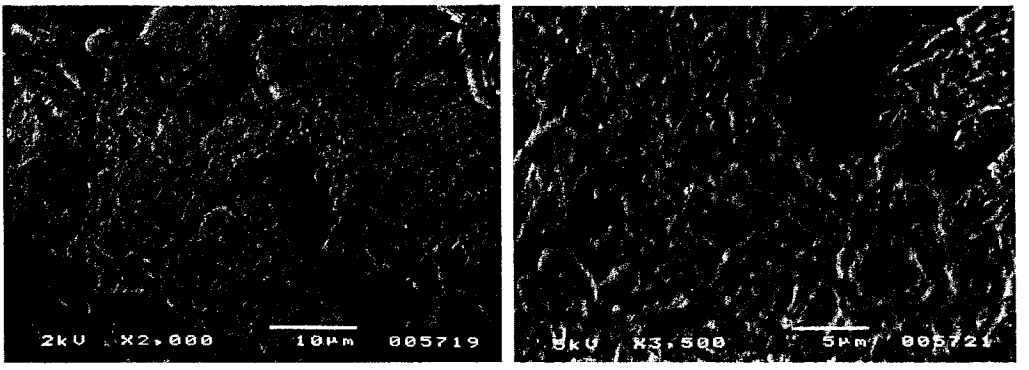

Fig. 3a; 3b SEM-microelectronograms of pea resistant starch concentrate after 24-hour hydrolysis by pancreatic alpha-amylase.

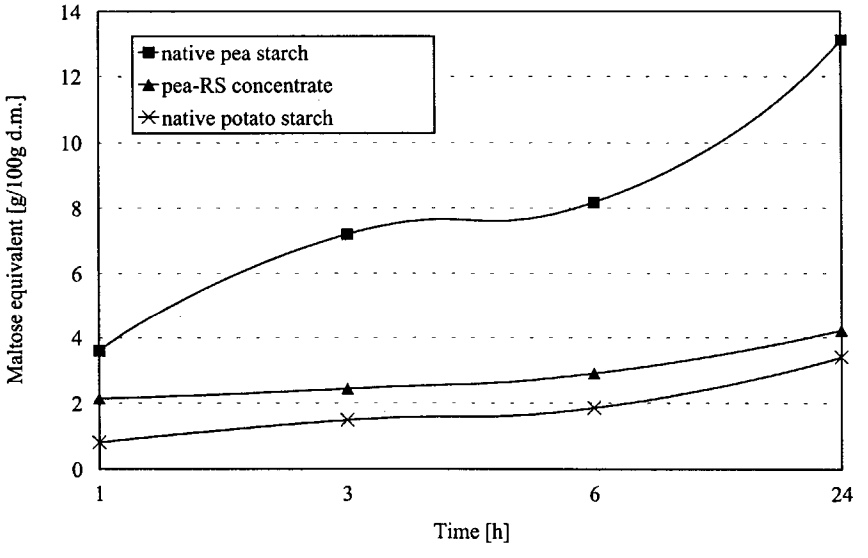

Fig. 4. The kinetics of hydrolysis of native pea starch and pea resistant starch concentrate.

Table 2

Sorption of the bile acids by native and pea resistant starch concentrate[1].

Sample	Cholic acid [%]	Deoxycholic acid [%]	Taurocholic acid [%]
Native pea starch	12.79 ±4.0	4.30 ±2.2	3.83 ±1.5
Pea-RS concentrate	0	11.62 ±3.6	0

[1] Values given are means of four replications; ± standard deviation

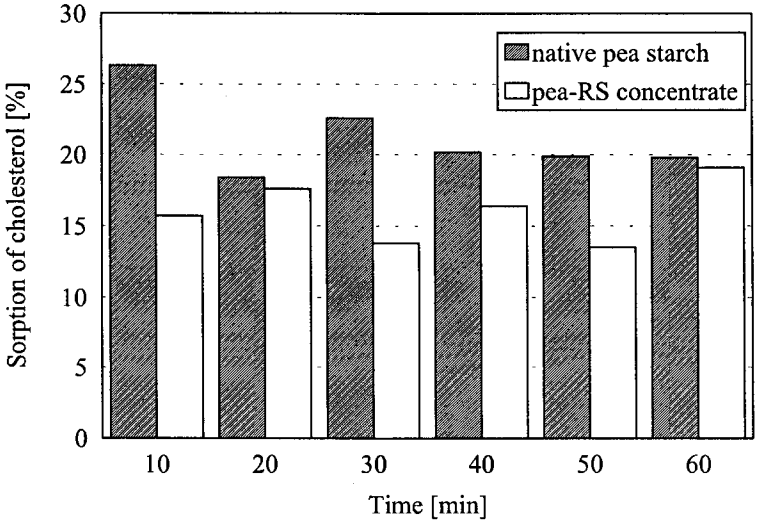

Fig. 5. Sorption of cholesterol by native pea starch and pea resistant starch concentrate.

Therefore, it was interesting to estimate the affinity of pea starch and experimental pea-RS concentrate to some hydrophobic substances such as certain bile acids and cholesterol. The results obtained for sorption of bile acids indicated considerable sorption of cholic acid by pea starch and three times waeker sorption of deoxycholic and taurocholic acids (Tab. 2). The sorption of deoxycholic acid by pea-RS concentrate was significant. The sorption of bile acids on pea-, wheat-, and potato-RS preparations is significantly different despite that the pea preparation resembled the potato-RS in this respect and in the sorption of deoxycholic acid [12]. Sorption of this acid may be of great importance in prevention of the large intestine diseases, especially in the case of patients after cholecysteomy. Present results together with our previous study [14] indicate that pea starch subjected to physical modification or physico-biochemical process has great affinity to bile acids, especially deoxycholic or taurocholic acids, which are considered as carcinogenic agents in the environment of the human intestine.

The sorption of cholesterol by native pea starch was satisfactory already after 10 min (Fig. 5). Less cholesterol was bound by the pea-RS concentrate, reaching the maximum within 1 hour. As attempted in our previous paper [8], a hypothetical model of interaction between processed starch and cholesterol is suggested here to explain the formation of specific complex with the hydrophobic tunnel domains.

Conclusions

1. Pea starch can be a good source for resistant starch concentration. Technological process with thermostable alpha-amylase provided the preparation containing up to

70% of resistant starch. It contained admixture of mineral and organic nitrogen compounds.

2. Experimental pea-RS concentrate had the affinity to secondary bile acid, deoxycholic, which considered as cancerogenic agent. Such RS-preparation has the ability for cholesterol sorption, although weaker than that of native pea starch.
3. Results of this investigations suggest that pea resistant starch concentrate may have the regulatory properties towards some hydrophobic substances. Its health-promoting properties suggest the potential use as a food component in special diets or for preventive, prophylactic, and therapeutic purposes.

Acknowledgements

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SKROBIA AMYLAZOOPORNA POCHODZENIA GROCHOWEGO

Streszczenie

Badane były możliwości uzyskania z izolowanej skrobi grochowej koncentratu skrobi amylazoopornej (RS - resistant starch) oraz określenie zdolności sorpcji niektórych substancji hydrofobowych. Stwierdzono, że skrobia grochu może być dobrym źródłem do koncentracji skrobi amylazoopornej (RS). Zastosowanie w procesie technologicznym termostabilnej alfa-amylazy umożliwia uzyskanie preparatu o zawartości ok. 70% RS. Koncentrat RS zawiera domieszkę składników mineralnych i organicznych azotowych. Wzorzec struktury krystalicznej jest nietypowy, i nie należy ani do typu A ani do typu B. Zaobserwowano, że koncentrat ten charakteryzuje się powinowactwem do kwasu żółciowego - deoksycholowego, któremu przypisuje się właściwości kancerogenne. Wykazuje też zdolność sorpcji cholesterolu, jednak nie większą niż natywna skrobia grochowa. Tak więc, grochowy koncentrat skrobi amylazoopornej (RS) może wykazywać potencjalne właściwości regulacyjne i może być użyty jako komponent do żywności w dietach specjalnych lub znaleźć zastosowanie profilaktyczno-terapeutyczne.

J.J.G. VAN SOEST*, Y. DZIECHCIAREK, A.P. PHILIPSE

STARCH-BASED MICROPARTICLES: A PRELIMINARY STUDY OF THE STRUCTURE AND PROPERTIES

Abstract

Starch is a cheap and abundant polysaccharide, which is found in nature as water insoluble semicrystalline granules with sizes in the range of 0.5-70 μm. Although starch is easily gelatinised or dissolved in water, it is not possible to obtain stable suspensions or colloidal systems from native starches. This inherent disadvantage of starch has limited its applications.

In this study potato starches were processed to obtain fully biodegradable microparticles, which behave as microgels or colloids in aqueous suspensions. The synthesis process is based on the unique combination of gelatinization and cross-linking performed in water-oil emulsions. The obtained starches are very stable in water and show an interesting shear-thinning behaviour even at high solid contents. In particular, the rheological behaviour of the new starches is unique. The starches offer new possibilities for preparing starch colloids with a range of properties. A range of starch microparticles was obtained opening the door to numerous food and non-food markets (paints and coatings, inks and pigments, superabsorbent polymers, food additives, personal care products, pharmaceuticals, ceramics, paper additives, adhesives, thickeners, emulsifiers, ...).

In order to make a wide-scale industrial use of these new materials possible, it is necessary to acquire detailed knowledge about the structure and properties of the prepared particles. By a multidisciplinary approach, a start was made on their process-structure-property relationships. The final goal of this work is to establish the relationships between synthesis parameters and the structural, colloidal and rheological features.

Particles were prepared using epichlorohydrin and trisodium trimetaphosphate as cross-linkers. In this paper important reaction parameters, such as temperature, time and composition of the reactants (starch, cross-linker, hydroxide), which influence the structure of the microparticles during synthesis, were identified. Using Bohlin reometry the formation of the starch network structure was studied. Particle sizes of the microgels are in the range of 60 nm up to 10 μm. The synthesised particles were slightly negative (in the range of - 5 to - 45 mV). Features such as size and charge of the particles depended on the type and amount of cross-linker used. Descriptions of the rheological properties of starch-based microparticles in aqu-

*corresponding author.

*Agrotechnological Research Institute (ATO), P.O. Box 17, 6700 AA Wageningen, The Netherlands. E-mail: J.J.G.vanSoest@ato.wag-ur.nl

A.P. Philipse, Van't hoff Laboratorium for Physical and Colloidal Chemistry, Padualaan 8, 3584 CH Utrecht, The Netherlands.

eous suspensions, both in dilute and concentrated systems, were given. The microgel-type particles showed a behaviour that is typical for (slightly) charged materials or polyelectrolytes.

Introduction

Fully biodegradable starch-based microparticles only became the subject of detailed studies over the last decades [1, 2]. Microparticles can be applied in a range of products. Typical applications are: copying paper, detergents, adhesives, cosmetics, foods, biomedical and pharmaceutical products, controlled release systems, ceramics, et cetera [3-6]. Microparticles give the opportunity to obtain unique colloidal and rheological properties when applied in suspensions or emulsions. The stability of suspensions is increased and mixing of immiscible ingredients is facilitated. Starch and most starch derivatives are favoured over synthetic polymer based materials because they are biodegradable and biocompatible [7].

Most native starches are composed of 20–30% amylose, an almost linear polysaccharide consisting of α-1,4-linked anhydro-glucose with a molecular mass of 10^3–10^6 g/mol, and of 70–80% amylopectin, a highly branched, high molecular mass (10^8 g/mol) polymer consisting α-1,4 linked anhydro-glucose and α-1,6 linked anhydro-glucose at the branching points [8]. Native starches are isolated from various sources like roots, tubers and seeds in the form of semicrystalline, cold-water insoluble granules. At present, the use of starches for many applications is limited. Native starches need to be heated before use to free the starch polymers. Gelatinized or melted starches show gelation, phase separation, retrogradation or crystallisation. Products from starch show problems with regard to their stability during storage or use. The properties of starch-containing materials are highly water and temperature sensitive. It is not possible to obtain highly stable aqueous starch suspensions from unmodified starches. Processing of starches is difficult because of the high viscosity and shear-thickening of starch already at low solid contents. The range of functional properties of the starches is limited even for chemical modified starches with high degree of substitution [9, 10].

Preparation of microparticles dates back to the fifties, where they were used in carbon-less copying paper [11]. Several manufacturing methods were developed since then using a broad range of monomeric as well as polymeric starting materials [12]. In particular, emulsion polymerisation and cross-linking (X-linking) methods are used and well understood for synthetic materials but also several biomaterials, such as agarose and cellulose [13-14].

There are several options available for preparing starch-based microparticles based on water-in-oil emulsion X-linking. In the first option, soluble starches are grafted with active groups, which can be X-linked. Usually this renders the materials non-

biodegradable or non-biocompatible. The second route makes use of chemically modified starches, such as carboxy methyl starch, containing groups, which can be internally X-linked by heat-treatment or complexation with salts. These materials are usually not very stable in aqueous environment. In the third route, the hydroxyl groups of the starch chains or other chemically introduced groups react directly with a X-linking agent in a water-in-oil emulsion. An extensive list of X-linkers is known for starches among which phosphoryl chloride, epichlorohydrin (ECH) and trisodium trimetaphosphate (TSTP) [15-17].

The X-linked starch microparticles are swellable in water and can be considered as microgels. Although no detailed studies have been performed on the influence of structural features, such as the influence of X-link density on rheological properties, some studies have been performed over the last decades for instance poly(methyl methacrylate) microgel systems [18-19].

In this paper, microgel particle materials will be prepared using emulsion X-linking of potato starches. A start was made in characterising and understanding these materials. The preliminary results will be presented. The influence of processing conditions will be discussed in relation to the structure and properties.

Materials and methods

Materials – Starches (Native *(Farina)* potato starch (PN), Paselli SA2 (Pa2) and Paselli SA6 (Pa6)) were supplied by Avebe (Foxhol, The Netherlands). The moisture contents of the starches were determined gravimetrically as 17.5, 15.0 and 15.0% for PN, Pa2 and Pa6, respectively.

Preparation of the particles – Two main types of X-linkers were used in this study, namely TSTP and ECH. The typical lay-out of the preparation of the various microparticles is shown in Figure 1.

Route one was typically used to prepare only TSTP X-linked materials. Starch (x g) was dispersed in 250 ml demiwater (deionized water).by stirring. Subsequently x g of TSTP was added as the X-linking agent. Cyclohexane (650 ml) was heated to 50°C after which 16.5 g Span-65 (sodium tristearate) was added. The hydrophobic and water phases were emulsified using a Branson emulsifier for 5 min. The white emulsion was stirred with a magnetic stirrer to prevent coagulation. During this x ml of 2 M sodium hydroxide (OH) was added. Reaction proceeded over night after which a stable slightly blue dispersion was obtained. Separation of the water phase from the hydrophobic phase was done with the addition of acetic or octanoic acid and shaking with excess water. The water phase was separated by centrifugation (Beckman centrifuge, Avanti J-251) at 10,000 rpm (3–4 times with intermediate washing with demiwater or sodium chloride solution). The purification was improved by adding ethanol. The white starch sediment was air-dried.

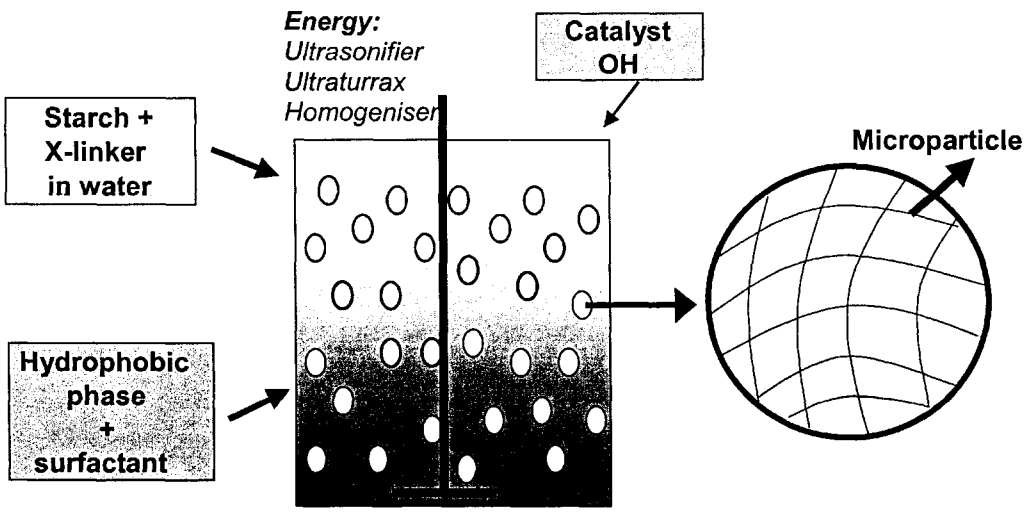

Fig. 1. General scheme of the synthesis route used for the preparation of microparticles.

ECH based (and some TSTP materials as comparison materials) particles were typically prepared by dispersing x g starch in 100 g OH (25 mM) aqueous solution. The mixture was stirred at 800 rpm until the starch was homogeneously distributed. ECH (x g) was added. After homogenisation, the water phase was poured into a 2-neck round-bottom flask containing ca. 5–7 g Symperonic NP5 (poly-oxyethylene nonphenyl ether) surfactant in 200 ml cyclohexane. An emulsion was obtained with an Ultra-Turrax (Janke & Junkel, IKA, type T25) initially operated at 13000 rpm. The speed was increased to 20000 rpm over a period of 10 min. The emulsion was cooled to ensure that the temperature was not higher than 45°C.

The reaction took place at room temperature under constant stirring (magnetic stirrer at 800 rpm). After 20 h, 250 ml demiwater/acetic acid was added to separate the cyclohexane phase from the water phase. The pH of the water phase was adjusted to 7. The aqueous milky suspension was centrifuged at 10000 rpm for 30 min. The particles were washed at least 3 times with demiwater. The washed materials were usually dialysed (membrane diameter 28.6 mm, MWCO 12–14 kDa, Fischer Scientific) at 4°C.

The exact compositions of the reactants are expressed in x grams of the various components used per 100 g water used in the emulsion during reaction. For example: the material referred to as **20**Pa2-**1**ECH-**1**OH means that 20 g of Paselli SA2, 1 g of ECH and 1 g NaOH was used during synthesis.

Structural analyses – Light microscopy was used to make rough estimates of the particle size and shape using a Zeiss axioplan MC1000 microscope. More detailed

studies were performed using a transmission electron microscopy (TEM; Zeiss) and a cryo-scanning electron microscope (SEM; Philips type 5.15). An environmental-SEM (ESEM; Philips) was used to study the swelling of particles (containing 15% water) at various relative humidities (RH = 20–100%).

X-ray diffractometry (XRD) was performed on dry materials with a Philips PV-ARD diffractometer (model PW 3710) [20].

Dynamic light scattering (DLS) was performed at the University of Utrecht (van't Hoff Laboratory for Physical and Colloidal Chemistry) with an Ar laser at 541.1 nm (Spectra Physics Series 2000). Auto-correlation functions were measured with a Malvern multibit K7025 128 correlator at scattering angles between θ = 35–120°. Static light scattering (SLS) were also studied in Utrecht with a Fica-50 LS photometer using vertically polarised incident and detected light at a wavelength of 546 nm. The scattering angle was varied between 30–150°θ. The Guinier approximation was used to calculate the radius of gyration (R_g). The samples were filtered through a 10 μm millipore filter. All measurements were performed at 25°C.

Rheology – Dynamic oscillation viscosity measurements were performed with a Bohlin VOR Rheometer using a cylindrical geometry. The starch gels were covered with a thin layer of paraffin oil to prevent water from evaporating. By measuring the elasticity modulus (G') of the polymer solution versus time, during which the X-linking reaction reactions of TSTP and ECH with starch takes place, it is possible to estimate the number of X-links formed and the efficiency of the reaction [15-17].

High-shear rheological experiments were performed on concentrated suspensions (1–10% w/w) using a Contraves Rheomat 115 Viscometer at room temperature with a shear rate (γ) in the range of 1–500 s^{-1}.

Low shear viscosity measurements were carried out with a constant shear Contraves LS40 Rheometer in a MS 41/1S Couette geometry at a constant temperature (20.0 ± 0.1°C). The shear rate (γ) was in the range of 0.1–10 s^{-1}. Using a Finn pipette, 2 ml of the samples were put into the measuring device. The flow curves were measured successively for increasing and decreasing shear rates. The relative viscosity, η_r, was determined as the slope of a linear fit of the viscosity data for the dispersion, η_{disp}, and the solvent (*i.e.* water), η_{solv}:

$$\eta r = \frac{\eta disp}{\eta solv} \tag{1}$$

Electrophoretic Light Scattering (ELS) – The Zeta-(ζ)-potential of the starch particles in salt-free and sodium or potassium chloride suspensions were measured with a Coulter Delsa 440 SX electrophoresis meter. From these measurements the ζ-potential was calculated using the Smoluchowski relation [21].

Results and discussion

X-linking reaction of starch

To be able to study the differences in the X-linking reaction as a function of starch and X-linker type and amounts, and the amount of hydroxide used, the G'-modulus was measured in time. This part of the research is important to understand the structural properties of the microparticles in terms of X-linking density and charge density of the hydrogel network. The assumption here is made that the particles can be visualised as microgels. In Figure 2 (top) some typical examples are shown of the increase in G'-modulus during reaction of starch (Pa-2) with ECH. The initial reaction rate is clearly dependent on hydroxide concentration. An increase in reaction rate is found with an increase in the OH concentration from 0.1 to 0.3 g/l. However at higher OH concentrations no further increase in the rate of the reaction is observed. The effect of starch and X-linker concentration and source shows a similar behaviour (data not shown) [16-17]. With increasing starch and X-linker concentration the initial reaction rate increases up to a certain starch concentration.

Usually a plateau or maximum in the G'-modulus is observed. The height of this maximum or plateau is determined by the composition of the reactants and temperature of the reaction. A decrease in the G'-modulus is observed at higher temperatures and reaction times and at high hydroxide concentrations. This is most likely due to molecular breakdown (hydrolysis) of the starch or X-links.

As a first approximation the Flory-Rhener swelling equilibrium theory is used to determine the X-linking efficiency [16-17]. According to this theory, the plateau G'-modulus is linear correlated with the number of elastically covalent entanglement points and inversely with the molecular mass between two entanglement points. Some preliminary results are shown in Figure 2 (bottom). It is shown that the effectiveness of the X-linking of starch with TSTP and ECH is low. The highest values obtained within this and other studies are 15% for ECH and 48% for TSTP, respectively [16-17]. The X-linking efficiency increases with increasing starch and X-linker concentration. An initial increase is found with increasing OH concentration. However, at high OH concentration the efficiency decreases. The effectiveness of TSTP seems to be higher than of ECH.

The low efficiency of the X-linking of starch is thought to be partly due competing hydrolysis of starch and the X-links and the formation of (short) free chain ends (*i.e.* single sided glycerol-linkages and phosphate mono-esters with different chain lengths). Part of the X-linker could have not reacted with starch at all. Furthermore the deviation from an ideal network attributes to imperfections in the gels, such as micro-heterogeneities, which can be the result of loops, loose ends (amylopectin outer branches), physical entanglements, and micro-aggregation of X-links.

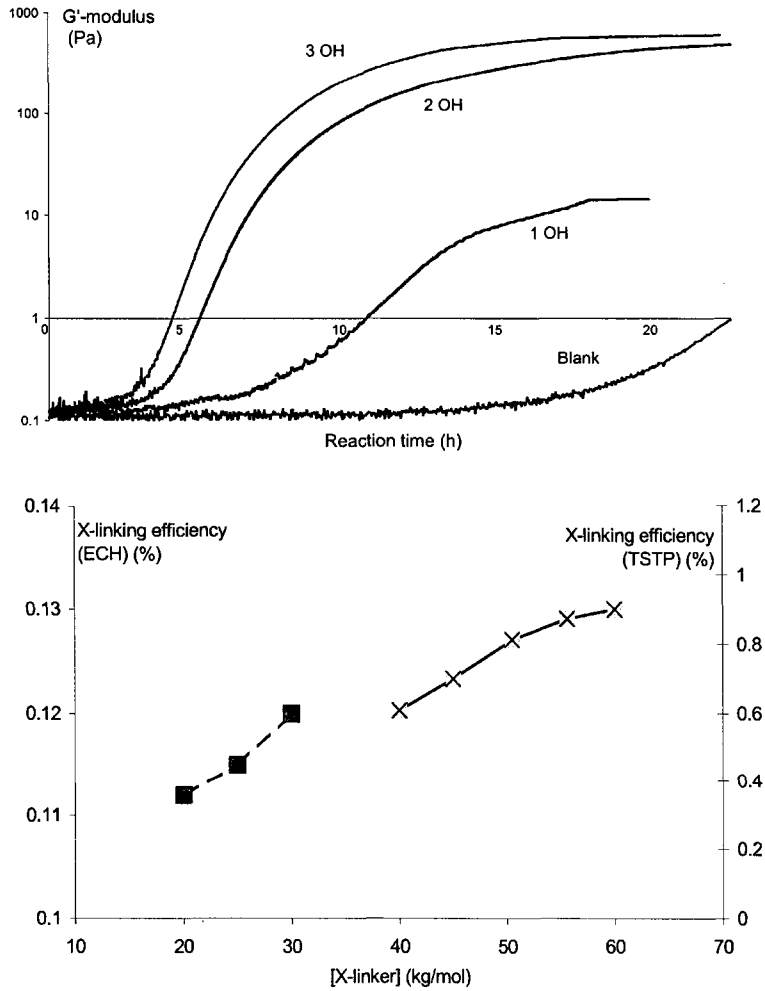

Fig. 2. Bohlin measurements. Top: The influence of hydroxide concentration (OH) on the G'-modulus during reaction. The blank is without the X-linker. The material composition used was: **20**Pa2-**2**ECH-*xOH*. Bottom: Estimation of the X-linking efficiency (expressed as the ratio of the number of calculated or effective X-links and the number of theoretical X-links times 100%) calculated on the basis of Bohlin reometry measurements using the Flory-Rhener approximation. A comparison of ECH and TSTP X-linked materials (**20**Pa2-**2**OH).

Structural characterisation

The starch-based microparticles were analysed by XRD. It was shown that the potato starch based materials were completely amorphous after purification and drying.

Apparently the X-linking reduces the retrogradation of the starch, which typical occurs during processing of gelatinized starches at high water contents.

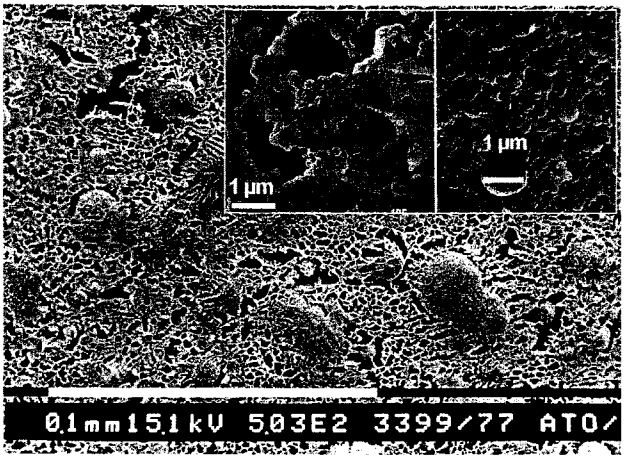

Fig. 3. EM photograph of TSTP-based microparticles after dispersing and swelling in demiwater. Inserts on the top right: EM photograph of TSTP-based microparticles after dispersing in water and freeze-drying the dispersion to about 12% water. The particle size is in the range of 150-800 nm.

Several microscopic techniques were used to study the size and shape of the microparticles. With light microscopy it was shown that the particles in suspension have a size below 10 µm. Figure 3 shows a typical example of a SEM study showing the appearance of a TSTP X-linked material after swelling in demiwater. Typical sizes are found for this material prepared with a low amount of X-linker in the range of 1–5 µm. A cryo-SEM is shown of more densely X-linked TSTP particles in Figure 3 (inserts on the right top side). More detailed studies showed that the particle size is depending on the starch/X-linker ratio. The particle size is clearly dependent on the degree of swelling in water, which was confirmed by ESEM at various RH. With increasing RH the microparticles increase in size as is seen in Figure 4. The studied ECH-based particles were spray-dried to obtain rather large and dented particles. The spray-dried material is shown in Figure 4 (Top left). The particles swell and form aggregates by increasing the humidity. By increasing the ECH concentration during synthesis (to 4.9 g/100 ml), it was shown that the swelling of dried particles was reduced significantly.

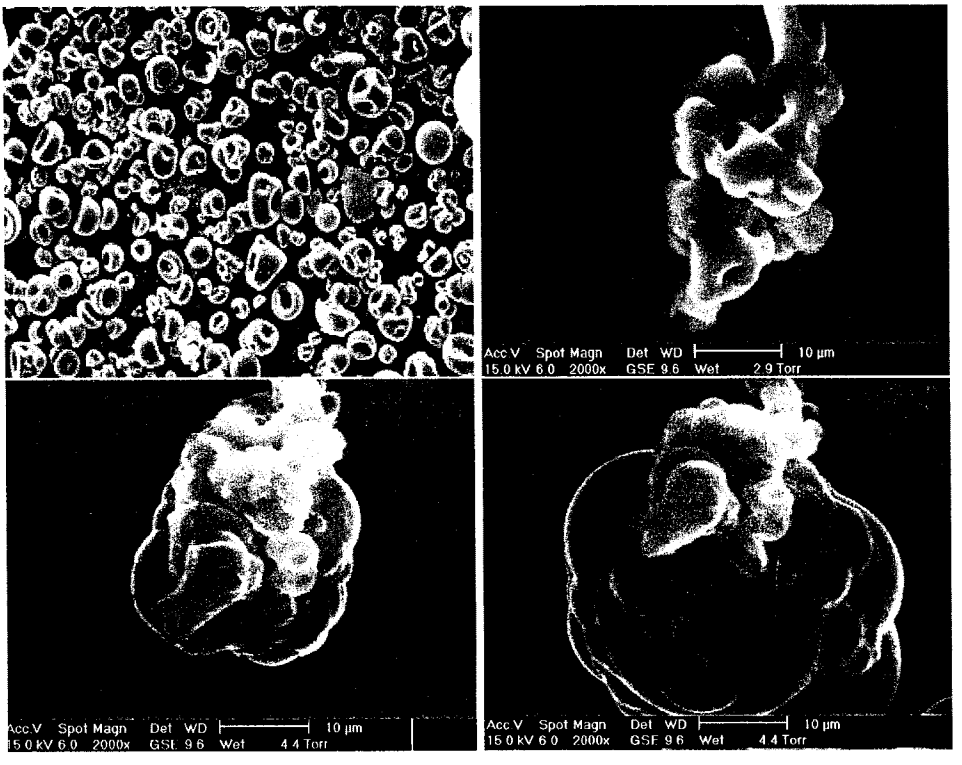

Fig. 4. (E)SEM photographs of the swelling behaviour of ECH X-linked microparticles (**20Pa2-1ECH-2OH**). Top left: SEM of spray-dried material. Top right, bottom left and bottom right: ESEM with increasing RH.

Table 1

The particle radius of two TSTP X-linked microparticles.

Composition	Average R_g (nm)	Polydispersity (%)
15Pa6-2TSTP-1OH	154	33
2.5PN-2TSTP-1OH	263	27

Using light scattering techniques typical particles sizes were measured in the range of 60–1000 nm. Two typical examples of the particle sizes measured with DLS of TSTP X-linked materials are given in Table 1. Characteristic SLS particle sizes of ECH X-linked materials as a function of amount of ECH are shown in Table 2. No linear relation is found. The X-linking efficiency of ECH with starch is low (as is shown in previous section) and not linearly related to the amount of reactants used over the whole range.

Table 2

The particle sizes of ECH X-linked materials (**20**Pa2-*x*ECH-**2**OH) as a function of ECH concentration during synthesis.

ECH (x)	ECH (weight fraction)	R_g at low angles (nm)	R_g at high angles (nm)	R_g ratio
0.5	0.004	90	204	0.44
0.5	0.004	76	140	0.55
1.0	0.008	70	129	0.54
2.0	0.016	69	140	0.49
4.0	0.032	62	189	0.33
4.9	0.039	84	192	0.44

It was shown that typically ECH X-linked microparticles had a ζ-potential in the range of -1 to -10 mV. While TSTP-based microparticles were in the range of -15 to -45 mV. The materials are thus slightly charged. Because of the presence of the phosphate groups in the TSTP-based materials, these materials are somewhat higher in charge density. Because of the inaccurate results obtained no clear relationships were found with the structural features of the various microparticles. The swelling of the particles in water is mainly due to the presence of the charges (osmotic pressure effect).

Rheological properties of starch microparticles

In Figure 5 (top) the viscosity as a function of concentration of TSTP-based microparticle (**40**Pa6-**8**TSTP-**1**OH) suspensions is shown for concentrated systems. The dispersions show shear-thinning behaviour at relatively high solid contents. The influence of salt concentration on the viscosity of ECH-based (**22.2**Pa2-**1.9**ECH-**1.1**OH) suspensions is shown in Figure 5 (bottom). The viscosity is significantly lower than the viscosity of the TSTP-based suspension shown in the Figure, which can be explained by the larger particle size and hydrodynamic volume of the TSTP-based microparticles. Visible is the effect of introducing low amounts of salt. The viscosity is reduced by a factor two at a shear rate of approximately 100 s^{-1}. Also the suspensions become less shear thinning. Because of the slightly negative particles, the hydrodynamic particle size is reduced by the addition of the salt. This is in agreement with the observed reduction of the swelling capacity of starch cross-linked hydrogels with increasing salt concentration [14-16]. This effect is even more pronounced for TSTP-based systems. The cross-linked microparticles show a characteristic behaviour of a ionic polymer.

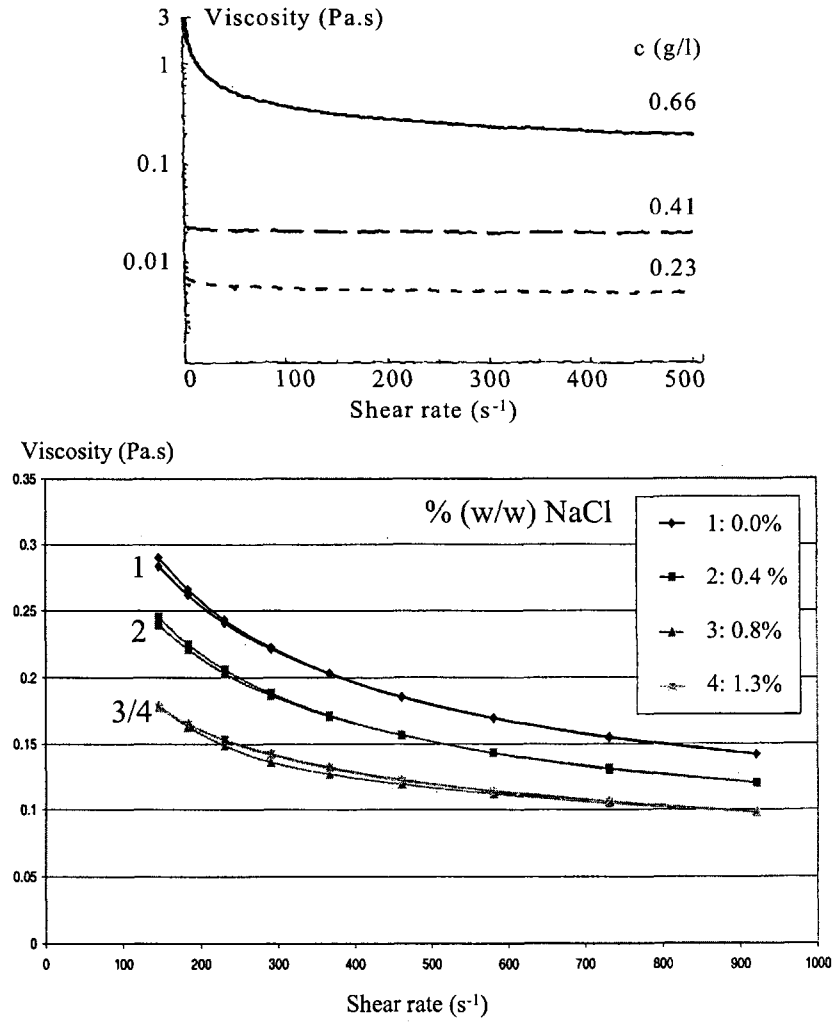

Fig. 5. Rheology of TSTP-based microparticle suspensions (**40**Pa6-**8**TSTP-**1**OH): Top: viscosity vs. shear rate. Bottom: Influence of sodium chloride concentration on the viscosity of a dialysed starch microparticles (**22.2**Pa2-**1.9**ECH-**1.1**OH) suspension (7.3 g/l). The amount of sodium chloride (%w/w NaCl) is indicated in the insert.

In Figure 6 an example of the relative viscosity (η_r) as a function of concentration is given obtained from low shear rheological experiments on dilute aqueous suspensions of ECH-based microparticles (with a size of circa 100 nm). For these samples a linear behaviour of the shear stress versus shear rate is obtained in the range of 0.1–50 s^{-1}. The specific viscosity shows a linear dependence on the concentration in dilute

systems. The slope of is depending on the amount of X-linker used during synthesis of the microparticles as is seen in Figure 6 (insert).

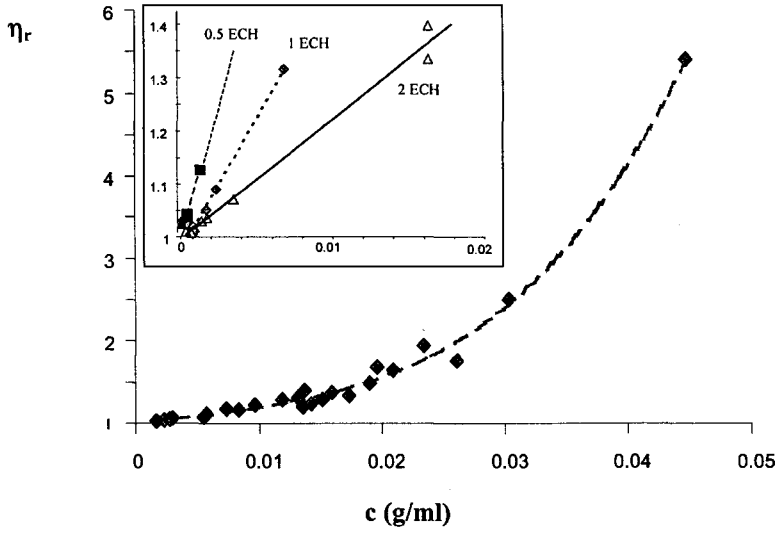

Fig. 6. Relative viscosity of ECH-based materials (20Pa2-1ECH-1OH) in dilute aqueous systems. The insert shows the dependence of ECH concentration during synthesis.

Conclusions

By emulsion X-linking of starches interesting microparticles are obtained. The particles can be considered as microgels. The (rheological) behaviour is characteristic of colloidal polyelectrolytes.

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MIKROCZĄSTECZKI SKROBIOWE WSTĘPNE BADANIA BUDOWY I WŁAŚCIWOŚCI

Streszczenie

Skrobie poddano obróbce w celu otrzymania w pełni biodegradowalnych mikrocząsteczek, które w roztworze wodnym zachowywałyby się jak mikrożele lub koloid. W tym celu połączono kleikowanie z sieciowaniem w emulsji wodno olejowej. Takie skrobie były bardzo stabilne w wodzie, wykazując właściwości rozrzedzania ścinaniem nawet w roztworach o dużej zawartości frakcji stałej. Są to właściwości bardzo unikalne w przypadku skrobi. W ten sposób można otrzymać nowe koloidy o zróżnicowanych właściwościach. Skrobie takie mogą znaleźć zastosowanie do celów spożywczych i niespożywczych (farby i pokrycia, tusze i pigmenty, superabsorbenty, dodatki do żywności, środki higieny osobistej, farmaceutyki, ceramika, dodatki do papieru, zagęstniki, emulgatory i inne zastosowania).

Cząteczki otrzymano stosując jako czynniki sieciujące epichlorohydrynę z trimetafosforanem trisodowym. Badano wpływ istotnych parametrów reakcji na przebieg syntezy i właściwości produktów.

MAŁGORZATA WRONKOWSKA, MARIA SORAL-ŚMIETANA

PEA STARCH AS A SOURCE OF PHYSICALLY MODIFIED PREPARATION WITH POTENTIAL HEALTH-PROMOTING ACTIVITY

Abstract

Industrially isolated pea starch was physically processed using: gelatinization, autoclaving and cooling cycles, and dehydration by spray-drying. The experimental pea starch preparation, with crystallographic pattern of the B-type had 30% d.m. of resistant starch as the RS_2/RS_3-type and showed a hydrophilic-hydrophobic character. During *in vitro* studies the sorption of some biologically active components was recognised. Specially, the sorption of deoxycholic or taurocholic acids and ions of toxic metals as lead and cadmium was noteworthy. Also, the sorption of cholesterol was observed. Physically modified pea starch preparation is suggested as additive to 'functional foods' and as agent preventing the colon diseases.

Introduction

By the end of '90s, searching for functional foods, having health-promoting functions, has attracted the attention. The digestion process in the human gastrointestinal tract can be controlled through modification of diet. The diet components affect the composition and metabolic functions of intestinal microflora. Thus, one of the most promising areas for the development of functional foods lies in modification of the activity of the gastrointestinal tract by the use of probiotics, prebiotics and synbiotics, which are already used as food ingredients.

Food saccharides, are an important source of energy. The results for population studies carried out in fourteen countries showed that complex saccharides and degradation products of starch can decrease the risk of civilisation diseases [3]. On the basis of these studies, it has been found that the frequency of colon cancer is negatively correlated (r = -0.76) with starch intake. Monosaccharides, disaccharides and starch are

M. Soral-Śmietana, M. Wronkowska, Polish Academy of Sciences, Institute of Animal Reproduction and Food Research, Division of Food Science, Olsztyn, Poland, ul. Tuwima 10, 10-747 Olsztyn.

defined as 'digestible' carbohydrates because they are digested and absorbed in the human small intestine, but dietary fibre and resistant starch, are named 'indigestible' since they are not digested by the intestinal endogenous enzymes. All poorly digestible saccharides, which are not absorbed in the small intestine, reach the large intestine and can be fermented there by the colonic microflora. A decrease in the pH of the intestine modifies the composition and character of existing microflora, what is strongly correlated with the bile acid concentration in faeces [11]. In recent years, 'resistant starch' i.e. starch resistant to the hydrolytic activity of the human gastrointestinal enzymes is of great interest for nutritionists and physiologists. According to Björck and Asp [2], resistant starch may exert potentially beneficial influence on the human digestive tract by: lowering the pH in the colon, generating the short-chain fatty acids in the colon, increasing glucose tolerance, lowering the blood lipid level. Resistant starch is an inert component in the small intestine but in the large intestine, it can have the prebiotic function as a potential energy source for colonic microflora.

Legume seeds are generally considered as a good source of starch with beneficial features arising from remarkably high levels of slow-released and resistant starch fractions. Food processing modifies the chemical nature of starch, by the way of hydration and disruption of the organized granule structure.

The objective of the study was to indicate the possibility of application of pea starch in the form of physically-modified preparation, having the resistant starch and sorptive activity towards some biological compounds.

Materials

Pea starch 'Nastar' was kindly gifted by Cosucra S.A., Belgium. It originated from native starch extracted from the kernels of yellow smooth pea. The following enzymes were used: solid amyloglucosidase (Fluka 10115, 70.7 U/mg, from *Aspergillus niger*); solid alpha-amylase (Sigma A-3176 [EC 3.2.1.1], 28.6 U/mg, from porcine pancreas). Other reagents used in the experiments were as follows: cholic acid (Sigma C-6445), deoxycholic acid (Sigma D-4297), taurocholic acid (Sigma T-9034), L-alpha-lecithin (Sigma P-5394), reagent kits for the enzymatic determination of cholesterol (P.O.CH., Gliwice cat. No 178132140). These were prepared in the solution of 0.05 M phosphate buffer, composed of monobasic sodium phosphate and dibasic sodium phosphate at various pH (from 6.0 to 7.6). Glucose was determined using glucose oxidase-peroxidase (GOPOD) and chromogen kits from Cormay following the manufacturer's instructions.

Experiment

The starch preparation was obtained from granular commercial pea starch. This preparation was made in a laboratory scale using some physical processes. The suspen-

sion of pea starch in distilled water (1:3.5) was autoclaved (121°C/1 h) and then cooled at 4°C/12 h. The autoclaving/cooling cycles were performed three times. The retrograded gel was homogenized with distilled water and spray-dried at 130°C inlet temperature and 50°C outlet temperature.

Methods

Chemical components: nitrogen was determined by Kjeldahl method and ash was determined after mineralisation in muflon oven at 700°C according to standard chemical methods [1]. Starch content was analysed according to ICC Standard No. 123, by hydrochloric acid dissolution [8].

Functional properties: water holding capacity (WHC) and oil sorption were assessed according to Soral-Śmietana et al. [12].

The resistant starch analysis was carried out using the Champ's procedure (the A-method) [4]. The sample (100 mg) was incubated with 500 U porcine pancreatic alpha-amylase at 37°C for 16 h. The products of hydrolysis were extracted with 80% ethanol and the extracts were discarded. Undigested material was dissolved in 3 ml of 2 M KOH and hydrolysed with amyloglucosidase (20 U) at 65°C for 90 min. Free glucose was analysed using the oxidase-peroxidase glucose test, measuring the absorbance at 500 nm in 1 cm cuvette.

The *in vitro* digestibility of starch preparations was determined using 200 U of porcine pancreatic alpha-amylase per 1 gram of sample. The enzyme solution was prepared in 0.05 M phosphate buffer pH 6.9 with the addition of $CaCl_2$ (3 mM). The sample (200 mg) was suspended in phosphate buffer pH 6.9 (20 ml) and alpha-amylolysis was carried out for 1, 3, 6, 24 hours at 37°C. Prior to hydrolysis, isopropanol (100 µl) was added to the sample to inhibit the growth of microorganisms during incubation. At the determined time intervals, the sample (1 ml) in centrifuge capped tube was mixed with 95% ethanol (4 ml) to inactivate the enzyme. The kinetics of hydrolysis was measured as an equivalent of maltose.

Estimation of the sorption properties towards metal ions: Pb^{2+}, Cd^{2+}, was performed by the electrochemical method (polarography DPP ASV), within the potentials of -1550 to -160 mV. The rate of the potential change was 10mV/s. Before the measurement, oxygen was removed from the samples by 10-min perfusion with argon. The measurement was performed at 36°C and lasted 20 h. Samples (100 mg /10 ml) were placed in buffered solutions of pH 6.4 and 2.2. Sorption was rapid and completed after 20 min; being irreversible after 20 h.

The cholesterol sorption was measured by *in vitro* analysis. The sample (100 mg) was combined with an emulsion (lecithin, sodium salt of deoxycholic acid and cholesterol) prepared in 0.1 M phosphate buffer of pH 6.8 (2 ml). The 1-h incubation was

carried out on shaking at 37°C. The kinetics of the cholesterol sorption by 20 μl emulsion was analysed in 10-minute intervals using reagent kits. The results were expressed as per cent of cholesterol sorption by the sample at each time.

The sorption of bile acids (cholic, deoxycholic, taurocholic) was measured by the *in vitro* analysis. The sample (100 mg) was treated with solution of each bile acid (10 ml). The solutions were prepared in 0.1 M phosphate buffer pH 7.6 for each bile acid in 2 μM/ml concentration. The samples and blanks were incubated at 37°C for 30 minutes. Centrifugation was carried out at 2000 x g for 5 min. The sample (50 μl) was treated on agitation with 70% sulfuric acid (5 ml) and freshly prepared solution of furan-2-aldehyde (2.3 g/l) (1 ml). Absorbance was measured at 510 nm after 80 minutes. The results were expressed as per cent of the bile acid sorption.

The scanning electron microscope (SEM) micrographs were obtained after spraying the dry starch preparation with gold in a JEE 400 vacuum evaporator and visualised in JSM 5200 at the acceleration of 10 KeV.

Results and discussion

The chemical compositions of native pea starch and pea starch preparation are presented in Table 1. The microstructure (SEM) of native pea starch, the source of investigated preparation is presented in Fig. 1, and the structure of pea starch preparation is presented in Figs. 2 a-b.

Table 1

Chemical composition of native pea starch and its starch preparation[1].

Sample	Starch [% d.m.]	Nitrogen [%d.m.]	Ash [%d.m.]	RS content	
				of sample [% d.m.]	of total starch [%]
Native pea starch	99.5 ±1.25	0.19 ±0.02	0.12 ±0.01	42.6 ±2.9	42.8 ±1.9
Pea starch preparation	84.9 ±1.87	0.13 ±0.02	0.30 ±0.02	29.8 ±1.9	35.0 ±2.2

[1] Values given are means of four replications ± standard deviation

The SEM-pictures of the preparation subjected to autoclaving and spray-drying were characteristic of starch gel particles dehydrated in the flow of the drying air. Different size and shape of characteristic collapses in the central part in large particles could be observed. These new structures are typical of dehydrated starch gels of different origin [10, 14, 15]. The process of rapid dehydration of colloidal suspension of such organic polymers as proteins of plant and animal origin has been previously observed with similar results for microstructure [6, 7, 9, 13]. Thus, the microstructure

image after spray-drying of colloidal organic polymer suspensions is characteristic rather of the process than dried material.

Fig. 1. SEM-electronogram of native pea starch.

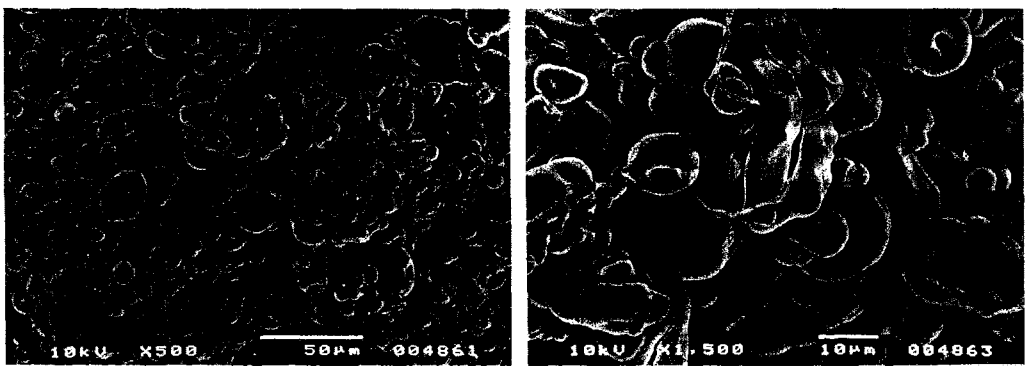

Fig. 2 a, b. SEM-electronograms of pea starch preparation.

The chemical analysis of pea starch (Table 1) and SEM-pictures (Fig. 1), clearly indicate high purity of the material. Additionally, it should be stated that the physical processes of gelling, retrogradation and dehydration used in the study caused that the resistant starch content in the experimental preparation was about 30% (Table 1). The pea preparation had crystallographic pattern of type B, as measured by X-ray diffraction [G. Lewandowicz, non published data]. On the basis of the kinetics of 24-h hydrolysis with pancreatic alpha-amylase, it was stated that the preparation has the properties similar to these of very resistant starch. The degree of hydrolysis within 6 to 24-h was insignificant (Fig. 3). It was interesting to observe the effect of the physical processes used on the affinity of the pea starch preparation towards water and oil (Table 2).

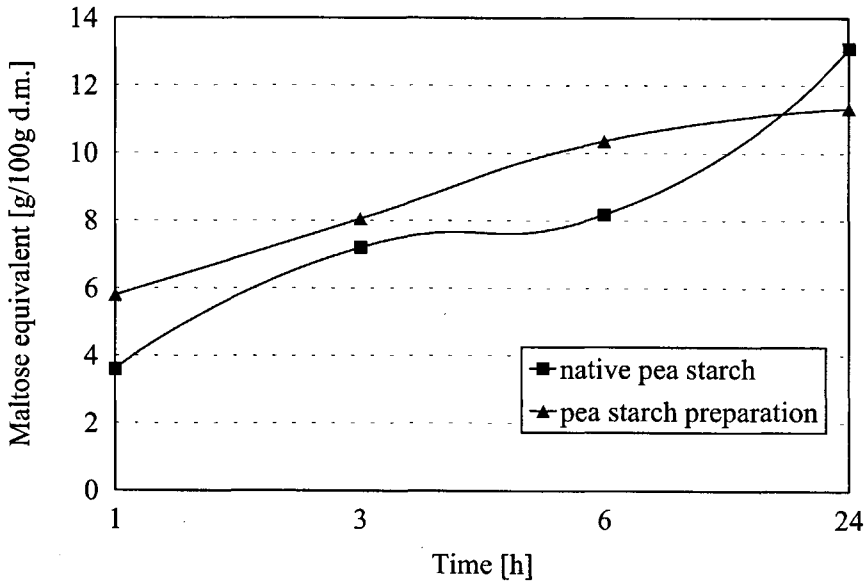

Fig. 3. The kinetics of hydrolysis of the native pea starch and its starch preparation by pancreatic alpha-amylase.

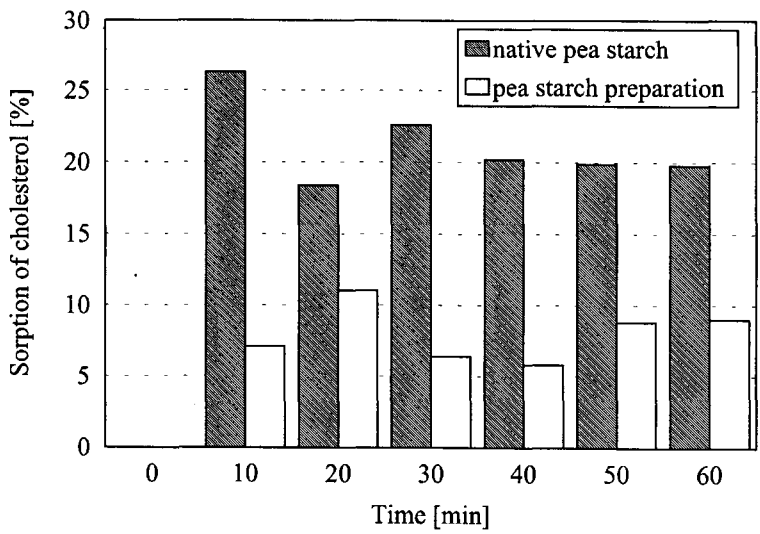

Fig. 4. Sorption of cholesterol by native pea starch and its starch preparation.

Table 2

Some functional properties of native pea starch and its starch preparation[1].

Sample	Water holding capacity [g water/g d.m. sample]	Oil sorption [g oil/g d.m. sample]
Native pea starch	0.96 ±0.02	1.50 ±0.01
Pea starch preparation	1.07 ±0.03	1.74 ±0.01

[1] Values given are means of four replications ± standard deviation

Assessment of the functional properties revealed that the starch preparation increased slightly the ability for binding water and oil. Taking into consideration the microstructure of pea preparation, it can be suggested that this ability is affected by the size and surface of the particles as well as by the chemical nature of the preparation. However, comparing the sorption of water and oil, the starch preparation seems to have the hydrophilic-hydrophobic character but with increased number of hydrophobic domains. This phenomenon has already been described, when the analysis of the possibility of forming complexes between physically-modified starches and nutrients was undertaken [5].

In the present study, experimental preparation of pea starch containing about 30 % RS was investigated considering its sorption properties towards cholesterol, bile acids and selected metals.

The cholesterol sorption ability of the experimental pea starch preparation was poorer than that of native starch (Fig. 4). However, the maximum cholesterol sorption for the starch preparation reached about 11 % after 20 min of reaction, while the native starch reached the maximum already after 10 min with about 26% sorption of this substance.

Sorption of bile acids by the tested preparation was determined on the basis of interaction with the following acids: cholic, deoxycholic and taurocholic (Tab. 3). The starch preparation had high sorptive ability towards deoxycholic and taurocholic acid, as compared to native pea starch. Special attention should be paid to the affinity of the pea starch preparation to these acids, since they are the secondary bile acids being the degradation substrates of primary bile acid. They have been shown to be involved, as a promoting agents, in the adenoma-carcinoma sequence of colorectal cancer [3]. Therefore, significant affinity of our experimental preparation to secondary bile acids may be important in prevention of the large intestine diseases.

Table 3

Sorption of the bile acids by native pea starch and its starch preparation[1].

Sample	Cholic acid [%]	Deoxycholic acid [%]	Taurocholic acid [%]
Native pea starch	12.79 ±4.0	4.30 ±2.0	3.83 ±1.5
Pea starch preparation	9.35 ±3.2	46.32 ±5.5	9.94 ±3.4

[1] Values given are means of four replications ± standard deviation

Table 4

Complexing of lead and cadmium by native pea starch and its starch preparation (μg/100 mg of sample).

Sample	Pb^{2+}		Cd^{2+}	
		r^2		r^2
Native pea starch	16.0	0.91	0.32	0.89
Pea starch preparation	22.0	0.88	0.45	0.87

r^2- regression coefficient

In the study, complexing of lead and cadmium as the antagonists of such important bioelements as magnesium and zinc was also investigated. The amounts of complexed Pb^{+2} and Cd^{+2} are presented in Table 4. In the case of the pea starch, higher sorption ability towards both ions was clearly seen. There are some unproven assumptions suggesting that both these ions occur in minimum concentrations in living organism where they, probably, play a positive physiological function [6]. On the other hand, lead and cadium ions are regarded toxic and the sorptive properties of the examined pea preparation towards these ions can also be used in health prophylaxis.

Conclusions

1. It should be emphasised that the physical modification of pea starch produced the preparation with the resistant starch content reaching 30%.
2. The preparation of pea starch, containing average amount of indigestible starch, has the affinity to such secondary bile acids such as deoxycholic and taurocholic acids, and to lead and cadmium ions.
3. The properties of pea starch preparation may suggest its use as additive to 'functional foods' to promote health and prevent the colon diseases.

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SKROBIA GROCHOWA JAKO ŹRÓDŁO FIZYCZNIE MODYFIKOWANEGO PREPARATU O POTENCJALNEJ AKTYWNOŚCI PROZDROWOTNEJ

Streszczenie

Izolowaną przemysłowo skrobię grochu poddano procesom fizycznym takim, jak: kleikowanie, cykle autoklawowania, cykle chłodzenia oraz odwodnieniu poprzez suszenie rozpyłowe. Eksperymentalny preparat skrobi grochowej, o strukturze krystalograficznej typu B, wykazujący charakter hydrofilowo-hydrofobowy, zawierał 30% skrobi amylazoopornej typu RS_2/RS_3. W wyniku badań *in vitro* oznaczono właściwości sorpcyjne w stosunku do niektórych biologicznych komponentów. Odnotowano szczególną aktywność sorpcyjną w stosunku do drugorzędowych kwasów żółciowych, deoksycholowego i taurocholowego oraz jonów metali toksycznych, jak ołów i kadm. Zaobserwowano też zdolność do sorpcji cholesterolu. Mając na uwadze uzyskane wyniki, można sugerować otrzymanie preparatu skrobi grochu fizycznie modyfikowanej, który może znaleźć zastosowanie w 'żywności funkcjonalnej', jako składnik o znaczeniu zapobiegawczym w chorobach jelita.

RAFAŁ ZIOBRO, ANNA NOWOTNA, HALINA GAMBUŚ, ANTONI GOLACHOWSKI, KRZYSZTOF SURÓWKA, WERNER PRAZNIK

SUSCEPTIBILITY OF STARCH FROM VARIOUS BIOLOGICAL SOURCES ON DEGRADATION DUE TO EXTRUSION PROCESS

Abstract

In order to evaluate the importance of biological source in starch extrusion we processed wheat, rye, triticale, oat, corn and potato starch in a single screw extruder and compared the products. They varied mainly in hardness, density, solubility and water binding capacity. On the other hand, molecular weights and paste properties of the extruded starches showed a low dependence on the type of raw material.

Introduction

There are several important factors which influence the properties of starch extrudates. The type of an extruder (single or twin-screw), screw geometry and configuration provide a specific mechanical energy. Moisture content of the raw material (usually 10–30% wet basis) [1] regulates the ratio of melting and gelatinization of starch granules which take place simultaneously at high temperatures set in barrel sections. It has also an impact on viscosity of the melt being formed in the extruder barrel and therefore it is responsible for the extent of molecular degradation caused mainly by a mechanical shear [11]. Generally, it is hard to predict which of these factors will play a decisive role in a given situation [5], but all of them could be relatively well controlled.

However, some parameters which could influence an extrusion process are much more difficult to handle. It concerns physical and chemical properties. The proper selection of a starch source is therefore of great importance for obtaining a good product.

Amylose content is the main factor in determining starch susceptibility to extrusion [2], but it is not clear whether it is due only to the differences in molecular mass between amylose and amylopectin, but also to the presence of 1-6 glycosidic bonds in

R. Ziobro, A. Nowotna, H. Gambuś, A. Golachowski, K. Surówka, Agricultural University of Cracow, Agricultural University of Wroclaw, W. Praznik, Universität für Bodenkultur Vienna.

amylopectin. Moreover, amylose and amylopectin from different plants are not identical. Besides, other starch features such as crystalline structure, content of integral lipids and inorganic components also depend on botanical source. Thus, it would be reasonable to estimate the influence of these features on the important properties of the extruded products such as expansion ratio, density, textural properties, water solubility and water binding capacity and the behaviour of pastes.

In order to evaluate the importance of a starch source on extrudate properties we compared wheat, rye, triticale, oat and corn starch processed under the same conditions.

Materials and methods

The extrusion was performed in a single screw extruder (Brabender 20 DN). Water content in the material was adjusted before the extrusion to either 16% or 24% on a dry basis. The temperatures of 3 barrel sections were 80, 120 and 150°C and a screw speed was maintained at 210 rpm. The hardness, density and expansion ratios were measured for the obtained products, as it was described before [4]. After milling the extrudates the measurements of phosphorus content [6], water solubility, water binding capacity and viscosity were performed. Solubility and water binding capacity of the extrudates, measured in a standard way as for native starch [9] were unreliable. For this reason the method was modified and the sample was dissolved in the form of a homogenous dispersion. The method of Morrison and Laignelet [7] was modified to measure an apparent content of amylose in 5 ml fractions obtained by size exclusion chromatography [12] and these values were used to calculate a molecular weight distribution of the amylose and the amylopectin present in the samples, basing on the pullulan calibration. To measure paste properties we prepared dispersions in cold water and heated them up rapidly allowing to boil for 5 minutes. The flowcurves $\tau(\gamma)$ at a constant shear stress were measured using a Rheolab MC 1 rotational rheometer with a standard measuring system DIN 53019. After cooling the pastes to 20°C. The parameters n and K were calculated using the Ostwald model.

Results

The extrudates prepared at different moisture contents varied much in hardness, expansion and density (table 1.) as we reported before [4]. The expansion was lower for the samples extruded at 24% of water in the raw material. As expected these samples had a higher density and hardness (for potato this parameter was out of the measurement range), their solubility was lower at 30°C while at higher temperatures they became the same, or even more soluble (fig. 1). The results clearly show that there is no simple relation between expansion and solubility. The highly expanded (and pre-

sumably more degraded) starches have more components, which are water soluble at a low temperature, but while heating it becomes less important and at 90°C solubility of the starch extruded at 24% of moisture is almost the same or even higher (potato). Water binding capacity was higher for the samples with a higher initial water content, which is believed to be more gelatinized and less melted. As for potato starch extrudates, which were almost completely dissolved at 60°C, water binding capacity could not be measured (fig. 2).

Table 1

Physical characteristics of corn, wheat and potato starch, extruded at different moisture levels.

Origin of starch	water content in a raw material	Hardness	Expansion ratio	Density
Wheat	16	36	3.5	0.12
	24	90	2.9	0.28
Corn	16	51	3.2	0.23
	24	93	3.1	0.30
Potato	16	150	3.1	0.32
	24	out of range	1.7	0.52

Table 2

Physical characteristics of starches from various botanical sources extruded at 16% of moisture.

Starch origin	Hardness	Expansion	Density
Wheat	36	3.5	0.12
Rye	98	2.8	0.18
Triticale	77	2.8	0.33
Oat	70	2.9	0.18
Corn	51	3.2	0.23
Potato	151	3.1	0.32

Better textural parameters were obtained for the starches extruded at 16% water content, so they were chosen for further studies. Physical properties of the samples, are shown in table 2. It is worth to noticing that the expansion of the product does not correlate with density and hardness, but under these conditions (which we suppose are close to optimal) it is comparable for all the studied starches. Some dependence could be observed between hardness and density, but it needs a larger data set to be proved.

In all the extruded starches, especially oat one, phosphorus content was higher than in native ones (table 3), which suggests some inadequacy of the method. Perhaps, to some extent, it could be due to the loss of the chemically combined water from the

starch granules during extrusion, which would reduce dry mass the samples. This seems likely, because similar effects of extrusion on mineral components content were already reported [10].

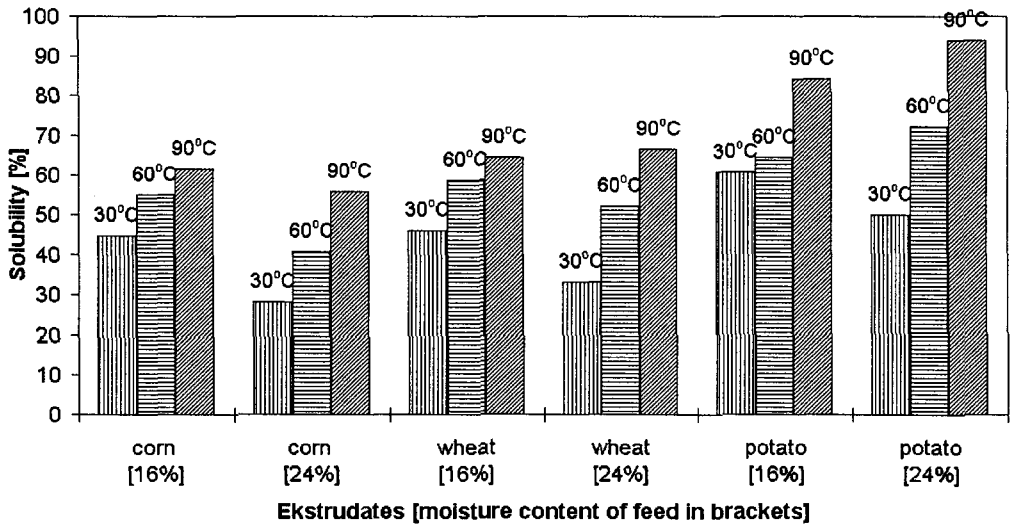

Fig. 1. Solubility of starch extrudates obtained at different moisture levels.

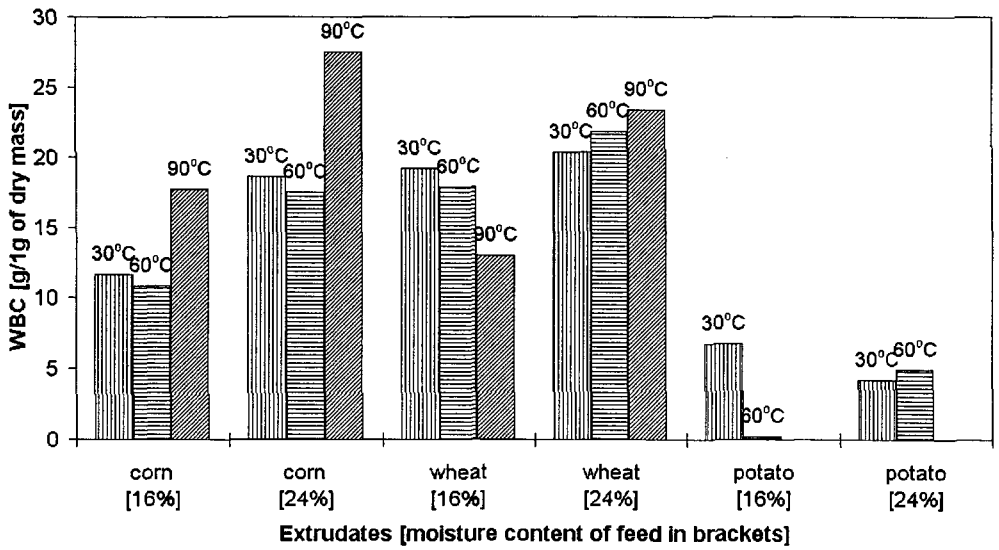

Fig. 2. Water binding capacity of starch extrudates obtained at different moisture levels.

Wheat, rye and triticale starch extrudates did not differ significantly in their solubility but lower values were measured for oat and corn starches. All the extrudates were much more soluble, than native starches, especially at low temperatures (fig. 3).

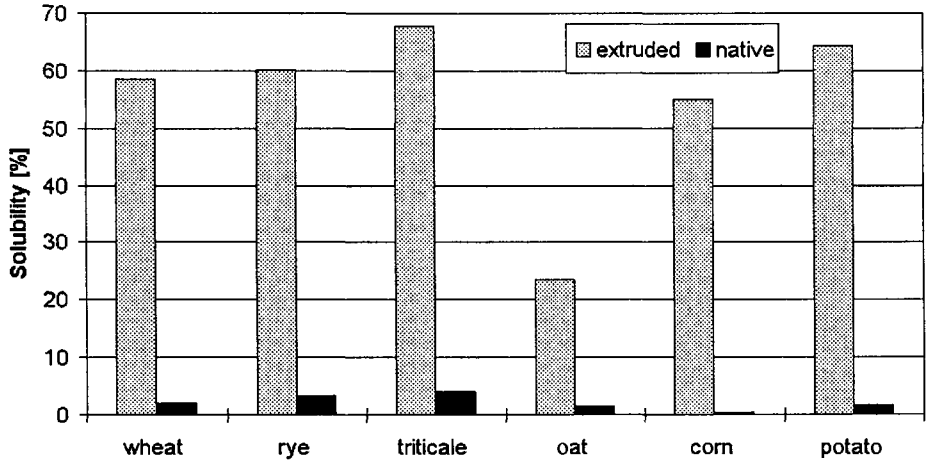

Fig. 3. Solubility of starch extrudates of different botanical origin obtained at 16% moisture content.

Table 3

Phosphorus content in native and extruded starches.

Starch type	Phosphorus content [mg %]		
	native	extruded	
		16 %	24 %
Rye	30	36	-
Triticale	47	51	-
Oat	80	101	-
Wheat	64	59	60
Corn	19	21	22
Potato	68	70	79

The obtained amylose content was confirmed by many other experiments. However, we obtained a bimodal distribution of iodine-stained glucans, as previously reported by Chinnaswamy and his colleagues [3]. While accepting the view of these authors that some part of amylopectin can give blue complexes, we did not try to separate this fraction from the apparent amylose. The results of weight-average molecular

weights of the branched fraction present in native starches were similar (table 4) and lower, than the usually reported data. We believe this is due to a few reasons: the insufficient separation in this range of glucan sizes, the use of the pullulan calibration, and last, but not least the described method of amylose/amylopectin signal separation, which gave wide distributions of these glucans. Potato, corn and rye starches had slightly higher masses than the other ones. The extrusion at the described conditions caused in all the cases significant reduction of molecular weight of amylopectin, but smaller of amylose which is in agreement with the reports of Politz et al. [8].

Table 4

Molecular characteristics of native and extruded starches.

Origin of starch	Amylose content[%]		M_w of amylose [$\times 10^6$]		M_w of amylopectin [$\times 10^6$]	
	native	extruded	native	extruded	native	extruded
Corn	22.5	18.6	1.8	1.1	8.3	3.7
Wheat	18.9	18.0	1.5	0.7	6.4	3.6
Rye	24.6	19.5	1.1	1.4	8.3	3.5
Oat	16.5	17.9	2.0	1.2	6.7	4.7
Triticale	23.3	21.2	2.0	2.0	7.8	4.3
Potato	27.6	22.4	2.3	2.2	9.2	4.1

Table 5

Ostwald model constants n and K for 3% pastes of native and extruded starches.

Starch origin	Native		Extruded starch	
	K	n	K	n
Wheat	0.08	0.77	0.003	1.23
Corn	0.87	0.44	0.014	1.04
Triticale	0.59	0.54	0.005	1.13
Rye	0.01	1.03	0.006	1.13
Oat	1.76	0.46	0.003	1.21

Native starches varied significantly in their paste properties. The Ostwald model constants n ranged from 0.4 to 1 (table 5.). The structural degradation caused by extrusion was reflected by these values. In all cases n value was close to 1, which is characteristic for Newton type fluids. The loss of structural viscosity was reflected in a dramatic decrease of K values.

Conclusions

The most important differences between the extrudated starches from different botanical sources are: hardness, density, solubility and water binding capacity. All these properties are also affected by the extrusion parameters, so it seems possible to optimize them for special purposes.

On the other hand, molecular weights and gel properties of the extruded starches are not so much different, as not to be used interchangeably for many applications.

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PODATNOŚĆ SKROBI RÓŻNEGO POCHODZENIA BOTANICZNEGO NA DEGRADACJĘ SPOWODOWANĄ PROCESEM ELESTRUZJI

Streszczenie

W celu określenia istotności związku pochodzenia botanicznego a ekstruzją skrobi, procesowi temu (w jednoślimakowym ekstruderze laboratoryjnym) poddane zostały informacje skrobie pszenna, żytnia, pszenżytnia, owsiana, kukurydziana i ziemniaczana. Największe różnice zaobserwowano w twardości, gęstości, rozpuszczalności i wiązaniu wody uzyskanych produktów. Wszystkie te cechy zależały w znacznym stopniu od parametrów ekstruzji. Z drugiej strony masy cząsteczkowe i właściwości kleików ekstrudowanych skrobi wykazywały niską zależność od rodzaju surowca.

WILLI WITT, HANS-PETER GOLDAU

MODERN METHODS OF SEPARATION THE COMPONENTS OF WHEAT

Abstract

The isolation of starch and gluten out of wheat requires sophisticated techniques. In the past it was mainly hand operated. Two methods: Martin process and Hydrocyclone process, as well as the advantages and disadvantages of them are described.

The modern methods of separation the components of wheat involve a three phase separator and/or decanter or the combination of the traditional and novel methods. The usage of three phase separators and decanters gives a lot of advantages to improve the wheat starch and gluten processing. It is possible to adjust the process to different raw materials and to optimise the yield of products and by-products, as well as reducing the quantity of effluent per ton of flour.

Introduction

The extraction of starch from renewable raw materials such as corn, manioc, potato, tapioca, wheat and others are accomplished by releasing starch granules from cell walls of plants and extraction. Different techniques are adapted to the specific raw material such as wet milling and dry milling. The separation procedures of foreign matters, such as fibres, proteins, fats and other non-starch components, as well as washing of the starch, are of particular importance.

Starch is used in the food and non food industry. For the food industry the starch is mainly converted into different kinds of glucose syrups. Besides native starch, after chemical and/or physical modification the starch is used in the non food industry.

General aspects of wheat starch production

The world starch production is about 46 mio. tons. Eighty-one per cent is produced out of corn and 8% from wheat.

W. Witt, H.-P. Goldau, Westfalia Separator AG, Oelde, Germany.

Especially in Europe the wheat starch production is of importance. From the total starch production of 8,2 mio. tons per year, about 30% is expected to be wheat starch.

During the last 20 years the wheat starch became more and more of importance and the wheat starch production increased more than 2500%.

The most important by-product of wheat starch production is wheat gluten.

Comparison of the composition of different raw materials

One of the important differences between the various raw materials is the starch content. Potato contains most of starch compared to corn and wheat. This based on the dry matter. Only the wheat flour has a higher potential of recoverable starch, but during the milling some starches are lost into the fibres (Fig. 1).

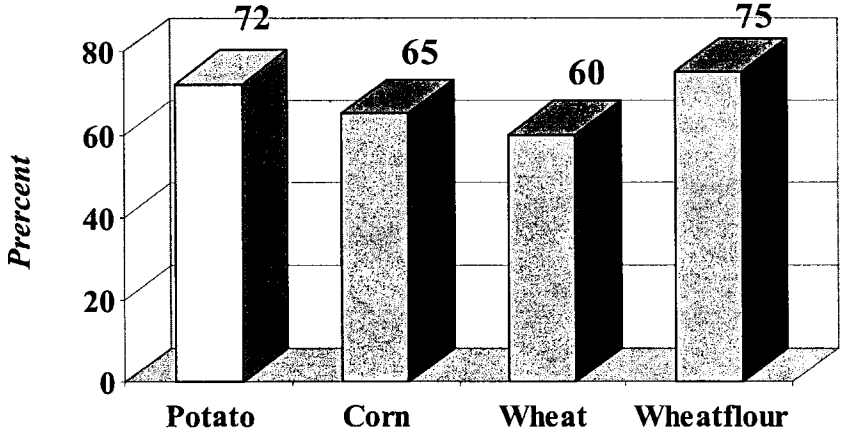

Fig. 1. Starch content on dry substance in relationship to different raw materials.

The comparison of the main components of corn and wheat (Fig. 2) shows that the endosperm in both raw materials is equal. The main difference is the content of germs and of the aleurone layer. Wheat contains a less amount of germs and a higher amount of aleurone layer and fibre.

The processing of a starch containing raw material will not only result in the starch, but also in a by product. The potato will give the lowest amount of by products based on dry matter, but contains approx. 75% water. This is a very big disadvantage.

Production of starch out of wheat will give the highest amount of by-products and the lowest amount of starch. With approx. 9,2% wheat gluten has a higher value than starch and other by-products (Fig. 3).

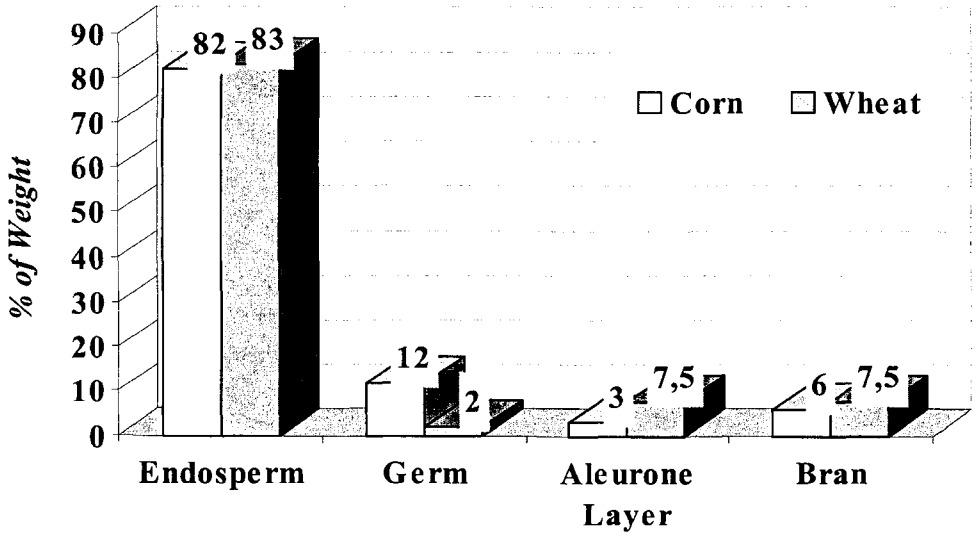

Fig. 2. Composition of the morphologic structure of wheat and corn.

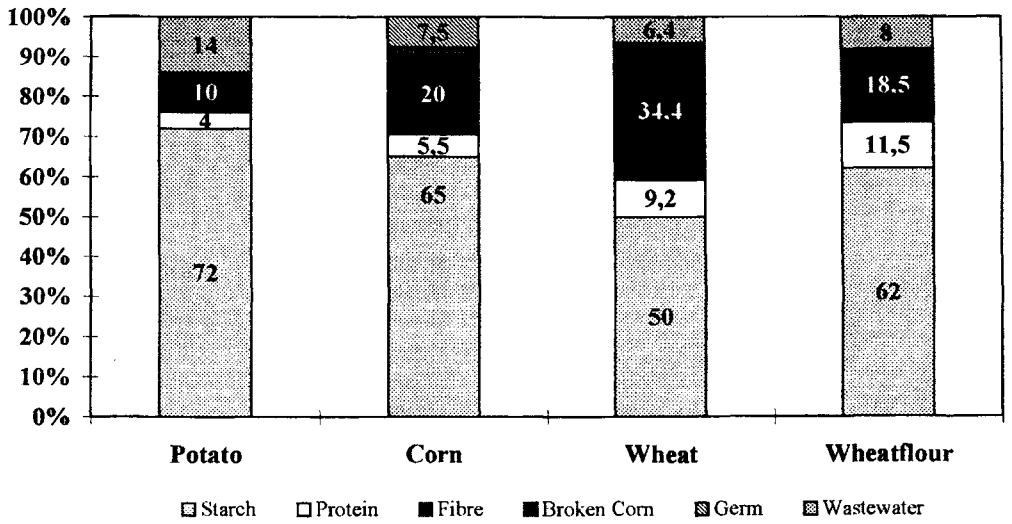

Fig. 3. Contents of starch and by products for different raw materials.

Using wheat flour, the starch yield will increase to a level of approx. 62%, the gluten yield to 11,5%, whereas the fibre, C-starch and B-starch as a by-product decrease to 18,5 %. The increase of the higher value main product resulted from the by

product fibre of the flour mill. It is not possible to process wheat directly to wheat starch and wheat gluten without getting a native high value wheat gluten.

Comparison of the different methods for starch processing

The most important difference in the processing of corn, potato and wheat is as follows:

a) corn has to be wet milled,
b) wheat has to be dry milled,
c) potato and tapioca are rasped because of the high water content.

Corn has a horny endosperm. The protein matrix must be softened before wet milling and the protein does not form a viscoelastic mass. Starch has only one starch spectrum that means that corn starch is one modular.

In comparison to corn, wheat protein forms a viscoelastic mass – the so called gluten. The dry milling before starch recovery is necessary, to be able to recover the high added value wheat gluten. Out of wheat two starch fractions are obtained – the A-starch and the B-starch. A third starch fraction is present – the so called C-starch. This contains the hemicellulose and pentosanes. In a spin test in lab the b- and C-starch will be one fraction.

A unique characteristic of wheat starch granules is the bi-modularity of its mass distribution, that does mean that starch of wheat consist out of 2 main size-populations of starch granules (Fig. 4).

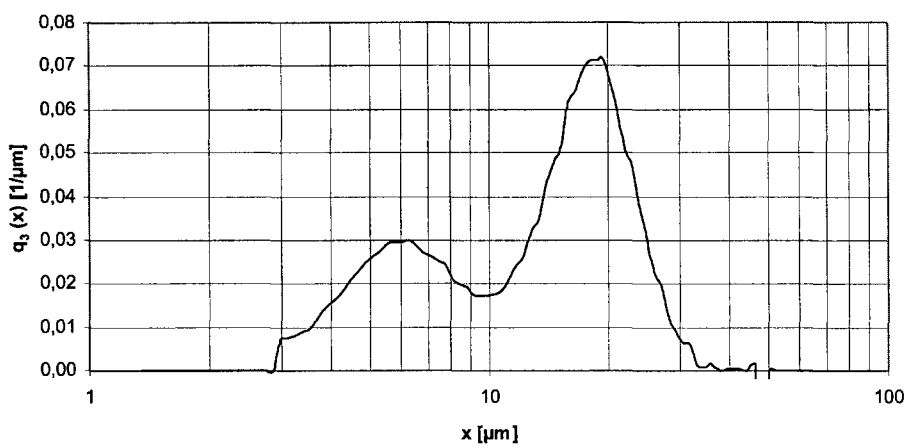

Fig. 4. Massdistribution of a wheat starch depending on its particle size.

The unique characteristics of wheat are leading to a sophisticated technique to isolate starch and gluten out of wheat flour. Two special features are the preconditions for the application of processing techniques. Firstly the ability of parts of its protein fractions to swell up with water and form, under influence of mechanical energy input, a cohesive viso-elastic mass termed gluten and, secondly, the characteristic presence of two fractions of starch granules which differ considerably from another in size and shape.

Due to these reasons the technical solution to recover starch and gluten from flour is much more complicated than from corn and potato.

Besides that another important point is that wheat and wheat flour contain a relative high amount of hemicellulose and pentosanes. These fractions do have viscous behaviour. During processing wheat flour to starch and gluten will influence the recovery of starch, in particular the small granule starches.

Starch processing out of wheat flour

Starch processing out of wheat flour is a several hundred years old technique. In the past it was mainly hand operated. One of this process was the so called Martin process.

It is very important to mill wheat to wheat flour, before processing it to starch and gluten, in order to obtain the wheat protein as native wheat gluten.

Martin process

The characteristics of the Martin process to recover starch and gluten out of wheat flour is, that a wheat flour is mixed with water in a ratio of 1:0,6. In a mixer the flour and water are kneaded to a stiff dough. After this procedure the dough is washed with a high amount of fresh and process water in a special starch and gluten separator. This washer, a so called extractor, has several sections. At the bottom of these sections screens are located, where the starch milk passes through. The dough is moved from section to section by a shaft assembled with paddles. Fresh water is used in the last section of the extractor to wash the gluten. Wash water that passed through the screens is used in counter current in the section before. Crude starch milk is leaving the washer from the two first sections with a concentration of approx. 8 ° Be.

The so extracted suspension contains all components of the flour besides the extracted wet gluten. In consequence the crude starch milk contains the total recoverable starch, the A- and B-starch, the Hemicellulose and the (soluble and insoluble) Pentosanes, the Solubles together with the Proteins and nonstarch Carbohydrates.

The difference in the apparent density of wheat components can be utilised to partially separate the dough into its fractions by means of centrifugal forces. When such a separation is carried out in a centrifuge beaker, two to four, clearly separable

layers are formed. Figure 5 gives an overview about a spin test of a crude starch milk from potato, corn and wheat starch. The heavy fraction stands for the separable starch of each raw material. The medium fraction contains the corn gluten, the fine fibre and ore the C-starch. The light fraction contains the water, the soluble solids and the very light suspended solids.

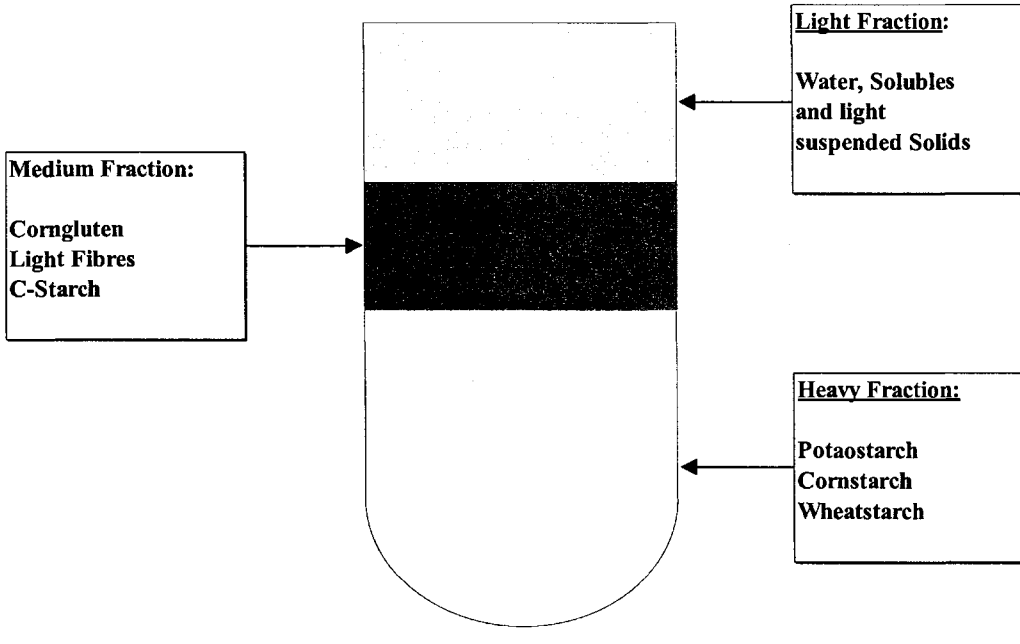

Fig. 5. Overview about a spin test of different crude starch milks.

The task of separation and washing of starch milk is to achieve the purified starch and to separate the fibres, proteins, fats, soluble like nonstarch carbohydrates, sugars, protein, fats and minerals from the starch.

This aim seems to be relatively difficult for the Martin process, because of the vast mixture of suspended components in the crude starch milk. Due to the presence of the pentosanes, which influence the behaviour and the agglomeration of the wheat gluten, much more water is needed for diluting the pentosanes. That is one reason for the high fresh water consumption by using that separation technique. The second explanation for a higher fresh water consumption, is the necessity to reduce the soluble protein content in the final starch. This value has to be reduced down to 50 mg/100 g starch.

Hydrocyclone and other processes

Another method to isolate starch and gluten out of wheat flour is the so called Hydrocyclone process.

In this process diagram a hydrocyclone system find its application after the dough preparation. The heavy A-starch is separated and leaves the hydrocyclone unit with the underflow. The overflow contains the wet wheat gluten, the B-starch, C-starch, like hemicellulose and pentosanes, and the soluble solids, like proteins, fats, sugars, etc. vall together.

The disadvantage of this process is similar to the Martin-process, that the gluten cannot be isolated directly from hemicellulose and pentosanes. On account of that gluten can be found together with these components in the overflow of the hydrocyclone system. The presence of the pentosanes will also influence the behaviour and the agglomeration of the gluten. That does mean that more fresh water for diluting this components will be necessary.

Westfalia separator 3-phase-decanter process

Westfalia Separator developed the three phase decanter process. This process was installed in three German wheat starch factories in 1984 at the same time.

In the years before 1984 have been performed various trials to separate, after using a homogeniser in order to agglomerate the wheat gluten, a wheat-flour-water-slurry with a decanter. Anyhow at that time it was not possible to achieve a good separating efficiency.

Flour milling

The Westfalia Separator decanter process also requires wheat flour for the separation of starch and gluten.

World-wide there do exist different possibilities to produce flour out of wheat. One way is to consume standard bakery flours, extracted with a normal mill design. The flour can be extracted by a normal mill in purpose to produce bakery flour. Sometimes a so called short flour mill is used. In this design less roller mill stages are included. Another possibility is to use a normal hammer mill, for example a so called ultra rotor.

A typical analysis of a wheat flour is listed according to Table 1.

Besides these normal characteristics, it is very important, that the used flour has a good gluten agglomeration. Only under these circumstances it is possible to achieve a high gluten recovery and fewer losses of gluten proteins within the different starch fractions.

Table 1

Typical analysis of a wheat flour for starch processing.

Water	approx.	14,50
Protein (N x 5,7)		11,50
Ash		0,63
Fat		1,50
Crude Fibre		1,50
Starch		80,00
(all Numbers are based on Dry Matter)		
Wet Gluten		28,00
Amylogram		500 BU
Falling number		250,00

Slurry preparation

The wheat flour before processing it in the wet starch process should have a relative short resting time in the flour storage silos. The storage time should not exceed more than 72 hours.

During this time the flour passes its maturation in outdoor flour silos. From there it is conveyed into a small bin in front of the flour dosing system. The dosing system can be a weigh belt feeder or a loss and weight dosing system. It is from significant importance to realise a continuous and not fluctuating dosing of flour and water. The flour is fed directly from the weighing into the flour-water mixer. Together with the flour process- or fresh-water enters the mixer.

The optimum flour-water-ratio should be established with about approximately 1:0,85–0,95.

The task of the mixer is to hydrate all flour particles in order to have a slurry which is free of lumps.

The temperature of the slurry should be among 30 and 40 centigrade, to achieve the best gluten agglomeration (aggregation).

After the mixer the slurry enters a homogeniser. This homogeniser is well known in the milk industry because of its duty to homogenise the protein and fat in milk. Figure 6 shows a cross section of a homogenising valve.

The slurry is fed by an eccentric screw pump into the homogeniser. Pistons in the homogeniser are pumping the slurry through the homogenising valve. The slit distance of the valve is adjustable, so that the back pressure can be risen up to 100 bar. The pressure in the slurry increase than up to 100 bar and after the valve the pressure drops

down to a normal pressure. Due to different kinds of stressing, like relaxation and the shear effect in the valve, the energy input is introduced into the protein matrix of the flour-water slurry and agglomerates the gluten proteins.

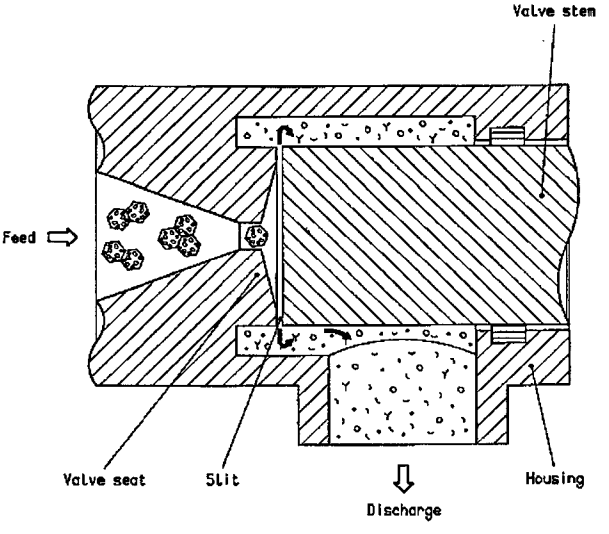

Fig. 6. Section of a homogenizing valve.

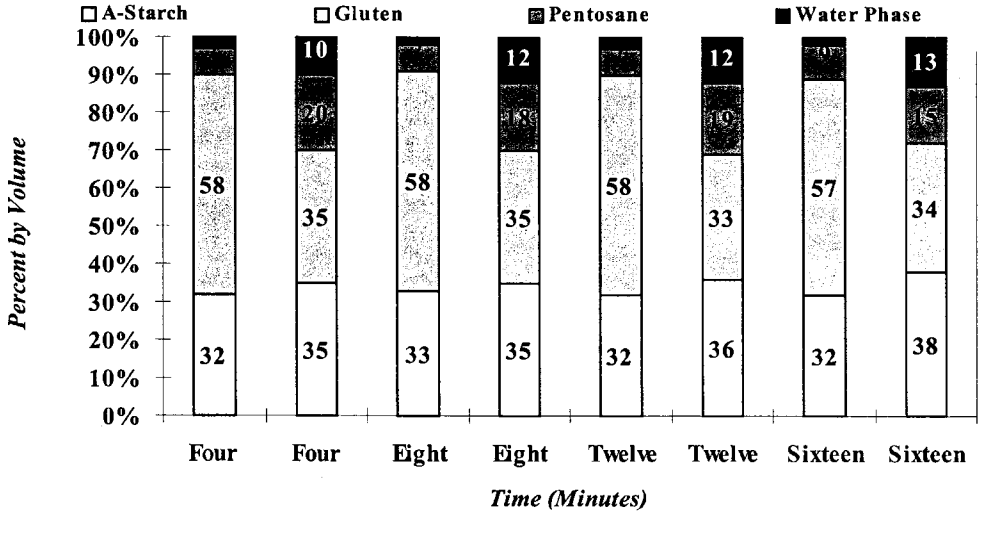

Fig. 7. Influence of the homogeniser on the gluten formation and separation efficiency.

To show the influence of the homogeniser on the gluten agglomeration and the separation of the components of a wheat flour, samples were taken in front of the homogeniser and direct after the homogeniser. After four, eight, twelve and sixteen minutes the slurry was centrifuged in a laboratory centrifuge. The percentage of volume of the different layers have been observed and the results are shown in Fig. 7.

It was evident that the separation effect of the components could be improved due to the homogeniser and that the best results have been achieved processing the combination of a homogeniser and a resting time of sixteen minutes. On the other hand it was obvious that a certain energy input was necessary to obtain a better gluten agglomeration, in order to improve the separation of wheat components. Without any extra energy input into the slurry and only a certain resting time it is not possible to achieve a good separation.

This is the reason, that in real processes the slurry has to be treated with a system to transform the right energy into the slurry and to install after the gluten agglomeration a resting tank to stabilise the protein matrix. One machine could be a homogeniser.

Separation the components of wheat

An eccentric screw pumps the slurry into a three phase decanter.

A decanter (Fig. 8) is a horizontal separator with an installed conveying screw. This scroll is operating with a differential speed to the revolutions of the bowl. The g-force is approx. 2500 to 3500 and the differential speed approx. 60 rpm.

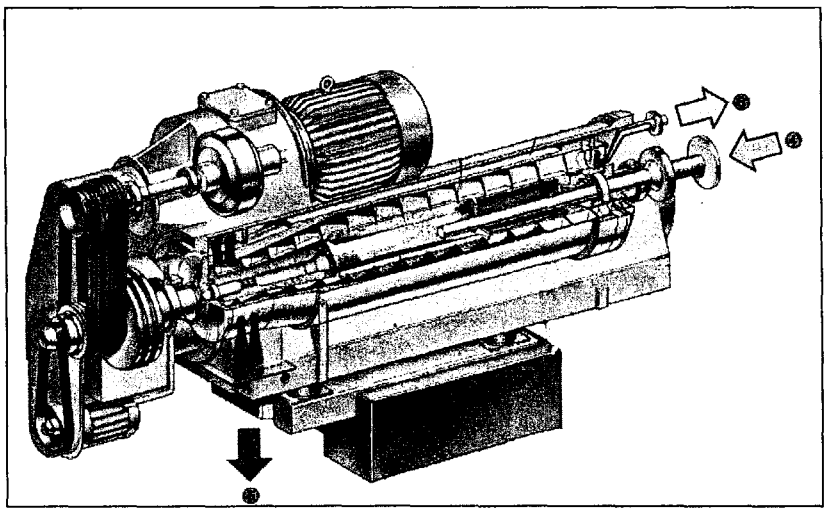

Fig. 8. Decanter type CA 505 with centripetal pump and 2-gear-drive.

Figure 9 shows a 3-phase-decanter type CA 755 with a 2-gear-drive and an installed centripetal pump.

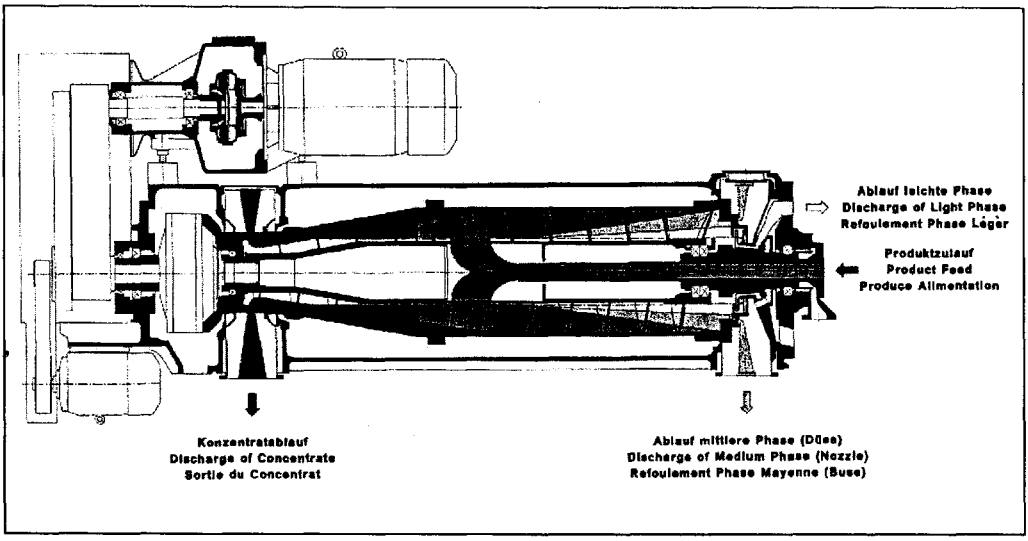

Fig. 9. 3-Phase decanter type CA 755 with 2-gear drive and installed centripetal pump.

This decanter is equipped with a 2-gear-drive. This 2-gear-drive was developed by Westfalia Separator and is patented at home and abroad. The 2-gear-drive allows the adjustment of the differential speed of the scroll depending on the torque developing at the motor. The torque depends on the infeed quantity, concentration and particular characteristics of the solids. The solids as particles with the highest density are separated and conveyed with the scroll at the discharge of the decanter.

The overflow of the decanter is leaving the machine by an installed centripetal pump. This installation is leading to two main advantages. On the one hand it will influence the processing of foamy products positively and on the other hand there is no need to install another pump after the decanter.

The difference of the 3-phase-decanter is based on the possibility to separate a third phase, the so called medium fraction. This fraction can be influenced in quantity, by mounting one or more nozzles, and qualitatively by changing the relative position of the nozzle.

In front of the decanter a further amount of fresh and/or process water for diluting the slurry is added. Normally this amount of water is about 0,3 to 0,9 m^3 per ton of flour. The temperature should be adjustable from 25 to 45 degrees centigrade.

When the slurry and the diluting water are fed into the decanter, the wheat components like the agglomerated gluten, the starch and the pentosanes, will be separated

in the decanter bowl. Due to the centrifugal force the starch, because of its highest apparent density, is separated from the liquid phase and settles down on the inner surface of the bowl. The so separated starch will be carried out of the decanter by transferring it with the scroll towards the concentrate discharge.

The gluten and B-starch and pentosanes are leaving with the nozzles of the medium fraction. In the light fraction most of the pentosanes, which are representing the sticky carbohydrates of the wheat flour, and some very small size starch granules are leaving the decanter with the installed centripetal pump.

Figure 10 gives a good impression about a spin test of the different fractions of the 3-phase-decanter.

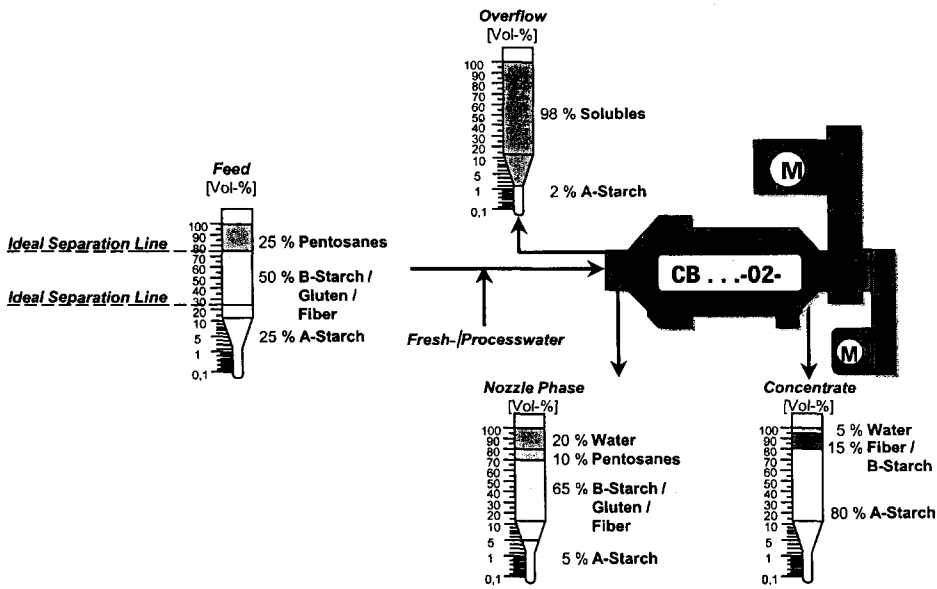

Fig. 10. Spin test of the different phases of 3- phase decanter.

It is of great significance, that pentosanes, as fraction of the lowest apparent density in the slurry, can be found mainly in the overflow of the 3-phase-decanter. This fraction is characterised with a remarkable rheologic property, because of its viscous behaviour.

One of the most important advantages of the three phase decanter process is, that this viscous mass is separated from the gluten in a very early stage of processing. That does mean that this pentosane fraction will not influence the gluten and the behaviour while separating the gluten and the B-starch.

Figure 11 shows the mass distribution of a wheat flour around a three-phase-decanter. On the discharge of the concentrate more than 85% of the A-starch leaves the

decanter. Together with the gluten approx. 10% of the A-starch leaves through the nozzles of the decanter. This starch fraction contains mainly small granule starches with an amount of approx. 60% of starch granules smaller than 10 µm.

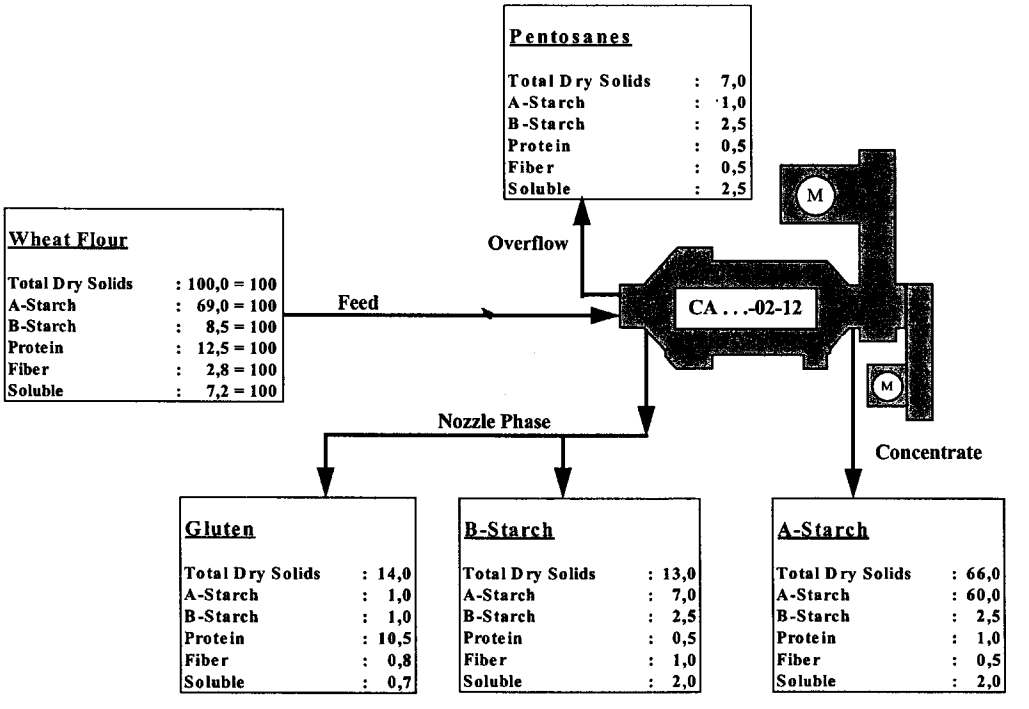

Fig. 11. Distribution of the wheat flour around the 3-phase decanter.

One important point can be seen from these numbers. The concentrate with the A-starch contains only 28% of the soluble solids of the incoming wheat flour. This will give a very positive effect for the washing of the starch in the further steps.

Afterwards the different fractions of the three-phase-decanter can be treated separately, as shown in Figure 12.

The concentrate and the sometimes the pentosane fraction too should be sieved to recover very small gluten lumps in order to feed them back to the medium fraction.

In a following process step the medium fraction is transferred to a screening device to separate the gluten from the B-starch milk. The gluten is then treated by a gluten washer to improve the protein content. From the separating device the B-starch milk passes once more a sieving section to recover small gluten particles.

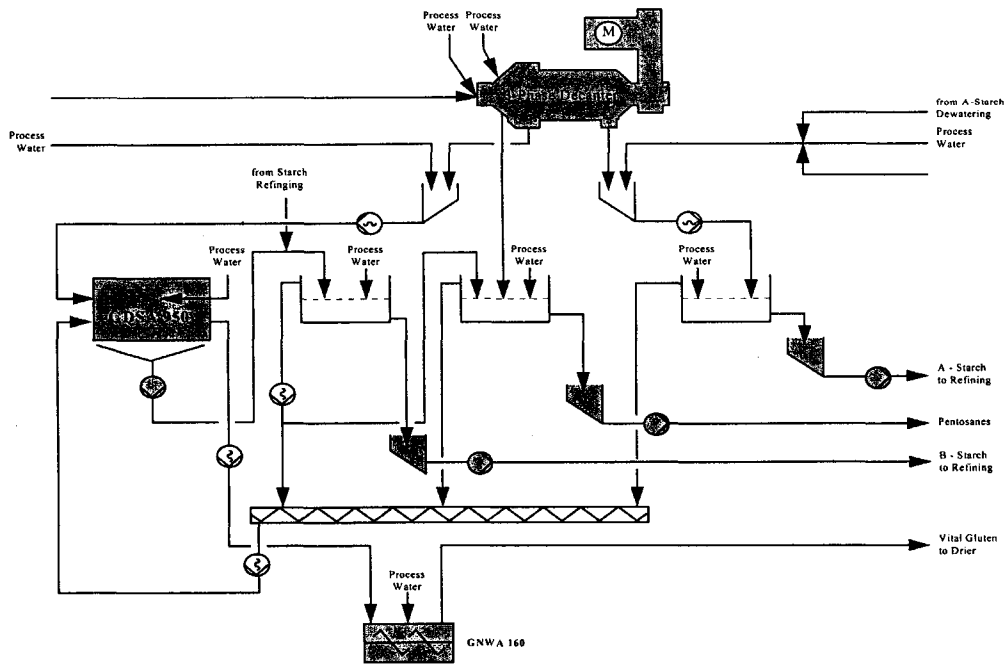

Fig. 12. Possibility to treat the different discharge fractions of a 3-phase-decanter.

Starch washing with three phase separators

The concentrate with the A-starch from the decanter is diluted with fresh water or normally with process water. The starch milk is pumped to centrifugal screens and screened. These screens should have approx. 60 μm mesh to remove fine fibres from the A-starch.

The washing of the starch milk with fresh water can be done with hydrocyclones, separators or a combination of both. To be able to reduce soluble solids in the A-starch milk down to an acceptable value, normally a 12 stage hydrocyclone system is introduced.

A hydrocyclone system has a very high disadvantage for the application of washing of wheat starch. The presence of a high amount of starch granules smaller than 10 μm and the necessity to separate the fine fibres and the pentosanes, are leading to difficulties during performance. For a hydrocyclone unit it is well known, that there can be losses with the overflow, because of its difficulties to separate particles which are smaller than the separation boundary. The application of a three phase separator improves this situation.

A separator type DA 100 is shown in Figure 13. This indicates the principle of a two phase separator.

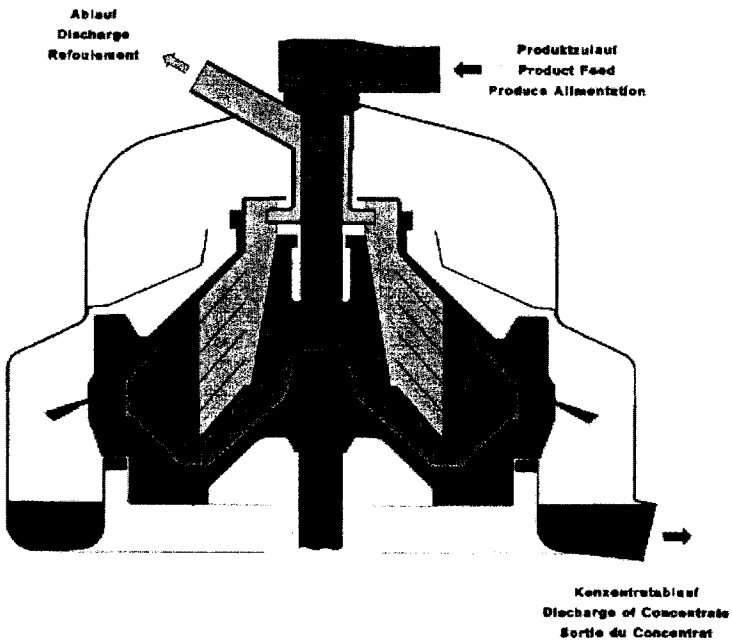

Fig. 13. Cross section of the 2-phase-separator DA 100.

This separator is a vertical centrifuge with a disc stack as clarification area, nozzles for discharging the concentrate and the discharge of the overflow with an installed centripetal pump. The acceleration of the bowl is up to 5000 rpm and the g-force up to 9000. The starch milk enters the separator and by means of centrifugal acceleration distributed at the outer area of the disk stack. The starch granules are separated from the liquid phase and will be concentrated in the outer area of the bowl, where they will be discharged passing the fitted nozzles.

In the disk stack the separation of small suspended solids and small starch granules will take place. If this machine is only used as thickener with up to 12 to 14 degree Baumé in the concentrate, the suspended solids will be concentrated and are leaving the machine through the nozzle phase.

If this separator is used as concentrator with up to 20 ° Bé in the underflow, the small suspended solids will leave the machine with the overflow and will enter the disc stack. In this case the load with suspended solid of the disc stack is very high.

This problem can overcome with the three phase separator SDA 90 (Fig. 14), the newest invention of Westfalia Separator AG for the starch industry.

This machine is equipped with a washing device on the bottom and a special separation disc on top of the disk stack.

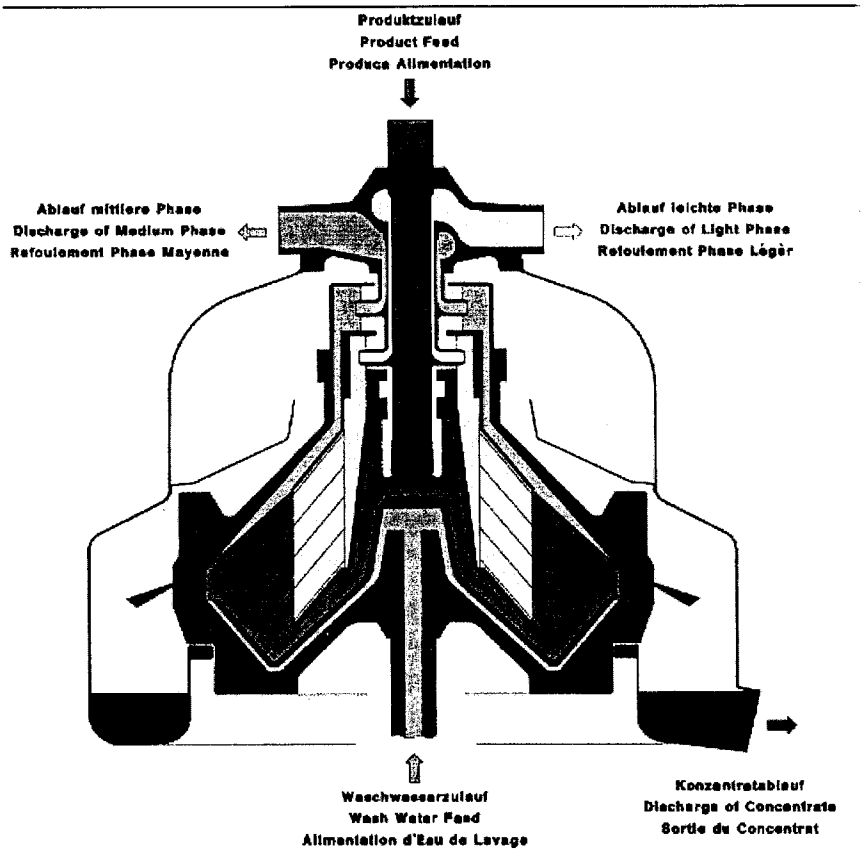

Fig. 14. 3-phase-separator type SDA 90 with washwater device.

The starch milk that enters the machine, is distributed in a channel in the outer area of the disc stack. All the suspended solids are separated from the liquid phase and concentrated in the bowl in front of the nozzle. The A-starch leaves the nozzles together with a smaller part of B-Starch. In comparison to the two phase separator, the concentrate will have a reduced amount of B-starch. The other amount of the B-starch, hemicellulose and pentosanes will enter the channel above the separation disc stack and be discharged over the second installed centripetal pump into the medium phase of the separator. Under these circumstances the liquid phase entering the disc stack has a reduced amount of soluble solids. That means that the load of the disc stack with suspended solid is less and the efficiency is much higher.

With the washing device it is possible to displace the soluble solids directly with fresh water. In Figure 15 is shown the relation between the density of the concentrate, the pressure of the washing water and the amount of washwater.

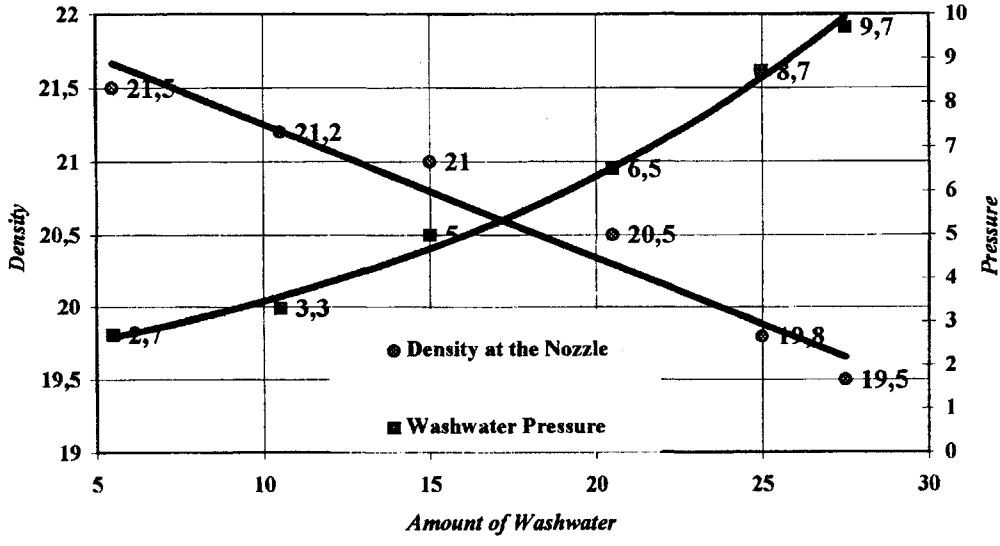

Fig. 15. Density and pressure in relation to the amount of washwater.

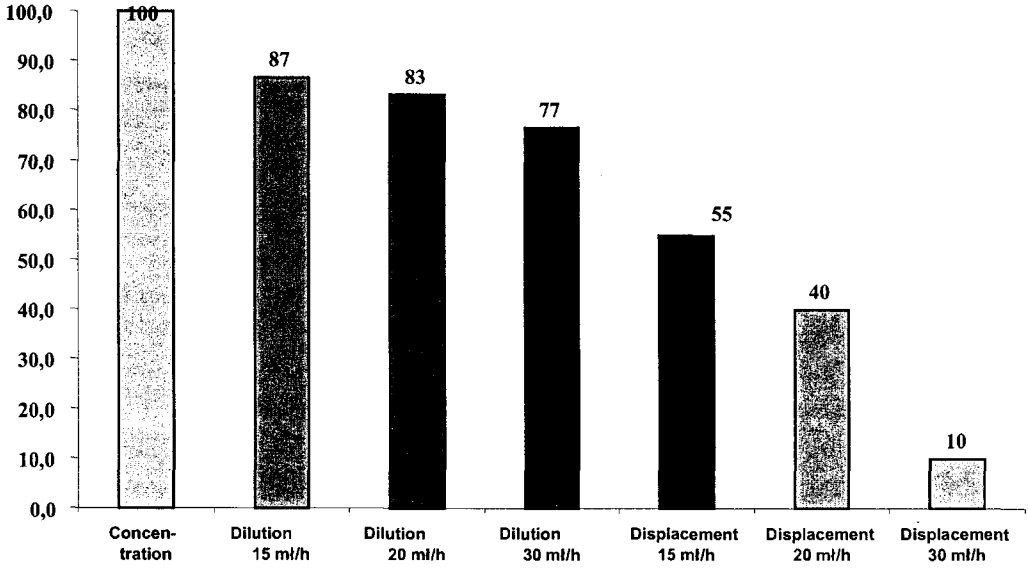

Fig. 16. Reduction of soluble protein content with different washing procedures.

A comparison of the reduction of soluble proteins in relation to the amount of washwater is shown in Figure 16. It can be seen that the dilution of a starch milk to reduce the concentration of soluble proteins is more efficient with the washing device of the three phase separator. Here the soluble solids are directly displaced. These solids are put together with the liquid in the overflow and medium phase. With an amount of 30 m^3/h fresh water, it is possible to reduce the content of solubles down to 10%. If the suspension will be only diluted, the reduction can be only 77%.

In Figure 17 is shown an overall view of the different phases of a three phase nozzle separator. It can be seen that the non starch solids are mainly distributed in the medium phase. The overflow of the machine is relatively free of suspended solids and can be used as process water.

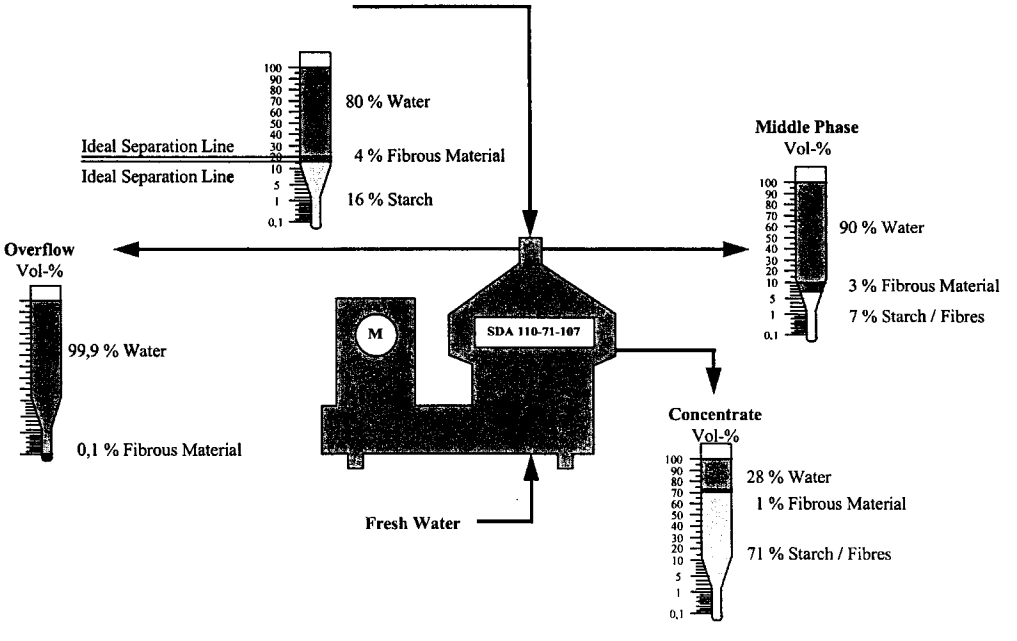

Fig. 17. Spin test of the different phases of a 3-phase nozzle separator.

A starch washing line can be composed out of two three phase separators for the A-starch washing and one three phase separator for the starch recovery. This separator recovers starch milk from the nozzle phase of the three phase decanter and out of the nozzle phase of the first starch washing separator (Fig. 18).

The introduction of an extra three phase separator for the starch recovery has the advantage that the medium phase will discharge the main part of the hemicellulose and pentosanes. These components are coming from the nozzle phase of the decanter and the first washing separator. There can be found as well some small size granule starches. They can be recovered by separators and/or decanters to produce a special fraction of small granule starches.

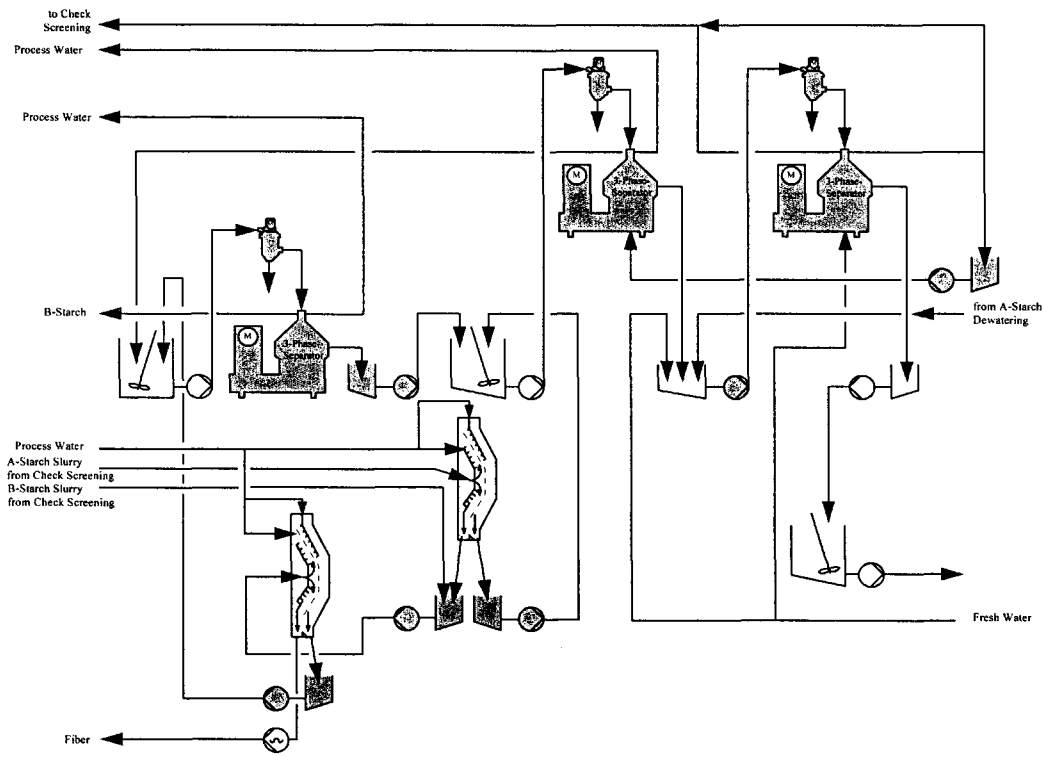

Fig. 18. Example for a possibility to wash starch with a combination of three 3-phase-separators.

Instead of using a second three phase separator in the washing line, a hydrocyclone system can be used. The disadvantage of the hydrocyclone unit is the lower g-force and more losses of small size granule starches in the overflow. This results in high amount of recycling starch in the process (Fig. 19).

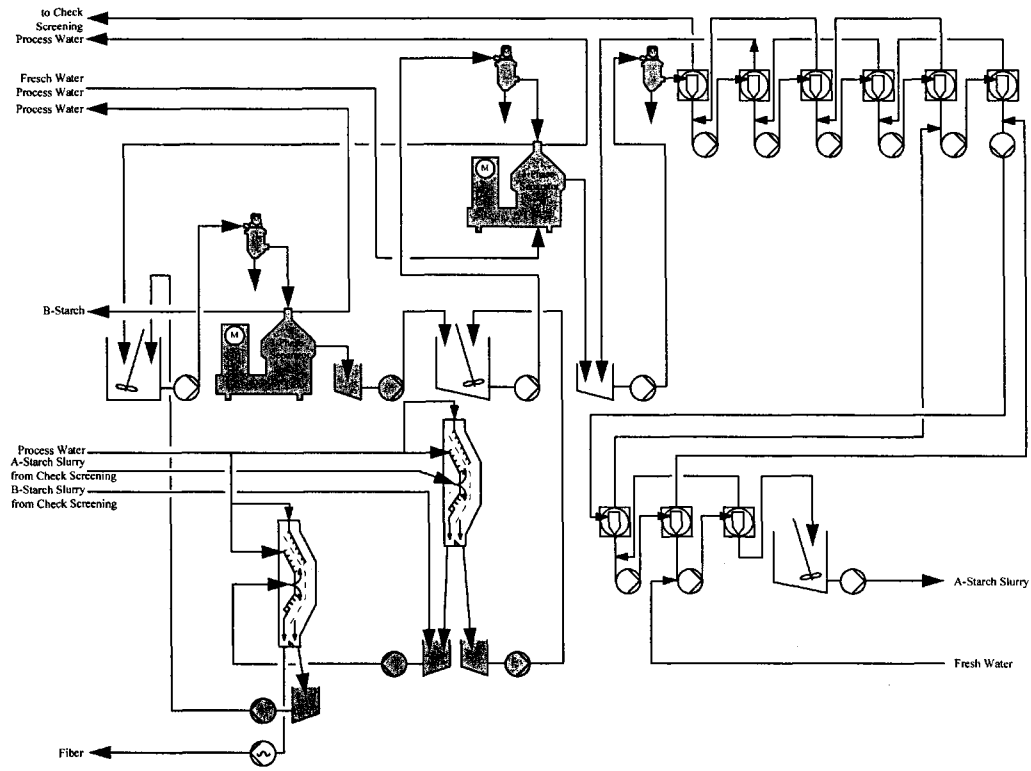

Fig. 19. Washing of starch with two 3-phase-separators and a hydrocyclone unit.

Concept for small granule starch recovery; 3 separate lines

Westfalia Separator AG has developed a modern concept for recovering small granule starch (Fig. 20). The starch washing; concentration and recovery is separated into 3 different lines; the A-plus starch line, the A-minus starch line and the C-starch line. Beginning from the 3-phase decanter, the A-starch coming out of the solid phase of the 3-phase decanter is passing a fibre screen and than is fed to a 3-phase separator. The separator is splitting the feed into an A-plus starch fraction, an A-minus starch fraction and as well as a process-water phase.

The A-plus starch afterwards is washed countercurrently by a multistage hydrocyclone unit. The A-minus starch including fine fibres is fed to the second washing line and enters first a further 3-phase separator. The starch milk from gluten washing is added too. The nozzle discharge of the separator contains the A-minus starch and is washed and concentrated by starch washing separator or decanter.

The medium phase of the 3-phase separator containing very small granule starch and other separable material like fine fibres are directed to the C-starch washing line.

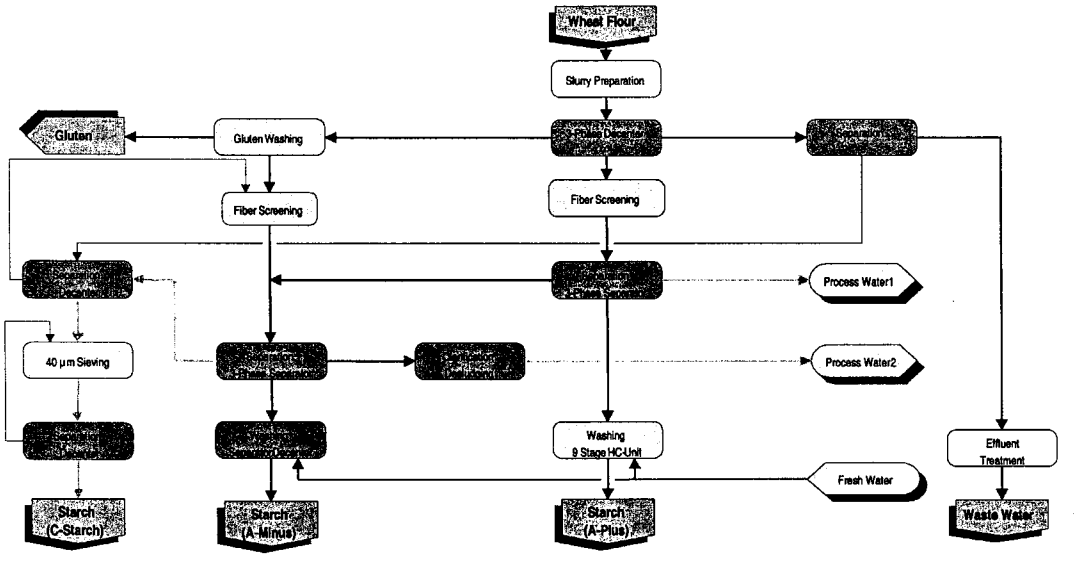

Fig. 20. Concept for small granule starch recovery; 3 separate lines.

A separated small granule starch fraction, collected from the pentosane phase of the 3-phase decanter is also fed to the C-starch washing line. This C-starch fraction is separately washed screened and concentrated by separators/decanters and screens.

This system enables certain advantages e.g.:

1st high yield of starch,

2nd less recycling of small granule starch and other separable particles into the system,

3rd less freshwater consumption.

Situation of the by-products

The pentosane fraction of the three phase decanter can be handled as a liquid by-product for animal feed. Another possibility is to treat it with enzymes, decanting the heavy particles away and concentrate it in an evaporator together with the waste water. Consequently the concentrate of the evaporator can be dried together with fibres or any other dry by-products.

Waste water

The waste water of the plant can be treated in different ways. For example evaporation, the anaerobic or aerobic treatment in a waste water treatment plant.

Yields of the components out of wheat

The yields of the components are of a very big interest for every starch producer. This depends on the recoverable starch, the distribution of the size of starch granules and the content of gluten protein in the flour. The recoverable A-starch can be extracted to an amount up to 85% and it is possible to recover an extra amount of small granule starches up to 12%.

The potential of recoverable gluten protein of the flour can be extracted up to 85 % into the gluten as final product.

Conclusion

This lecture shows that the usage of three phase separators and decanters gives a lot of advantages to improve the wheat starch and gluten processing. It is possible to adjust the process to different raw materials and to optimise the yield of products and by-products, as well as reducing the quantity of effluent per ton of flour.

NOWOCZESNE METODY ROZDZIAŁU SKŁADNIKÓW PSZENICY

Streszczenie

Wyodrębnianie skrobi i glutenu z pszenicy wymaga specyficznych metod. W przyszłości wykonywano to w zasadzie ręcznie. W referacie opisano dwie główne metody (Martin'a i hydrocyklonową) oraz ich zalety i wady.

Nowoczesne metody rozdzielania składników pszenicy obejmują zastosowanie trójfazowego oddzielacza i dekantera lub też połączenia tradycyjnych i nowych metod. Użycie trójfazowego oddzielacza oraz dekantera korzystnie wpływa na proces otrzymywania skrobi i glutenu z pszenicy. Umożliwia też dostosowanie procesu do różnych surowców i optymalizację wydajności produktów i produktów ubocznych oraz zmniejszenie ilości wycieku w przeliczeniu na tonę maki.

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 5211; 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
Dr hab. Krzysztof Surówka Oddział Małopolski	ul. Podłużna 3 (AR), 30-239 KRAKÓW Tel. (+22) 425 28 32
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 hab. Danuta Kołożyn - Krajewska Oddział Warszawski	ul. Nowoursynowska 166 (SGGW), 02-787 WARSZAWA Tel.: (+22) 843 90 41 w. 1200
Prof. dr hab. Janusz Czapski 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
Dr Karol Krajewski Ekonomiczna	ul. Nowoursynowska 166 (SGGW), 02-787 WARSZAWA Tel.: (+22) 843 90 41
Prof. dr hab. Teresa Smolińska Technologii Mięsa	ul. Norwida 25/27 (AR), 50-375 WROCŁAW Tel.: (+71) 205 284; Fax.: (+71) 205 273
Prof. dr hab. Krzysztof Krygier Technologii Tłuszczów	ul. Grochowska 272 (SGGW), 03-849 WARSZAWA Tel.: (+22) 810 18 42; e-mail: tzyktznoiks@alfa.aggw.waw.pl
Prof. dr hab. Wacław Leszczyński Technologii Węglowodanów	ul. Norwida 25, 50-375 WROCŁAW (Katedra Technologii Rolnej i Przechowalnictwa) Tel.: (+71) 320 5221
Mgr Ewa Mrówka Młodej Kadry	ul. Rakowiecka 36 (Instytut Biotechnologii Przem. Rolnego i Spoż.), 02-532 WARSZAWA Tel.: (+22) 606 38 18

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