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Szanowni Państwo,

Przekazujemy Państwu kolejny numer 2(7) kwartalnika „Żywność. Technologia. Jakość.", który ma charakter specjalny. Zamieszczamy w nim bowiem teksty referatów prezentowanych na VII International Starch Convention (VII Międzynarodowej Konferencji Skrobiowej), której współorganizatorem był Oddział Małopolski PTTŻ.

Ponieważ Konferencja miała charakter międzynarodowy wydrukowaliśmy teksty w języku angielskim, ze streszczeniami w języku polskim.

Wyrażamy nadzieję, że zamieszczone w tym numerze naszego kwartalnika materiały przybliżą Państwu aktualny stan badań nad skrobią i zostaną przyjęte z zainteresowaniem.

Tadeusz Sikora

Kraków, czerwiec 1996 r.

This collection of papers is a regular issue of the "Żywność. Technologia. Jakość." ("Food. Technology. Quality.") journal and, at the same time, this volume plays a role of the VII Starch Convention Proceedings held in Cracow on 12-14 June 1996.

The volume of this issue significantly exceeds the standards of this journal being due to printing facilities available. Nevertheless, the editor could not manage with collecting in one issue all papers submitted to the Conference Board. Therefore, the Editorial Board would like to apologize to the contributors and to the readers – participants of the VII ISC for this inconvenience. The subsequent issue of ZTJ which is quarterly available will contain the second, final collection of the conference contributions. All participants of the VII ISC will be supplied with the following issue of ZTJ as soon as possible.

Prof. Dr. Piotr Tomasik, D.Sc.
(Editor of this issue)

HIDEAKI ANDO[1], NAOYOSHI INOUCHI[1], MASAKO ASAOKA[1],
KAZUTOSHI OKUNO[2], HIDETSUGU FUWA[1]

THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON DISTRIBUTION OF SHORT CHAINS OF RICE AMYLOPECTIN

Abstract

The effects of the environmental temperature during the early development of seeds on the characteristics of the endosperm starch were investigated using near- isogenic lines of rice plants grown under temperature controlled conditions. The components of starch and pasting characteristics of starch granules varied by the environmental temperature. The lower temperature increased the amylose content and the ratio of short chain to long chain of amylopectin. Moreover, the starch granules of rice plants grown under the lower temperature were more gelatinized than those grown under the higher temperature. By high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) of *Pseudomonas*-isoamylase-debranched amylopectins we found the amylopectin of Taichung 65 (waxy) grown under the lower temperature (25°C) has decreased amounts of chains with degree of polymerization (DP) of 8 and 9, and slightly increased amounts of chains with DP of 6, 11, 12 and 13 compared with the amylopectin of the same near-isogenic plants grown under the higher temperature (30°C).

Introduction

The amylose content, distribution of α-1,4 chains of amylopectin, and some properties of starch granules in the cereal endosperm are affected by the endosperm mutation and the environmental condition. Environmental effects on properties of endosperm starch are, in general, not as much as effects associated with genetic factors, such as species and varieties.

The effects of the environmental temperature,at the milky stage on properties of endosperm starch in rice have been studied. We showed that the lower temperature (25°C) during the filling period of rice grains increased the amylose content of endosperm starches of non-mutant [1-4] and mutants [4]. In this connection Sano et al. [5] reported that the starch-granule bound *Wx* protein level in the rice endosperm related to the amylose contents was affected by the environmental temperature. Moreover, the

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amylose of rice plants grown at the lower temperature in either the growth chambers or the paddy field gave higher weight-average degree of polymerization (DPw) values than those of plants grown at higher temperature [6]. We also showed that the lower temperature decreased the amount of long B chains of amylopectin and increased that of short B chains, as compared with the higher temperature (30°C) [3, 7]. The pasting characteristics of the starch granules were also affected by the environmental temperature under which the rice plants were grown. Namely, the onset and conclusion temperatures of gelatinization and heats of gelatinization determined by differential scanning calorimetry (DSC) of the endosperm starch from the rice plants grown under the lower temperature were lower than those starch from the plants grown under the higher temperature [1-4, 6, 7].

This paper deals with the determination of structure characteristics by high performance liquid chromatography (HPLC) and high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) of isoamylase-debranched amylopectins of near-isogenic rice plants grown under temperature conto rolled conditions (25°C and 30°C) after heading to elucidate detailed chain-length distributions of endosperm starches of rice plants grown under the different environmental conditions.

Materials and methods

Experimental plants

Near isogenic lines of Taichung 65 for the waxy (*wx*) locus were used. The rice plants having spikelets which flowered within two days after heading were grown in a temperature-controlled growth chamber. The temperature conditions during the grain filling period are shown in Table 1. Heat summation during the grain filling period was 1,000°C. The light intensity for the 12-hr-daylength was about 40,000 lux.

Table 1

Temperature Conditions for Rice Plants (Cultivar; Taichung 65, waxy) during the Grain Filing Period

Group I	Temperature condition	Group II	Temperature condition
I-1	25°C 5 days 30°C 29 days	II-1	30°C 5 days 25°C 34 days
I-2	25°C 10 days 30°C 25 days	II-2	30°C 10 days 25°C 28 days
I-3	25°C 15 days 30°C 21 days	II-3	30°C 15 days 25°C 28 days
I-4	25°C 20 days 30°C 17 days	II-4	30°C 20 days 25°C 22 days
I-5	25°C 40 days	II-5	30°C 34 days

Preparation of rice starch granules

Starch granules were prepared from milled rice by the cold and dilute alkali method of Yamamoto et al. [8, 9].

High performance liquid chromatography (HPLC) of isoamylase-debranched amylopectin

Gelatinized amylopectin (2 8 mg) in 3. 5 ml of pure water at 100°C for 6 min was added 100 µl of 1 M acetate buffer (pH 3. 5) and 10 µl of *Pseudomonas* isoamylase (10 µg/10 µl, 590 units/µg protein), and incubated at 45°C for 2.5 h. The reaction mixture was added 200 µl of 0.1 N sodium hydroxide solution, 1.0 ml of 0.5M phosphate buffer (pH 8.5)-0.1 % sodium azide solution and 190 µl of pure water, and filtered through a 0.22 µm filter (Millipore). The filtrate (1.0 ml) was subjected to a HPLC apparatus (LS-8000, Tosoh Co. Ltd., Tokyo, Japan) with 5 columns; TSKgel G3000PWxr, (7.6x300 mm, Tosoh), Asahipak GS-320H (7.6x250 mm, Asahi Kasei Co. Ltd., 'Kanagawa, Japan)x2, TSKgel G2500PWxL, (7.6x300 mm, Tosoh), and TSKgel G-Oligo PW (7.6x300 mm, Tosoh). Detectors were a differential refractometer (RI-8011, Tosoh) and a low-angle laser light scattering photometer (LS-8000, Tosoh) with an interface (IF-8000, Tosoh) and a data processor (software, PC- LALLS vol. 1.03). Chromatograms were eluted with 0.1 M phosphate buffer (pH 8.5)-0.02 % sodium azide solution at 40°C with a flow rate, 0.5 ml/min.

High performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) of isoamylase-debranched amylopectin

Gelatinized amylopectin (5 mg) in 4.69 ml of pure water at 100°C for 6 min was added 1 00 µl of 1 M acetate buffer (pH 3. 5) and 10 µl *Pseudomonas*-isoamylase (10 µg/10 µl, 590 units/µg protein), and incubated at 45°C for 2.5 h. The reaction mixture was added into 200 µl of 0.1 N sodium hydroxide solution and filtered through a 0.22 µm filter (Millipore). The concentration of the filtrate used for analysis was 0.5 mg/ml.

Chain-length distribution was determined by a Dionex model DX-300 system (Dionex Corp., Sunnyvale, CA, USA) and a Model SC-PAD II pulsed amperometric detector consisting of an amperometric flow-through cell with a gold working electrode, a silver-silver reference electrode, and potentiostat according to the method described by Koizumi et al. [10-12] with a minor modification. Briefly, the system was equipped with a Dionex Carbopac PAl column (4x250 mm) in combination with a Carbopac PAl Gurad column (4x15 mm). Repeating sequences of potentials (volts) and durations (ms) on the PAD were as follows: E_1 0.10 (t_1 300), E_2 0.60 (t_2 120), E_3

-0.80 (t_3 300). The sample-injection loop size was 50 µl. Results were recorded on a SC 8020 integrator (Tosoh).

Eluent A was 0.15 M NaOH, and eluent B was 0.15 M NaOH containing 0.5 M sodium acetate. The gradient program was as follows; 40 % of eluent B at 0 min, 50 % at 5 min, 60 % at 20 min, 70 % at 26 min, and 80 % at 40 min. All separations were carried out at ambient temperature with a flow rate of 1 ml/min.

The degree of polymerization (DP) of oligomers was assigned by spiking samples with maltohexaose and standard response curves were prepared by using a mixture of malto-oligosaccharides (Fuji-oligo G67, Nihon Shokuhin Kako Co. Ltd., Fuji, Japan).

The individual peak area obtained from chromatograms of the isoamylase- debranched amylopectin was corrected by dividing the relative detector response. Using the corrected peak area, the exact distributions of chain length (6-17) of amylopectins were obtained [11].

Other method

The procedure for differential scanning calorimetry (DSC) was described earlier [13].

Results and discussion

Effects of environmental temperature on chain-length distribution of amylopectins by HPLC

Rice starch samples under study consisted of nearly 100 % amylopectin as shown by HPCL of iso- amylase-debranched materials. Table 2 shows some of their HPLC characteristics. The amylopectin of group I-1, group II-2, -3, -4 and -5 have decreased

Table 2

HPLC Characteristics of Isoamylase-debranched Materials Obtained from Endosperm Starches of Rice Plants Grown at Different Temperature after Pollination (Cultivar; Taichung 65, waxy)

Group I	BL %	BS + A %	BS + A / BL	Group II	BL %	BS + A %	BS + A / BL
I-1	28.6	71.4	2.5	II-1	26.2	73.8	2.8
I-2	27.1	72.9	2.7	II-2	28.5	71.6	2.5
I-3	26.1	73.9	2.8	II-3	29.1	70.9	2.4
I-4	25.6	74.4	2.9	II-4	29.3	70.7	2.4
I-5	25.8	74.2	2.9	II-5	30.2	69.8	2.3

BL – long chains, BS – short B chains, A – A chains.

amounts of long B chains and increased amounts of short chains as compared with the amylopectin of the group I-2, -3, -4 and -5, and group II-I. Accordingly, the ratio of short chains to long B chains for the amylopectin affected lower temperature (25°C) and higher temperature (30°C) are in a range from 2.7 to 2.9 and from 2.3 to 2.5, respectively.

The development stage, when the chain-length distribution of rice amylopectin was affected by the environmental temperature, was 5-15 days after anthesis (Table 2) and the same as the stage which was the most effective on determination of the amylose content in the endosperm starch in rice seeds [3, 7].

Effects of environmental temperature on short chain-length distribution of amylopectins by HPAEC-PAD

To elucidate detailed distributions of the amylopectin short chain-length, isoamylase-debranched materials of the starch obtained from the near-isogenic waxy rice plants grown under temperatue controlled conditions (25°C and 30°C) by HPAEC-PAD (Figs. 1 and 2).

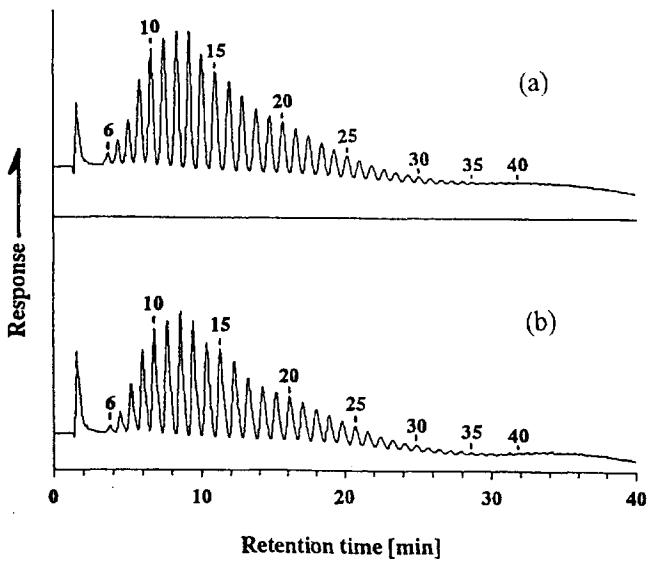

Fig. 1. HPAEC-PAD traces for isoamylase-debranched materials of starches obtained from rice plants grown under different temperature conditions after anthesis.
(a) group I-4; and (b) group II-4.

The amylopectin of Taichung 65 (*wx*) grown at 25°C has decreased amounts of chains with DP of 8 and 9, and slightly increased amounts of chains with DP of 6, 11, 12, 13 compared with the amylopectin of the same near-isogenic plants grown at 30°C.

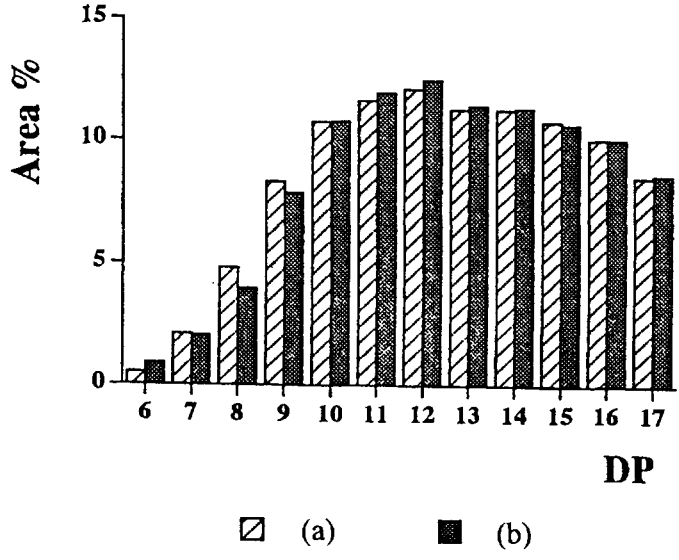

Fig. 2. Chain-length distributions of debranched amylopectins of rice plants grown under different temperature conditions after authesis.
(a) group I-5; and (b) group II-5.

Effects of environmental temperature on DSC characteristics of rice starches

As shown in Table 3, starch granules of the groups I-1, and group II-2, -3, -4 and -5 have higher: onset (T_o) and conclusion (T_c) temperatures and heats of gelatinization (OH) determined by DSC as compared to the groups I-2, -3, -4 and -5, and II-1.

Table 3

DSC Characteristics of Endosperm Starches of Rice Plants (Cultivar; Taichung 65, waxy) Grown at Different Temperature after Pollination

Group I	T_o [°C]	T_p [°C]	T_c [°C]	ΔH [J/g]	Group II	T_o [°C]	T_p [°C]	T_c [°C]	ΔH [J/g]
I-1	64	70	84	14.6	II-1	60	66	80	10.9
I-2	60	–	82	10.0	II-2	64	70	85	12.6
I-3	59	–	81	11.3	II-3	65	71	85	14.2
I-4	61	64, 72	78	11.3	II-4	65	72, 78	85	15.1
I-5	60	66, 73	80	10.0	II-5	65	72	84	14.2

T_o, onset, T_p, peak, and T_c, conclusion temperatures of gelatinization,
ΔH, heat of gelatinization.

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WPŁYW TEMPERATURY OTOCZENIA NA ROZMIESZCZENIE KRÓTKICH ŁAŃCUCHÓW W AMYLOPEKTYNIE RYŻOWEJ

Streszczenie

Na podstawie blisko izogenicznych linii ryżu wzrastającego w kontrolowanej temperaturze zbadano wpływ temperatury otoczenia na charakterystykę skrobi w endospermie ziaren w okresie wczesnego wzrostu. W okresie tym wraz z temperaturą otoczenia zmieniały się składowe skrobi i charakterystyka kleikowania gałeczek skrobiowych. W niższych temperaturach wzrasta zawartość amylozy i stosunek liczby łańcuchów krótkich do długich w amylopektynie. Ponadto gałeczki skrobiowe ryżu wzrastającego w niższej temperaturze kleikowały lepiej niż te wzrastające w temperaturze wyższej. Posługując się wysokosprawną chromatografią anionowymienną z pulsacyjnym detektorem amperometrycznym (HPAEC-PAD) w badaniach amylopektyn, w których odcięto łańcuchy boczne izoamylazą *Pseudomonas* stwierdzono, że amylopektyna woskowego Taichung 65 rosnącego w niższej temperaturze (25°C) zawierała mniej łańcuchów o stopniu polimeryzacji (DP) 8 i 9 i nieco więcej łańcuchów o DP 6, 11, 12 i 13 w porównaniu z amylopektyną z tych samych roślin blisko izogenicznych wzrastających w wyższej temperaturze (30°C).

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KATARZYNA BARTOSZEWICZ, BEATA PRABUCKA

RELATIONSHIPS BETWEEN THE TISSUE STRUCTURE AND β-ENDOGLUCANASE LOCALISATION IN GERMINATING TRITICALE SEEDS

Abstract

The mechanism of some enzyms taking part in the degradation of grain polysaccharides during the initial period of triticale germination is discussed. This concerns the function of the high pI α-amylase which is suggested to be the most important isoform active in the starch hydrolysis as well as the 1-3, 1-4-β-glucane-4-glycosidase which seems to be one of the most important enzymes degrading the cell wall structural components - β-glucanes. The use of two triticale varieties differentiated in their susceptibility to pre-harvest sprouting allowed to discuss both the enzyme activities and the internal structure changes evidenced in the light and electron microscope in relation to the various processes taking part in those samples. Most interesting observations seem to be those concerning the aleurone layer where the characteristic channels and perforations in the cell wall were formed specially in case of samples demonstrating the elevated activities of β-endoglucanase. Similar effects od starch digestion were observed in samples where the high pI-α-amylase isoform was present. Microscopic observations on the loosening of the cell wall structure seem to be particularly interesting as this could be the mechanism facilitating the enzyme and metabolite translocation through the grain tissues, thus accelerating the metabolic processes.

There are many factors which can more or less directly influence the seed germination. Thus, they are connected with the pre-harvest sprouting, quite common in cereals, particularly in rye and triticale. These factors are either of metabolic character, bound with the synthesis or activation of hydrolytic enzymes or of structural character connected with the tissue permeability to the enzymes and metabolites. The most important enzymes participating in the degradation of the storage components in order to produce low molecular weight metabolites needed for the embryo development are: the α- and β-amylases [EC 3.2.1.1 and 3.2.1.2] degrading starch, endo- and exopepti-

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dases, hydrolyzing the storage proteins to form the amino acids as well as the acid phosphatases which mobilize the P_i form from their organic complexes. However, recently attention was also paid to the other hydrolases, the activity of which might influence the changes in the internal tissue structure of seeds by the degradation of some insoluble cell components. The enzymes of this type may include the β-1→3; 1→4-endoglucane-4-glycosidase [β-endoglucanase - EC 3.2.8.73] together with the related ones of other specificity to decompose the glycosidic bonds as well as the phytate hydrolase [phytase - EC 3.1.3] [26].

Starch is the basic energy substrate, which degradation is needed for the developing embryo as the ATP source. Its hydrolysis is carried out in the plant cell by the α– and β-amylases (together with some related enzymes, e.g. α-glucosidase). α-Amylase, the enzyme playing main part in the starch degradation, is synthetized to some extent already during the cereal grain maturation (Beck, Ziegler 1989; Sitarski et al. 1992; Andrzejczuk-Hybel et al. 1994). This synthesis is taking place in the scutallum and the enzyme is quickly transferred to the aleurone layer. However, the predominant increase of the β-amylase activity takes place at the very initial germination stages, mostly as the result of the transformation of the bound to the active form and, to a lesser extent, due to the enzyme protein synthesis (Sitarski et al. 1992). The main α-amylase isoform taking part in the starch hydrolysis during the germination process is supposed to be that of the high isoelectric point (hpI-α-amylase). The synthesis of that isoform also begins in the last period of grain maturation, parallel to some other isoforms of low pI (Maresh, Gale 1990; Prabucka et al. 1995). As it has already been demonstrated by the above mentioned authors, some balance of both α-amylase isoform exists in the mature grain, which, however, depends on the variety resistance to the pre-harvest sprouting; the susceptible Malno variety demonstrates the predominance of the hpI-α-amylase activity as compared to the resistant Lasko variety. It results from the cited investigations (Table 1 – Prabucka et al. 1995) showing that the hpI-α-amylase reveals the higher affinity to the native potato starch as compared to the soluble one, a fact that was additionally confirmed on the commercial barley high pI-α-amylase Sigma type VIIIA. Using this preparation it can be demonstrated that this isoform presents a 2.6 times higher affinity to the native potato starch as compared to the soluble one. This behaviour might be connected with the partial unfolding of the helical structure of the native starch (as a result of breaking numerous hydrogen bonds in the course of its preparation). The observed affinity difference of hpI-α-amylase to both starch forms and the various values of this affinity determined after the successive germination times prove indirectly that the level of the hpI isoform varies depending on the variety, milling fraction and germination time. Therefore, the appli-

cation of the definite starch sample as the substitute should be of high importance in the determination of the total α-amylase activity (or the total amylolytic one). In selecting the substrate form the native starch should be anticipated as the one producing higher results. This may be of a special significance when activities of the amylase isolated from the germinating or spouting grains are determined.

Table 1

Activities of α-amylase determined on the native – n and soluble – s starch as well as β-endoglucanase activitities in selected milling fractions of Triticale grains of various pre-harvest sprouting resistance: Lasko (resistant) and Malno (susceptible)[a]

Germination time h	α-amylase activity mg starch · min^{-1} · g dry $matter^{-1}$						β-endoglucanase u · g dry $matter^{-1}$		
	Lasko			Malno					
	native	soluble	n/s	native	soluble	n/s	Lasko	Malno	M
	Milling bran								
0	52.8	75.1	0.70	100.0	77.7	1.25	3.7	5.7	1.5
24	81.2	88.8	0.91	135.1	79.2	1.70	13.9	19.4	1.4
48	242.1	168.2	1.44	348.1	183.4	1.90	25.4	35.8	1.4
72	542.6	261.0	2.02	707.5	413.9	1.71	32.2	36.5	1.1
	Outer endosperm								
0	34.3	92.7	0.37	49.4	76.8	0.64	2.7	2.8	1.0
24	31.9	81.4	0.39	51.0	77.4	0.66	3.2	3.6	1.1
48	144.4	133.0	1.08	220.9	127.4	1.73	9.9	12.1	1.2
72	332.5	256.2	1.30	476.4	363.9	1.31	15.7	15.0	0.9

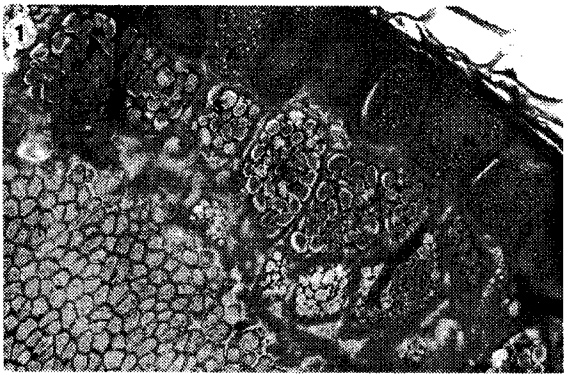
[a] In case of α-amylase the n/s ratio is the measure of the hpI-α-isoform as compared to the total, the pure hpI-α-amylase from barley (Sigma) has the n/s ratio = 2.60.

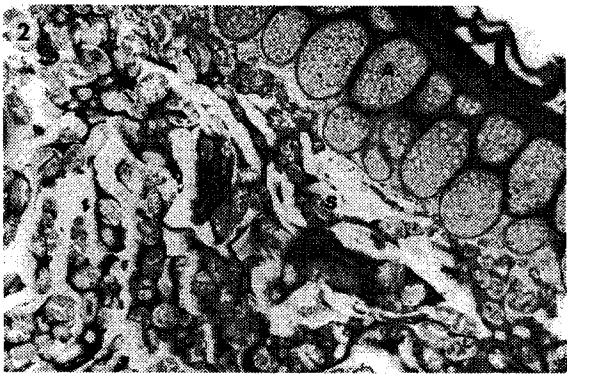
Our recent data obtained in the experiments on the triticale pre-harvest sprouting have confirmed that some amounts of the hpI-α-amylase are really formed during the final maturation period (Sitarski et al. 1992; Andrzejczuk-Hybel et al. 1994). However, the highest increases were observed during the initial 24 h (48 h in Lasko) of germination depending on the varietal susceptibility to the pre-harvest sprouting. Comparing the two investigated varieties it was demonstrated that the metabolic activity was at least one day slower in grains of the resistant variety Lasko as compared to the susceptible Malno. It seems, therefore, that the synthesis velocity of the isoform hpI-α-amylase may be the important factor affecting the pre-harvest sprouting resistance trait (Prabucka et al. 1995). At present, some more detailed investigations on the α-amylase isoform composition (together with some related enzymes) during grain germination were carried out in our laboratory.

Parallel to the hpI-α-amylase synthesis the changes of 1→3, 1→4-β-glucane-4-glycosidase (β-endoglucanase - EC 3.2.8.73) were investigated in the developing and germinating triticale grains. They are enzymes degrading β-glucanes which are the main components of the cell wall. The opinion exists that this enzyme (together with many related ones of other specificities) takes part in the preliminary digestion of the cell wall glucanes and pentosans (Stuart et al. 1986; Cordes, Henry 1989; Fincher 1989; Fry 1995). Their actions can, therefore, facilitate the transport of other digestive enzymes and their contact with substrates. Their action against the cell aleurone layer may be of particular significance facilitating the communication between the scutellum and endosperm. Admittedly the preliminary analysis did not exhibit much difference between the β-endoglucanase activities in the whole grains of various pre-harvest sprouting resistance during the first two days of germination (Niziołek et al. 1994). However, when these activities had been examined in particular milling fractions, some high differences were observed, particularly between those fractions which contained scutellum and aleurone cells; the β-endoglucanase activity in the susceptible variety Malno after 18 h germination was about 50 % higher than in the resistant Lasko (14.8 u and 10.4 u respectively per g dry matter but also at the zero time the difference amounted to 5.7 and 3.7 u, respectively). Hence the activity increase during the initial 18 h amounts to 9.7 u in Malno and 6.7 u in Lasko, i.e. it is about 1.5 times higher.

These results allowed to expect some consequent structural changes between the cross-sections of cell fragments of both varieties and imbibition times (0 and 18 h) which might be registered as different microscope images. The investigations were carried out in the light microscope UN 2, Zeiss, Jena and electron microscope JEM 100C (Jeol) and on respective enlargements of 150-450x and 5,000-50,000x. The material for the investigation included kernels germinated in the conditions described earlier (Bartoszewicz et al. 1993); the grain humidity for Malno amounted to 7 and 44 % and for Lasko 7 and 33 %, respectively at the zero and 18 h.

There were observed at least two changes differentiating the investigated samples in relation to the susceptible Malno variety after 18 h of germination which could not be observed either in both samples at zero h or in the resistant Lasko variety after 18 h of germination. At least 50 different elements of each sample were analysed in order to confirm the preliminary observations. The typical border cells between the aleurone layer starchy endosperm in the Malno variety at zero time and after 18 h germination are presented in Figs. 1 and 2, respectively. The basic differences can already be observed in the light microscope after 18 h germination as compared to the ungerminated sample. They include the reduced thickness of the cell wall, the partial digestion of the

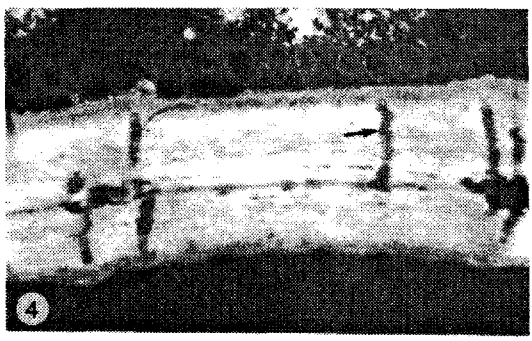




Figs. 1, 2. The cross section of the aleurone cells of variety Malno t = 0(1) and t = 18 h(2); light microsciope, enlargement 150 x.

aleurone granules as well as the partial digestion and desorganization of the starch globules.

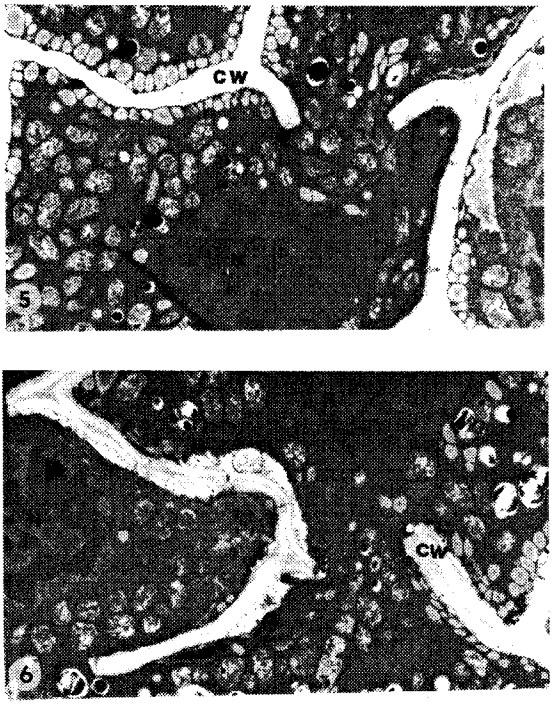
The phenomenon clearly visible in the electron microscope concerns the formation of the numerous channels penetrating into the walls of the aleurone cells at various angles (Figs. 3 and 4) which is assumed to be generated by the partial digestion of the β-glucane wall components. No such channels can be found either in both Lasko samples (0 h and 18 h) or at 0 h in the Malno variety. In the latter case only the plasmodesmata of similar shape could be observed. The even more interesting second phenomenon seems to be the appearance of a distinct discontinuity of the cell walls resulting from the complete breaking of the β-glucane components which is demonstrated in Figs. 5 and 6. Such an impairement allows the direct contact between the neighbouring cells and the mechanical exchange of the cell contents such as cytomyxia. Some direct translocations of starch granules through the recently formed slits in the cell wall were also clearly visible (Figs. 5 and 6). It could be presumed that the



Figs. 3, 4. Two objects of the cell wall sections, variety Malno 18 h showing the channels penatrating them. Electrone microscope, enlargement 50000 x.

demonstrated structural changes (the perforations of the cell wall) resulted from the action of β–endoglucanase which is 50 % more active in those samples. For comparison, Figs. 7 and 8 demonstrate that in the Lasko variety neither the channels nor the perforations of the cell wall can be observed.

Finally the comparison of Figs. 9 and 10 presenting the border region between the scutellum and endosperm in both Lasko and Malno varieties after 18 h germination shows the significant differences in the degree of digestion of the external starch granules. The enzyme layer is strictly adhering to the starch granules which in the Lasko variety are not much changed in their shape whereas in the susceptible Malno variety they are almost fully digested forming the almost amorphous matter. Similar comparison is presented in Figs. 1 and 2 showing the starch granules in the Malno variety at 0 and 18 h of germination but in this case the granules are digested to the lesser extent as compared to Figs. 9 and 10. The main site of the α–amylase and β-endoglucanase synthesis as well as peptidases particularly on the initial day of germination is located in the scutellum cells. During 2nd and 3rd day of germination the site of the second highest activity of the mentioned enzymes was situated in almost all

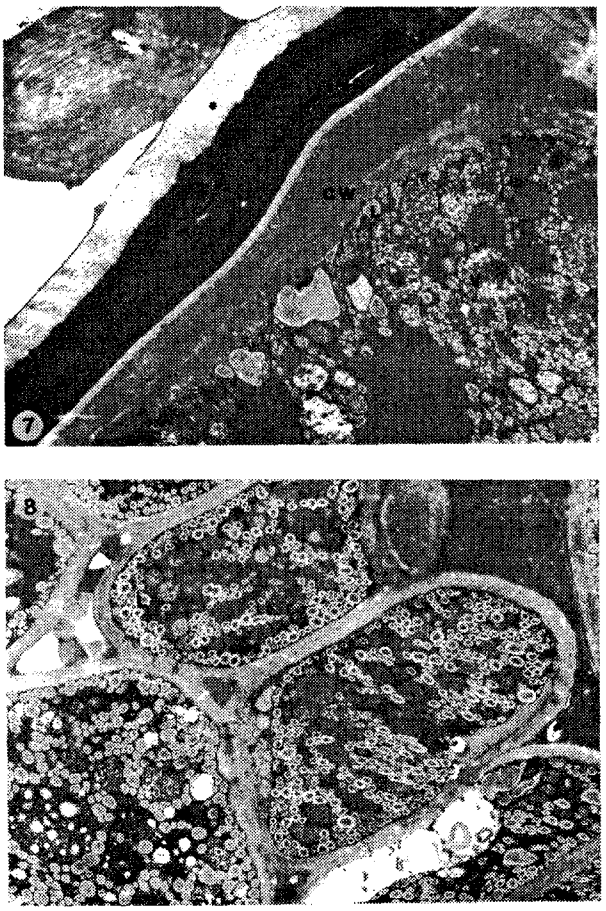


Figs. 5, 6. Two objects of the cell wall sections, variety Malno 18 h with the holes. Electron microscope enlargement 5000 x; the flow of starch granules trought the recently formed holes.

cases in the milling bran fraction. This fraction contains the majority of all the grain aleurone cells (Niziołek et al. 1994; Prabucka et al. 1995).

The presented results in relation to both the enzymatic character and microscope observations reveal that the accelerated production of the hydrolytic enzymes which are active in the degradation of the energy substrate (starch) as well as the storage proteins takes place at the very beginning of germination which is demonstrated in Figs. 1 and 2. This fact is well known from literature (Beck, Ziegler 1989; Kermode 1990; Shutov, Waintcaub 1987). Recently also the action of other hydrolases was reported which take part in the decomposition of the structural components of the cell wall, e.g. the β-endoglucanase (Cordes, Henry 1983; Fincher 1989; Prabucka et al. 1995). There was also suggested the ability of these enzymes to loosen the cell structure and thus to facilitate the internal transport of enzymes from the site of their synthesis (scutellum) to the regions of their substrate localisation as well as the metabolite translocations between the tissues of the germinating grains.

The investigations of the triticale resistance to the pre-harvest sprouting has been carried out for five years revealing, among others, that the initiation time of at least

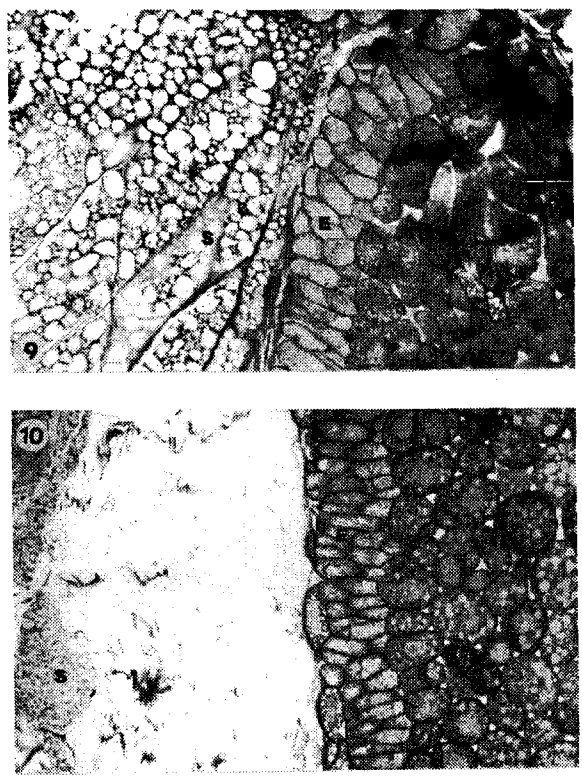


Figs. 7, 8. For comparison the two objects of Lasko variety t = 0 are presented. Electrone microscope; no channels holes penetrating the cell walls can be observed.

some processes directly connected with germination is simply related to the grain morphology changes and the resistance of the variety. The further experiments were carried out on two varieties well differentiated in relation to that feature – the susceptible Malno and resistant Lasko, which were developed in the direction of the activity and action mechanisms of some glycosidases. The comparison of the activity determination of the high pI α-amylase on two types of starch substrates – native and soluble potato starch allows the conclusion reached in the indirect way that the synthesis of this isoform (most important to starch degradation) in the susceptible variety overtakes that of the resistant variety by at least one day. Therefore, the isoform hpI in the initial germination period (up to 48 h) predominates in the aleurone layer but later it is translocated to the endosperm. This transport is also faster in the susceptible variety than

in the resistant one. The increase faster by about 50 % of the β-endoglucanase activity in the aleurone layer was also demonstrated during the first 24 h of germination of the susceptible variety as compared with Lasko. However, also in that case the enzyme translocation to the endosperm cells was delayed though not as much as in case of α-amylase. That last observation could suggest that the function of β-endoglucanase in the endosperm cells should be rather of limited significance if any at all.

The above considerations could be confirmed to some extent by the microscopic investigation in which the unsprouted (t = 0) and germinating samples (18 h) were compared as well as those of both susceptible and resistant varieties. From the technical point of view the preparation of kernels more humid than 40 % was too difficult. The significant structural differences were demonstrated between samples of t = 0 and 18 h of each variety as well as between parallel times and inter-sections of Malno and Lasko. It seemed to be most interesting to investigate the suspected effects of the β-endoglucanase action on the aleurone cell walls, expressed by the formation of charac-



Figs. 9, 10. Two objects of bordering cells between the scutellum and endosperm of Lasko (9) and Malno (10) varieties t = 18h; the differences in the digestion degrade are observed. Light microscope enlargement 150 x.

teristic channels visible on the wall sections as well as the decline of the cell fragments (perforations) providing the exchange of the contents of the neighbouring cells such as the cytomyxia. The findings of such changed structures among the elements of the susceptible variety Malno after 18 h of germination and the total lack of such structures at 0 time in Malno and at both times (0 and 18 h) in Lasko allow the suggestion that those channels or holes can be treated as the effect of the higher activity of the enzymes degrading the cell wall components (β-glucane) during the initial period of germination of the variety susceptible to the pre-harvest sprouting. The facilitated translocation of enzymes and metabolites in the grains of the susceptible variety is confirmed not only by the changes in their activities determined chemically in the milling fractions but also by comparing the structures shown in Figs. 1 and 2 or 9 and 10, where significant starch digestion in Malno cells after 18 h of germination is compared to that in Lasko at 0 time and 18 h of germination. The presence of channels formed in the cell wall as the effect of the β-endoglucanase action had already been suggested (Stuart, Fincher 1987) but the microscopic documentation of this phenomenon concerning the cereal seeds is lacking so far. Thus the comparison of structures presented in our research can serve as the possible confirmation of that suggestion also in relation to other seeds. On the other hand, the information of the existence of holes or wanes in the cell wall formed as the result of the hydrolytic degradation of polysaccharides can already be found in the literature, however it was suggested to be rather the effect of the cellulose hydrolysis (Fry 1995). Nevertheless, the observations of such structural modification facilitating the exchange of the cell contents of the cytomyxia type seems to be very interesting. However, in our opinion the existence of many glycosidases situated inside the plant cell wall makes the lysis of its components much more complicated but probably β-endoglucanase is playing a rather important part in that process. All these and similar phenomena have not been so far connected with the grain susceptibility to the pre-harvest sprouting. The investigations of these relations should be further continued as the possible diagnostic tool or in some other directions.

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ZALEŻNOŚĆ MIĘDZY STRUKTURĄ KOMÓRKI I UMIEJSCOWIENIEM β-ENDOGLUKANAZY W KIEŁKUJĄCYCH ZIARNACH TRITICALE

Streszczenie

Omówiono mechanizm działania pewnych enzymów uczestniczących w degradacji polisacharydów ziaren w czasie początkowego kiełkowania triticale. Dotyczy to roli wysokiego pI α-amylazy co sugeruje, że jest ona w hydrolizie skrobi najważniejszą aktywną izomorfą oraz 1-3,1-4-β-glukano-4-glikozydazy, która zapewne jest najważniejszym enzymem degradującym β-glukany będące składnikami strukturalnymi ścian komórek. Badanie dwu odmian triticale różniących się podatnością na wzrost do momentu zbioru pozwoliło poznać rolę aktywności obu enzymów. Badania mikroskopowe, także z użyciem mikroskopu elektronowego, pokazały wewnętrzne zmiany strukturalne zachodzące pod wpływem poszczególnych procesów. Najbardziej interesujące obserwacje dotyczą warstwy aleuronowej, w której powstały charakterystyczne kanały i perforacje, szczególnie widoczne w próbkach o podwyższonej aktywności β-endoglukanazy. Podobne efekty trawienia skrobi obserwowano w próbkach z wysoką izomorfą pI α-amylazy. Obserwacje mikroskopowe zaniku struktury ścian komórkowych pozwalają przypuszczać, że ten zanik jest częścią mechanizmu ułatwiającego przemieszczanie się enzymu i metabolitów przez komórki ziaren, co przyspiesza procesy metabolityczne.

CHENG-YI LII[1,2], MIN-FENG LAI[1], MEI-LIN TSAI[2]

STUDIES ON STARCH GELATINIZATION AND RETROGRADATION WITH DYNAMIC RHEOMETRY – THE INFLUENCE OF STARCH GRANULAR STRUCTURE AND COMPOSITION

Abstract

The study on the starch gelatinization and retrogradation with dynamic rheometry are reviewed. Three typical varieties of rice starches, including indica (KSS7), japonica (TNu67) and waxy rice (TCW70) are used during the discussion. The amylose contents are 24-26 % for KSS7, 15-16 % for TNu67, and 0.8-1.0 % for TCW70. The heating and cooling behaviours of the individual starch, the combination of two starches, and the addition of amylose to the starch systems are discussed.

The correlation between swelling power, amount of water soluble, blue value, and λ_{max}, as well as gelatinization temperature, and the dynamic rheogram are applied for the elucidation of relationships between the starch molecular and granular structures, and the gelatinization and the retrogradation mechanisms. Generally, the G' increases in gelatinization process of starch is mainly governed by the granular characteristics, which include the rigidity of swollen granule and the interaction between these close-packed granules. However, the G' in starch retrogradation is influenced by the interaction between leached-out or external added amylose itself and swollen granule, in addition to the property of swollen granule. As for the mixed starch system, the combination of waxy and non-waxy starches will decrease G' drastically. Whereas the addition of amylose molecule will decrease the G' for the gelatinization process, but will increase G' during cooling and aging profoundly. Hence, it is suggested that the starch granular properties and characteristics are the major factor for the starch rheological behavior, followed by the leached-out amylose during gelatinization process, especially in the high concentration system.

Introduction

Changes in physicochemical properties of starchy product are usually described by the gelatinization and retrogradation behaviours of starch. When the aqueous starch suspension is heated above the gelatinization temperature, an irreversible swelling will occur. This irreversible swelling is accompanied by the loss of order, the loss of crystallinity, and the release of amylose into solution [1-4]. Starch gelatinization in excess

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water has been considered as a two-stage process, consisting of the initial swelling of the granule and its eventual dissolution. Such behavior follows pseudo-first-order Arrhenius kinetics [5]. At sufficient concentration, the hot starch suspension will behave as a viscoelastic dispersion [6-8]. During cooling, the paste may be transformed into an opaque elastic gel [3, 9-11]. The SEM for starch paste or gel indicates a coarse network formed by the solubilized amylose and amylose-linked swollen granules [12]. Thus, the paste and the gel are often considered as a composite material with the swollen starch granules filling through the polymer solution [9-11, 13-17]. Miles and his co-workers have suggested that the initial stages of gelation of starch is dominated by the gelation of the solubilized amylose [10]. However, the results from Evans and Haisman (1979) showed that the material outside the swollen granules (eg. amylose) had little effect on the rheology of the starch suspensions [18]. Factors influencing on the rheological properties of starch gel or paste include the starch granules (concentration, granule size and distribution, swelling pattern, rigidity and deformability of granule) [7, 19-22], amylopectin (fine structure) [23-26], leached-out amylose (amount, type and entanglements), interactions between the components (granule-granule contact and granule-amylose interactions) [25, 27], temperature, heating and cooling rates, mechanical treatments [6-8, 19], additives, etc. The structure of gel or paste controls the texture and quality of starchy foods.

Many studies concerning these behaviours have been made using differential scanning calorimetry, Brabender viscoamylography, x-ray diffractometry, β-amylase-pullulanase method [28-31], etc. However, parts of studies on gelatinization and retrogradation behaviour observed from change in viscosity of starch dispersion involved the behaviour of highly sheared starches with extensively disrupted granule. It is important to explore the contribution of leached-out amylose chain, swollen granule and the interaction through the system on the starch rheology with an undamaged, precise and simultaneous approach. Recently, a small angle oscillatory rheometer has been adopted as one of excellent tools, because it provides information about the rapid change of viscoelastic properties of starch dispersion during heating and cooling with negligible interference on the formation of a gel network [6, 8, 20, 22, 27, 32-40]. Hence, the effect of molecular and granular structures of rice starch on the dynamic rheological properties of starch dispersion system during heating and cooling are reviewed, in order to clarify the mechanisms of starch gelatinization, gelation, and retrogradation.

Pyysical and structural characteristics of rice starch

Rice (*Oryza sativa* L.) can be classified into three categories – indica, japonica and waxy rice [41]. Hsieh and his co-worker [42] indicated that the granular sizes of

both indica and japonica rice starches were 2~10 μm; and waxy rice starch, 2~8 μm. The mean sizes of these starches are in the range of 4~5 μm. Rice starch is polygonal in shape [42, 43-44]. The apparent amylose contents in starches from indica, japonica and waxy varieties are in the ranges of 14~29 %, 14~19%, and 0.8~2.0 %, respectively [8, 22, 45]. The powdered X-ray diffractogram of rice as well as other cereal starches shows a typical A-type [28].

Studies on the gelatinization temperatures (GT) for various rice starches by DSC measurements indicate that no significant differences are observed among these three varieties. The result implies that the GT of rice starch may be principally governed by the starch granular structure and by the chain length of amylopectin rather than by the amylose content [44-46]. Brabender viscoamylograms of all non-waxy rice starches display a moderate restricted-swelling pattern [44] and belong to type-B according to Schoch's classification [47]. And the setback viscosity have a close correlation with the amylose content. A type-A viscoamylogram with a high pasting peak is shown for the waxy variety.

Table 1

Molecular characteristics and some physical properties of rice starches

	Indica KSS7	Japonica TNu67	Waxy TCW70
Amylose (%)[a]	24.1~25.6	14.8~15.7	0.78~0.99
DP_n[b]	1075	1004	–[g]
Amylopectin DP_n[c]	2743	8812	9101
Physical property			
WBC, %[d]	93	118	–
Iodine affinity, %[d]	4.95	3.73	–
Gelatinization[e]			
T_o-T_p-T_c, °C[f]	72.0-76.6-89.2	64.4-71.0-82.3	64.0-71.9-85.0
ΔH, cal/g	3.37	2.94	3.20
Retrogradation ΔH, J/g	~10	~9.0	~8.0

[a] apparent amylose content was determined by the method of Lii et al. (61)
[b] exerpted from ref. 48
[c] excerpted from ref. 45
[d] excerpted from ref. 49
[e] excerpted from ref. 8 & 22
[f] T_o, T_p, T_c, and ΔH designated for the onset, peak, and completion temperatures; and the enthalpy for gelatinization
[g] not detected

The compositions and physical properties of three typical rice starches, including indica (Kaohsiung Sen 7, KSS7), japonica (Tainung 67, TNu67) and waxy (Taichung waxy 70, TCW70) varieties, are excerpted from the investigations of Chen [45], Lii and his co-workers [8, 22, 45, 48, 49, 62] and are listed in Table 1. Apparent amylose contents of KSS7, TNu67 and TCW70 are 24~26 %, 15~16 % and 0.8~1.0 %; and the number-averaged degree of polymerization (DP_n) of the KSS7 and TNu67 amyloses are 1075 and 1004 (glucose units) [48], respectively. And, the DP_n of KSS7, TNu67, and TCW70 amylopectins are 2743, 8812, and 9101, respectively [45]. Indica starch possesses lower water binding capacity (WBC) than that of japonica starch [49]. And, the temperatures and enthalpies of gelatinization measured with DSC are 72~89°C and 3.37 cal/g for KSS7; 64~82°C and 2.94 cal/g, TNu67; and 64~85°C and 3.20 cal/g, TCW70 [8, 22]. The retrogradation enthalpies for 20 % concentration (w/w) stored at 5°C are KSS7 > TNu67 > TCW [45]. Generally, the high-amylose KSS7 starch possesses the amylopectin with significantly lower DP_n, higher iodine affinity, higher gelatinization temperature and retrogradation enthalpy than the other two starches.

Table 2

Structural properties of rice amylopectins [45]

		Indica KSS7	Japonica TNu67	Waxy TCW70
Average chain length, $\overline{CL}$	(g.u.)	20.2	17.5	17.6
Exterior chain length, $\overline{ECL}$	(g.u.)	14.5	12.6	12.2
Interior chain length, $\overline{ICL}$	(g.u.)	4.67	3.86	4.39
β-Amylolysis limits (%)		62.1	60.8	58.0
Chain distribution [a] (%)	Extralong (a)	3.74	nd[b]	nd
	Long (b)	35.6	34.5	34.2
	Short (c)	60.7	65.5	65.8
	(a+b)/c	0.648	0.527	0.519
λ_{max} (nm)		592	538	533

[a] measured with GPC
[b] not detectable

The structural properties of these three rice amylopectins are listed in Table 2 [45]. The average chain length ($\overline{CL}$), average exterior chain length ($\overline{ECL}$) and average interior chain length ($\overline{ICL}$) of amylopectin from KSS7 starch are all higher than those from TNu67 and TCW70. The gel permeation chromatograph of KSS7 amy-

lopectin, which is of lower DP_n, shows less amount of short chain fraction, but with a small amount of extralong chain fraction. And, no extralong chain fraction is detected in the other two amylopectins. Consequently, KSS7 amylopectin displays a higher degree of β-amylolysis and λ_{max} than the other two.

Dynamic rheological studies on starch gelatinization mechanism

Figure 1 displays the changes of the storage moduli (G') of KSS7 and TNu67 starches during heating at a rate of 1°C/min [8]. The temperature at which G' increases drastically and instantaneously is designated as $T_{G'}$ [8]. The increments of starch concentrations from 5 to 30 % can increase the gradient of G' notably and the value of maximum G' (G'_{max}), but with a lower $T_{G'}$. Both G' and $T_{G'}$ of TNu67 are higher than those of KSS7 at same starch concentration. The value of G' for TCW70 is very small, even with concentration up to 30 %. The results coincided with the fact that starch granule with low amylose content is less rigid and tended to disintegrate easily when swollen intensely and overcrowded [50].

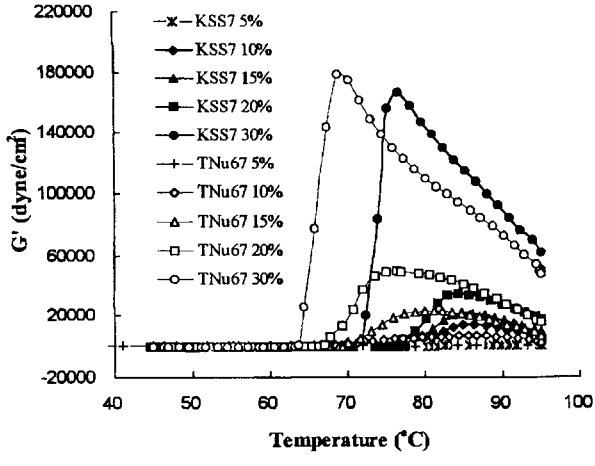

Fig. 1. Storage modulus (G') measurements of KSS7 and TNu67 at different concentrations during heating (frequency, 1Hz; strain, 0.015; heating rate, 1°C/min) [8].

The developments of loss modulus (G'') for these rice starch dispersions at different concentrations with elevating temperature are displayed in Figure 2 [8]. The influence of temperature on G'' of the rice starch is similar to that on G' (Fig. 1).

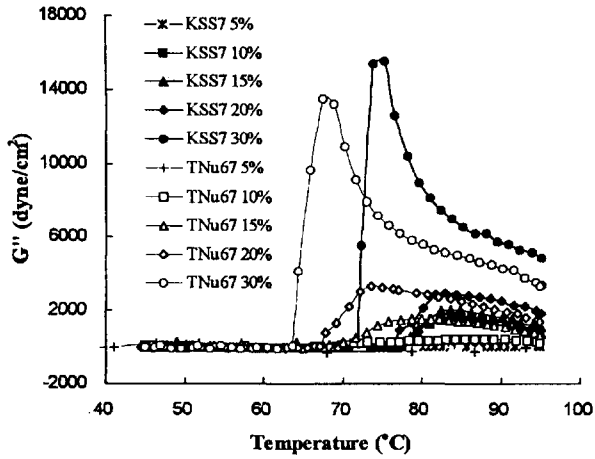

Fig. 2. Loss modulus (G'') measurements of KSS7 and TNu67 at different concentrations during heating (frequency, 1Hz; strain, 0.015; heating rate, 1°C/min) [8].

For further elucidating the heating behaviour of starch, some indices from Figure 1, including $T_{G'}$, $T_{G'max}$ (temperature at G'_{max}), $(dG'/dT)_{max}$ (the maximum slope of increasing G' against temperature), G'_{max} (maximum G'), G'_{95} (G' at 95°C) and $tan\delta_{95}$ (ratio of G'' to G' at 95°C) are applied and listed in Table 3. While the concentration increasing from 10 % to 30 %, the values of $T_{G'}$ for KSS7 and TNu67 decrease from 78.7 to 72.4°C and 69.9 to 64.4°C; and $T_{G'max}$, from 86.4 to 76.6 and 85.6 to 68.8°C, respectively. The $(dG'/dT)_{max}$ of KSS7 and TNu67 raise with the increment of starch concentration up to the temperature of G'_{max} ($T_{G'max}$). The G' on continuous heating after $T_{G'max}$ will decrease to a certain level, depending on starch variety. The loss tangent ($tan\delta$), which is an index of viscoelastic property, of KSS7 and TNu67 at 95°C reduce from 0.28 to 0.08 and 0.10 to 0.07, with the increase of concentration. These results indicate that TNu67 shows higher G'_{max}, lower G'_{95}, and more elastic than KSS7 during heating. The G'_{95} of 30 % TCW70 is only 950 dyne/cm^2. However, the value of $tan\delta$ (~0.4) implies that TCW70 in the concentration of 20–30 % tends to be elastic, or solid property, rather than liquid property.

Heating above $T_{G'}$ will promote the interactions between swollen granules and/or leached-out amylose and granule. Finally, G' reaches a maximum value (G'_{max}). After that, further heating provides the energy to breakdown the residual crystalline structure of the granule accompanying with releasing amylose [6, 8], and to enhance Brownian mobility. Consequently, swollen granules become softer and G' drops down. Keetels and Vliet (1994) [6] suggested that the initial increase of the G' could attribute to the

degree of granule swelling just to fill the whole available volume of the system. And G'_{max} has been considered as one of the rheological parameters of swollen granule tightly packed.

Figure 3 demonstrates the concentration dependences of $T_{G'}$, $T_{G'max}$, $(dG'/dT)_{max}$ and G'_{max} for KSS7 and TNu67 starches. There is a linear logarithmic relation between $(dG'/dT)_{max}$ or G'_{max} and starch concentration, especially for TNu67. For KSS7, $(dG'/dT)_{max}$ or G'_{max} can be detected at concentration as low as 5 %. The $(dG'/dT)_{max}$ of KSS7 is in proportion to ($\propto$) $C^{3.90}$, and is close to that of TNu67 ($C^{3.95}$). However, the G'_{max} of KSS7 is $\propto C^{3.22}$, which exponent is larger than that of TNu67 ($C^{2.97}$). $T_{G'}$ of 10 % and 15 % KSS7 or TNu67 starches are similar to each other. Above 15 %, $T_{G'}$ decreases with the increment of concentration by the slopes of -0.419 (R^2 = 0.996) and -0.374 (R^2 = 0.994), respectively. This result implies that the temperature of the swollen granule just fulfilling the system will drop down with the increase of starch concentration at above 15 %. Whereas, the linear correlation be-

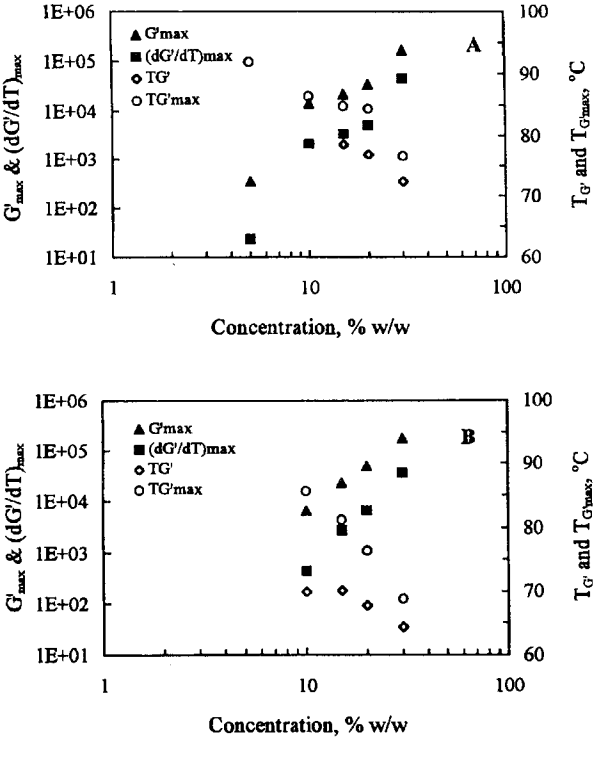

Fig. 3. Concentration dependences of rheological parameters and critical temperatures of KSS7 and TNu67 starches during heating (1°C/min) [51].

tween $T_{G'max}$ and concentration is observed with the gradient of -0.552 (R^2 = 0.935) for KSS7 or -0.841 (R^2 = 0.997) for TNu67. This suggests that the tight-packing temperature of swollen granule decreases with the reduction of moisture content.

Correlations between granular swelling and rheological properties of starch

The viscoelasticity of gelatinized starch dispersion is a result of a complicated combination of parameters involving the viscoelasticity of the continuous phase, the volume fraction of the dispersed phase, and the shape and deformability of the swollen particles [7]. Thus, it will be of great interest to learn the correlations between the dynamic rheological properties of starch dispersion and the swelling-solubility properties, degree of swelling, and the close-packing temperature of starch granule.

The swelling-solubility property of starch granule

The swelling-solubility properties, including swelling power, water soluble index (WSI), blue value and λ_{max}, of 1 % KSS7, TNu67 and TCW70 starch dispersions are shown in Figure 4 [51]. The changes in swelling power and WSI for these three starches suggests a two-stage process of swelling which fits the kinetics model mentioned by Kokini et al. (1992) [5]. The temperatures of first notable increase in swelling power are 65~75°C for KSS7 and 55~65°C for the other two (Figure 4A, C, E). The second stage of swelling drastically, accompanying with remarkable WSI increase, all occur at above 85°C. And this temperature is slightly higher than both T_c of gelatinization and $T_{G'max}$ in rheogram for 20 %. The degree of swelling power is TCW70 >> TNu67 > KSS7, where the swelling power of TCW70 is almost as high as two times of the other two. Both blue value and λ_{max} of KSS7 and TNu67 increase extensively at 65~75 and 55~65°C, respectively (Figure 4B&D). The changes in λ_{max} of KSS7 and TNu67 are from ~570 to ~630 nm with elevating temperature. As for TCW70, the blue value and λ_{max} increase slightly at above 75°C from ~0.0 to 0.1 and 520 to 570 nm, respectively (Figure 4F).

From the above results, the swelling for KSS7 and TCW70 starches in excess water are similar to the corn and tapioca starches, respectively [5], and the TNu67 behaves between them. These starches swell in different manners, reflecting varietal differences in the molecular organization within the granule. KSS7 granule is the most close-packed, and TCW70, the most unrestrained. This result is in accordance with the data of viscosity [45]. Generally, non-waxy starch (KSS7 or TNu67) has higher WSI and blue value than those of waxy starch (TCW70). It also indicates that the amount of water solubles of KSS7 at 95°C is much less than its amylose content (~25 %). That is, only one half of amylose molecules is leached out, and the residual amylose remains

entangling with amylopectin within granule. Moreover, the amount of residual amylose within starch granule will increase with the increment of starch concentration [7, 15]. This entanglement (not crystallization) inside of the gelatinized KSS7 granule may be responsible for its higher G'_{95} than that of TNu67 (Table 3). Such phenomenon hints that the rigidity of swollen KSS7 granule can improve with the amylose fraction retaining within the granule.

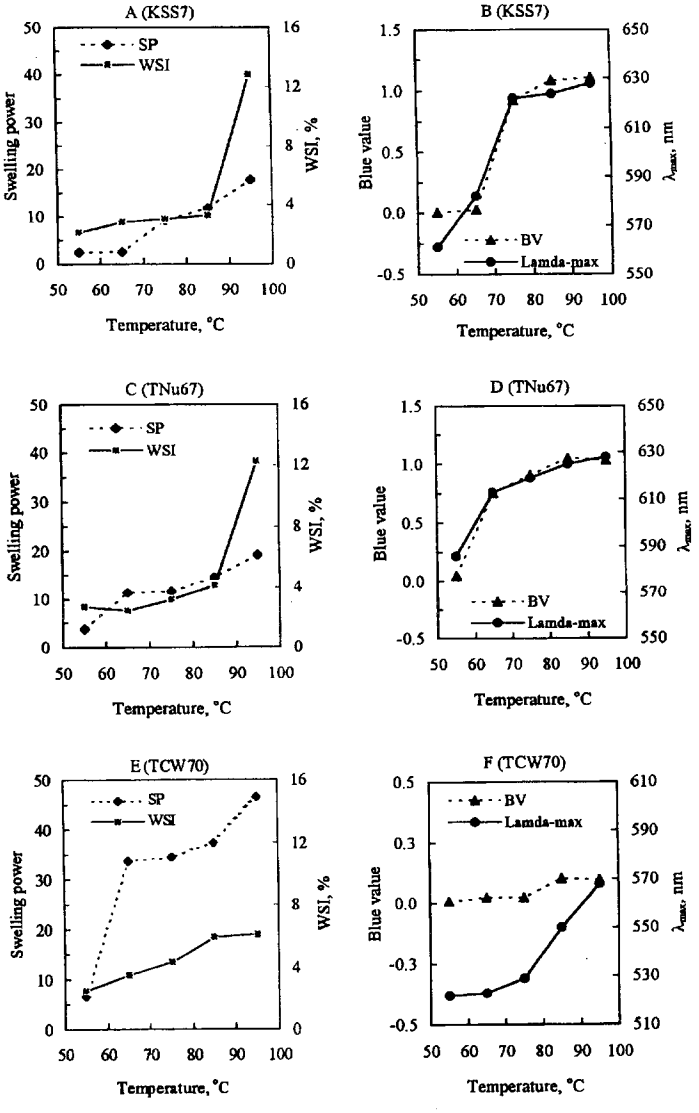

Fig. 4. The swelling power, WSI, blue value and λ_{max} as a function of temperature for 1 % (w/w) KSS7 (A, B), TNu67 (C, D) and TCW70 (E, F) starch dispersions [51].

Table 3

Effect of starch concentration on rheological properties of rice starches in water during heating [8]

Starch	Concn. %	$T_{G'}$ °C	$T_{G'max}$ °C	$(dG'/dT)_{max}$ dyne/cm^2·°C	G'_{max} dyne/cm^2	G'_{95} dyne/cm^2	$\tan\delta_{95}$
Indica- KSS7	5	nd [a]	91.9	22.9	346	182	0.28
	10	78.7	86.4	2135	14230	1619	0.17
	15	78.6	84.8	3436	21800	5746	0.12
	20	76.9	84.4	5144	34630	19680	0.11
	30	72.4	76.6	44736	167300	54760	0.08
Japonica-TNu67	5	nd	nd	nd	nd	479	nd
	10	69.9	85.6	439	6630	1651	0.10
	15	70.1	81.2	2759	23100	6486	0.09
	20	67.8	76.4	6584	49520	14970	0.09
	30	64.4	68.8	36006	178500	47150	0.07
Waxy--TCW70	5	nd	nd	nd	nd	nd	nd
	10	nd	nd	nd	nd	nd	nd
	15	nd	nd	nd	nd	nd	nd
	20	nd	nd	nd	nd	391	0.40
	30	nd	nd	nd	nd	950	0.39

[a] not detectable

For understanding the influence of WSI on the rheological properties of starch, it is important to clarify what molecular characteristics of the water soluble fraction is. Hizukuri [52] has demonstrated that the $\overline{CL}$ of water-soluble amylose is increased with elevating extraction temperature from 60–80°C. It may contribute the higher λ_{max} with higher temperature until granule breakdown during heating. The studies on 14 rice amylopectins by Chen [45] further prove such phenomenon. A linear relationship between averaged chain length ($\overline{CL}$) and λ_{max} (nm) for indica amylopectin is $\overline{CL}$ = 0.063(λ_{max}) - 16.845 (R^2 = 0.955) (Figure 5A). And the ratio of extralong (a) and long (b) to short chains (c) is also in proportion to the λ_{max} for all 14 amylopectins and can be expressed by the equation of (a+b)/c = 0.002 (λ_{max}) - 0.575 (R^2 = 0.909) (Figure 5B). Hence, it may conclude that the higher the λ_{max}, the greater is the $\overline{CL}$ of the leached-out molecules.

The λ_{max} of amyloses from KSS7 (DP_n=1075), TNu67 (DP_n=1004), wheat (Dp_n = 500~790) and potato (Dp_n = 4360~6990) starches are 650, 653, 645 and 665~670 nm, respectively; and λ_{max} of amylopectins are 579 nm for KSS7, 537 nm, TNu67, and 534 nm for waxy rice [52, 53]. Hence, the values of λ_{max} for three gelatinized starch dispersions shown in Figure 4 reveal that the average molecular sizes of leached-out matter are small for TCW70 and intermediate for KSS7 and TNu67. These swelling-solubility properties and the swollen granular structure are responsible for the dynamic rheological characteristics during gelatinization.

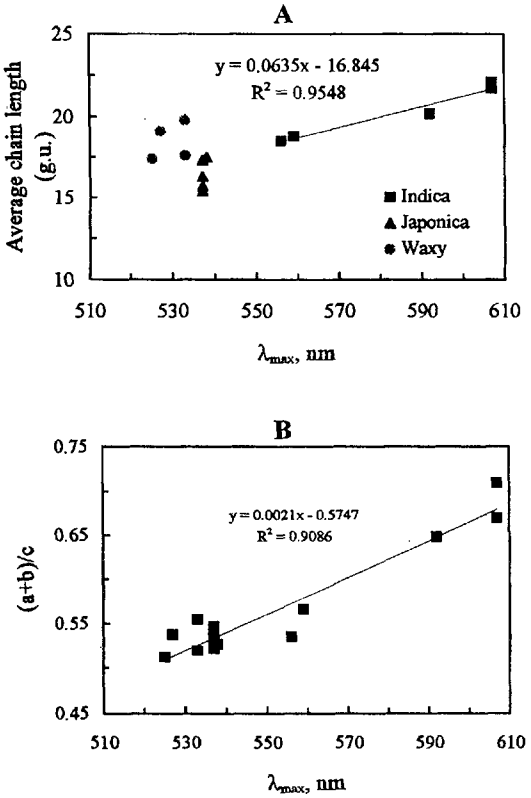

Fig. 5. Correlations between the average chain length (A) as well as extralong (a) + long chains(b)/short chain (c) (B) and λ_{max} for 14 rice amylopectins [45].

Contribution of the rigidity of swollen granule to dynamic rheological property of starch

Doublier, Ring and their co-workers [7, 15] have proposed that the rigidity of swollen granule can govern the rheological property of concentrated starch system, and the degree of influence is increased significantly with the increment of concentration. The rigidity of swollen granule can be simply estimated by the inverse of swelling value [7, 19]. The effects of the degree of granular swelling and the amount of leaching-out material on dynamic rheological properties have been conducted by heating the 20 % KSS7 dispersion up to 70~95°C, followed by cooling to 25°C. The rheological parameters including G'_{T_f} (G' at final heating temperature T_f), tan δ_{Tf} (tanδ at T_f) on heating, and the $(dG'/dT)_{max}$ (maximum slope of G' increase), G'_{25} (G' at 25°C), tan δ_{25} (tanδ at 25°C) as well as G'_{25}/G'_{T_f} during cooling are listed in Table 4 [8]. The $(dG'/dT)_{max}$ or G'_{25}/G'_{T_f} can be applied as a retrogradation index. No modulus development is observed while the starch dispersion is heated up to 70°C (Table 4). When the

temperature is raised up to 75°C (near to the T_P of gelatinization), a negligible G'_{75} shows on rheogram, but with a significant G' development on cooling. It leads to a high G'_{25}/G'_{75} ratio, 16. When the starch dispersion is cooked up to 80 or 85°C (higher than the T_P of gelatinization), the respective G'_{80} (40710 dyne/cm^2) and G'_{85} (41660 dyne/cm^2) are higher than the other temperatures. Consequently, high $(dG'/dT)_{max}$ and G'_{25} on cooling are obtained. Heating at 85°C can result in the starch system of the highest G'_{Tf}, G'_{25}, $(dG'/dT)_{max}$, elasticity (i.e. lowest tanδ) and G' declines at the late stage of cooling among the heating-cooling processes examined. Above 85°C (higher than T_c of gelatinization), elevating temperature will decrease G'_{Tf}, $(dG'/dT)_{max}$, and G'_{25}. However, tanδ reduces from 0.08~0.11 to 0.03.

Table 4

Effect of final heating temperature on rheological properties of 20 % KSS7 sratch [8]

Final Heating Temperature T_f, (°C)	Heating		Cooling			
	G'_{Tf} (dyne/cm^2)	$\tan\delta_{Tf}$	$(dG'/dT)_{max}$ (dyne/cm^2·°C)	G'_{25} (dyne/cm^2)	$\tan\delta_{25}$	G'_{25}/G'_{Tf}
70	nd[a]	nd	nd	nd	nd	nd
75	74	0.48	22	1186	0.39	16.0
80	40710	0.08	458	65890	0.03	1.6[b]
85	41660	0.08	504	71880	0.03	1.7[b]
90	31770	0.09	490	63630	0.03	2.0
95	17320	0.11	395	45010	0.03	2.6

[a] not dectectable

[b] G' drops at the late stage of cooling

From the data of Figure 4 and Table 4, the starch heated up to 70°C does not show any notable modulus is due to not gelatinized yet. High G'_{25}/G'_{75} ratio for the 75°C is the result of interaction between partially gelatinized, moderately swollen granule and a fairly small amount of shorter-chain solubilized material. The granule of 85°C with an appropriate degree of swelling and some residual crystallites, and a small amount of longer-chain solubles are responsible for the maximal G'_{Tf}, $(dG'/dT)_{max}$ and G'_{25}. Above 90°C, the melting of remaining crystallites inside granule and most amylose releasing from granule cause the swollen granules to become softer. This softening of swollen granule should be the reasons of low modulus and $(dG'/dT)_{max}$. Furthermore, a large amount of long-chain amylose leaching-out into solution at 90~95°C (Figure 4A) tends to improve the G'_{25}/G'_{95} on cooling (Table 4). The impor-

tance of rigidity of swollen granule on the dynamic rheological properties is also shown for concentrated starch system [6, 15].

Relations between the temperatures of granular close-packing and G' increase

Bagley & Christianson (1982) [19] have introduced the product of granular swelling capacity (Q) and starch concentration (C) in a suspension free of solubilized material as a true measurement of whether there is excess water between the swollen granules (CQ < 1) or not (CQ > 1). When excess solvent is present, CQ is less than unity and is equivalent to the volume fraction (ϕ) of swollen granule in the system. CQ can be greater than unity for deformable granules. Taking into account that part of starch is solubilized, Doublier et al. (1987) [7] have further expressed the relationship among the volume fraction of the swollen granule (ϕ), concentration, swelling power and solubility as the following equation:

$$\phi = C \cdot Q \cdot (1 - S/100) \quad (1)$$

Where C is starch concentration expressed in g/g; Q, swelling power in g/g; and S, WSI in %. When $\phi < 1.0$, the swollen granules are dispersed in excess water, and $\phi = 1.0$, the swollen granules just fill up starch system [7, 19, 21]. The temperature at which the swollen granules just fill up the system is designated as $T_{\phi=1}$ [22].

The mass fraction (ϕ) of starch granule at a certain concentration and temperature can be calculated from equation (1) by introducing the values of swelling power and WSI. $T_{\phi=1}$ for a known concentration can be derived by interpolation method from plot of ϕ *versus* temperature. The relations between the $T_{G'}$ in rheogram and $T_{\phi=1}$ for 5~40 % KSS7 concentrations are displayed in Figure 6 [51]. Both $T_{G'}$ and $T_{\phi=1}$ decrease with the starch concentration increases. There are two concentration

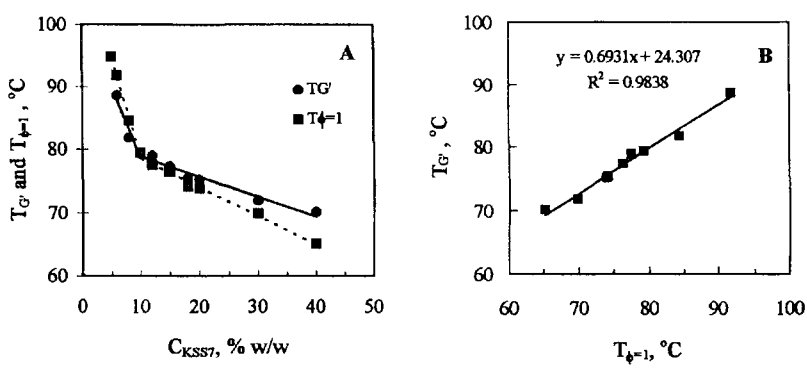

Fig. 6. Concentration dependences (A) and correlation (B) of the critical temperatures $T_{G'}$ and $T\phi=1$ of KSS7 starch [51].

dependences of $T_{G'}$ or $T_{\phi=1}$ for KSS7 starch dispersions with a turning point at 10 % concentration. Below 10 %, $T_{\phi=1}$ is higher than $T_{G'}$; and above, $T_{\phi=1}$ is lower than $T_{G'}$. This result reveals that the restricted swelling of starch granule occurs at above 10 %, which is also in accordance with the data of Figure 3. $T_{G'}$ and $T_{\phi=1}$ should be identical, if the initial increase in storage modulus is contributed by the swelling of starch granules to occupy the available volume of the system as proposed by Keetels and Vliet [6]. The discrepancy between $T_{\phi=1}$ and $T_{G'}$ may be due to the influence of the starch concentration on Q and S in eq (1).

It has been manifested swelling power and WSI will decrease with the increment of concentration [7, 15]. Nevertheless, the degrees of influence from swelling power and WSI on rheological properties of starch system may vary with different concentrations. The granule of 5 to 10 % concentration may swell as freely as that of 1 % before close packing, because of still excess water in the system. Consequently, Q value should not be affected by the concentration. One can attribute the high $T_{\phi=1}$ to over-estimate of S value applied in the eq (1) under these low concentration systems. At concentration higher than 10 %, both $T_{G'}$ and $T_{\phi=1}$ are lower than 80°C. The value of WSI (S) is very small. The result of low $T_{\phi=1}$ from over-estimate of Q value is anticipated.

At low concentration (< 10 %), generally, the value of WSI should be considered for interpreting the pasting behaviour of starch system; and at high concentration (> 10 %) the swelling power should be taken into account. In addition, measurements of both $T_{G'}$ and $T_{\phi=1}$ may be affected by the rigidity of swollen starch granule, although such influence is difficult to prove. It is interesting to point out that a linear relation between $T_{G'}$ and $T_{\phi=1}$ of KSS7 is detected, irrespective of starch concentration. The relationship can be expressed as the equation of $T_{G'} = 0.69T_{\phi=1} + 24.31$ ($R^2 = 0.984$) (Figure 6B).

Rheological characteristics of starch during retrogradation

During cooling, the G' for gelatinized KSS7 and TNu67 starches at different concentrations increase steadily with decreasing temperature as shown in Figure 7 [8]. The increments of slope of G' against cooling temperature for the two starches are in proportion to the concentration. And KSS7 exhibits higher slope and G' than those of TNu67. The decline of G' for 30 % starch dispersions may be due to rapid cooling during the measurement. However, further investigation is required for the elucidation of such peculiar phenomenon.

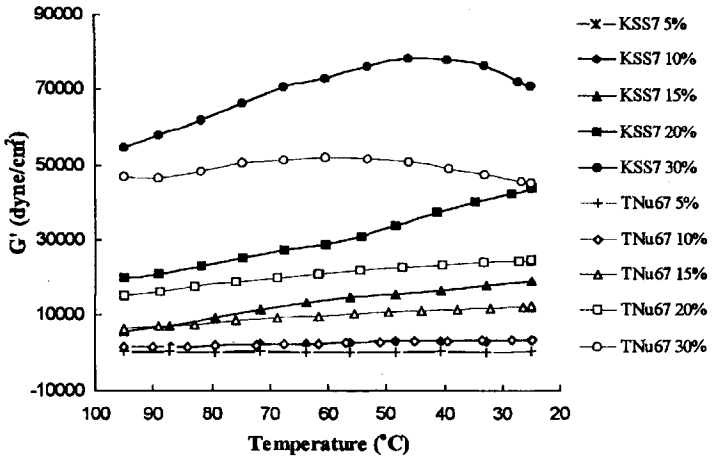

Fig. 7. Storage modulus (G') measurements of KSS7 and TNu67 starches at different concentrations during cooling (5°C/min) [8].

$(dG'/dT)_c$ (gradient of raising G' during cooling), G'_{25} (G' at 25°C), $\tan\delta_{25}$ ($\tan\delta$ at 25°C), and G'_{25}/G'_{95} of KSS7, TNu67 and TCW starches are applied for the indices of rheological properties during retrogradation. At low concentration (5~10 %), the gelatinized TNu67 shows higher $(dG'/dT)_c$ and G'_{25}, but lower $\tan\delta$ than those of KSS7 and TCW70. However, at high concentration (15~30 %), $(dG'/dT)_c$ of KSS7 (193~499 dyne/cm^2·°C) is at least as high as two times of TNu67 (81~139 dyne/cm^2·°C). Consequently, the G'_{25} of KSS7 (19060~68200 dyne/cm^2) is significantly greater than that of TNu67 (12380~45720 dyne/cm^2). TCW70 exhibits very slow rate of retrogradation and low G'_{25} (442~1107 dyne/cm^2 for 15~30 %). The values of $\tan\delta_{25}$ for 15~30 % starch concentrations of KSS7, TNu67 and TCW70 are 0.03~0.04, 0.05~0.06 and 0.39~0.54, respectively. These data indicate that the elasticity of retrograded starch dispersion is KSS7 > TNu67 >> TCW70.

The concentration dependences of $(dG'/dT)_c$ and G'_{25} for the three starches follow power laws as depicted in Figure 8 [51]. The result of regression analysis indicates that $(dG'/dT)_c$ of KSS7 is in proportion to $C^{3.9}$ (R^2 = 0.927), which exponent is close to that of TNu67 ($C^{3.9}$) (R^2 = 0.996). And, G'_{25} of KSS7 increases linearly with $C^{3.2}$ (R^2 = 0.968), which exponent is much larger than that of TNu67 ($\propto C^{2.4}$) (R^2 = 0.970). As for TCW 70, the G'_{25} is in proportion to $C^{1.4}$ (R^2 = 0.920) [8]. The concentration dependence of TCW70 paste is very similar to that of 4.2~16.7 % potato starch ($C^{1.5}$) [40], implying both starches have resembling pasting behaviours. Biliaderis and Juliano [38] reported that the moduli of rice starches are related with $C^{2.2\sim2.9}$

And, the dependence of moduli for 1.5~7.0 % amylose gels is $C^{3.1}$ [54] or $C^{7.0}$ [55]. Thus, the exponent value for starch dispersion may depend on the chain size distributions of amylose and amylopectin, the integrity as well as rigidity of swollen granule [7], and the range of polymer concentration measured, gel preparation [54], and measurement conditions, etc. The low concentration dependence of modulus for polysaccharide system has been considered as a result of high degree of polymer network defect due to the extensive entanglement among molecular chains [56].

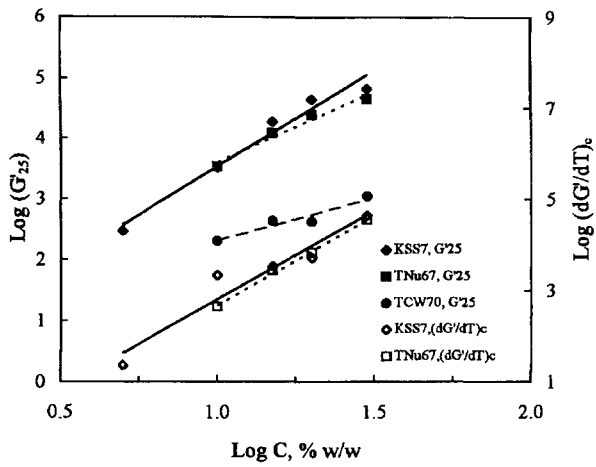

Fig. 8. The storage modulus (G') and $(dG'/dT)_c$ of different rice starches as a function of concentration during cooling [51].

The cold starch gel is more rigid than the hot starch gel [57], and KSS7 or TNu67 starch gel can be developed at heating stage for a starch system with an enough concentration. The G' increases during cooling, may be driven by the interactions, including hydrogen-bond formation favored at lower temperatures (exothermic), of starch constituents [8, 10, 11, 39, 54]. It mainly due to the retrogradation of amylose in short period [3, 10, 11, 15, 33, 54, 58]. The facts that KSS7 gel with higher G' than TNu67 during cooling, and TCW70 only makes a paste may attribute to the differences in amylose/amylopectin composition and fine structure of amylopectin in the swollen granule [54]. It has been found that the cereal amylopectins have a reduced rate of retrogradation due to their shorter average chain-length [24]. Similar results were also found in the studies of 14 rice amylopectins [45].

Frequency dependence of rheological properties for starch gel and paste

The effects of frequency from 0.01 to 20 Hz on the values of G' for 20 % KSS7 and TNu67 at 25°C during measurements are little and similar (Figure 9A) [51]. But

the G' of TCW70 gains drastically with the raise of frequency at above 5 Hz. And the frequency dependence of G'' is KSS7 < TNu67 < TCW70 (Figure 9B). Due to the G''/G, ratio is of the order of 0.01~0.1 and the variation in G, with frequency is small, the 20 % KSS7 and TNu67 can be considered as "true gel" [8, 51, 59]. And, 20 % TCW70 exhibits a very weak gel with a G''/G' > 0.1, resembling a concentrated entangled solution as described by Morris and Ross-Murphy (60); and can be classified into "pseudo gel" [59] or "paste" [8].

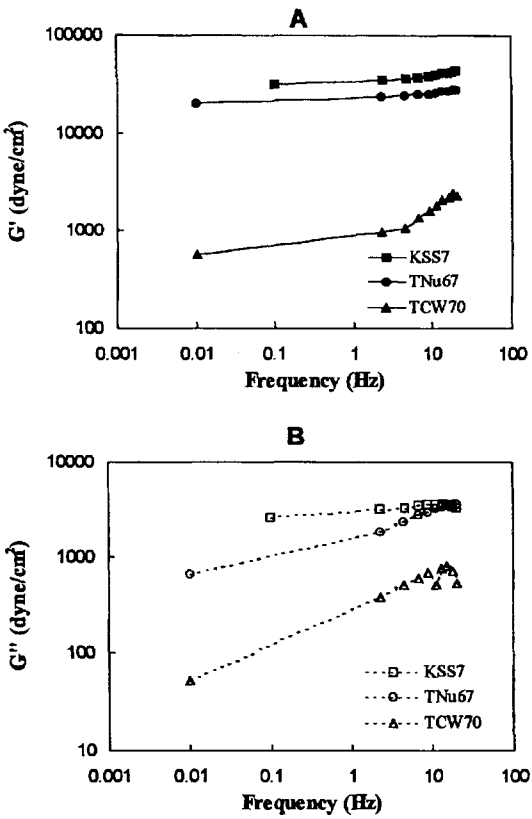

Fig. 9. Frequency dependence of 20 % (w/w) KSS7, TNu67 and TCW70 rice starches at 25°C. (samples prepared by heating up to 95°C (1°C/min) and then cooling down to 25°C (5°C/min); strain, 0.015) [51].

Influence of heating rate on the rheological properties of starch dispersion

Lower heating/cooling rate applied for the 20 % KSS7 starch dispersion can give higher G'_{95} and G'_{25} of the resulting gel (Table 5) [8] due to providing enough time

for the granule swelling, the amylose leaching-out, and enhancing the interactions among amyloses and swollen granules. The starch treated by a heating-cooling rate of 2°C/min has a lower G'_{95} than that of 1°C/min, but both values of G'_{25} are similar after cooling. However, high G'_{25}/G'_{95} ratio, one of retrogradation indices, is observed for fast heating/cooling rate. One may ascribe this high ratio to the rigidity of swollen granule because of restricted swelling. Moreover, it is possible that quick cooling causes "immobilization" of chains in the gel network and, consequently, low modulus values [54].

Table 5

Effect of heating/ cooling rate on rheological properties of 20 % KSS7 gel at 95°C and 25°C [8]

Heating/Cooling Rate (°C/min)	Up to 95 °C		Down to 25 °C		G'_{25}/G'_{95}
	G'_{95} (dyne/cm^2)	$\tan\delta_{95}$	G'_{25} (dyne/cm^2)	$\tan\delta_{25}$	
1	17320	0.11	45010	0.03	2.60
2	13660	0.12	45640	0.03	3.34
5	9697	0.14	38350	0.03	3.95

Interactions between amylose and starch granule

Since the content and average chain length of solubilized amylose, the content and fine structure of amylopectin, granular rigidity and swelling capacity, all can influence the rheological properties of starch dispersion during gelatinization and retrogradation. The effect of addition of amylose or amylopectin, as well as mixing starches on the rheological behaviour of starch during heating are investigated in order to illustrate the interaction mechanisms among them.

Mixed starch system – KSS7/TCW70

During Heating

The rheological properties of the mixed starch samples with different ratios of KSS7 and TCW70 during heating are shown in Figure 10 [22]. The figure shows that 20 % KSS7 starch system gives the highest G', and 20 % TCW the lowest. A peculiar phenomenon is also detected from the figure. Although the total starch concentration of the mixture of 15 % KSS7 and 5 % TCW70 sample was higher than that of 15 % KSS7 alone, the G' of the former is smaller than the latter during heating.

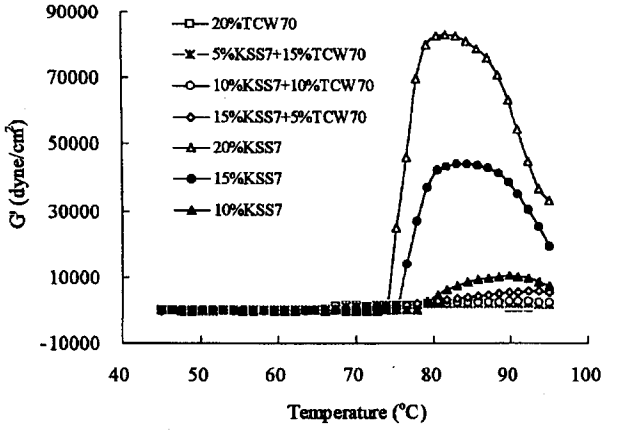

Fig. 10. Storage modulus (G') measurement of mixed KSS7/TCW70 starch systems at different ratios (frequency, 1 Hz; strain, 0.015; and heating rate, 1°C/min) [22].

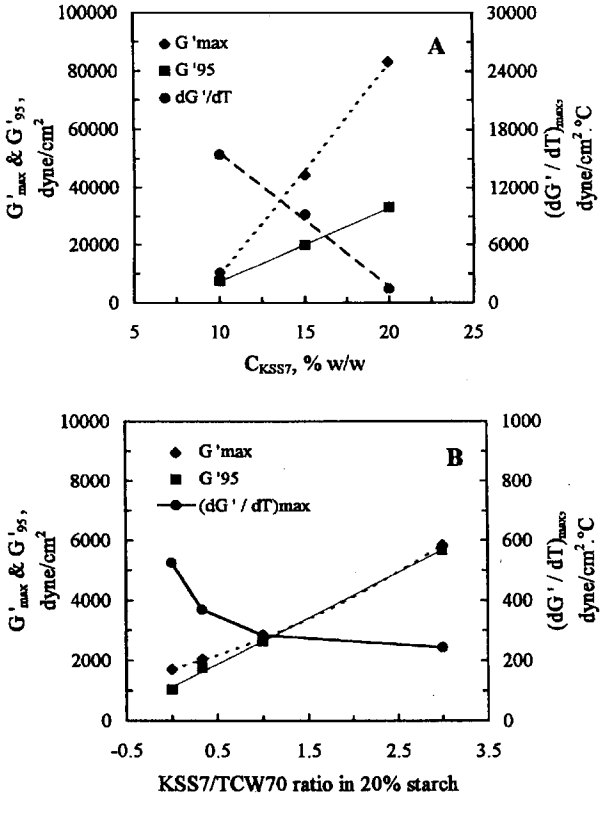

Fig. 11. G' and $(dG'/dT)_{max}$ of KSS7 at different concentrations (A) and of mixed KSS7/TCW70 at different ratios (B) during heating [51].

The rheological characteristics between KSS7 alone and KSS7/TCW70 mixed systems are varied significantly. G'_{max} and G'_{95} of KSS7 alone are in proportion to the starch concentration (C_{KSS7}) (Figure 11A). The effect of concentration on G'_{max} is much higher than on G'_{95}. And, $(dG'/dT)_{max}$ (the maximum slope of G' vs. T) of KSS7 starch is in inverse proportion to C_{KSS7} linearly. For KSS7/TCW70 mixed system (Figure 11B), both G'_{max} and G'_{95} value are relatively low and very close when the ratio of KSS7 to TCW70 $\geq$ 1. And higher proportion of KSS7 in the starch mixture will affect the dG'/dT more.

The concentration dependences of $T_{G'}$ and $T_{G'max}$ for mixed KSS7/TCW70 are also different from those of KSS7 alone (Figure 12). Both $T_{G'}$ and $T_{G'max}$ for KSS7 alone can be presented as a linear function of its concentration (C_{KSS7}) (Figure 12A); $T_{G'} = -0.4C_{KSS7} + 82.8$ ($R^2 = 0.893$), and $T_{G'max} = -0.76C_{KSS7} + 96.8$ ($R^2 = 0.951$). However, both $T_{G'}$ and $T_{G'max}$ for KSS7/TCW70 mixture increase nonlinearly with the raising ratio of KSS7/TCW70

(Figure 12B). $T_{G'}$ for all mixed ratios are in the range of 65 to 70 °C and much lower than those of 10~20 % KSS7 alone. And $T_{G'max}$ of mixture increases exponentially with the increment of KSS7/TCW70 ratio. $T_{G'max}$ of mixed 10 % each of KSS7 and TCW70 system is similar to that of the 10 % KSS7 alone. Moreover, $T_{G'max}$ of KSS7/TCW70 ratio < 1 is lower, but >1 is higher, than that of KSS7 alone at same C_{KSS7}. Generally, $T_{G'max}$ is more feasible to be affected by starch concentration or the ratio of mixture than $T_{G'}$.

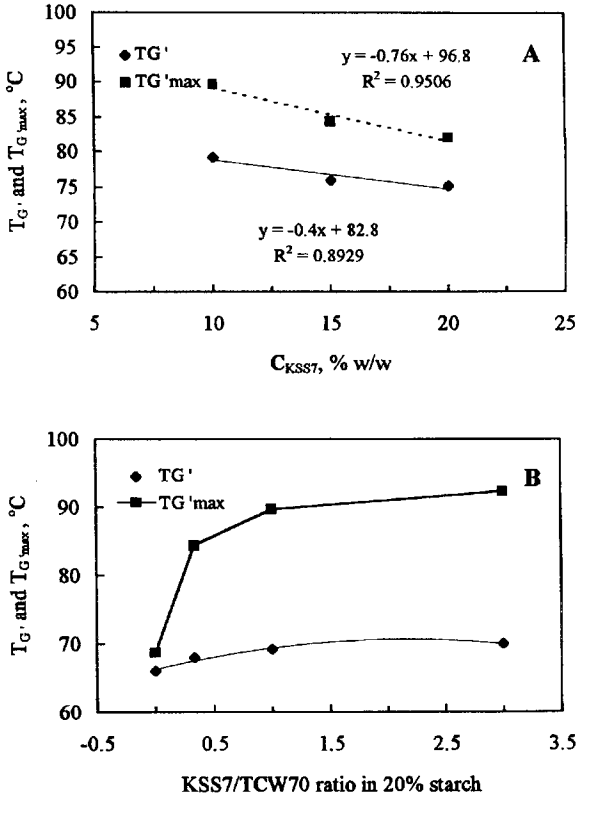

Fig. 12. $T_{G'}$ and $T_{G'max}$ of KSS7 at different concentrations (A) and of mixed KSS7/TCW70 at different ratios (B) during heating [51].

Because that neither significant modulus (G'_{max} or G'_{95}) nor $T_{G'}$ or $T_{G'max}$ is developed in TCW70 even up to 30 % concentration during heating, the rheological properties of the KSS7-TCW70 mixture (Figure 11 & 12) may attribute mainly to the heating behaviours of KSS7 starch with the influence of swollen TCW70. This influence may come from two actions. First, TCW70 starch granule swells much earlier and binds higher amount of water than does KSS7. Consequently, it retards the swelling of granule and leaching-out of amylose for KSS7. Secondly, the unswollen KSS7 granule may be excluded by the swollen TCW70 granule, resulting in a phase-separated mixed gel of very low moduli. These phenomena are resembling that the addition of amylopectin will restrict starch granules from swelling as suggested by Svegmark et al.[39].

During Cooling

Figure 13 shows the change of G' for KSS7/TCW70 mixed system during cooling [22]. The gradient of G' against temperature in KSS7 alone as well as the mixed

KSS7/TCW70 increases with the increment of C_{KSS7}. The dependences of G'_5 and $(dG'/dT)_{max}$ on starch concentration for both systems are exhibited in Figure 14. G'_5 and $(dG'/dT)_{max}$ as a function of C_{KSS7} for KSS7 system alone can be expressed as: $G'_5 = 5374\ C_{KSS7} - 40353$ ($R^2 = 0.994$) and $(dG'/dT)_{max} = 61\ C_{KSS7} - 552$ ($R^2 = 1.000$) (Figure 14A). As for the KSS7/ TCW70 mixed system, equations are $G'_5 = 3309\ R_{KSS/Tcw} + 1144$ ($R^2 = 1.000$) and $(dG'/dT)_{max} = 23.3\ R_{KSS/Tcw} - 1.45$ ($R^2 = 0.999$) ($R_{KSS/Tcw}$, the concentration ratio of KSS7 to TCW70 in 20% starch) (Figure 14B). The results demonstrate that G' decreases profoundly in the mixed system, but linear relationship between the G' or $(dG'/dT)_{max}$ and C_{KSS7} still exists.

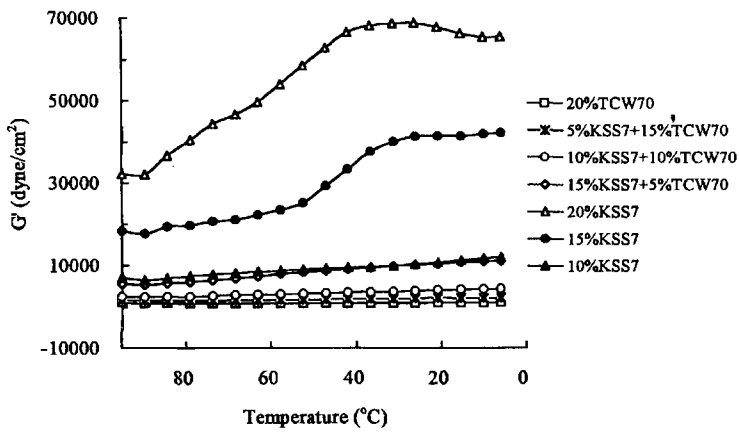

Fig. 13. Storage modulus (G') measurements of mixed KSS7/TCW70 starch systems during cooling (5°C/min) [22].

The 15 % KSS7 alone shows the highest G'_5/G'_{95} among all KSS7 and KSS7/TCW70 systems measured (Figure 15A) [51]. For the mixed KSS7/TCW70, G'_5/G'_{95} increases steadily with elevating C_{KSS7} (5~15 %) (Figure 15B). And those of 10 % KSS7 alone and 10 % each of KSS7/TCW70 are similar to each other.

Addition of KSS7 amylose

On KSS7 starch

The effects of added amylose on the rheological properties of KSS7 starch during heating, cooling and aging stages are listed in Table 6 [22]. Where the DP_n of isolated amylose is about 1000 (Table 1). Generally, Table 6 shows that those with addition of 2% amylose gives lower G'_{95}, but similar G'_5, and higher G'_{5A} (aging at 5°C for 1 h), except for the 20 % KSS7+2 % AM system. The G'_5 and G'_{5A} of the 20 % KSS7+2 % AM are slightly lower than those of 20 % KSS7 alone, reflecting an effect presumably via the competition of added amylose for water against the starch. Moreover, the val-

ues of $\tan\delta_5$ and $\tan\delta_{5A}$ for added amylose systems are higher than those of KSS7 alone. This result suggests that a phase separation may occur in this gelatinized mixture as examined by the TEM of amylose/potato starch system [39]. However, the degree of incompatibility between KSS7 starch and amylose is far less than that of KSS7 and TCW70 starches (Figure 14).

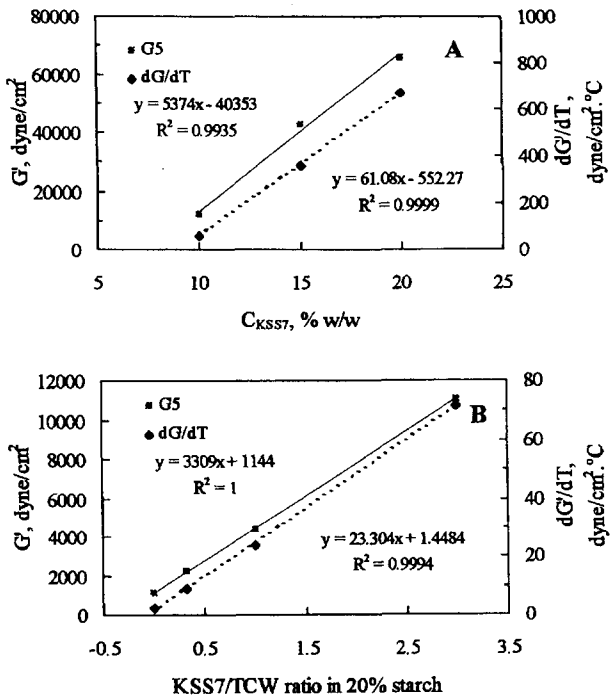

Fig. 14. G' and dG'/dT of KSS7 at different concentrations (A) and of mixed KSS7/TCW70 at different ratios (B) during cooling [51].

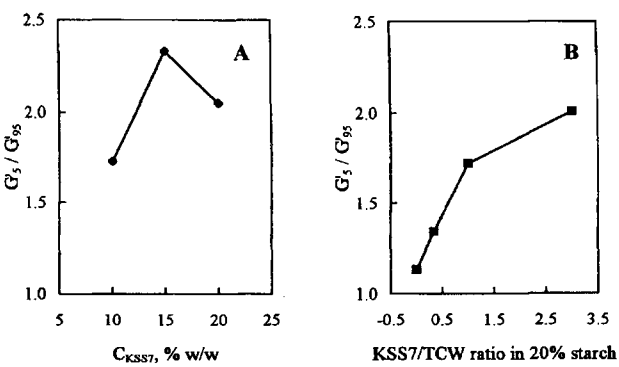

Fig. 15. Values of G'_5/G'_{95} of KSS7 at different concentrationd (A) and of mixed KSS7/TCW70 at different ratios (B) during heating-cooling cycle [51].

Table 6

Effect of added amylose on the rheological properties of KSS7 dispersions during heating, retrogradation and aging [22]

	Heated to 95 °C		Cooled to 5 °C		Aging at 5 °C for 1 hr	
	G'_{95}[a]	tan δ_{95}	G'_5	tan δ_5	G'_{5A}	tan δ_{5A}
5% KSS7	507±8[b]	0.10±0.00	563±12	0.06±0.00	578±24	0.11±0.01
5% KSS7 +2% AM[c]	188±5	0.18±0.01	557±10	0.14±0.02	1710±283	0.06±0.00
10% KSS7	6995±190	0.08±0.00	12730±156	0.04±0.00	17120±230	0.03±0.00
10% KSS7 +2% AM[c]	4072±269	0.10±0.02	12730±2140	0.05±0.00	19050±1715	0.04±0.00
20% KSS7	32210±645	0.07±0.00	65960±255	0.04±0.00	66290±270	0.03±0.00
20% KSS7 +2% AM[c]	33450±2311	0.07±0.00	60530±2185	0.04±0.00	61250±2267	0.04±0.00

[a] G' (dyne/cm^2).

[b] Mean ± standard deviation. n = 3.

[c] 2 % KSS7 amylose added.

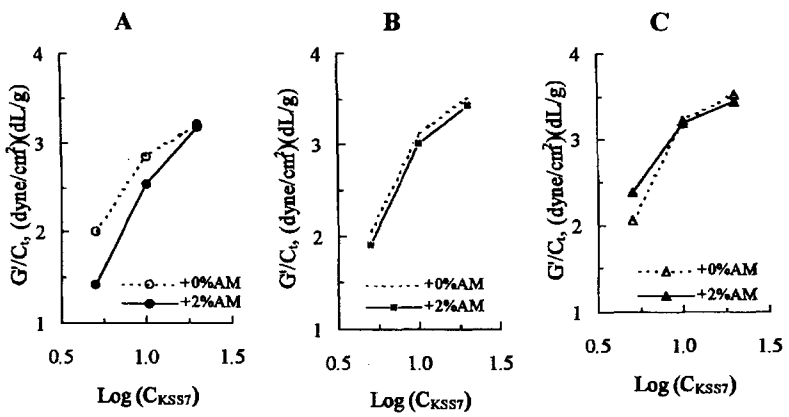

Fig. 16. Effect of added amylose on the G'/C_t at 95°C (A), 5°C (B), and aged at 5°C for one hour (C) for KSS7 (C_t, the total starch concentration) [51].

The ratio of G' to total concentration of the whole starch system (C_t) can be applied to further illustrate the influence of added amylose on the rheological properties [51]. The G'/C_t at 95°C for the 5~15 % KSS7 alone are significantly higher than those with 2 % added amylose (Figure 16A). The higher the C_{KSS7}, the smaller is the difference in G'/C_t between the samples with and without addition of amylose. No significant difference of G'/C_t is detected while C_{KSS7} raises up to 20 %. Although the

addition of amylose to the KSS7 starch at 5~15 % brings a lower G'/C_t at 95 °C, the similar value of G'/C_t is obtained when it is cooling down to 5°C (Figure 16B) or after aging (Figure 16C). 5 % KSS7+2 % AM demonstrating a higher G'/C_t after aging for one hour is an exception. The result imply that the enhancement of retrogradation is only found in the system with high ratio of added amylose to starch.

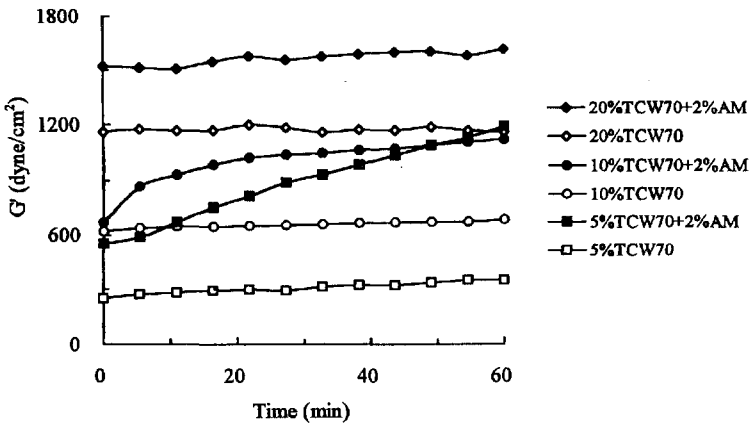

Fig. 17. Effect of added amylose on the time dependence of storage modulus (G') of TCW70 starch during aging at 5°C [22].

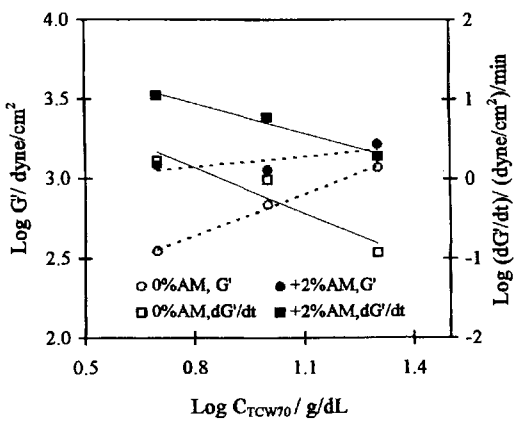

Fig. 18. Effect of added amylose on the concentration dependence of G' and dG'/dt for TCW70 starch during aging at 5°C [51].

On TCW starch

The influences of added amylose on the rheological properties of TCW70 paste can be observed in the stage of cooling and aging. The addition of 2 % amylose raises G' largely during cooling. Subsequently, it improves G' and dG'/dt (where t is the

aging time, min) of aged sample (Figure 17 & 18), especially at concentration of 5 and 10 % [22]. This increment may attribute to the gelation of amylose [10, 11, 33]. However, the G' does not increase remarkably in the concentrated TCW70 system (20 %). Svegmark et al. (1993) [39] also indicated that 1–10 % potato starch swollen in the amylose solution exhibits a lower complex modulus (G^*) than the starch swollen in water during heating and a sharp increase in G^* is observed due to amylose gel formation during cooling.

Conclusion

The changes in rheological properties of starch dispersions during gelatinization and retrogradation controlled predominately either by the swelling-solubility properties or the interaction between starch constituents and water molecule can be identified. At low concentration (< 10 %), the rheological properties of starch dispersion during heating and cooling are governed mainly by the amount and chain length of water-soluble constituents. The significance of the granular characteristics such as the degrees of granular rigidity and close-packing, molecular sizes and distribution of amylopectin branches, and the presence of residual microcrystallite on the pasting behaviour is raising with increment of starch concentration. All these parameters can influence the concentration dependence of modulus for starch dispersion, and the formation of whether "gel" or "paste". Among the starches studied the KSS7 starch gel possessing the highest G' and dG'/dT in the heating-cooling cycle can attribute to the high ratio of extralong and long chains to short chain in amylopectin, the presence of soluble with longer chain length, the amount of unreleased amylose entangled within granule, and high rigidity of granule.

The effects of mixing different starches and the adding amylose into a system on shear modulus of the mixed dispersion depends on the swelling-solubility properties, and/or the composition and structure of starch granule. The addition of 2 % KSS7 amylose can decrease the modulus development of starch dispersion during heating, but improve significantly the modulus and retrogradation rate of KSS7 and TCW70 starches at a moderate concentration during cooling and aging. Further investigation is required to clarify the mechanisms of contributions from the degree of granular rigidity, the volume fractions and moduli of continuous and dispersed phases in the system, and amounts of amylose inside and outside of granule to the rheological properties of starch dispersion.

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BADANIA NAD KLEIKOWANIEM I RETROGRADACJĄ SKROBI ZA POMOCĄ REOMETRII DYNAMICZNEJ – WPŁYW STRUKTURY GAŁECZEK I SKŁADU

Streszczenie

Dokonano przeglądu badań nad kleikowaniem i retrogradacją skrobi za pomocą dynamicznej reometrii. Dyskusja dotyczy badań nad trzema odmianami skrobi ryżowej: indica (KSS7), japonica (Tnu67) i woskowej (TCW70). Zawierają one odpowiednio 24–26 %, 15–16 % i 0.8–1.0 % amylozy. Omówiono zachowanie się tych skrobi w czasie ogrzewania, chłodzenia i dodawania amylozy.

Do wyjaśnienia mechanizmu retrogradacji i kleikowania oraz zależności pomiędzy strukturą cząsteczkową skrobi i strukturą gałeczek skrobiowych posłużono się korelacjami pomiędzy pęcznieniem, rozpuszczalnością w wodzie, reakcją z jodem, temperaturą kleikowania i dynamicznymi reogramami. Na ogół wzrost G' w czasie kleikowania zależy od charakterystyki gałeczek, tj. sztywności pęczniejących gałeczek i oddziaływania między upakowanymi gałeczkami. Jednakże G' w retrogradacji skrobi zależy ponadto od oddziaływań między amylozą, która wypłynęła z pęczniejących gałeczek lub amylozy dodanej w trakcie doświadczeń i spęczniałymi gałeczkami. W przypadku układu mieszanego, np. skrobi woskowej z niewoskowymi G' drastycznie maleje. Dodatek amylozy wyraźnie obniża G' dla kleikowania, ale podwyższa G' w trakcie chłodzenia i starzenia. Dlatego wydaje się, że ziarnistość skrobi i charakterystyka gałeczek są głównymi czynnikami wpływającymi na reologiczne zachowanie się skrobi. Kolejnym istotnym czynnikiem jest amyloza wydobywająca się z gałeczek w czasie kleikowania, szczególnie w przypadku układów o dużym stężeniu.

K. BRUNT, P. SANDERS

INTERNATIONAL COLLABORATIVE STUDY CONCERNING THE IMPROVED GAS CHROMATOGRAPHIC DETERMINATION OF ADIPATE IN STARCH

Abstract

A draft protocol in ISO format for the improved gas chromatographic method for the determination of the total and the free adipate content in acetylated adipyl cross-linked starches is evaluated by an international collaborative study. The improvements in the method provide an analytical protocol in which the amount of organic solvent needed for each determination was reduced tenfold and the daily capacity of the analyses was increased threefold.

This international collaborative study led to the following results: (1) for the total adipate determination the repeatability (r) and reproducibility (R) were respectively 50 and 90 ppm adipic acid and (2) for the free adipate determination the repeatability (r) and the reproducibility (R) were respectively 12.6 and 27.2 ppm adipic acid.

Introduction

Acetylated adipyl cross-linked starch is a modified starch used in food applications. The adipyl content in these cross-linked starches can be determined by gas chromatography as described by Mitchell et al. [1] in 1982. According to this method the sample is saponified with alkali in the presence of an internal standard glutaric acid (pentanedioic acid). During the saponification the adipyl group is hydrolyzed from the starch and forms free adipate. After acidifying the hydrolysate, the resulting adipic acid (hexanedioic acid) and the internal standard, glutaric acid, are extracted with ethyl acetate. After removal of the ethyl acetate, the organic acids are silylated to their corresponding trimethyl silylesters. These are quantified by gas chromatography using a packed column with silicone oil as the active phase.

This method is laborious and uses large quantities of ethyl acetate (300 ml per determination).

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For environmental and economic reasons, and for improved efficiency, we have miniaturized the analytical method, especially with respect to the amount of organic solvent needed for each determination [2]. Provided that the samples of cross-linked starch to be analyzed are homogeneous, the sample weight can be decreased considerably. Consequently, the amount of organic solvent can be decreased, resulting in a considerable reduction of time needed for the evaporation to dryness of the ethyl acetate extracts.

Moreover, we have used pimelic acid (heptanedioic acid) as an internal standard instead of glutaric acid. The solubility of pimelic acid in water, and its extraction behaviour, is more similar to adipic acid than glutaric acid.

This improved methodology results in a reduction of about 90 % in the use of ethyl acetate needed for each determination, and in an increase of about 200 % in the daily capacity of analysis.

This improved method has been discussed in ISO/TC93 WG3 "Starch (including derivatives and by products) – Chemical functions". A draft protocol in ISO format of this method was prepared and an international collaborative test study has been started to evaluate this method for the determination of adipic acid content of acetylated distarch adipates.

In this paper the results of this international collaborative study are presented and discussed.

Material and methods

Chemicals

The following chemicals were used:

- concentrated hydrochloric acid (Merck, Darmstadt)
- sodium hydroxide (Merck, Darmstadt)
- ethyl acetate (Merck, Darmstadt)
- adipic acid (hexanedioic acid) (Merck, Darmstadt)
- pimelic acid (heptanedioic acid) (Merck, Darmstadt)
- acetonitrile (Lab-Scan)
- bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) which includes 1 % trimethylchlorosilane (TMCS) (Pierce)
- nitrogen gas (Hoek Loos, Schiedam)

Apparatus

- glass reaction tubes (100 x 16 mm) with screw cap fitted with PTFE covered rubber seals were used for the saponification, the extraction, the evaporation, and the silylation of the sample, respectively, the analyte.
- rotary shaker.
- adjustable Finn pipettes 0.1 – 1.0 ml
- waterbath adjusted to 30 °C
- evaporation device, based on solvent removal with a stream of nitrogen (e.g. Pierce Reacti-Vap III)
- ultrasonic bath
- gas chromatograph, accomodating capillary columns, fitted with a flame ionisation detector, on-column injector, and a (computer) integration system. Typical chromatographic conditions are as follows:
  Carlo Erba Vega gas chromatograph equipped with a cold on-column injection system, and a flame ionisation detector (temperature 300°C, hydrogen pressure 0.5 bar, air pressure 1.0 bar). The separation was performed on a WCOT fused silica CP-sil 5CB capillary column (length 50 m, internal diameter 0.32 mm, film thickness 0.12 mm) with helium as the carrier gas (pressure 0.7 bar). During the separation the temperature of the column oven was programmed as follows: after injection the temperature was kept constant at 130°C for 1 minute, then the temperature was raised at 5°C/min. up to 190°C, immediately followed by a fast temperature rise of 25°C/min. to 290°C. The temperature was kept at 290°C for 5 minutes, and then the oven was cooled down to 130°C in order to get the instrument ready for the next injection. The retention times of adipic and pimelic acid derivatives are 10.3 min. and 12.2 min. respectively.

Analytical Methods

Total adipate

Sample preparation

50 mg of the acetylated adipic cross-linked starch sample is weighed accurately in a glass reaction tube, and 1.5 ml distilled water, and 1.0 ml aqueous solution containing 0.05 mg pimelic acid/ml are added. The reaction tube is shaken to disperse the sample and 2.5 ml of 4 M sodium hydroxide solution are added. Agitation of the reaction tube is continued in order to dissolve the starch sample. The reaction tube is closed and the adipyl-starch ester bond is saponified by continuous rotating the tube with the rotary shaker during at least 5 minutes. Then 1.0 ml of concentrated hydrochloric acid is added and the mixture is homogenized. 5 ml of ethyl acetate are added, the tube is closed, and shaken vigorously for at least 1 minute to extract the adipic and

pimelic acid into the ethyl acetate. After phase separation the upper, ethyl acetate layer is transferred with a glass Pasteur pipette into a clean glass reaction tube. The ethyl acetate extraction of the aqueous solution is repeated three times and the ethyl acetate fractions are collected. These collected fractions are evaporated to dryness with a nitrogen stream in a Pierce Reaction-Vap Evaporator at a temperature of 30°C in a water bath. Then 0.3 ml of acetonitrile is added to the dry residue, and the reaction tube is placed in an ultrasonic bath for several minutes to dissolve the residue. 0.3 ml of BSTFA/1% TMCS solution is added, and the mixture is homogenized again in the ultrasonic bath for several minutes. After a reaction time of at least 30 minutes in a water bath at a temperature of 30°C, 0.3 ml of the reaction mixture is injected in the capillary gas chromatograph.

Calibration

Four 50 mg samples of waxy corn starch are weighed into four glass reaction tubes. 1.0 ml aqueous pimelic acid solution containing 0.05 mg pimelic acid/ml is added into each tube followed by the addition of 0.25, 0.50, 0.75, and 1.00 ml aqueous adipic acid solution, containing 0.05 mg adipic acid/ml, into the respective tubes. The volume is adjusted to 2.50 ml with distilled water and the procedure as described in the sample preparation section, beginning with "The reaction tube is shaken to disperse the sample..", is carried out.

Free adipate

Sample preparation

100 mg of the acetylated adipic cross-linked starch sample is weighed accurately in a glass reaction tube, and 4.0 ml distilled water, and 1.0 ml aqueous solution containing 0.05 mg pimelic acid/ml are added. The tube is closed and the free adipate is extracted by agitating the closed reaction tube for 16 hours using a rotary shaker. Then the tubes are centrifugated for 5 minutes at 1100 g in a laboratory centrifuge. The clear supernatant liquid is transferred into a clean glass reaction tube and 50 ml 12 M aqueous solution hydrochloric acid and 5 ml ethyl acetate are added. The tube is closed and shaken thoroughly for 1 minute. After phase separation the upper, ethyl acetate layer is transferred with a glass Pasteur pipette into a clean glass reaction tube. The ethyl acetate is evaporated completely under a steam of nitrogen. Then the silylation and gas chromatographic determination are conducted as described for the total adipate beginning with "Then 0.3 ml of acetonitrile is added to the dry residue..".

Calibration

Four 500 mg samples of waxy corn starch are weighed into four glass reaction tubes. 1.0 ml aqueous pimelic acid solution containing 0.05 mg pimelic acid/ml is added into each tube followed by the addition of 0.25, 0.50, 0.75, and 1.00 ml aqueous adipic acid solution, containing 0.05 mg adipic acid/ml, into the respective tubes. The volume is adjusted to 2.50 ml with distilled water and the procedure as described in

the sample preparation for the determination of free adipic acid content section, beginning with "The tube is closed and the free adipate is extracted by agitating the closed reaction tube for 16 hours..", is carried out.

Expression of results

The peak areas for the pimelic acid and the adipic acid in the prepared calibrant solutions are determined. A graph with the different amounts of adipic acid (mg) added to the waxy maize starch on the x-axis and the corresponding ratios of the area of the adipic acid peak to the pimelic acid peak on the y-axis is plotted. The best fitting curve is derived by using linear regression analysis.

For each sample analyzed, the ratio of the area of the adipic acid peak to the pimelic acid peak is calculated and the corresponding amount of adipic acid is derived from the graph.

The adipic acid content in the samples is expressed in ppm (mg/kg) of adipic acid in the dry matter of the sample. The bound adipic acid content is obtained by the difference between the total adipc acid and free adipic acid content in the sample. The dry substance content in the starch samples is determined by using the oven drying method according to ISO 1666.

Experimental set-up of the collaborative study

To meet ISO requirements eleven randomly numbered samples, being five blind duplicates and a test sample of known content for practising the method, were sent to the participants of this study. For the statistical evaluation single analysis were required on each sample of the five blind duplicates. The participants were in alphabetical order: Amylum in Belgium, AVEBE in The Netherlands, Cerestar R & D in Belgium, National Starch and Chemicals in the USA, Netherlands Institute for Carbohydrate Research TNO in The Netherlands (NIKO-TNO), Roquette Frères in France, and Zolltechnische Prüfungs- und Lehranstalt in Germany. Both at Cerestar and National Starch a double set of samples have been analyzed by different persons at different days.

In accordance with resolution 35 of the 8th meeting of ISO TC93 WG3 on May 1993, the samples were analyzed according to the draft protocol entitled "Determination of adipic acid content of acetylated di-starch adipates", based on a proposal of NIKO-TNO [2]. The results were reported on the form provided together with the method.

Results and discussion

Determination of the total and free adipate content

As described before [2] the most laborious and time consuming steps in the analytical procedure are the extractions with ethyl acetate followed by the complete evaporation of the organic phase.

By decreasing the sample weight from the original 1 gram to just 50 mg for the determination of the total adipate content, the saponification and the extractions can be carried out in small volumes of a few ml in screw-cap glass reaction tubes.

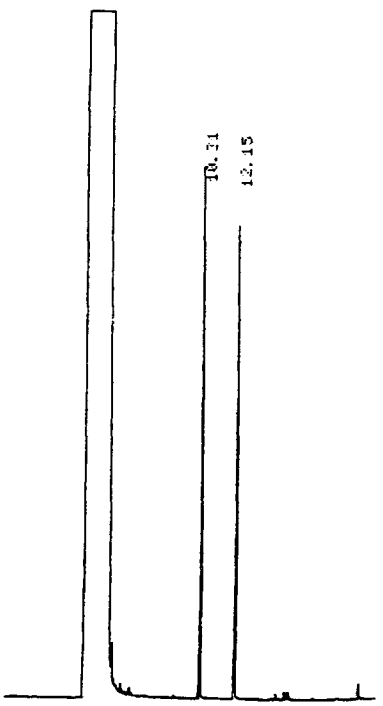

Fig. 1. Chromatogram of a calibration standard to which 1.00 ml adipic acid and 1.00 ml pimelic acid solution was added, measured with the typical chromatographic conditions as given in the protocol. Retention time of the adipic acid derivative is 10.3 min. and of pimelic acid derivative 12.2 min.

Total extraction of the analyte and the internal standard pimelic acid into the organic phase was achieved by four successive extractions with 5 ml of ethyl acetate. Moreover by performing the extractions in closed reaction tubes placed in a rack, it is possible to do 20-30 extractions simultaneously. 27 samples can be evaporated to dryness in a nitrogen stream simultaneously by applying a Reaction-Vap Evaporator (Pierce). Then the adipic acid and the pimelic acid in the residue are dissolved in acetonitrile (instead of pyridine), derivatized with the BSTFA/TMCS reagent to form their corresponding trimethylsilyl derivatives, and then separated and quantified by capillary gas chromatography (Figure 1). A calibration graph based on standard addition of adipic acid to waxy corn starch is used for quantification.

Advantages of the improved method [2] with respect to the original method are:

1. increase in daily analysis capacity of about 8 samples to about 25 samples,
2. considerable decrease in the consumption of organic solvent per determination; instead of 300 ml, just 20

Table 1

Applied GC conditions by the various laboratories

LAB nr.	Column type, length, ID film thickness	Temp. program	Injection
1	HP 1, 12 m, 0.2 mm 0.33 µm	1 min. 100°C 25 °C/min. to 250°C 8 min. 250°C.	Gerstel CI S3, cooled injection system
3	Quadrex, 15 m, 0.25 mm 0.1 µm	not reported	injector 200°C
4	DB1, 30 m, 0.32 mm 0.25 µm	init. 100 °C 7 °C/min. to 290 °C.	injector 300 °C, split 50 ml/min.
5	not reported	not reported	not reported
6	CP-SIL 5CB, 10 m, 0.32 mm, 0.12 µm	1 min. 100 °C 25 °C/min. to 290 °C.	on-column
7	HP 1, 5 m, 0.53 mm, 2.65 µm	2 min. 60 °C 15 °C/min. to 300 °C 2 min. at 300 °C.	not reported
8	not reported	not reported	split injector splitless mode
9a	not reported	not reported	not reported
9b	not reported	not reported	not reported

ml ethyl acetate is needed per determination,

3. improved repeatability.

It should be noted that, as summarized in Table 1, most participating laboratories used somewhat different GC conditions than the typical chromatographic conditions as given in the protocol. An example of such a chromatogram is given in Figure 2.

Total adipic acid

The contents of total adipate in the five blind duplicate samples of acetylated adipyl cross-linked starches as measured by the participating laboratories are presented in Table 2. In this table the duplicate difference and the average duplicate value of the blind duplicates are given also. At the bottom of this table for each participating laboratory is calculated the value of the average of all data and the sum of the duplicate averages for that laboratory.

With the Dixon Q-test no outliers could be detected in these calculated averages of all data and sums of the duplicate averages for each laboratory, indicating that no severe systematic errors are present.

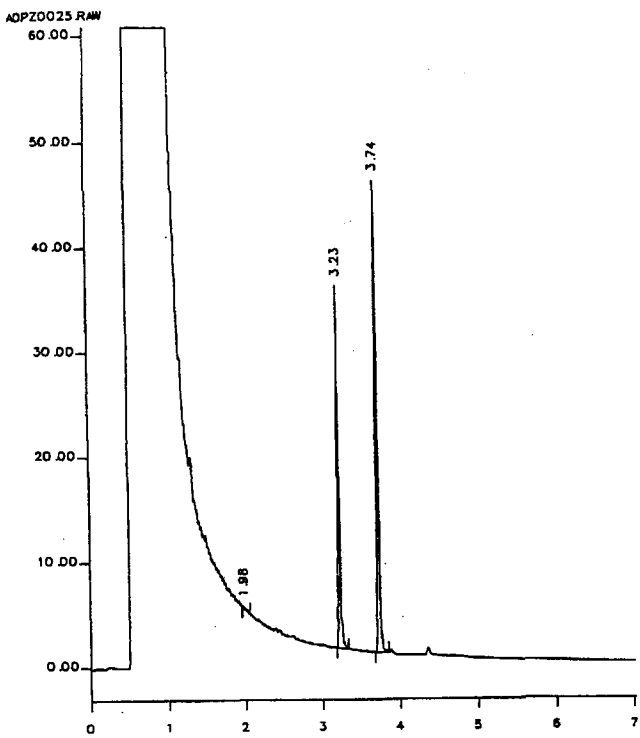

Fig. 2. Chromatogram of an acetylated adipyl cross-linked corn starch sample, measured with a modified temperature program and 10 m column (Table 1, lab nr. 6). Retention time of the adipic acid derivative is 3.2 min. and of pimelic acid derivative 3.7 min.

The Cochran maximum variance test ($p = 0.05$) and the one-tailed Dixon Q-test were used to evaluate the within sample duplicate differences and the within sample duplicate averages of the laboratories. It appeared that the duplicate difference of the samples 545/949 of laboratory 7 and 041/551 of laboratory 8 were outliers. And by one-tailed Dixon Q-test ($p = 0.05$) it was shown that the duplicate averages of samples 556/161 of laboratory 5 and of sample 041/551 of laboratory 8 were outliers also. Therefore these results have been rejected before further statistical evaluations were made.

The within laboratory standard deviation was calculated using the duplicate differences and is given also at the bottom of Table 2. With the Cochran maximum variance test ($p = 0.05$) no outliers could be detected in these within laboratory standard deviation.

The analytical results of each blind duplicate as measured by the different laboratories were statistically evaluated by one-way analysis of variances. The results of this evaluation are presented in Table 3. Of each duplicate sample (excluding the outliers) the following parameters have been calculated:

- the average measured total adipate content,
- the total standard deviation (S_{Tot}) in the average,
- the within sample standard deviation (S_r),
- the between sample standard deviation (S_R).

Table 2

Measured content of total adipate (in ppm adipic acid) in the five blind duplicate samples of acetylated adipyl cross-linked starches

sample		Laboratory number								
		1	3	4	5	6	7	8	9a	9b
557		333	414	400	529	430	394	421	454	412
664		404	433	390	539	418	403	467	471	428
	difference	71	19	10	10	12	9	46	17	16
	average	368	423	395	534	424	399	444	463	420
455		313	377	400	435	399	370	326	422	386
006		381	373	400	410	399	358	369	421	404
	difference	68	4	0	25	0	12	43	1	18
	average	347	375	400	423	399	364	348	422	395
556		631	633	620	849	666	676	607	692	653
161		572	656	625	731	642	615	635	705	645
	difference	59	23	5	118	24	61	28	13	8
	average	602	645	623	790	654	647	621	699	649
041		86	74	100	102	79	87	124	105	91
551		84	69	85	108	85	89	185	103	98
	difference	2	5	15	6	6	2	61	2	7
	average	85	72	93	105	82	88	155	104	95
545		369	384	390	482	380	354	396	434	392
949		359	374	400	499	390	434	394	436	398
	difference	10	10	10	17	10	80	2	2	6
	average	364	379	395	490	395	394	395	435	395
average all data		353	379	381	468	389	378	392	424	391
sum average data		1766	1894	1905	2342	1944	1890	1962	2122	1954
within lab standard deviation		36	10	8	12[a)]	9	22[b)]	24[c)]	7	9

[a)] excluding outlying data laboratory 5,
[b)] excluding outlying data laboratory 7,
[c)] excluding outlying data laboratory 8.

The overall total, within laboratory, and between laboratory standard deviations have been calculated by pooling the respective standard deviations of the duplicates. The results are given at the bottom of Table 3.

According to ISO 5725 the repeatability (r) and the reproducibility (R) can be calculated by multiplying both the corresponding pooled within laboratory standard deviation and the pooled between laboratory standard deviation by a factor 2.8. Thus this collaborative study results for the total adipate determination in a repeatability r = 50 ppm and the reproducibility R = 90 ppm adipic acid.

Table 3

Statistical evaluation of the data of Table 1

Sample duplicate	outlying laboratory	average	S_{Tot}	S_r	S_R
557/664	-	430	50	22	45
455/006	-	386	32	21	25
556/161	5	642	34	24	23
041/551	8	90	11	5	12
549/949	7	405	40	7	40
Pooled Standard Deviation			36	18	32

The free adipic acid content

The contents of the free adipic acid in the five blind duplicate samples of acetylated adipyl cross-linked starches as measured by the participating laboratories are presented in Table 4.

Just as in Table 2, also the duplicate differences and the average duplicate values of each blind duplicate for all the laboratories are given. The average of all data and the sum of the duplicate averages for the individual laboratories are given at the bottom of Table 4. The Cochran maximum variance test and the one-tailed Dixon Q-test were used to evaluate the within sample duplicate differences and the within sample duplicate averages of the laboratories. The duplicate difference of sample 545/949 of laboratory 8 appeared to be an outlier ($p = 0.05$) and the duplicate average values of the samples 557/664, 556/161, and 545/949 of laboratory 5 are outliers. Although the within laboratory standard deviation of laboratory 5 is very good, the duplicate averages are systematically much too high. This is clearly demonstrated by the values of the average of all data and the sum of the duplicate averages as listed at the bottom of Table 4. Looking at the analytical data of laboratory 7, it has to be concluded that these data are systematically much too low. Possibly a dilution error of a factor 2 has been made.

The within laboratory standard deviation was calculated by using the duplicate differences and is given at the bottom of Table 4 also. With Cochran maximum variance test ($p = 0.05$) no outliers in these within laboratory standard deviation could be detected.

For the above mentioned reasons all the analytical results of laboratory 5 and 7 were rejected just as duplicate 545/949 of laboratory 8. Also the analytical results of

Table 4

Measured content of free adipic acid in the five blind duplicate samples of acetylated adipyl cross linked starches

sample		Laboratory number								
		1	3	4	5	6	7	8	9a	9b
557		26	43	35	56	32	17	29	21	37
664		35	32	35	56	33	17	27	21	37
	difference	9	11	0	0	1	0	2	0	0
	average	31	38	35	56	33	17	28	21	37
455		110	120	110	164	104	60	120	80	126
006		110	102	100	169	110	58	113	91	125
	difference	0	18	10	5	6	2	7	11	1
	average	110	111	105	167	107	59	117	86	126
556		10	18	20	31	19	10	15	14	17
161		14	19	20	29	19	10	20	13	22
	difference	4	1	0	2	0	0	5	1	5
	average	12	19	20	30	19	10	18	14	19
041		10	17	15	26	16	8	9	10	17
551		14	10	20	28	17	8	11	10	17
	difference	4	7	5	2	1	0	2	0	0
	average	12	14	18	27	17	8	10	10	17
545		98	110	105	154	103	53	101	66	121
949		93	104	100	153	102	54	83	63	120
	difference	5	6	5	1	1	1	18	3	1
	average	96	107	103	154	103	54	92	65	121
average all data		52	58	56	87	56	30	53	39	64
sum average data		260	288	280	433	278	148	264	195	319
within lab standard deviation		3.7	7.3	3.9	1.8	2.0	0.7	3.2[a)]	3.6	1.6

[a)] excluding outlying data laboratory 8

laboratory 9a seem systematically too low. Statistically it is on the edge of significance. Therefore these data are given the benifit of the doubt.

The analytical results of each blind duplicate as measured by the different laboratories were statistically evaluated by one-way analysis of variances. The results of this evaluation are presented in Table 5.

Of each duplicate sample (excluding the outliers) the following parameters have been calculated:

- the average measured total adipate content,
- the total standard deviation (S_{Tot}) in the average,
- the within sample standard deviation (S_r),
- the between sample standard deviation (S_R).

Table 5

Statistical evaluation of the data of Table 3

Sample duplicate	outlying laboratory	average	S_{Tot}	S_r	S_R
557/664	5, 7	31.6	6.4	3.8	5.1
455/006	5, 7	108.6	13.2	6.7	11.3
556/161	5, 7	17.1	3.5	2.2	2.7
041/551	5, 7	13.8	3.7	2.6	2.7
549/949	5, 7, 8	98.8	18.8	5.5	18.6
Pooled Standard Deviation			10.5	4.5	18.6

The overall total, within laboratory, and between laboratory standard deviations are calculated by pooling the respective standard deviations of the duplicates. The results are given at the bottom of Table 5.

According to ISO 5725 the repeatability (r) and the reproducibility (R) can be calculated by multiplying the corresponding pooled within laboratory standard deviation and the pooled between laboratory standard deviation both by a factor 2.8.

Thus, this collaborative study results for the free adipic acid determination in a repeatability r = 12.6 ppm and the reproducibility R = 27.2 ppm adipic acid.

Considering the fact that most participating laboratories used somewhat different GC conditions than the typical chromatographic conditions as given in Table 1 and that they had no experience with the applied method, the results are very promising.

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MIĘDZYNARODOWA WSPÓŁPRACA PRZY ULEPSZONYM OZNACZANIU ADYPINIANU W SKROBI METODĄ CHROMATOGRAFII GAZOWEJ

Streszczenie

Międzynarodowa współpraca pozwala ocenić wartość roboczego protokołu w formacie ISO ulepszonego oznaczania metodą chromatografii gazowej całkowitego i wolnego adypinianu w acetylowanych skrobiach sieciowanych łańcuchem adipylowym. Ulepszenia metody pozwalają dziesięciokrotnie zmniejszyć ilość organicznego rozpuszczalnika stosowanego w oznaczeniach i trzykrotnie zwiększyć dzienną ilość oznaczeń.

Międzynarodowa współpraca dała następujące wyniki: (1) w oznaczaniu całkowitego adypinianu osiągnięto powtarzalność (r) na poziomie 50 ppm kwasu adypinowego i odtwarzalność (R) na poziomie 90 ppm tego kwasu; (2) w oznaczeniach wolnego kwasu adypinowego osiągnięto powtarzalność (r) na poziomie 12.6 ppm i odtwarzalność (R) na poziomie 27.2 ppm.

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RHEOLOGICAL PROPERTIES AND MICROSTRUCTURE OF MAIZE STARCH / MILK PROTEINS GELS

Abstract

Maize starch pastes whether containing milk proteins or not behave as shear thinning bodies. The increase in the heating temperature from 100 to 130°C provoked an important decrease in the maize paste viscosity measured at 60°C. The addition of milk proteins greatly changed the effect of heating temperature. A maximum viscosity at 60°C was observed for those pastes heated at 105-115°C for the milk protein concentrate, while for the sodium caseinate it was lowest at 110°C, whereas for the whey protein concentrate, the paste viscosity at 60°C was found to increase during heating.

The maize starch gels revealed two types of structure. In the maize starch gels some regions were composed of spherical particles, others of branched and flat filaments of several μm of length and 0.1 to 0.2 μm thick. The microstructure of mixed gels revealed an independent network for whey and starch fractions. These gels showed intermeshing networks, where each polymer developed its own network and no copolymer structure could be perceived. Sodium caseinate/starch solutions failed to form a continuous network and the gel presented multiple fractures.

Introduction

Over 40 % of starch produced in France is used for food [7]. One of the most important starch applications is in the preparation of milk desserts. Specific interactions between milk proteins and starch can take place during heating. Electrostatic interaction between potato starch and a-casein at pH 4 was shown by the electrolytic conductance method [8]. The viscosity, gelling and thixotropic properties of starch were much reduced in the presence of sodium caseinate [3]. Quite the opposite effect was observed by Lelievre et al. [5]. They found that sodium caseinate increased the viscosity of the starch pastes and a starch – sodium caseinate synergistic effect occurred. Similar results were obtained by Marzin et al. [6].

The aim of this work is to analyse the effects of heating temperature (100–130°C) and milk proteins on the rheological properties and texture of maize starch pastes and gels.

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Materials and methods

The following raw materials were used: maize starch (Sigma, Saint Quentin Fallavier, France), low heat milk powder (NIZO, Wageningen, Holland), sodium caseinate and whey protein isolate (Eurial, Herbignac, France). A controlled stress rheometer type Carri-Med CS100 (Rheo, UK) with a cone (4°, 6 cm) and plate geometry was used for rheological measurements.

Two hundred millilitres of water suspensions containing 5 % starch alone or in a mixture with 5 % milk proteins (Nx6.38) were heated during 30 minutes at 100 to 135°C in a small (250 ml) reactor vessel with magnetic stirring. Then the pastes were cooled to 90°C and transferred on to the plate of the rheometer for rheological measurement. For microscopic studies, the gel samples were dehydrated by the Critical Point Drying with CO_2 carried out in an Emscope CPD 750. These were coated with Polaron E5100 and then observed in a JEOL 35 CF Scanning Electron Microscope at 15 kV.

Results and discussion

For all samples analysed, for a shear rate range of 0.1 to 100 s^{-1}, the pastes containing either starch only or starch and milk proteins behaved at 60°C as shear thinning bodies (Fig. 1), with the stress (t) logarithm linearly related to the logarithm of the shear rate (g):

$$\text{Log}(t) = \text{Log}(K) + n\,\text{Log}(g) \quad (1)$$

where: K – consistency index or the stress level (in Pa) for g = 1 s^{-1} and n – structure or behaviour index.

For the pastes composed of the maize starch only, the logarithm of the shear stress was found to decrease with pasting temperature (Fig. 1A), following the Arrhenius type relation:

$$\text{Log}(t) = A + E / RT \quad (2)$$

where: A – hypothetical stress level for T – infinity, E – activation energy of flow (in J/mol), R – gas constant = 8.314 J/mol K, T – absolute temperature (K).

The maize starch / whey proteins pastes (Fig. 1B) increased their strength as the pasting temperature rose. Those pastes prepared from maize starch and the milk powder (Fig. 1C) showed a maximum stress level for pasting temperatures between 110 and 115°C. On the other hand, the pastes containing sodium caseinate (Fig. 1D) were 4 to 6 times softer at 60°C than all other pastes and they presented a minimum stress for at a pasting temperature of 110°C.

A lower viscosity in starch / sodium caseinate pastes heated at 90°C was observed by Hermansson [3]. The opposite tendency was observed by Lelievre et al. [5],

and Marzin et al. [6]. The reason for these differences could be the different sources of sodium caseinate and its composition [3]. The important differences in rheological behaviour of starch/protein pastes between the sodium caseinate and the milk protein, composed of 80 % of casein, might be the different structure of casein in milk and sodium caseinate. In absence of calcium in the sodium caseinate, the casein fractions (α-, β-, κ-) form oligomeres of a low degree of aggregation. These can interact with starch polysaccharides (amylose and amylopectine), by forming phosphate – hydrogen bridges. Thus associated polysaccharide – casein molecules became negatively charged at pH > 4.6 and would not aggregate any further. This could explain the important decrease in the paste viscosity in the presence of sodium caseinate within the heating temperature range of 100 to 110°C (Fig. 1D). Heating during half an hour at over 110°C might cause inter – molecular, protein – protein or protein – polysaccharide cross-linkage reactions between some reactive side groups of amino acids and sugars. These results in the viscosity increase of the pastes for heating temperature above 110°C (Fig. 1D).

In milk, in the presence of calcium, the casein fractions are aggregated into micelles made up of 105–107 molecules of caseins (α-, β- and κ-). The micellar casein has no "reactive" phosphate groups and because of the enormous "molecular / micellar " weight (~109 daltons) its reactivity is next to none. This would explain the differences in the rheological properties between the starch / milk proteins and the starch / sodium caseinate pastes (Fig. 1C and 1D).

Whey proteins (β-lactoglobulin, α-lactalbumin and serum albumin) are heat coagulating proteins and precipitate when heated above 70°C. The strength of the gel formed is a function of the degree of protein coagulation, being related to the heating temperature. This could explain the continuous increase in the viscosity of the starch / whey proteins pastes (Fig. 1B).

As typical of for the thixotropic products, the level of the consistency index (K) from the equation (1) was higher for the increasing than for the decreasing shear rate (Fig. 2A). It was lower for the second and third shearing cycle. This difference was more pronounced for the increasing than for the decreasing shear rate The addition of sodium caseinate provoked an important decrease in the consistency index if compared with the paste containing only starch. On the other hand, those pastes containing starch and whey proteins were about twice as viscous and much less thixotropic than samples of starch only paste. The addition of milk induced only minimal changes in the consistency index at 60°C but at 25°C it was much higher than at 60°C (Fig. 2B). This is due to the gelation process taking place during cooling. The greatest increase in the consistency index was observed for the starch / milk gels and the least for the starch / sodium caseinate pastes.

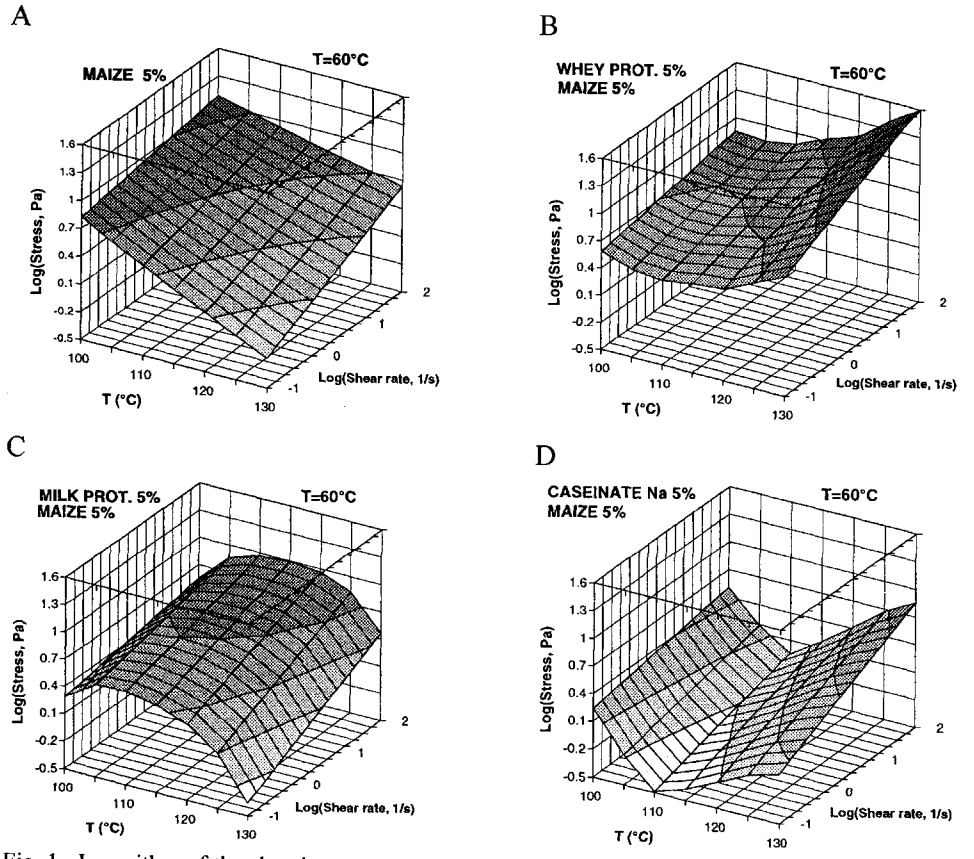

Fig. 1. Logarithm of the shearing stress as a function of the pasting temperature and the logarithm of the shear rate at 60°C for the 5 % maize starch only (A) and when mixed with: whey protein isolate (B), milk proteins (C) and sodium caseinate (D).

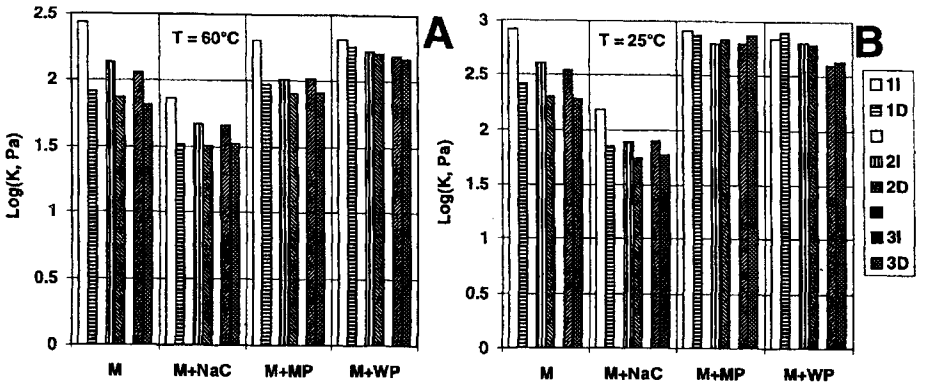

Fig. 2. Logarithm of the consistency index (K) at 60°C (A) and 25°C (B) for the pastes and gels of 5 % maize starch only (M) and when mixed with: sodium caseinate (NaC), milk proteins (MP) and whey proteins (WP) for the first three shearing cycles (1, 2, 3) for the increasing (I) and decreasing (D) shear rate.

The structure index (n) from equation (1) varied between 0.15 and 0.48 (Fig. 3). It was on average about 0.1 lower for the increasing shear rate. This is typical thixotropic behaviour. The structure index was slightly higher at 25°C for starch alone and starch/sodium caseinate pastes and slightly lower for the starch/milk and the starch/whey protein pastes. The structure index is equal to 1 for Newtonian bodies and is below 1 for so called shear thinning bodies. A similar level of the structure index ($0.4 < n < 0.6$) we observed previously for starch pastes heated at the temperatures < 100°C [4].

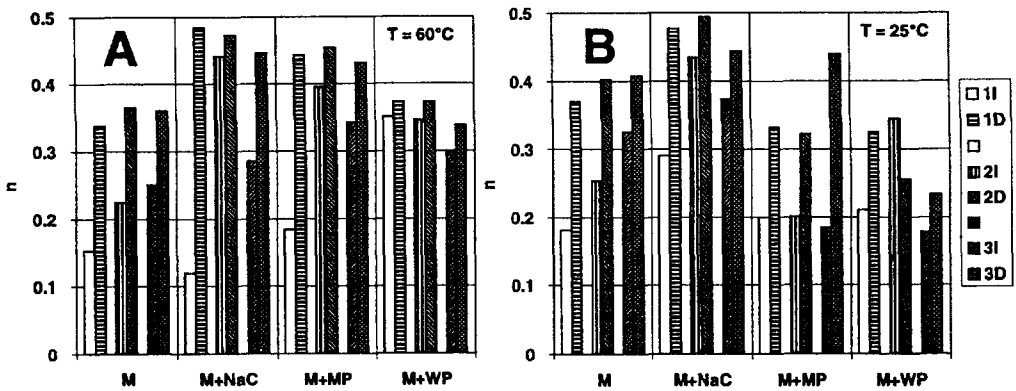

Fig. 3. Structure index (n) at 60°C (A) and 25°C (B) for the pastes and gels of the 5% maize starch only (M) and when mixed with: sodium caseinate (NaC), milk proteins (MP) and whey proteins (WP) for the first three shearing cycles (1, 2, 3) for the increasing (I) and decreasing (D) shear rate.

The activation energy of flow (E from the equation 2) varied between 5 and 20 kJ/mol depending on the composition of the paste (Fig. 4). It was lowest for the starch/sodium caseinate and highest for the starch/milk pastes. The activation energy of flow corresponds to structural changes during cooling between 60 and 25°C. This confirms the inhibiting effect of sodium caseinate on the retrogradation process.

As typical of thixotropic materials [2], there was a significant difference between the stress evolution of the increasing and that of the decreasing shear rate (Fig. 5). Stress decrease during shearing can be explained by a decrease in the extent of aggregation of the gel particles. These are also capable of assuming some structural reformation by flocculation. The thixotropic behaviour of both pastes and gels suggests that under continuous shear there is a continuous process of destruction/restoration of intermolecular linkages. This is a function of the product composition and structure, of the shearing time and of the shear rate and can be measured using the viscometric method. The thixotropy can be quantified as an absolute or a relative area of the hysteresis loop, between their values for the increasing and decreasing shear rate.

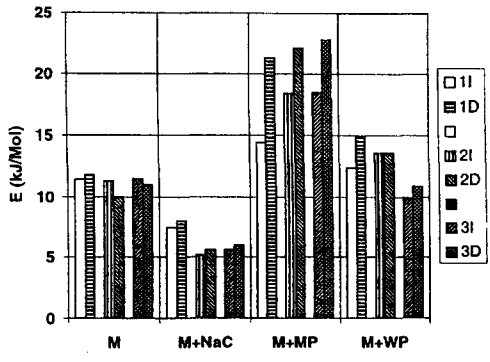

Fig. 4. Activation energy of flow (E) for the pastes and gels containing 5% maize starch only (M) and when mixed with: sodium caseinate (NaC), milk proteins (MP) and whey proteins (WP) for the first three shearing cycles (1, 2, 3) for the increasing (I) and decreasing (D) shear rate.

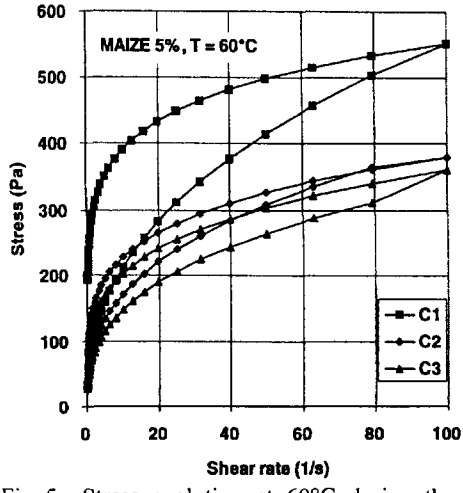

Fig. 5. Stress evolution at 60°C during the first three cycles of shearing (C1, C2, C3), for the 5% maize starch paste, for the increasing and decreasing shear rate.

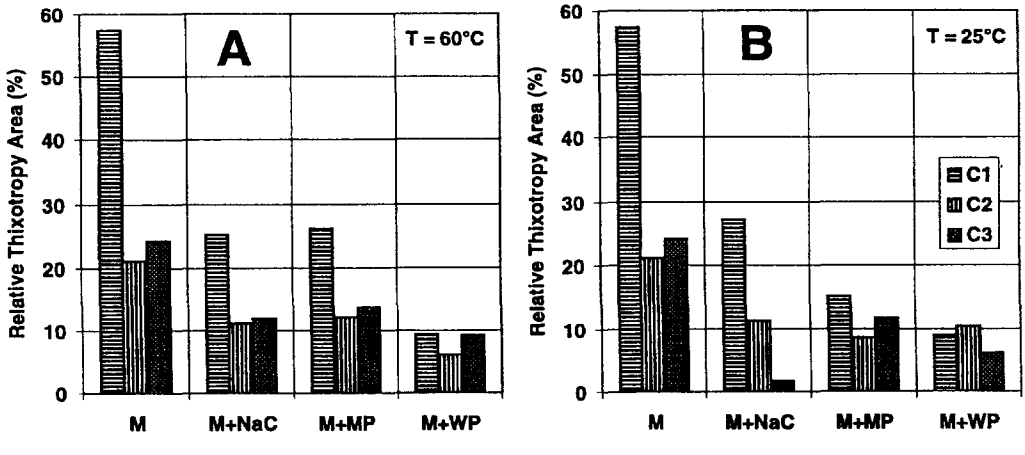

Fig. 6. Relative thixotropic area at 60°C (A) and 25°C (B) for pastes and gels containing 5% maize starch only (M) and when mixed with: sodium caseinate (NaC), milk proteins (MP) and whey proteins (WP) for the first three shearing cycles (C1, C2, C3).

The thixotropic properties of the starch/milk protein mixtures were much lower than of the starch only pastes and gels (Fig. 6). For the starch alone, the relative area of the thixotropic loop represented over 50 % of the total surface under the viscosity versus shear rate curve, for the first shearing cycle. For the second and third shearing cycles it represented only about 20 % for the starch only pastes and gels and about 10 % for the starch/milk proteins mixtures. This means that the addition of the milk proteins greatly changes of the structure of the resulting pastes and gels.

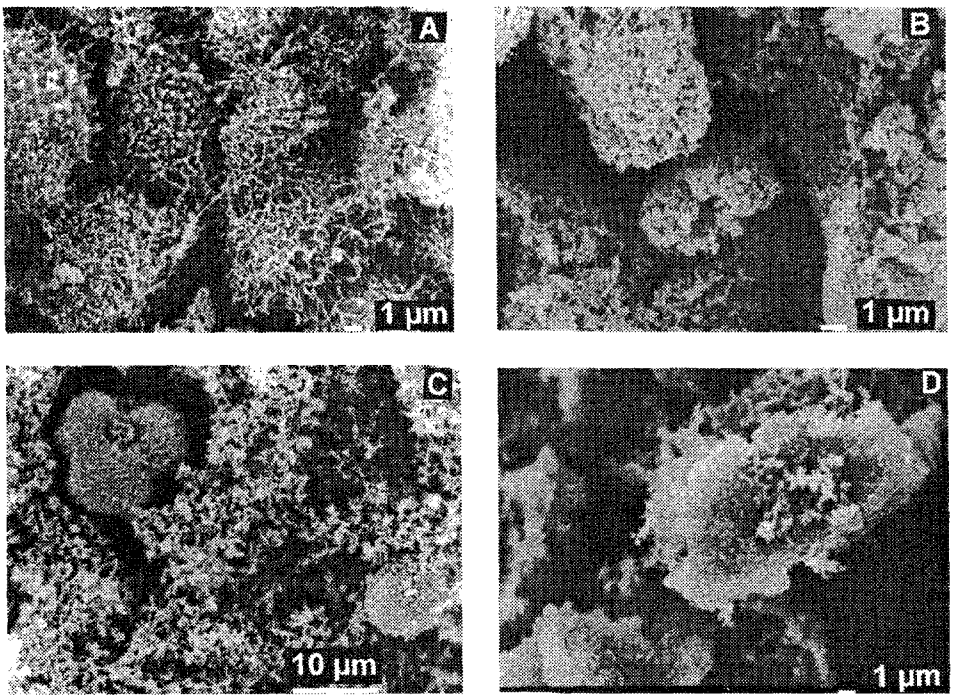

Fig. 7. Scanning electron micrographs of gels containing 5% maize starch only (A) and when mixed with: sodium caseinate (B), whey proteins (C) and milk proteins (D).

The starch only gel is made up of swollen starch granules having a filamentous structure, interconnected by a network of filaments 0.1–0.2 µm thick and several µm long (Fig. 7A). In the presence of the sodium caseinate (Fig. 7B) the swollen grain structure is no longer filamentous but granular and no intergrain filaments are observed. It is possible that the sodium caseinate sticks to the surface of the starch grains and inhibits the departure of amylose from the grains and prevents from forming the filamentous structure [6]. In the whey proteins/maize starch gel (Fig. 7C) the swollen starch grains are embedded in the continuous phase which is composed of irregular aggregates of coagulated whey proteins of variable dimensions. No interpenetration

between the protein and starch networks was observed. Similar results were obtained for manioc starch/whey proteins gels [1]. Mostly granular, similar to sodium caseinate (Fig. 7B) gels, but also partly filamentous structure is observed in the maize starch / milk protein gels (7D).

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REOLOGICZNE WŁAŚCIWOŚCI I MIKROSTRUKTURA ŻELI ZE SKROBI KUKURYDZIANEJ I BIAŁEK MLEKA

Streszczenie

Celem pracy było zbadanie wpływu temperatury obróbki cieplnej ($100 < T_{oc} < 130$, 30 minut) w trakcie żelatynizacji kleików ze skrobi kukurydzianej (C = 5 % w/w) i dodatku (5 %) białek mlecznych (pełne białko mleka, kazeinian sodowy, izolat białka serwatkowego) na lepkość (w zakresie prędkości ścinania od 0.1 do 100 s^{-1}), własności tiksotropowe kleików w temperaturze 60°C i 25°C oraz na strukturę mikroskopową otrzymanych z nich żeli.

Lepkość kleików ze skrobi kukurydzianej (5 %) z dodatkiem (5 %) lub bez dodatku białek mlecznych spada ze wzrostem prędkości ścinania. Zwiększenie T_{oc} ze 100°C do 130°C powoduje bardzo istotną obniżkę lepkości kleików mierzonej w temperaturze 60°C. Kleiki skrobiowe z dodatkiem 5% pełnego białka mlecznego wykazywały maksymalną lepkość dla T_{oc} = 105°C do 115°C. Natomiast z dodatkiem kazeinianu sodu wykazywały one minimalną lepkość przy T_{oc} = 110°C. Przy dodatku białek serwatkowych lepkość zwiększała się wraz ze wzrostem T_{oc}. Najwyższe własności tiksotropowe wykazywały kleiki skrobiowe bez dodatku białek mlecznych zaś najniższe z dodatkiem kazeinianu sodu.

Żel ze skrobi kukurydzianej jest zbudowany z napęczniałych ziarenek (10–30 μm) charakteryzujących się strukturą włóknistą. Włókienka o grubości 0.1–0.2 μm zbudowane prawdopodobnie z amylozy mają długość rzędu kilkunasu do kilkudziesięciu μm i łączą sąsiadujące ziarenka skrobi. Przy dodatku kazeinianu sodu struktura ziarenek staje się gąbczasta oraz znikają połączenia międzyziarnowe. W żelu z dodatkiem białek serwatkowych ziarenka skrobiowe są zatopione w gąbczastej strukturze skoagulowanego białka.

SARAH VEELAERT

PROPERTIES AND APPLICATIONS OF DIALDEHYDE STARCH

Abstract

The term dialdehyde starch is commonly used to describe the polyaldehyde material derived after periodate oxidative cleavage of the C2-C3 bond in starch. Upon oxidation of starch with periodate many inherent product characteristics are altered. The few things common between native potato starch and its dialdehyde form are the granular shape and the biopolymeric character. The newly introduced aldehydes strongly affect the inter and intramolecular interactions: helix formation becomes disrupted and crystallinity disappears. In particular, beyond a degree of oxidation of 40 %, dialdehyde starch granules were observed to be amorphous. Iodine staining and complexing with lysolecithine was also reduced pointing at a loss of helical shaped chains.

The aldehydes in dialdehyde starch are not present as such, but appear to be hydrated or to be involved in hemiacetal or eventually acetal linkages with neighbouring alcohol functions. These hemiacetal bridges can be formed intra or intermolecular resulting in the formation of an intragranular network. The knowledge of fundamental physico-chemical properties is the bases to explain many aspects of the behaviour of dialdehyde starch during production and applications. In particular the swelling capacity in water has been subject of interest, both at room temperature and during gelatinization. Throughout this research dialdehyde starches with different degrees of oxidation have been studied to screen deviating product properties.

Introduction

Dialdehyde starch is a promising starch derivative with the potential for many industrial applications, e.g. in paper, textiles, glues, coatings, etc. [1-7]. Especially in the paper industry, dialdehyde starch has been proven to work very efficiently as a wet-strength agent [8-15]. Dialdehyde starch is the product of the oxidative cleavage of the glycol groups present in the glucopyranose rings. The synthesis can be performed selectively with periodate as an oxidant [16-19]. Starch can be oxidized to predetermined dialdehyde content depending on the requirements with respect to chemical and physical properties. Dialdehyde starch is a cold water insoluble and biodegradable polymer. The rate of biodegradation depends on the degree of oxidation

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[20]. In spite of the wide scope of applications, the use of dialdehyde starch has been limited up to now owing to its high price. In order to reduce the production costs, processes have been designed to combine the oxidation of starch with the regeneration of periodate [1-5, 21-34].

The preparation, properties and uses of dialdehyde starch have frequently been reviewed [1-7]. Several chemical reactions have been reported with, e.g. sodium borohydride, bisulphite and a variety of amines (ammonia, urea, hydroxylamine, melamine). Although numerous derivatives are known, most of the industrial applications of dialdehyde starch are based on its cross-linking capacity. Various natural and synthetic polymers containing hydroxyl or amino groups have been treated with dialdehyde starch to improve the strength or to alter the solubility in water.

The application of dialdehyde starch to improve the wet-strength of paper products, such as wrappings, sanitary tissues, towelling maps, etc. is well documented [8-12]. During the paper making process, dialdehyde starch is mostly applied by wet-end addition: after being dispersed in water it is added to the suspension of paper pulp before sheet formation. Upon mixing and removal of water, hemiacetals are formed between the hydrated aldehyde groups of dialdehyde starch and the hydroxy groups of cellulose. These hemiacetals act like intermolecular cross-links, thus creating a reinforcement of the paper.

The general aim of this research at our institute is to revalue the use of dialdehyde starch for industrial purposes. Present scepticism about the industrial application of dialdehyde starch is mainly based on the unattractive price and the limited knowledge of fundamental product properties and efficient processing conditions. Certainly, a reduction of the actual price of dialdehyde starch is essential to compete with synthetic alternatives, e.g. in the paper industry. Moreover, it is important to achieve more experience with the characteristic product properties; especially the product stability and reactivity are important parameters that determine the efficiency of further use.

Materials and methods

A series of dialdehyde starches with a degree of oxidation (DO) varying from 1 to 99 % have been synthesized by periodate oxidation of potato starch as described earlier [35]. The oxidation has been performed in a 10 wt % aqueous solution at pH 3.5 and 25°C in the dark. After filtration and washing 5 times with the same volume of water, the products have been freeze-dried.

Chemical and structural analyses of the dialdehyde starches have been performed as described in a previous paper [35].

The swelling capacity of the products was determined by measurement of the increase in weight after suspending in water for 30 minutes, followed by isolation of the suspended granules by centrifuging [35].

The water absorption was calculated as the mass of water absorbed over the initial mass of dry material [35].

Viscosity changes during gelatinization have been measured in a Brabender Viskograph-E following a conventional gelatinization program [36]. Viscoelastic measurements during gelation were recorded with a Bohlin VOR Rheometer [36].

Abbreviations

DAS-X dialdehyde starch with a degree of oxidation equal to X, the number X referring to the percentage of oxidized anhydroglucose units (e.g, DAS-50 and DAS-100).

PS native potato starch.

Results and discussion

Product properties

With wide-angle X-ray scattering (XRD), differential scanning calorimetry and optical microscopy, all crystallinity and structural ordering has been proven to be lost

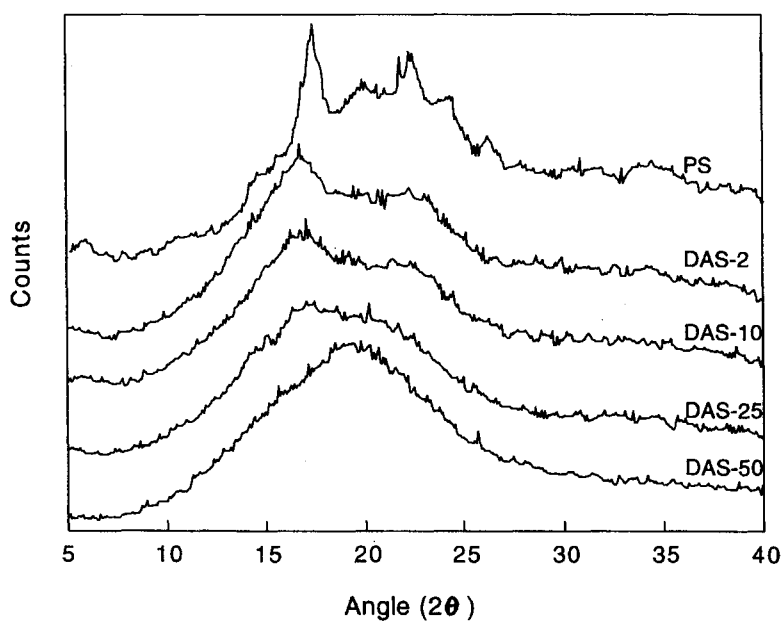

Fig. 1. X-Ray difractograms of different dialdehyde starches and native potato starch.

beyond degrees of oxidation of ±40 % [35]. Nevertheless the granular shape of the material was retained. The loss of B-type crystallinity in native potato starch, measured with XRD is illustrated in Fig. 1. The material was according to all applied analysis procedures amorphous at degrees of oxidation beyond 40 %. Also complexing with iodine or lysolecithine has been observed to be very low for higher degrees of oxidation [35]. The presence of aldehyde groups at C2 and C3 are expected to disturb helix formation. The effective helical chain length for complexing reactions diminishes gradually upon oxidation.

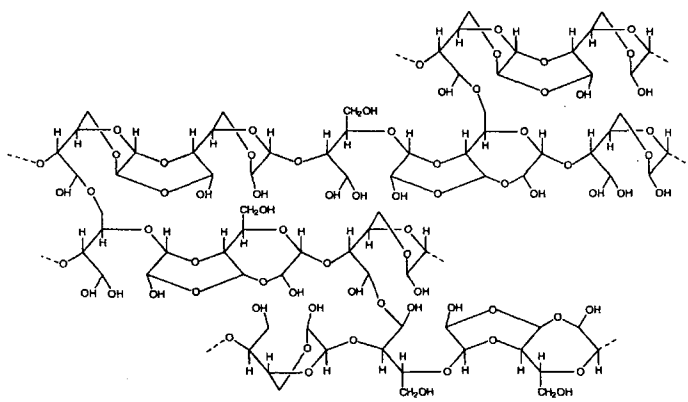

Fig. 2. Cross-linked dialdehyde starch fragment.

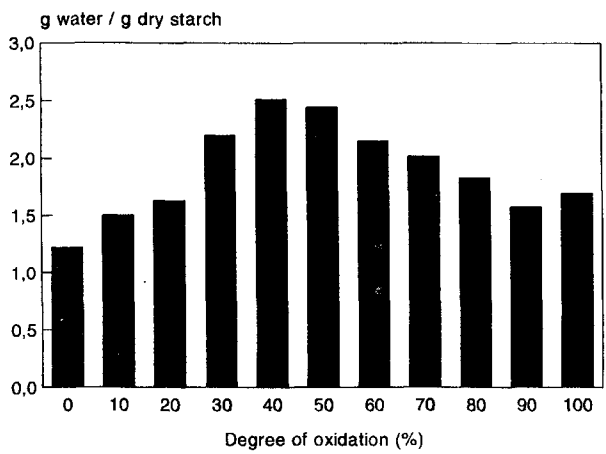

Fig. 3. Swelling capacity as function of the degree of oxidation.

The aldehydes in dialdehyde starch are not present as such but appear to be hydrated or to be involved in hemiacetal or eventually acetal linkages with neighbouring alcohol functions [37]. These hemiacetal bridges can be formed inter or intramolecularly resulting in the formation of an intergranular network. The molecular fragment presented in Fig. 2 illustrates the type of interactions that might occur and result in cross-linking.

Behaviour of dialdehyde starch in water

Loss of crystallinity enhances swelling whereas hemiacetalization (and cross-linking) of the aldehyde groups inhibit swelling. Swelling at ambient temperature is due to this counteracting effect maximal at intermediate degrees of oxidation (Fig. 3) [35].

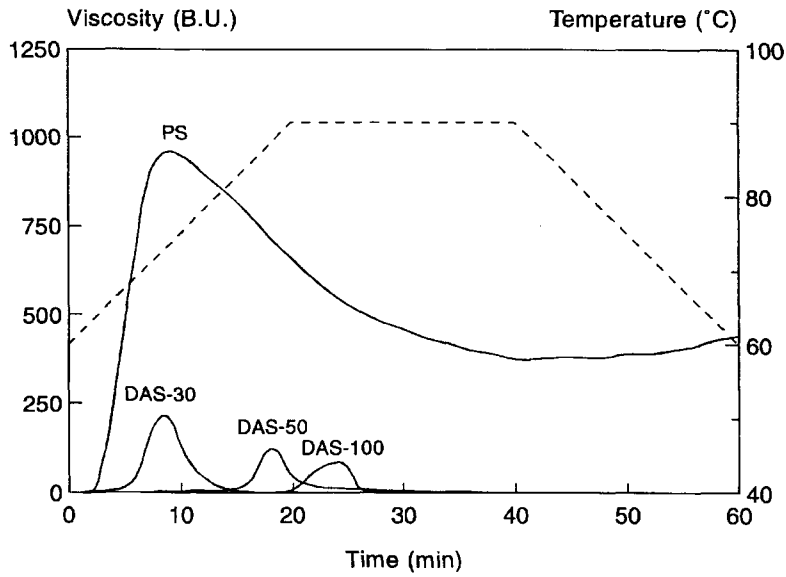

Fig. 4. Viscosity changes during gelatinization in a Brabender Viskograph.

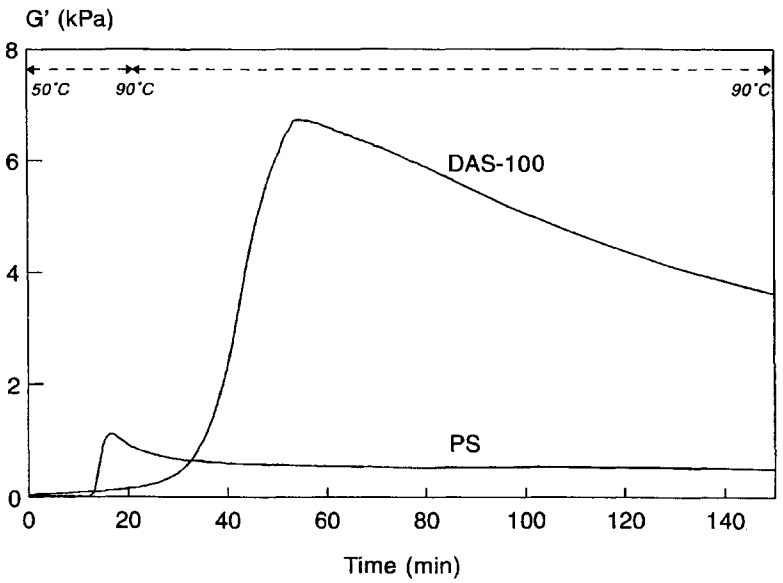

Fig. 5. The elastic response of DAS-100 and native potato starch at 20 wt % concentration during heating in a Bohlin Rheometer.

Swelling capacity upon heating was observed to be very low compared with native potato starch [36]. Above the melting temperature of crystallites, the swelling capacity of the starches diminishes with rising degree of oxidation (Fig. 4). The underlying effect is hemiacetal induced cross-linking in combination with an increased hydrophobic character. Above a critical concentration, highly visco-elastic particle gels were obtained, as soon as the particles were swollen into a close-packing stage [36]. The granules retained their rigid structure and were slightly deformable, in contrast with native starch granules, which swell considerably and therefore lose rigidity. This explains the high elastic response of the DAS gels relative to the native potato starch gels (Fig. 5). The critical concentration for gel formation (14 wt %), deviating from that of native starch, is attributed to a different conformation and functionality and a higher temperature lability of DAS.

Thanks to aldehyde induced hemiacetalization, the granules are reinforced, but the respective cross-links are pH sensitive. Granular swelling increases upon working at higher pH values. This effect can be advantageous for the removal of iodate from the reaction mixture during washing. But degradation, and the concurrent release of low molecular weight material, might cause problems during the separation of iodate from organic material.

The most important differences in properties between dialdehyde starch and native potato starch are compared in Table 1.

Table 1

The most important differences in properties between dialdehyde starch and native potato starch

	Dialdehyde starch	Native potato starch
Crystallinity	DO > 30: amorphous	semi-crystalline
Iodine staining	DO < 30: brown – yellow	amylose: blue; amylopectin: brown
Swelling at room temperature	low (high for intermediate DO)	low
Swelling upon heating	low at 70 – 90°C	high at ± 60°C
Viscosity 5 wt %	low	high
Visco-elasticity 15 wt %	high	low
Paste stability	no retrogradation	retrogradation
Molecular stability	> pH 5: low	higher

In the past only DAS-100 has been subject of interest with regards to applications. The use of DAS-50, however, might occasionally be advantageous. Table 2 gives an overview of the fundamental differences between DAS-50 and DAS-100.

Besides for a lower periodate consumption, the production of DAS-50 is advantageous with respect to the required reaction time to obtain the desired degree of oxi-

dation. The periodate oxidation of starch initially follows second order kinetics. A clear deviation from this second order model, however, was observed halfway the reaction. The involved reaction inhibition was attributed to the formation of hemiacetals between newly formed aldehyde groups and adjacent alcohol functions [37]. Un-oxidized diol units are thus protected for further reaction. Upon continued oxidation, the availability of diol units gradually decreases and the reaction rate slows down. Consequently, complete oxidation of starch to dialdehyde starch becomes a time-consuming process (> 24 h), unless a surplus of periodate is applied. The reaction time needed to prepare DAS-50 is considerably lower (± 1 h).

Table 2

Fundamental differences between DAS-50 and DAS-100

	DAS-50	DAS-100
Reaction time*	± 2 h	> 24 h
Periodate consumption per mol*	2 mol	1 mol
Washing efficiency after two washing cycles*	± 99 %	± 95 %
Swelling	high	low
Dispersibility	80°C	90°C
Particle rigidity	low	high

* The batch process.

Washing of the products is very important not only for purification but also for recovery of the periodate. The price of the process strongly depends on the recovery of iodate or periodate out of the reaction mixture. The periodate dissipation therefore has to be minimized. The washing efficiency of batch-wise prepared products has been investigated as function of the respective degree of oxidation. It was found that washing efficiency is maximal for intermediate degrees of oxidation (DAS-40 till DAS-60) [35]. This phenomenon was related to the swelling of the material, which was also observed to be maximal for the same products. Obviously, swelling has a positive effect on the accessibility of the granules: an increased water flow through the granules enhances the removal of iodate.

DAS-50 is dispersed more easily than DAS-100, due to the lower extent of hemiacetal induced cross- linking [35]. DAS-100, on the other hand is less alkali sensitive, at least with respect to the granular integrity [39]. Both DAS-50 and DAS-100, exhibit extensive molecular degradation when heated at pH 7. The stability of the viscoelastic particle gels was higher for DAS-100, as particle rigidity increased with increasing aldehyde content [35].

Conclusions

The broad based research described in this paper has led to interesting knowledge on the fundamental product properties of dialdehyde starches. Physical properties have been observed to be highly dependent on the degree of oxidation of the dialdehyde starch. A relation has been established between the chemical and physical structure and the behaviour of dialdehyde starch in aqueous suspensions under various conditions.

The preferential degree of oxidation depends on the desired performances of the dialdehyde starch. The production costs of DAS-50 are without any doubt lower than for DAS-100, not only for a reduced periodate consumption but also for a shorter reaction time and a higher washing efficiency. The surplus value of the higher aldehyde content in DAS-100 has to be weighed over the increased production costs.

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WŁAŚCIWOŚCI I ZASTOSOWANIE SKROBI DIALDEHYDOWEJ

Streszczenie

Pod pojęciem skrobi dialdehydowej zazwyczaj rozumie się polialdehyd otrzymywany przez spowodowane jodem (V) utleniające rozszczepienie wiązań C2–C3 w skrobi. Pod wpływem utleniania zmienia się szereg właściwości produktu. Należy do nich, między innymi, kształt gałeczek, polimeryczny charakter.

Wprowadzane grupy aldehydowe wyraźnie zaburzają oddziaływania wewnątrz- i międzycząsteczkowe. Nie może powstawać helisa skrobiowa i zanika krystaliczność. Szczególnie, w skrobi dialdehydowej o stopniu utlenienia 40 %, gałeczki są bezpostaciowe. Zanika ich zdolność barwienia się jodem i tworzenia kompleksu z lisolecetyną, co wskazuje na brak łańcuchów mogących się zwijać w helisę.

Aldehydy w skrobi dialdehydowej nie istnieją w stanie wolnym, lecz są albo hydratowanie albo istnieją jako hemiactale, a nawet acetale przy współudziale sąsiadujących z nimi grup hydroksylowych. Powstające mostki hemiacetalowe mogą mieć charakter wiązań wewnątrz i międzycząsteczkowych. W tym drugim przypadku sieciują się gałeczki. Szereg przejawów zachowania się skrobi dialdehydowej w czasie jej produkcji i zastosowania można wyjaśnić na podstawie podstawowych właściwości fizykochemicznych tego produktu. Ze szczególnym zainteresowaniem spotkała się w tym względzie zdolność do pęcznienia w wodzie zarówno w temperaturze pokojowej jak i w czasie kleikowania. W niniejszej pracy przebadano skrobie dialdehydowe o różnym stopniu utlenienia w celu ustalenia właściwości odróżniających te produkty od siebie.

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THERMAL STARCH TRANSFORMATIONS

Abstract

Studies on the radical, thermal decomposition of 11 starch varieties revealed that among amaranthus, cassava, oat, maize, waxy maize, rye, triticale, two varieties of potato and two varieties of wheat starch the oat starch was least stable and waxy maize starch was the most stable. The stability determination was based on the free radical count in the EPR spectra. The radicals appeared to be very stable against air as well as water and alcohol used for extraction of dextrins resulting from the thermolysis of starch. Simulations of the EPR spectra for 12 glucosyl radicals and the comparison of experimental and simulated spectra led to the conclusion that the delocalization of unpaired spin as well as steric hindrances are responsible for that stability.

In order to prepare novel dextrins of increased water binding capacity accompanied by low aqueous solubility thermolysis of starch was carried out under nitrogen, carbon dioxide, ammonia and hydrogen sulfide. The conventional and microwave heating of plain starch and its blends with formaldehyde, carboxyamides and esters were tested. The results are compared and discussed.

A majority of starch derivatizations involves elevated temperature as the source of energy. Starch containing foodstuffs are usually treated for consumption by either cooking, frying or roasting. Also the most common industrial starch modification which turns starch into dextrins frequently requires an elevated temperature, and eventually catalysts. Depending on the reaction conditions the derivatization proceeds according either to ionic or radical mechanisms.

It is well known that temperature above 200°C applied to mono- and oligosaccharides results in their radical decomposition [1]. Therefore, a considerable attention has been paid to the radical character of thermally processed food and food ingredients prepared thermally from saccharides, for instance, caramels. The most recent studies [2] have revealed that such evidently radical containing products are not mutagenic. The radicals are very stable, and when generated in starch as well as in

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cellulose only randomly add to polysaccharides and crosslink them [3]. Radicals can also be generated from polysaccharides on heating but neither their properties nor structure are well recognized.

Our studies on thermolysis of 11 varieties of starch up to 325°C for 30 to 120 min. revealed that radicals appeared when starch turned very dark brown. The radical count (number of spins) in starch of various origin dependent on the roasting temperature and time is given in Table 1.

Table 1

Number of spins, n, in starch of various origin[a], $n \cdot 1015/g^b$

Temp.	Time	$n \cdot 10^{15}/g$										
°C	min	PM	PD	T	O	WE	R	WH	M	C	WM	A
285	90				10							
	120			5	20	6	8	4	1		2	
300	90	5	7	2	10	2	2	6		6	3	5
	120	6	10	3	30	3	4	5	3	7	2	5
325	60				4							
	90	40	10	4	80	3	4	70	2	10	4	12
	120	200	80	8	1000	40	60	600	7	50	10	40

[a] PM and PD – potato starch of two origins; T – triticale starch; O – oat starch; WE and WH wheat starch of two origins; R – rye starch; M – maize starch; WM – waxy maize starch; C – cassava starch; A – amaranthus starch.

[b] $CuSO_4$ was taken as the standard.

One may see that oat starch was the least thermally stable, and both triticale and maize starch were the most stable in terms of the number of spins generated. This stability was in no relation to the amylose-to-amylopectin ratio because the number of spins in dextrins resulting from waxy maize starch should take a terminal position in the order of either decreasing or increasing spin count. The presence of noneluted ingredients such as protein and/or lipids in form of native starch complexes could have an essential influence on the thermal stability of starch. Proteins, as thermally unstable readily decomposed and accelerated the thermal generation of radicals from starch. It might be deduced from the significant weight loss of the oat starch on heating. The loss reached 40 % within 90 min heating at 285°C and almost 80 % after 120 min. heating at 325°C. This behavior of oat starch was unique because other starch varieties did not loose their weight on such heating. The stability of maize starch might result from the presence of lipids which resided in form surface and helical amylose com-

plexes. Distinctly more thermally stable lipids and their complexation to starch inhibited its thermal decomposition.

The analysis of the UVVIS absorption spectra of iodine complexes of resulting dextrins revealed that the extention of the damage of starch matrix as well as its macromolecules were proportional neither to time nor to temperature of roasting. Relevant information was brought by the observation of the shift of the absorption maximum around 625 nm in the visible spectra of the iodine complexes as well as the ratio of absorbance measured at 625 and 545 nm (Table 2).

Table 2

Selected characteristics of the visible spectra of iodine complexes with roasted starch

Roasting conditions		625 nm-band nm	A_{625}/A_{545}	$(A_{625}/A_{545})_0$-A_{625}/A_{545}[a]
Temp.,°C	Time, min			
Potato starch				
unprocessed		626	1.51	
170	30	645	1.63	-0.12
	60	625	1.71	-0.20
	90	625	1.68	-0.17
	120	625	1.62	-0.11
250	30	625	1.61	-0.10
	60	625	1.51	0.00
	120	588	1.03	0.48
270	30	613	1.51	0.00
	60	613	1.49	0.02
	90	588	0.94	0.57
	120	no band		
285	30	581	1.07	0.44
	60	585	1.51	0.36
	90	no band		
300	30	581	1.07	0.44
	60	no band		
325	30	578	0.86	0.65
Oat starch				
unprocessed		583	0.88	
170	30	575	0.92	-0.04
	60	571	0.84	0.04
	90	588	1.00	-0.12
	120	571	0.95	-0.07
250	30	595	1.08	-0.20
	60	581	0.92	-0.04
	90	no band		
270	30	588	0.91	-0.03
	60	575	0.87	0.01

	90	no band		
285	30	515	0.54	0.34
	60	no band		
300	30	543	0.62	0.26
	60	no band		
325	30	no band		
Maize starch				
unprocessed		598	1.06	
170	30	568	0.90	0.16
	60	588	1.10	-0.04
	90	588	1.11	-0.05
	120	588	0.95	0.09
250	30	562	0.88	0.18
	60	538	0.79	0.27
	120	556	0.65	0.41
270	30	588	1.00	0.06
	60	588	1.16	-0.10
	90	543	0.67	0.39
	120	543	0.69	0.37
285	30	565	0.82	0.24
	60	543	0.78	0.28
	90	588	1.08	-0.02
	120	no band		
300	30	588	1.04	0.02
	60	no band		
325	30	521	0.62	0.44
	60	no band		
Waxy maize starch				
unprocessed		614	1.20	
170	30	613	1.36	-0.16
	60	613	1.35	-0.15
	90	613	1.44	-0.24
	120	613	1.26	-0.06
250	30	588	0.97	0.25
	60	599	1.21	-0.01
	90	566	0.67	0.57
	120	566	0.79	0.41
270	30	588	1.02	0.08
	60	606	1.18	0.02
	90	543	0.66	0.54
	120	no band		
285	30	513	0.51	0.69
	60	549	0.71	0.49
	90	no band		
300	30	575	0.78	0.42
	60	no band		
325	30	515	0.48	0.72
	60	543	0.59	0.61

[a] $(A_{625}/A_{545})_0$ is the ratio for unprocessed starch.

The blue shift of the 625 nm-band signalized the damage of the starch structure to dextrins. The increase of the A_{625}/A_{545} ratio in respect to the original value spoke in favor of the priority of amylopectin in thermally decomposition. Neither the position shift of the 625 nm-band nor the A_{625}/A_{545} absorbance ratio variation were monotonous with the treatment time and temperature increase being a possible result of a repolymerization and reversion. The total damage of the starch structure into dextrins took place at temperatures lower than these required for the generation of radicals.

The radicals from starch were unusally stable. They survived a several months long air exposure and extraction with such hydroxylic solvents as cold (24 h) and hot (30 min) water as well as hot ethanol (24 h), although in an extreme case the concentration of unpaired spins decreased even by 80% (Fig. 1)

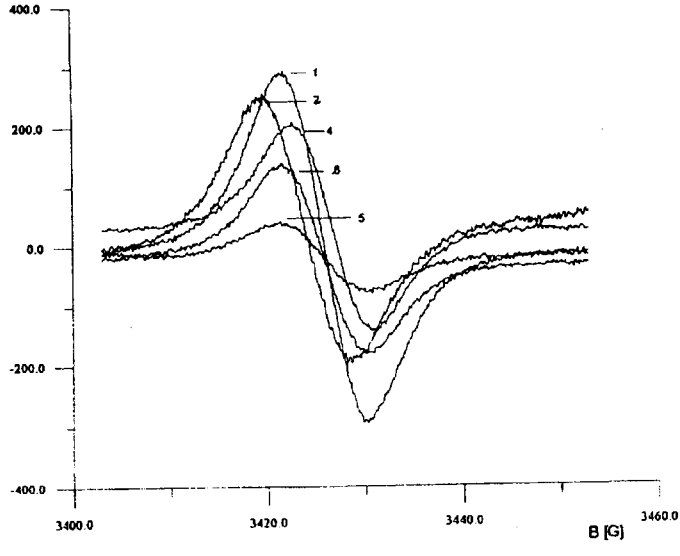


Fig. 1. Spin count decrease in roasted potato starch after extraction: 1 – sample (14.1 mg) prior to extraction; 2 – sample (13.8 mg) treated with cold water followed by evaporation to dryness (nonfiltered); 3 – dry sample (8.1 mg) after extraction (30 min) with hot water; 4 – dry sample (0.14.3 mg) after extraction (24 h) with cold water; 5 – dry sample (15.3 mg) after extraction (24 h) with hot ethanol.

This phenomenon could be explained as the result of either perfect delocalization of unpaired spin within the glucose unit radicals or/and steric hindrance of the unpaired spin from approaching spin scavenger. A simulation of EPR spectra for all possible glucosyl radicals (Fig. 2) was carried out with and without an assumption of intermolecular spin-spin interactions, respectively.

The resemblance of experimental spectra and those simulated under the intermolecular spin-spin interaction condition and lack of such similarity when this

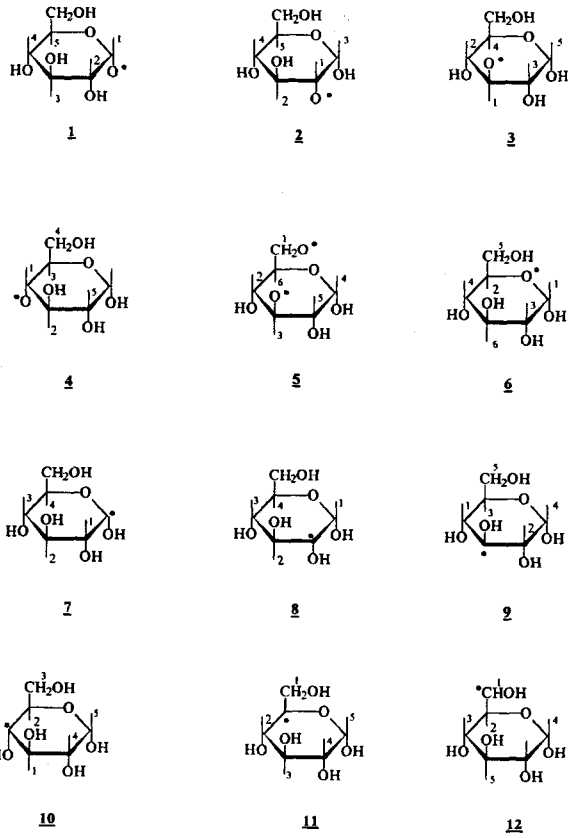


Fig. 2. Radicals for which the simulation of the EPR spectra wascarried out.

assumption was rejected (Fig. 3), pointed to an essential role of a migration of unpaired spins within the structure in the stabilization of radicals.

The thermal transformations of starch are commonly used for industrial manufacture of dextrins. Starch can be processed either as a plain material or with some catalysts added. There was also published a variety of laboratory procedures of dextrinization. Pyrolysis of starch to gaseous and liquid products was also described in the literature [4].

Since more than decade a great concern is noted about biodegradable plastics. Starch and its modificates are utilized as components of such materials [5]. Such and other modern applications developed studies on a facile starch modifications providing more hydrophobic products and, simultaneously, with a higher water binding capacity. Their electric compatibility to proteins is also an essential factor.

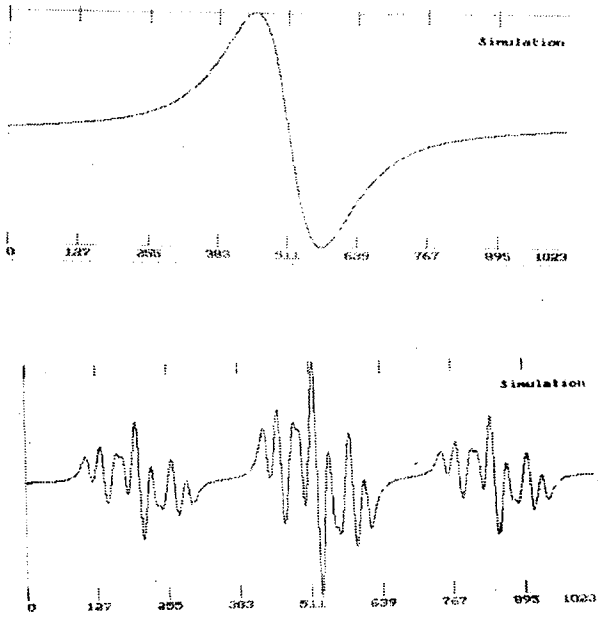


Fig. 3. Example of the EPR spectra simulated under assumption of the spin-spin interactions (left) and lack of such interactions (right). Compare the results with experimental spectra in Fig. 4.

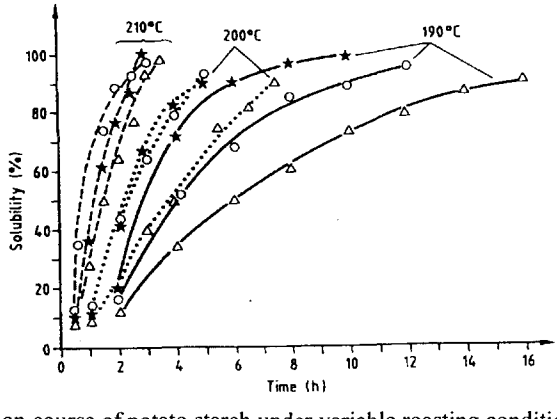


Fig. 4. The dextrinization course of potato starch under variable roasting conditions presented in terms of the aqueous solubility increase resulting dextrins with the rosting time (circles – in the air; stars – under nitrogen; triangles – under CO_2) (after [6]).

We have considered microwave heating of starch either plain or with certain reagents as well as conventional heating of starch in a oxygen-free atmosphere.

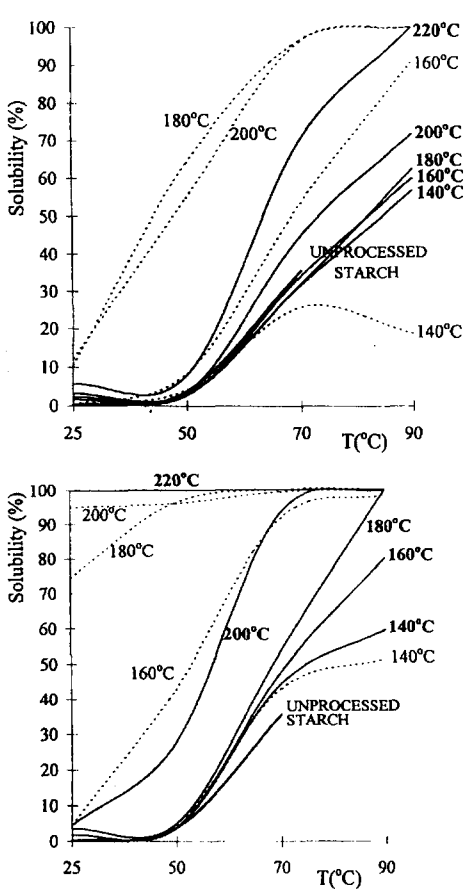
The latest group of experiments involved the starch dextrinization under nitrogen and carbon dioxide [6, 7], ammonia [8, 9] and hydrogen sulfide [10].

Table 3

Solubility, viscosity of 40 % aq. solutions and degree of thickening of British gums prepared under nitrogen, carbon dioxide, and in the air after twelveth hour of roasting at 190°C (according to [6])

Atmosphere	Solubility, %	Viscosity of 40 % aq. solutions (cP)	Degree of thickening
Air	90.0	173.0	165.0
Nitrogen	90.7	351.0	164.0
CO_2	90.6	1534.0	531.0

Fig. 4 [6] shows that the dextrinization carried out under CO_2 provided the least soluble dextrins which, simultaneously, differed from dextrins prepared under nitrogen and in the air in the viscosity of their aqueous solutions and degree of their thickening (Table 3).



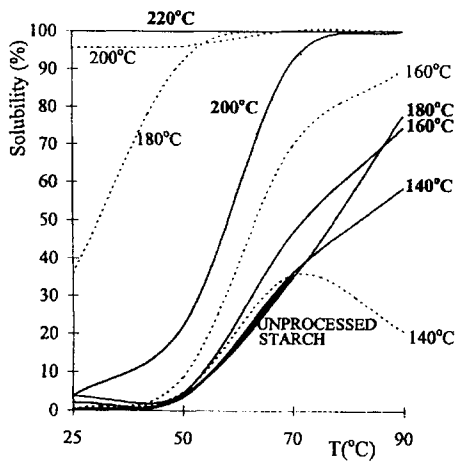


Fig. 5. The aqueous solubility (AS) of dextrins from potato starch roasted under gaseous ammonia [9].

These dextrins were acidic (pH 5.28 to 4.11) and for this sake their isoelectric point could be suitable for the formation of complexes with proteins.

Ammonia as the atmosphere of thermolysis retarded the polisaccharide destruction. Resulting dextrins, especially these prepared between 180 and 200°C had lower solubility (AS) and higher water binding capacity (WBC) as the corresponding products thermolyzed in the air (Figs. 5 and 6, respectively).

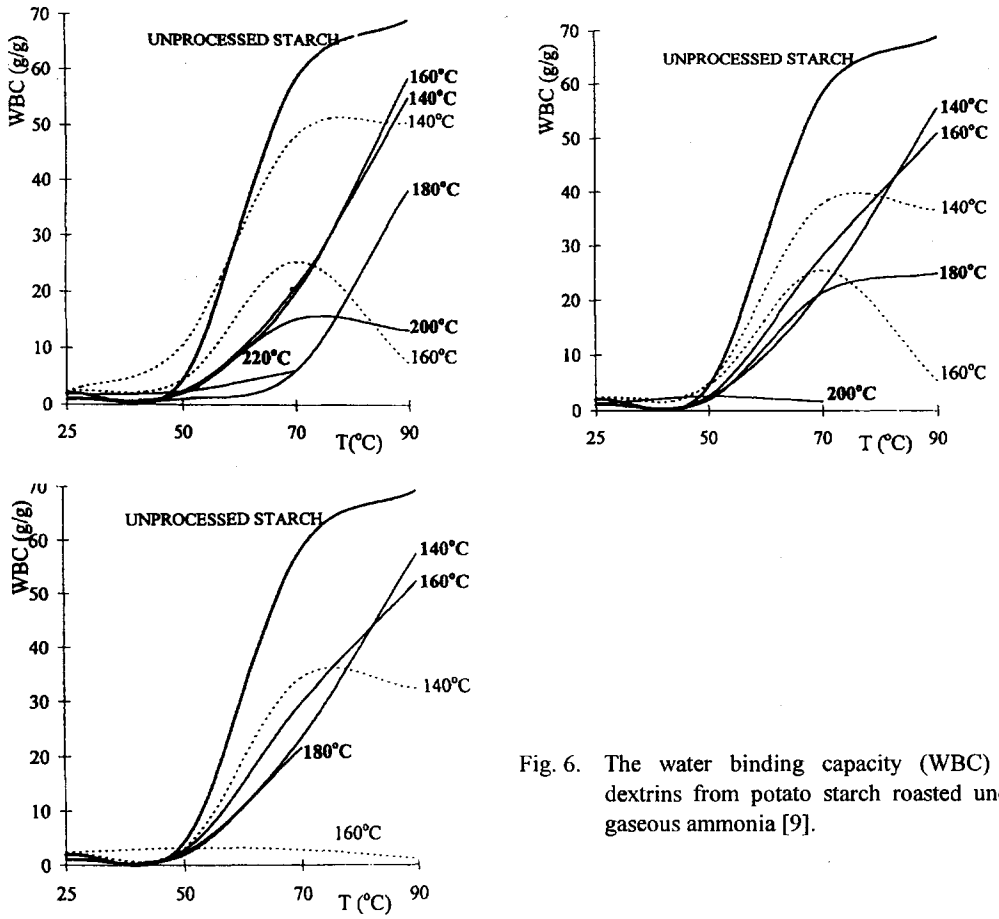


Fig. 6. The water binding capacity (WBC) of dextrins from potato starch roasted under gaseous ammonia [9].

Ammonia practically did not add to starch and only dextrins prepared in extremal conditions (220°C, 6 h) contained residual nitrogen (0.48 %) bound to starch. pH of these dextrins was between 7.00 and 5.80. Also hydrogen sulfide as the thermolysis atmosphere provided dextrins of suitable AS – temperature and WBC – temperature profiles (Figs. 7 and 8).

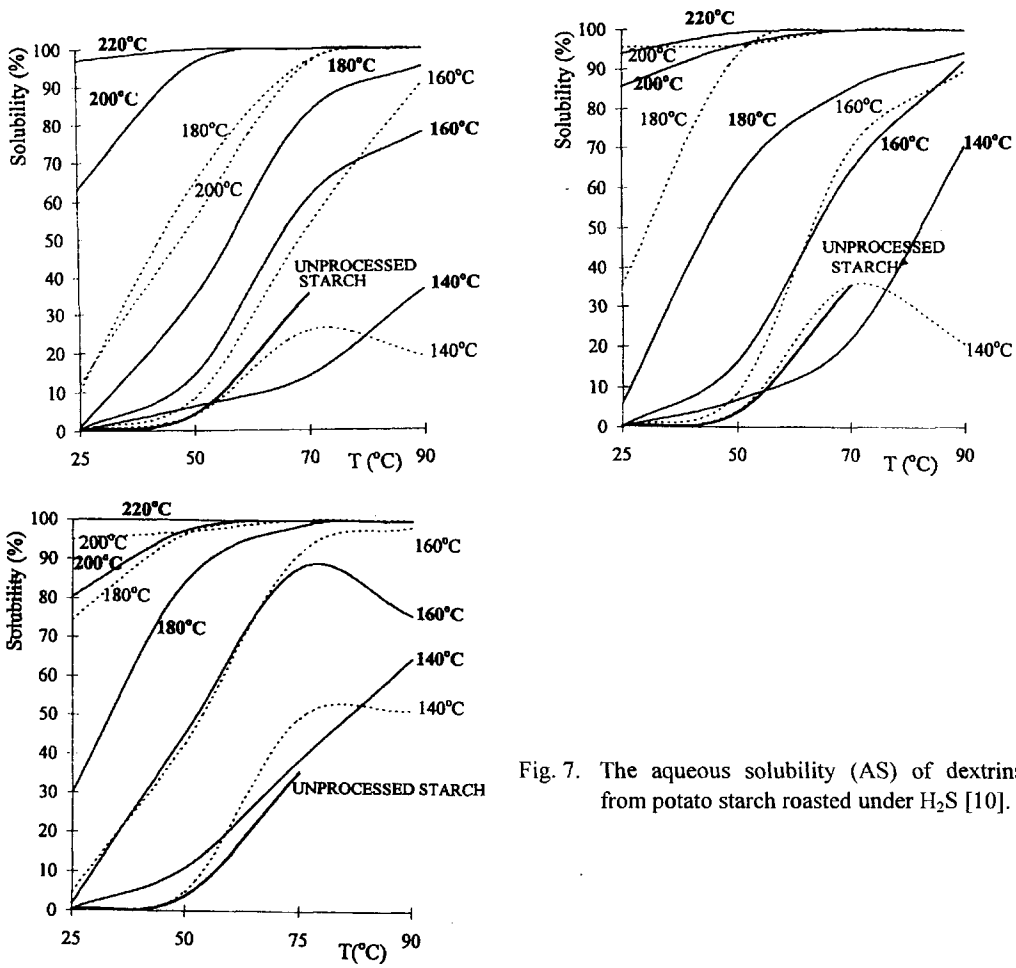


Fig. 7. The aqueous solubility (AS) of dextrins from potato starch roasted under H_2S [10].

Contrary to ammonia H_2S added to polysaccharide which after thermolysis contained from 0.4 to 0.9 % S. pH of these product varied form 5.25 to 4.0.

Our experiments with microwave thermolysis of starch involved plain, solid starch, starch sols, starch – formaldehyde mixtures [11] as well as starch blends with one of urea, (U), formamide, (FA), dimethylformamide, (DMF), phthalimide, (PI), ethhyl benzoate (EB) and dimethyl phthalate (DP) as well as a group of α-hydroxy and α-amino acids [lactic, (LA), tartaric, (TA), and citric (CA) acids as well as leucine, (Leu), serine, (Ser), asparagine, (Asn), and glutamic acid (GluA)].

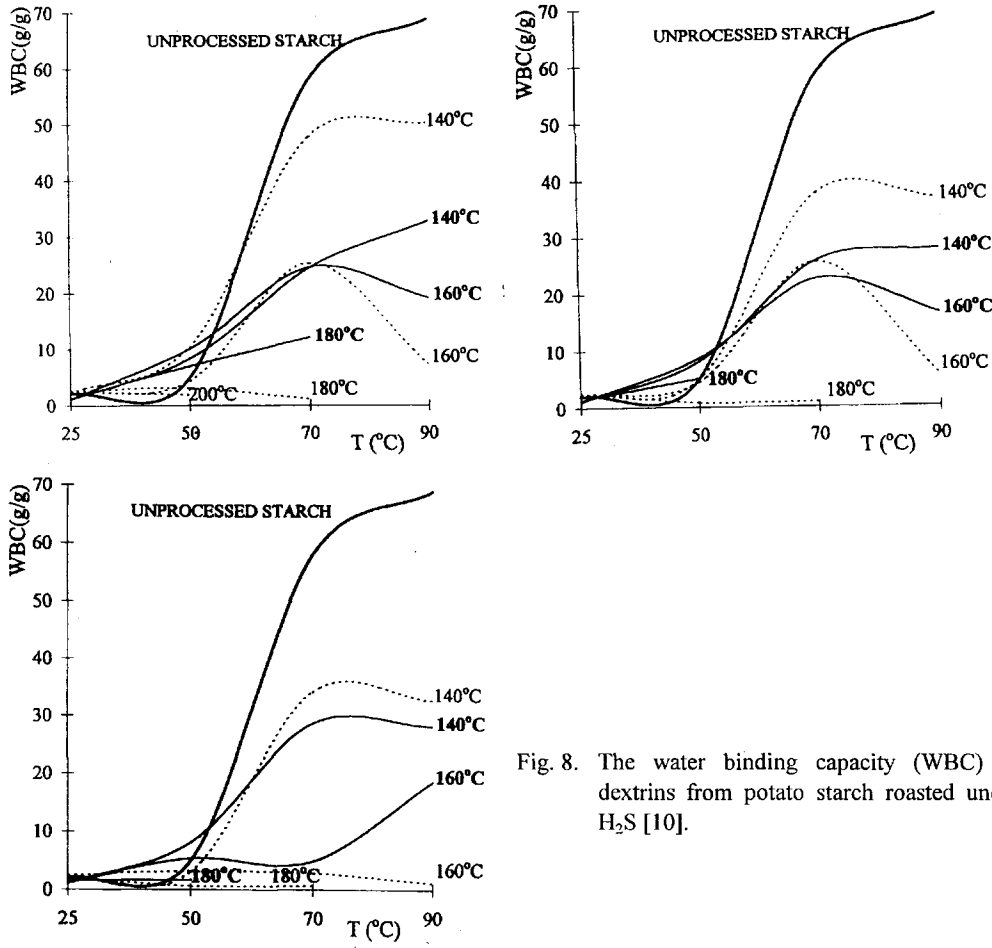


Fig. 8. The water binding capacity (WBC) of dextrins from potato starch roasted under H_2S [10].

The irradiation of air dried cassava, maize and potato starch showed their fast destruction even by the irradiation with the middle low energy. Fig. 9 shows the decrease of the viscosity of gels made of such gels.

The 15 min. irradiation of 4 % starch slurries with the middle low energy provided a dirty brown opaque gel of low reversion from maize starch, gummy clear product of low reversion from potato starch, and milky tixotropic stiff gel from cassava starch.

The starch crosslinking with formaldehyde on microwave irradiation was very successful. The reaction which required an acid catalyst when carried out on conventional heating was over without any catalyst within 5 to 20 min. of irradiation with low energy. The reaction time was dependent on the starch variety and volume of formaldehyde added as shown in Table 4 in terms of the viscosity of gels made of such products.

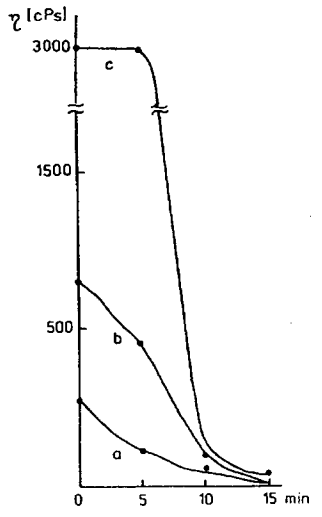


Fig. 9. The viscosity decrease of 5 % gels made of air-dried maize (a), cassava (b) and potato (c) starch irradiated for variable period with middle low energy microwaves (after [11]).

On microwave irradiation starch could also be crosslinked with acetylene. Hydrogen peroxide was used as the catalyst. The product of crosslinking of maize starch contained one double bond per 42 glucose units whereas the products from crosslinking of potato and cassava starch contained one double bond per 5 and 10 glucose units, respectively. In spite of crosslinking starch deteriorated to a significant extent under the reaction conditions (20 min. with low energy, 6 % of H_2O_2 added) and the gels made of the products had a low viscosity.

α-Hydroxy and α-amino acids added to starch prior to its irradiation with microwaves, except lactic acid which is liquid, practically did not dissociate under reaction conditions. They reacted with starch possibly by an addition hindering the macromolecule from deterioration. As shown in Figs. 10 and 11 such

Table 4

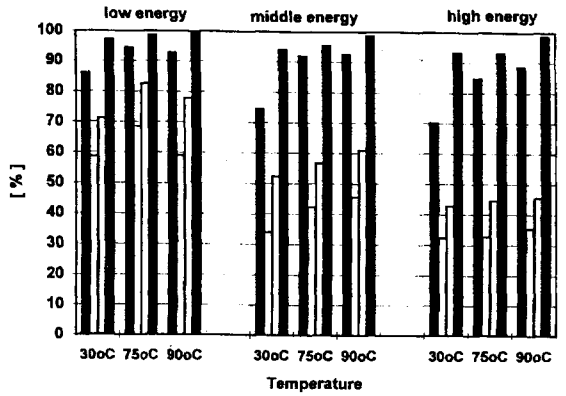
Viscosities, cP, at 25°C of 4-5 % gels made of starch crosslinked with 40 % aq. formaldehyde on irradiation with low energy [11].

Irradiation time, min.	Gel viscosity at 25°C, cP (gel. conc., %)		
	Maize	Potato	Cassava
1 weight % added			
0		330 (5)	280 (4)
5	650 (5)	4400 (5)	445 (5)
10	610 (5)	5200 (5)	370 (5)
15		3200 (5)	
2 weight % added			
5	480 (5)	1900 (4)	400 (4)
10	330 (5)	1900 (4)	330 (4)
20		2100 (4)	
25		800 (4)	
5 weight % added			
5	270 (5)	1200 (4)	800 (4)
10	365 (5)	1400 (4)	300 (3)
15	140 (5)	800 (3)	
10 weight % added			
5	170 (5)	1250 (4)	400 (4)
10	365 (5)	1300 (4)	400 (3)
15	270 (5)	1250 (3)	95 (1)

treatment increased WBC and, at the same time, hydrophobized starch. The products were anionic. α-Hydroxy acids gave better results in terms of all AS, WBC and pH. The latest decreased in the case of tartaric acid up to 2.7.

Carrying the reactions with amides and esters we assumed that the reaction of transesterification could be likely. In this manner amides and esters could act as acylating agents.

The microwave irradiation of potato starch blends with all esters and amides but phthalimide produced more hydrophobic dextrins with enhanced WBC which, however, in none of the cases exceeded 6 g of H_2O per 100 g of the product at 30°C as shown in Figs. 10 and 11.



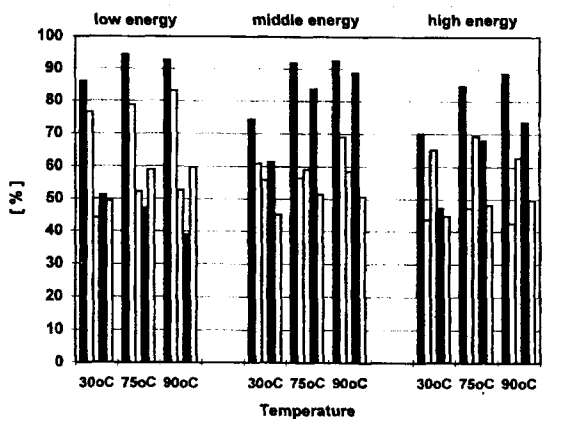
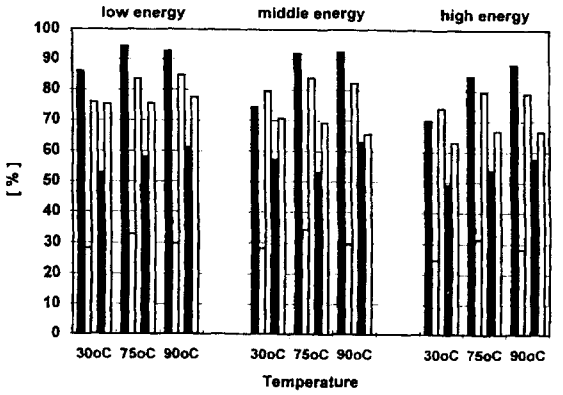


Fig. 10a. The aqueous solubility (AS) of dextrins from potato starch roasted with: 1 – α-hydroxy acids (columns from the left: plain starch, citric acid, tartaric acid, and lactic acid); 2 – α-amino acids (column from the left: plain starch, glutamic acid, asparagine, leucine and serine);



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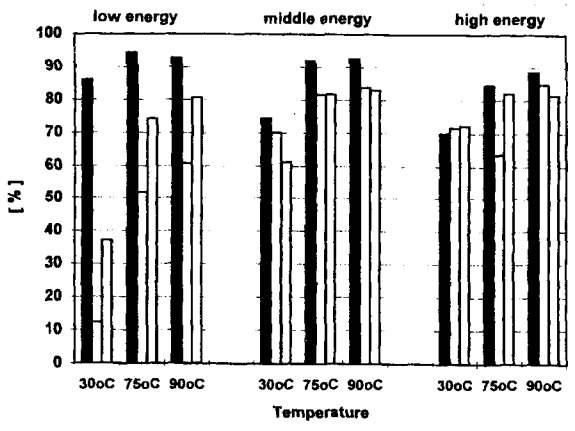

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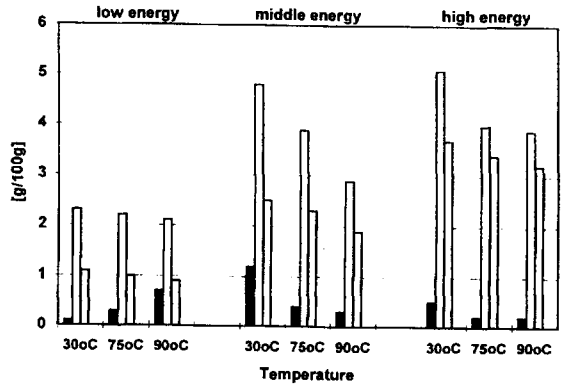
Fig. 10b. The aqueous solubility (AS) of dextrins from potato starch roasted with: 3 – amides (columns from the left: plain starch, urea, phthalimide, dimethylformamide, formamide); 4 – esters (columns from the left: plain starch, ethyl benzoate, dimethylphthalate). The 10 min irradiation with low, middle low and high energy microwaves in every case.



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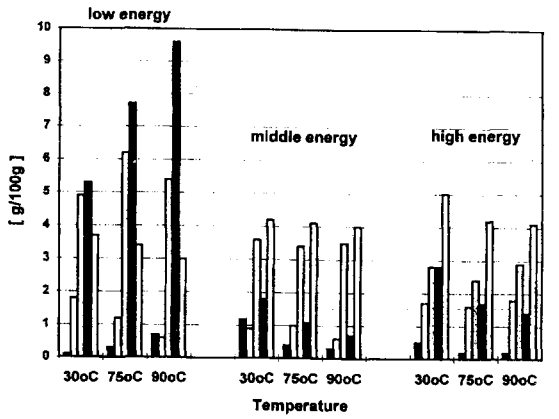

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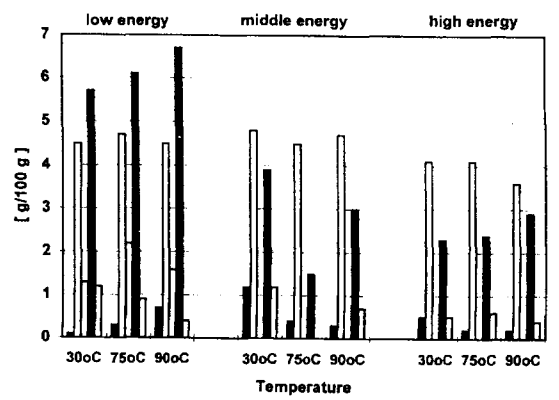

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Fig. 11a. The water binding capacity (WBC) of dextrins from potato starch roasted with α-hydroxy and α-amino acids as well as with amides and esters (see Fig. 10 for notation).

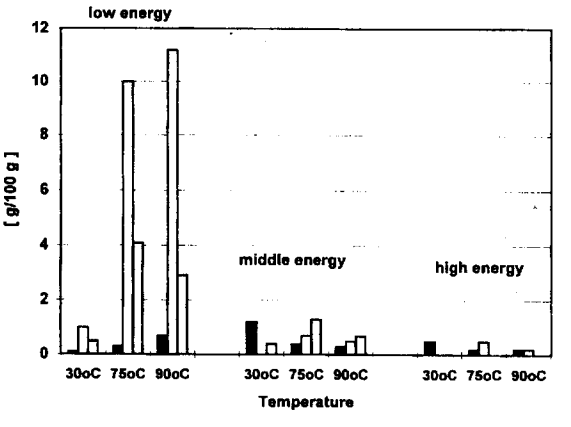

Fig. 11b. The water binding capacity (WBC) of dextrins from potato starch roasted with α-hydroxy and α-amino acids as well as with amides and esters (see Fig. 10 for notation).

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TERMICZNE PRZEKSZTAŁCENIA SKROBI

Streszczenie

Badania nad rodnikowym, termicznym rozkładem 11 odmian skrobi wykazały, że spośród skrobi z amarantusa, tapioki, owsa, kukurydzy, kukurydzy woskowej, żyta, triticale, dwu odmian skrobi ziemniaczanej i dwu odmian skrobi pszennej skrobia owsiana jest najmniej trwała, a skrobia kukurydziana woskowa jest najtrwalsza. Trwałość określono na podstawie liczby wolnych rodników wykazanych widmami EPR. Rodniki te były bardzo trwałe w kontakcie z powietrzem, wodą i alkoholem, którymi ekstrahowano powstające w trakcie termolizy dekstryny. Symulacja widm EPR dla 12 rodników gliukozy-

lowych i porównanie tych widm z widmem doświadczalnym doprowadziły do wniosku, że za trwałość rodników odpowiada delokalizacja niesparowanych spinów oraz osłony sferyczne.

W celu przygotowania nowych dekstryn o podwyższonej zdolności wiązania wody i obniżonej rozpuszczalności w wodzie termolizowano skrobię pod azotem, dwutlenkiem węgla, w amoniaku i siarkowodorem. Badano też sieciowanie skrobi formaldehydem, karboksyamidami i estrami przez ogrzewanie mieszanin reakcyjnych w polu mikrofalowym.

GRAŻYNA LEWANDOWICZ[1], JÓZEF FORNAL[2],
ALEKSANDER WALKOWSKI[1]

STRUCTURAL CHANGES OF TUBER STARCHES BY MICROWAVE IRRADIATION

Abstract

Time-temperature profiles of model starch water-systems, containing 0–35 % of water were studied, under microwave irradiation and effect of this treatment on physico-chemical properties of starch were recognized. Brabender rheological method, light microscopy, scanning electron microscopy and X-ray diffractometry were involved. Microwave irradiated starch containing more than 20 % of water underwent isothermal structural transformation. Consequently gelatinisation temperature rose and starch partially lost its solubility in water. The most dramatic change took place in case of potato starch. Its crystal structure changed from B into the A type. Tapioca starch changed to a lesser extent. Both tapioca and potato starch with less than 20 % of water solely lost their humidity on short irradiation then dextrinized.

Introduction

Microwaves deliver the nonionizing energy that causes a rise in temperature within a penetrated medium as a result of rapid electromagnetic field changes at high frequency. The design of microwave process involves not only thermal properties of foods, which are relative intensive to temperature differences, but also a number of interrelated electrical properties which vary extensively with the processing frequency and with product time-temperature profiles. At microwave frequencies, the most basic of these electrical properties – the dielectric constant and loss factor – are largely determined by product moisture and salt content [1]. Therefore, dry food components such as unmodified starch granules are thought to be electrically inert [2]. The most of experiments on starch – microwave irradiation interaction concern systems containing considerable amount of water [2–4]. Rashed Khan et al. hydrolyzed starch in water by heating with microwave energy at high temperatures and pressures inside the glass

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tubes. They obtained brown caramel-like starch hydrolysates containing oligosaccharides ranging from G_1 to G_8 [3]. Gordon and Davies examined in detail some physico-chemical properties of starch water – systems. They found that the distribution of variously swollen granules and the range in degree of swelling within the samples depend on the heating method. However, there are no structures unique to gels prepared, either by microwave irradiarion, or by convection heating [4]. Dielectric constants of modified and unmodified starches – water systems remained constant during heating, while dielectric loss factors and absorptivities decreased slightly during heating except during starch thermal transition times as measured by DSC [2]. There are also found dielectric properties of wheat starch powder containing 12.1 % of water (dielectric constant of 2.23 and dielectric loss factor of 0.23). Irradiation of air dried maize, potato and cassava starches leads to obtainment of dextrines [5]. The viscosity of its gels dramatically changes (decreases by one to three orders of magnitude), temperature of gelation slightly decreases and the colour becomes progressively yellow and brown. Reaction of starches with formaldehyde as well as with acetylene carried out by microwave irradiation gave products with enhanced viscosity [5].

Chemical modification reactions in solid state usually are carried out in rotating roasters. The design of this processes needs first of all detail knowledge about temperature and humidity of reaction mixture. The application of microwave ovens instead of rotating roasters seems to be attractive, but needs knowledge how microwave processing affects starch. The main purpose of the paper was to determine time-temperature profiles of model starch water-systems under microwave irradiation and the influence of microwave irradiation on physico-chemical properties investigated starches.

Materials and Methods

Commercial potato and tapioka starches were air-dried or humidified to obtain samples with required moisture values. 200 g starch samples were irradiated in 600 ml glass beakers covered (A-method) or not (B-method) with a special foil suitable for microwave ovens. DeLonghi microwave oven (Italy), 800W microwave output power and 2450 MHz microwave frequency were used to perform irradiation process. The power in experiments was set on minimum level (about 10 % output power). The temperature of starch layer was measured periodically with a mercury termometer after removing the beaker from the oven.

A gelatinisation course of starch samples was determined by a Brabender viscograph under the following conditions:

- measuring cartridge 700 cmg
- heating/cooling rate 1,5°C/min

- thermostating 30 min.

X-ray diffractometry was conducted by a X-ray diffractometer type TUR 62 Carl Zeiss Germany under the following conditions: X-ray tube CuKα (Ni filter); voltage 30 kV; current 15 mA; scanning from $\Theta = 2^{\circ}$ to 18°.

Starch samples subjected to examinations in a light microscope were prepared by a smear method. The starch suspensions were heated at temperatures corresponding to initial gelatinization temperature measured by a Brabender method and at 95°C. Drop of the starch paste was smeared on the microscope glass and after cooling smears were coloured by iodine according to Kaczyńska et al., [6] and observed in a Nicon FX light microscope. Starch samples examined in a scanning electron microscope were prepared according to Kaczyńska et al., [6] also Fornal [7] and observed in Jeol JSM 5200 microscope.

Results and Discussion

On the base of determined time- temperature profiles (pictures not shown) a strong correlation between water content in starch samples and time-temperature profiles character was found. Samples containing small amounts of water show quick and almost linear temperature rise. Higher water content in starch samples causes less vertical slope of the curves. This phenomenon is probably related to high water specific heat value. For starch samples containing over about 20 % of water one can observe plateau. Plateau interval length rises when water content in starch samples increases. This observation points to some kind of isothermal transformation which takes place in starch samples. More precise observation of changes between time temperaure profiles (pictures not shown) of samples irradiatied in covered and uncovered beakers shows that plateau interval lengths are higher for irradiated samples covered with foil. This observation points to critical role of water in isothermal transformation course mentioned.

If above hypothesis presented were true, starch samples irradiated with different water content should have different physico-chemical properties. In general a strong relationship between water content in irradiated starch samples and their Brabender curves course (pictures not shown) can be observed. Dry starch samples show high viscosity decrease after microwave irradiation, meanwhile the gelatinization temperature remains constant. Higher water content in starch samples up to 20 % causes less viscosity decrease. Starch samples containing approx. 20 % water show after microwave irradiation almost the same Brabender curves course in comparision to native starch. The situation is quite different for starch humid samples in the range from 20 % to 35 %. Higher water content causes viscosity decrease and the rise of the gelatinisation temperature value. More precise observation of changes between Brabender

curves course (pictures not shown) of samples irradiatied in covered and uncovered beakers shows that gelatinisation temperature rise is higher for irradiated samples covered with foil. This observation points to strict correlation between plateau interval length in time temperature profiles and gelatinisation temperature value of starch samples. That also leads to conclusion that above mentioned isothermal transformation which takes place in microwave irradiated starch samples causes changes in gelatinisation temperature and consequently perhaps in other physico-chemical properties. Intensities of Brabender curves course changes are higher for potato in comparision with tapioka starch. It is presented in the figure 1. The most important conclusion resulting from this figure is change in the Brabender curves character. Native potato starch reveals the course typical for tuber starches meanwhile the irradiated one (humidity of 35 %) is typical for cereal starches.

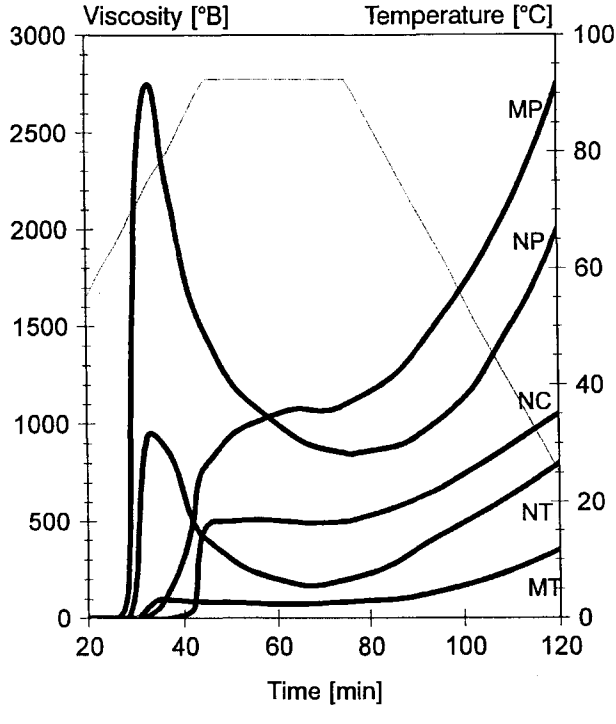

Fig. 1. Amylograph pasting curves for 8 % suspensions on native and microwave irradiated starch samples MP, potato starch microwave irradiated at 35% of humidity NP, native potato starch NC, native corn starch NT, native tapioca starch MT, tapioca starch microwave irradiated at 35 % of humidity.

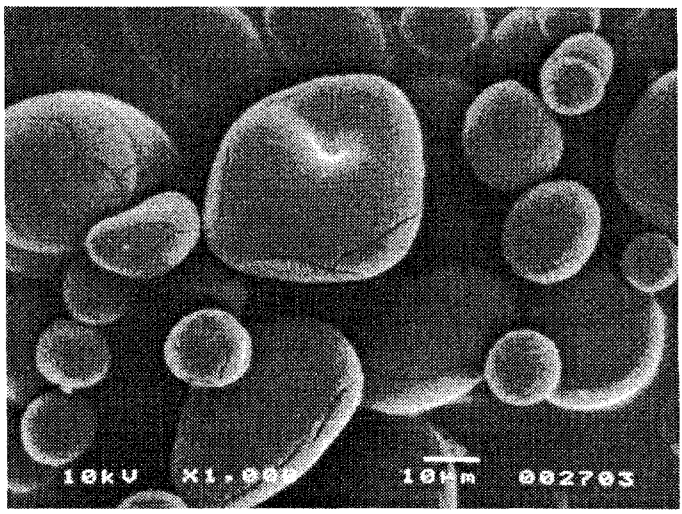

Fig. 2. Scanning Electron Micrograph dry potato starch after microwave irradiation.

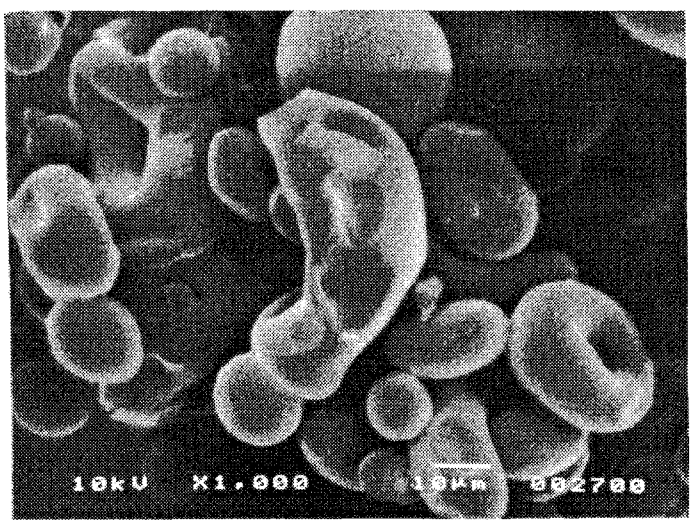

Fig. 3. Scanning Electron Micrograph of potato starch microwave irradiated at 35 % of humidity.

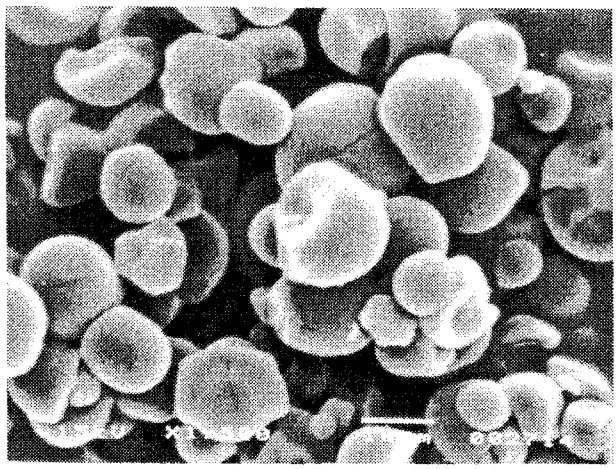

Fig. 4. Scanning Electron Micrograph of dry tapioca starch after microwave irradiation.

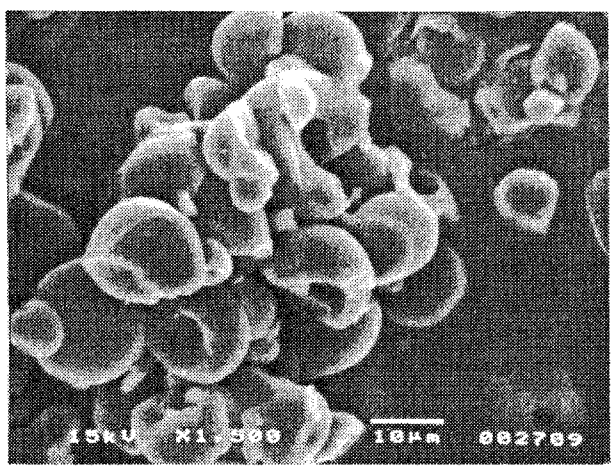

Fig. 5. Scanning Electron Micrograph of tapioca starch microwave irradiated at 35 % of humidity.

The light microscopy (photographs not presented) was used to examine other aspects of gelatinisation process. A typical image for initial gelatinisation period i.e. amylose efflux out of the starch granule is presented in the picture of native potato starch at the temperature of 68°C. At the temperature of 95°C native potato starch granules are almost solubled. The situation is different for microwaved starch. At the temperature of 68°C there are not any symptoms of gelatinisation process, when at the temperature of 90°C the image is the same like in the temperature 68°C for native starch. This facts reflect deep changes in the starch granule structure which cause some difficulties in the solubilisation process. In other words starch – starch intermo-

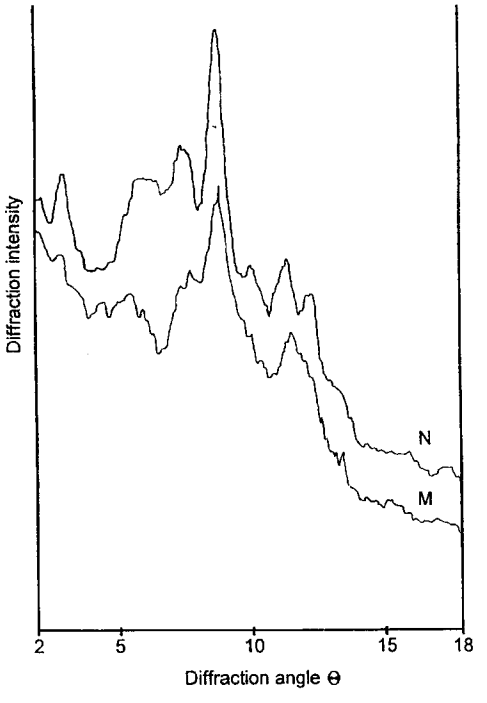

Fig. 6. X-ray diffraction patterns of potato starch samples N, native starch M, starch microwave irradiated at 35 % of humidity.

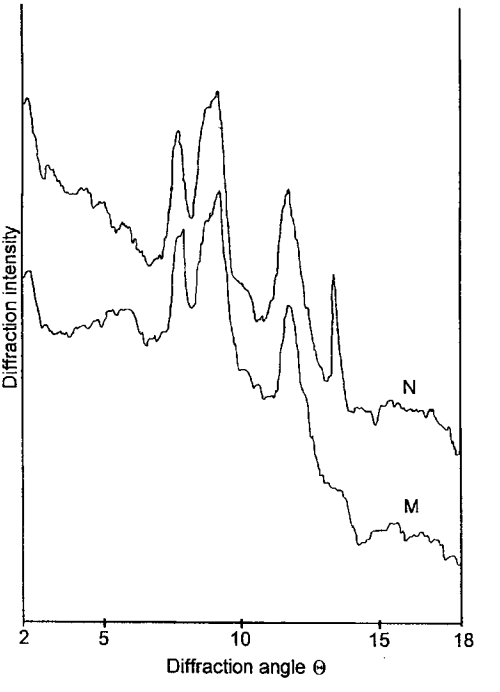

Fig. 7. X-ray diffraction patterns of tapioca starch samples N, native starch M, starch microwave irradiated at 35 % of humidity.

lecular interactions are stronger in microwave modified starch than in the native one. Tapioca starch shows lower structural changes reflected by light microscopy (photographs not shown). At the temperature of 68°C microwave modified tapioca starch shows low but appreciable amylose efflux out of the starch granule. At the temperature of 73°C the image is almost the same like at the temperature 68°C for native starch. Such radical changes in gelatinisation mechanism suggests essential alteration in the starch structure. SEM pictures of microwaved starches are shown in the figures 2–5. Predominant phenomenon in the case of dry starch samples is formation of superfacial cracks, what can be caused also by electrone beam. More interesting in our opinion are starch granules which collapse at the centre. Starch samples irradiated by high humidity show granules deformation first of all as a result of collapse at the centre. The results are similar to obtained by Kawabata et. al. for heat/moisture treated starches [8]. Heat/moisture treatment was described in detail first by Sair and later by Lorenz and Kulp et.al. It causes similar consequences [9–15]. Heat moisture treatment changes the physical properties of starches. The largest change takes place in the

root starches. The treatment changes sorption properties with corresponding changes in gelatinisation temperature, transluency, and pasting characteristics; in the case of potato starch, the B X-ray diffraction pattern is changed to the A pattern. Classical heat/moisture treatment is effected by heating in a pressure cooker at 100 % relative humidity; i.e. heating at 95°C for time periods from 2 to 18 hr., or by heating at temperatures 100–110°C for periods ranging up to 18 hr. In our microwave irradiation experiments the temperature of isothermal transformation is in the range of 80–90°C, and the time ranging up to 2.5 hr., so the conditions were milder. X-ray diffractometry investigations were carried out to check if by microwave irradiation takes place heat/moisture treatment. Figures 6–7 present X-ray diffraction patterns of microwave irradiated wet starch samples. The comparison of X-ray diffraction patterns of native and modified potato starches shows that there are changes from type B to type A. For tapioca starch which exhibits intermediate type of X-ray diffraction pattern, it is difficult to determine exactly it's type after microwave irradiation, but distinct differences between diffraction properties of native and microwaved ones were found.

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WYWOŁANE PROMIENIOWANIEM MIKROFALOWYM ZMIANY STRUKTURALNE W SKROBI Z ROŚLIN BULWIASTYCH

Streszczenie

Badano wpływ czasu i temperatury na właściwości fizykochemiczne modelowych układów skrobia – woda (0 do 35 % wody) ogrzewanych mikrofalami. W tym celu posłużono się wiskozymetrem Brabendera, mikroskopem, elektronowym mikroskopem skanningowym i rentgenografią proszkową. Skrobia zawierająca ponad 20 % wilgoci podczas ogrzewania mikrofalowego ulegała strukturalnym przemianom izotermalnym. W następstwie wzrastała temperatura kleikowania i malała rozpuszczalność w wodzie. Najwidoczniejsze zmiany zachodziły w skrobi ziemniaczanej. Jej struktura krystalograficzna zmieniała się z typu B na typ A. W mniejszym stopniu zmieniała się skrobia tapiokowa. Zarówno skrobia ziemniaczana jak i tapiokowa z zawartością wilgoci poniżej 20 % przy krótkotrwałym ogrzewaniu jedynie traciły wilgoć, a potem dekstrynizowały.

ZBIGNIEW K. BRZOZOWSKI

STARCH – ACRYLAMIDE COPOLYMERS

Abstract

Potato starch was modified by acrylamide under basic condition either via polyaddition or in acidic media via graft copolymeryzation.

Modified starch was applied as environmentally friendly agent for the following purposes: a formaldehyde scavenger from formaldehyde resins especially urea-formaldehyde resins used in chipboards or phenol-formaldehyde resins for binding of insulation mineral fiber-plates; agents reducing drag flow in pipelines for waste liquids, and reinforcement of drillings in oil and gas mines.

Elaborated method of production of formaldehyde emission decreasing agents is wasteless. Starch/acrylamide copolymer is completely harmless and nontoxic product, useful for environmental protection.

Introduction

Availability, low price, simple modification, atoxicity, simple degradation, possibility of different application are advantages of natural polymers and their derivations. Potato starch was modified either by 1-chloro-2,3- propanodiol and acryloamide in basic conditions or in acidic media by graft copolymeryzation of acryloamide. Modified starch (MS) was applied as environmental friendly agents to play a role of:

1) formaldehyde scavenger from formaldehyde resins used in urea-formaldehyde chipboards or phenol-formaldehyde resins used for binding of insulation mineral fiber-plates,
2) agents reducing drag flow in pipelines for liquid waste,
3) drilling muds.

Formaldehyde

Formaldehyde vapours are highly toxic. In man, irritant effects to nose, throat, lung and eyes could be seen on exposure of ≥ 0.5 mg/m^3 with acute effect at 10 mg/m^3.

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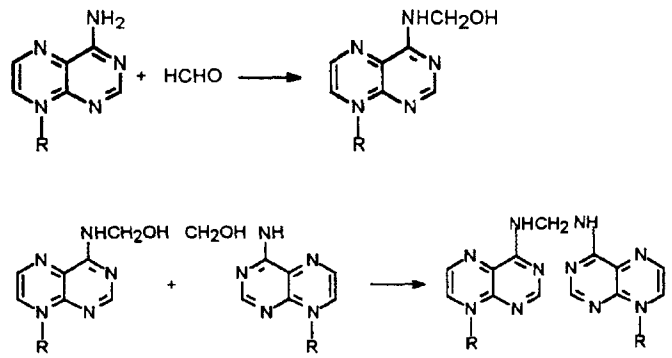


Fig. 1. Formation od DNA cross-links by formaldehyde.

In man, cutaneous sensitisation and allergic contact dermatitis have been well documented in individuals chronically exposed to formaldehyde – containing materials. A 2 % solution, however, does not produce acute skin irritant effects in man.

Formalin solution (26 %) has been shown to be severely irritant, producing permanent corneal injury in rabbits, guinea pigs and man. Splashes of a 4 % formaldehyde solution are highly irritant in man but no permanent effects have been reported.

A high reactivity of formaldehyde results in the formation of metylene cross-links between metylol groups by a condensation reaction between nucleotides of DNA in a single strand configuration as shown in Fig. 1.

Table 1

Trends in formaldehyde regulation

Period of time	Maximum formaldehyde content (mg/100g chipboard or plywood)
to 1970	100
1971-1973	65
1974-1975	45
1976-1977	30
1978-1981	25
1981-1986	20
1987-1990	> 10
1991-	>5

These and other biochemical reactions have led to the expectation of genotoxic potential of formaldehyde and hence potential for carcinogenicity

The standards concerning the amount of formaldehyde in chipboard or plywood are still tightening. This trend is shown in Table 1.

Modified starches (MS)

We investigated the modification of starch under

acidic or basic conditions. The reaction of starch with acrylamide is shown below:

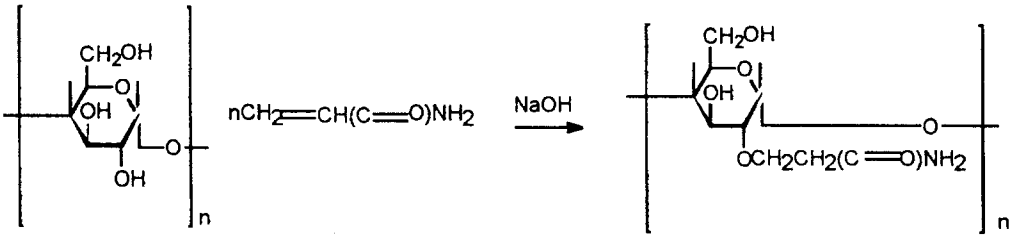


Fig. 2. Starch modification by acrylamide (S+AA).

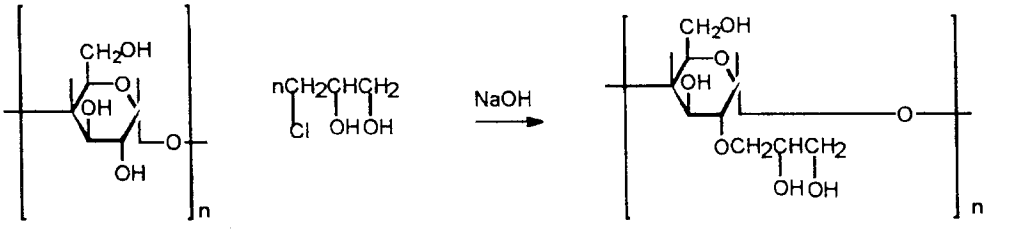


Fig. 3. Starch modification by chloropropandiol (S+CPD).

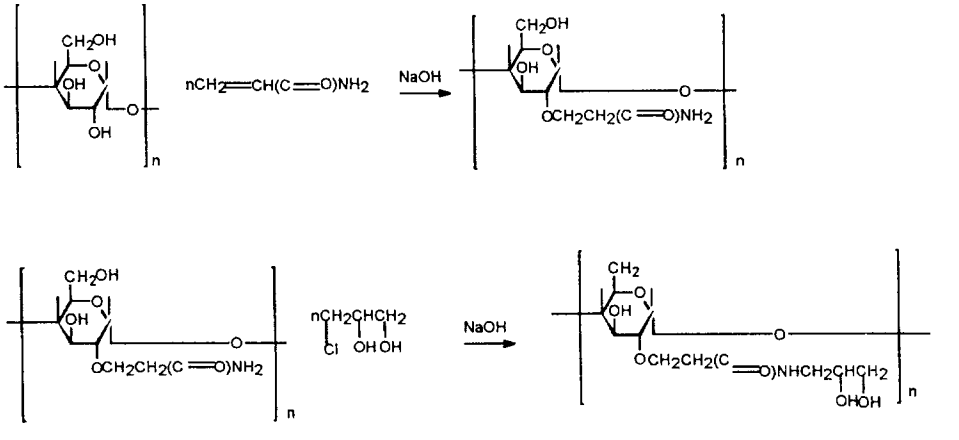


Fig. 4. Starch modification by acrylamide and chloropropandiol (S+AA+CPD).

Starch modification by the graft copolymerization with acryloamide.

Initiation

Me^{n+} + St–OH – Complex – St–O• + $Me^{(n-1)-}$ + H^+

St–O + M – St•–OM

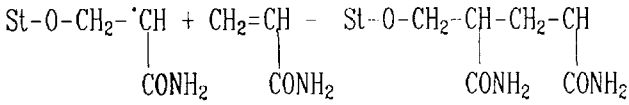
Polymerisation of acrylamide may proceed at the same time:

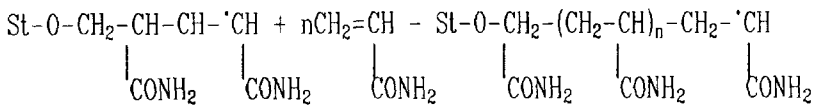
$Me^{n+} + M–M\bullet + Me^{(n-1)-} + H^+$

$M + M\bullet – M–M\bullet$

Chain extension

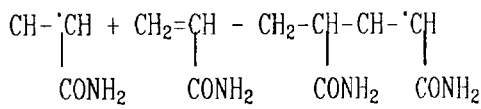
$St–OM\bullet + nM – St–OM–(M)_{n+1}M\bullet$

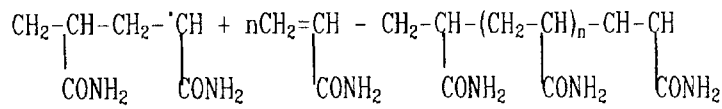




Chain extension of homopolimer is simultaneously formed.

$M–M\bullet + nM–(M)_n–M$





Chain termination takes place as below:

$St–O–M–(M)n–M\bullet + Me^n – St–O–M–(M)_n\text{-}M + Me^{(n-1)} + H^+$

$M–(M)_n–M + Me^{n+} – M–(M)_n–M + Me^{(n-1)+} + H^+$

Fig. 5. Starch modification by acrylamide in acidic condition.

Formaldehyde scavengers

The copolymer obtained was applied in aqueous solution as an additive to urea-formaldehyde resins. Following the technical guidelines we propose some binders consisting of urea-formaldehyde resin, curing agent (NH_4Cl-ammonium chloride), pure starch, diammonium phosphate and our graft copolymer in aqueous solution as a modifying agent.

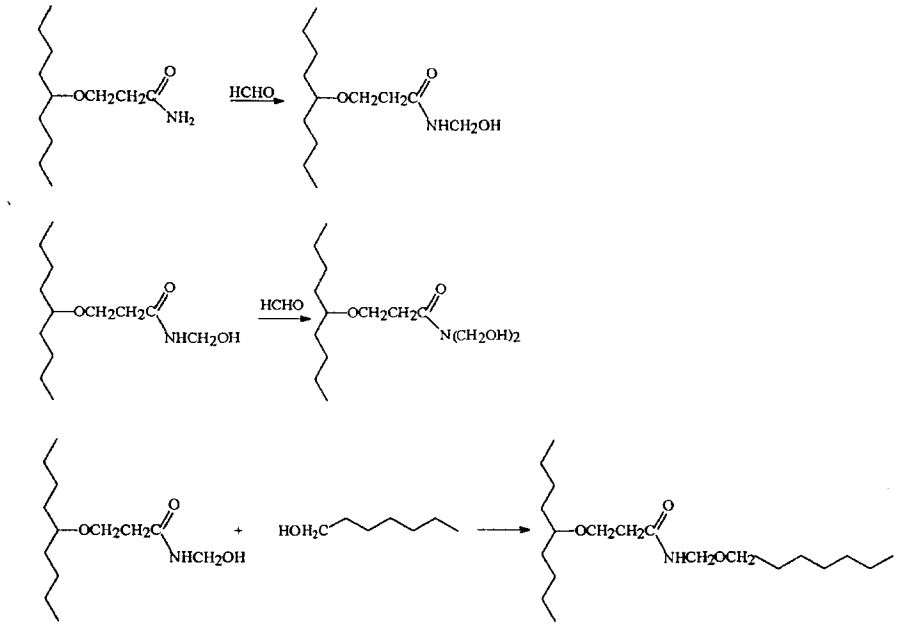


Fig. 6. The reaction of MS with formaldehyde occurs as follows:

We obtained chipboards using modifying agents with a free formaldehyde content below 10 mg per 100 g of chipboards (corresponding to E1 class for formaldehyde emission). These chipboards have the same physical and mechanical properties as chipboards prepared without a modifying agent. Thus, we eliminated about 58 % of the free formaldehyde emission, without altering the chipboards characteristics.

The Technical Chipboard Guidelines

1. The total content of dry substances in binding compounds ≥ 53% should be maintained.
2. 4.5 g of 20 % aqueous solution of curing agent has to be added per 100 g urea-formaldehyde resin.
3. The time of binding compound gelation should be between 90 - 190 s.

The composition of a typical binding compound is given in Table 2.

The physical properties of the modifying agent used in this experiment are presented in Table 3.

Table 2

Typical binding compound composition.

Components	Quantity [g]	Dry substance [g]	Gelation time [s]	Dry substance [s]
Resin	100	66		
Modifying agent	10	0.69		
Starch	–	–	100	59.5
NH_4Cl	4.19	0.838		
$(NH_4)_2HPO_4$	1	1		

Table 3

Modifying agent properties.

Parameter	Modifying agent value
Density [g/cm^3]	1.145
Viscosity [mPa s]	122
Dry substance	6.55
pH	< 2
Miscibility with water	

Chipboards presses with modifying agents satisfy the requirements of Polish Standard BN-80/7123-04-03 for the highest quality of chipboard.

The physical properties of the chipboards which were bound with the modifying agent were identical to those of chipboards which were bound with pure urea-formaldehyde resins. The reduction of free formaldehyde emission in pressed chipboards were as follows:

Table 4

Reduction of free formaldehyde in chipboards

Case	Content of free formaldehyde (in mg/100g chipboards)
Without modifying agent	23.0
With modifying agent	9.7

We obtained chipboards using modifying agents with a free formaldehyde content below 10 mg per 100 g of chipboard (corresponding to E1 class for formaldehyde emission).

These chipboards have the same physical and mechanical

properties as chipboards prepared without a modifying agent. We have thus eliminated about 58 % of free formaldehyde emission, without altering the chipboard characteristics.

Some properties of binding agents for chipboard before and after addition of MS are shown in Table 5.

Table 5

Chipboard properties and MS addition

Binding agent	Gelation time [s]	Emission of free formaldehyde [mg/100g]	Emission decrease [%]
Before MS addition	86	202	–
After MS addition	77	96	52

Formaldehyde scavengers based on modified starch have decreased the formaldehyde amount > 10 mg/100 g of chipboards or plywood without any depreciation of other properties.

Drag reduction of liquid flow

The special apparatus has been constructed for our laboratory investigations on the drag reduction of liquid flow – notably for the extraction of salt water from mines (Figure 7). The results are shown in Table 6.

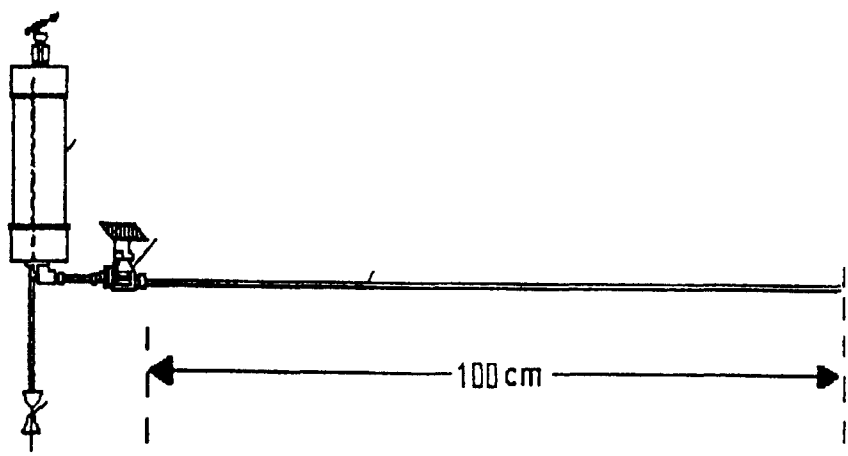


Fig. 7.

Table 6

(Density = 1026 kg/m^3, time = 34.2 sec.)

	Viscosity [m·Pa·s]	Mass [kg]	Re (Reynolds number)	Z (drag flow) [m]
Salt water	1.61	0.521	989	0.110
S+AA+CPD				
0.10 %	1.30	0.522	2453	0.060
0.8 %	1.37	0.526	2394	0.060
0.06 %	1.16	0.525	2815	0.050
0.04 %	1.14	0.527	2881	0.054
0.02 %	1.12	0.516	2850	0.051

The best results were obtained with the starch modified by acrylamide. Our products have identical reduction properties against drag flow as commercial products made by Allied Colloids.

Advantages of new modified natural polymers are as follows: availability, low price, simple modification, atoxicity, simple degradation, possibility of different application.

Elaborated production method of formaldehyde emission decreasing agents is wasteless. Starch/acrylamide copolymer is completely harmless and nontoxic for environmental protection and it evokes significant interest of environmentality.

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[2] Z.K. Brzozowski, K. Noniewicz: Polimery, **38** (11-12), 1994, 726-730.
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KOPOLIMERY SKROBIOWO-AKRYLOAMIDOWE

Streszczenie

Skrobię ziemniaczaną modyfikowano akryloamidem przez poliaddycję w środowisku zasadowym lub szczeponą kopolimeryzację w środowisku kwaśnym.

Skrobię modyfikowaną zastosowano jako czynnik bezpieczny dla środowiska naturalnego do następujących celów: wychwytywanie formaldehydu uwalnianego z żywic formaldehydowych, szczególnie żywic mocznikowo-formaldehydowych stosowanych do płyt wiórowych i żywic fenolowo-formaldehydowych do wiązania i izolowania elementów z włókien mineralnych, czynniki zmniejszające opory przepływu w rurociągach przesyłających ścieki oraz płuczki wiertnicze.

Opracowana metoda produkcji czynników zmniejszających emisję formaldehydu jest metodą bezodpadową. Kopolimer skrobi z akryloamidem jest zupełnie bezpieczny i nietoksyczny i nadaje się do zastosowania w działaniach chroniących środowisko naturalne.

A.A.C.M. BEENACKERS, G. LAMMERS, N.J.M. KUIPERS

NOVEL CONTINUOUS REACTORS FOR CHEMICAL MODIFICATION OF STARCHES WITH IMPROVED SELECTIVITY

Abstract

Two novel continuous processes for the chemical derivatisation of starch have been developed. A gas-solid fluidized bed process for modifications with gaseous substrates and a gel process in a static mixer reactor for handling concentrated pastes. Based on results obtained with hydroxyethylation (dry-process) and hydroxypropylation (gel-process) it can be concluded that both processes are very attractive relative to the classical slurry process with respect to improved reaction selectivity, much shorter residence times and superior controlability and safety.

Introduction

Traditionally, chemical modification of starch is carned out in batch processes. However, in the past decades, the production capacity of many starch modiflcation plants has increased to such a high level that this fact alone justifies the development of continuous processes. But there is more: continuous processes often allow for a more strict process control resulting in less variation in product quality and often in safer operation. The latter is particularly important ifpoisonous or unstable reactants are used such as alkyloxides in the alkoxyethylation of starch. In addition, depending on the application, specific extra advantages may be realised in optimised continuous processes such as reduced risks of contamination of the product and the opportunity to operate with reduced fractions of water which often results in improved reaction selectivities. Because of these considerations we decided about a decade ago to develop novel continuous processes for starch modification. So far we focused on developing a continuous static mixer reactor for in situ gelatinising concentrated aqueous starch slunries followed by chemical modification in the same reactor snd on a special type of fluidized bed reactor for (semi-)dry chemical modifications with gaseous reactants. Because of the extra drive for improved safety in processes with potentially unstable

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reactants such as propylene oxide and ethylene oxide, we selected these reactants as the first candidates for the novel gel and gas-solid starch modifications, respectively.

The continuous static mixer reactor

With respect to continuous chemical modification of gelatinised starch, most research concentrates on the application of extruders as a reactor, particularly for cationisation [1, 2], but in our Department also in the benzylation [3] and in the acetylation and hydroxypropylation of starch [4]. Potentially, extruders can operate with slightly more concentrated pastes than static mixers, though often at the expense of starch breakdown due to high shear forces, leading to lower starch molecular weight and lower viscosity pastes [2]. Further, extruders have some major draw backs relative to static mixers. In contrast to extruders, static mixer reactors are easy to scale-up to large capacities, if necessary can be fully temperature controlled (e.g. by applying Sulzers SMR mixers), have much better plug flow characteristics (often resulting in a more homogeneous substitution), are relatively cheaper and require less maintenance. Reason enough to concentrate on the development of a static mixer reactor for chemical modification of starch.

Static mixer selection

From those static mixers presently commercially available and suitable for high viscosity applications, only KTEK's [5], Kenics and Sulzer's [6] SMX and SMXL mixers are sufficiently documented to allow for a rational relative assessment. From these, the SMX mixer has the best mixing and heat transfer characteristics (see figs. 1

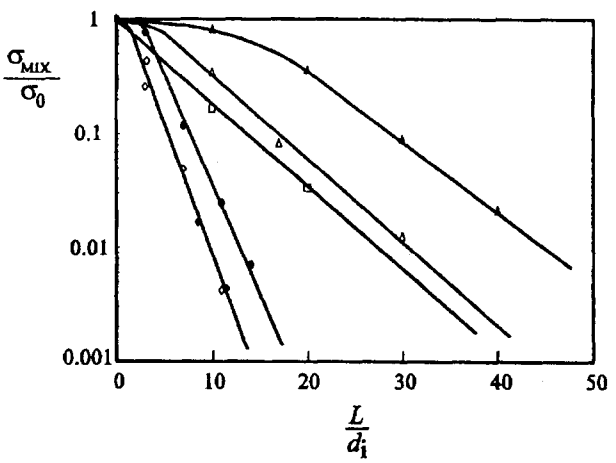


Fig. 1. Relative standard deviation in liquid composition at the mixer outlet due to incomplete mixing of two liquid flows entering the mixer, as a function of length over diameter ratio (L/d_i) of the mixer [7]; ◊, ♦, •: SMX; □: Kenics; △, ▲: SMXL.

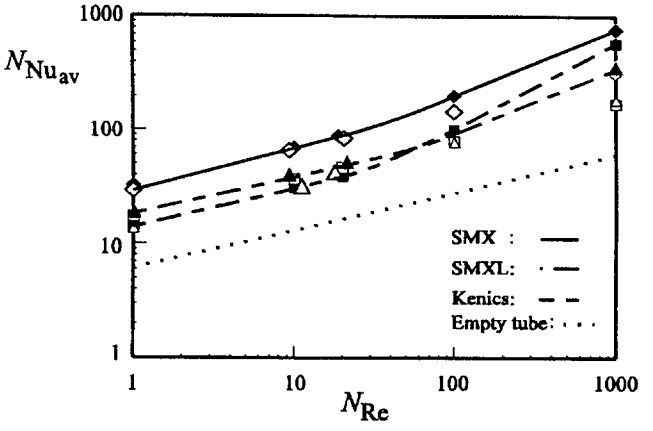


Fig. 2. Dimensionless average heat transfer coefficient to the wall as a function of the Reynolds Number; ◊, ♦: SMX; □: Kenics; △, ▲: SMXL.

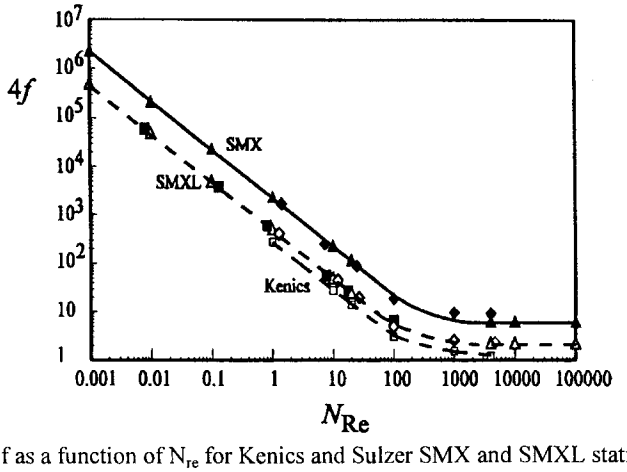
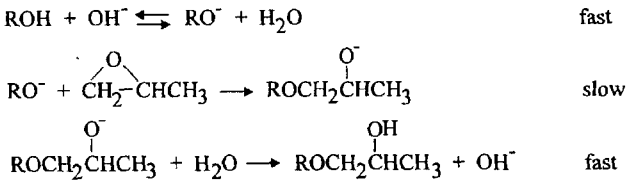


Fig. 3. Friction factor 4f as a function of N_{re} for Kenics and Sulzer SMX and SMXL static mixers [7].

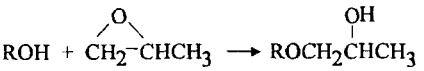
and 2, respectively), though at the expense of a slightly higher pressure drop (see figure 3). Therefore, we decided to apply SMX mixers.

Chemical reactions

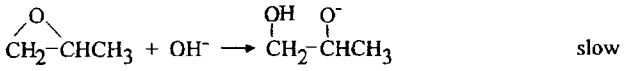
We applied the novel reactor for the alkali catalysed hydroxypropylation of potato starch. The following reactions occur:
Catalysed reaction with starch:

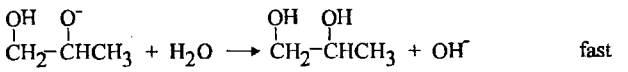


Uncatalysed reaction with starch:

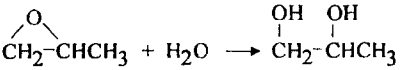


Catalysed hydrolysis:





Uncatalysed hydrolysis:



We have a selectivity problem because propylene oxide may either react with starch or water, leading to the desired product and 1,2 propane diol, respectively. For the reaction kinetics, see [8].

Pilot plant design

The design ofthe continuous pilot was based on the following requirements:

- Range of residence times suitable for obtaining conversions of propylene oxide of over 80 % in the temperature range ofinterest (343 to 373 K);
- Good plug flow characteristics;
- Good temperature control;
- Operation at starch concentrations ofup to 35 mass percent must be possible with regard to maximum allowable pressure drop;
- Possibility of monitoring the course of the reaction in the reactor.

The resulting scheme is shown in figure 4. The starch slurries were prepared in a stirred tank of 50 dm^3 (1). Driven by a Mohno pump (2) these slurries flow via a heating coil (4), a premixer (5) and a heating section (6) into the reactor. Just before the premixer, both an aqueous sodium hydroxide solution and liquid propylene oxide could be added to the starch slurry by two metering pumps. In the premixer, starch slurry, sodium hydroxide and propylene oxide were mixed by 10 Sulzer SMX mixers. Always, sufficient sodium hydroxide was added to get complete gelatinisation of the

starch immediately after the premixer. The heating section consisted of a ceramic tube, inserted in a wave pipe of a microwave oven (7). The reactor contained five so-called measurement sections (8) each equipped with a Pt 100 temperature sensor, a pressure sensor and an adapter for the insertion of a radial temperature sensor, consisting of five radially spaced 0.10 mm thermocouples. In each section the reaction mixture could be sampled. The reactor contained four so-called SMX sections (9), consisting of a jacketed tube, filled with 14 Sulzer SMX static mixer elements (d_{sm} = l_{sm} = 27.3 mm) to induce plug flow and to enhance heat transfer. The tube was thermostatted with water. The reactor outlet consists of a pressure controlling pneumatic valve (10). Total reactor volume: ± 1 dm^3.

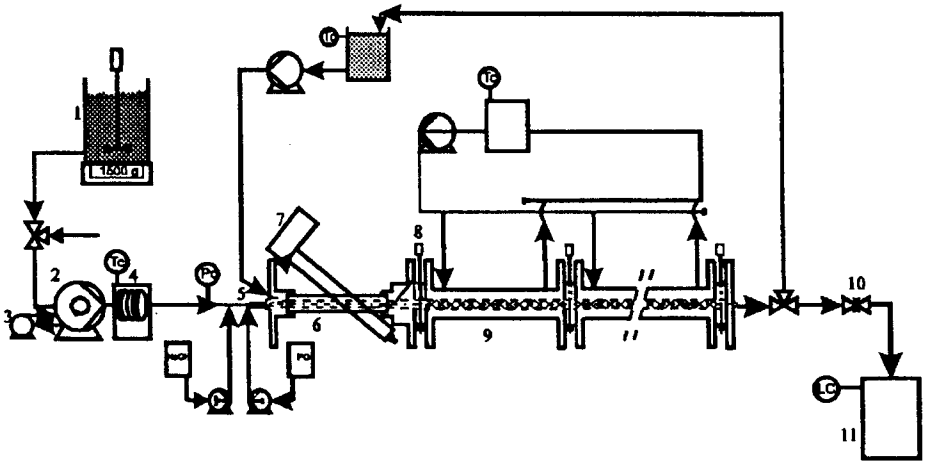


Fig. 4. Flow sheet ofthe static mixer reactor for the continuous chemical modification of concentrated starch pastes.

On chemical analysis methods applied, see [9].

Residence time distribution

Fig. 5 gives some results of residence time distribution measurements together with curves from a model presented elsewhere [7]. Indeed, the reactor shows excellent plug flow behaviour with Peclet numbers of typically: N_{pe}= 100 (or 63 per meter length of reactor) Notice that N_{pe}= 100 is equivalent to a residence time distribution obtained from 50 ideal mixers in series.

For comparison: Van Zuilichem [10] reported for single screw extruders $10 < N_{pe} \leq 20$ only.

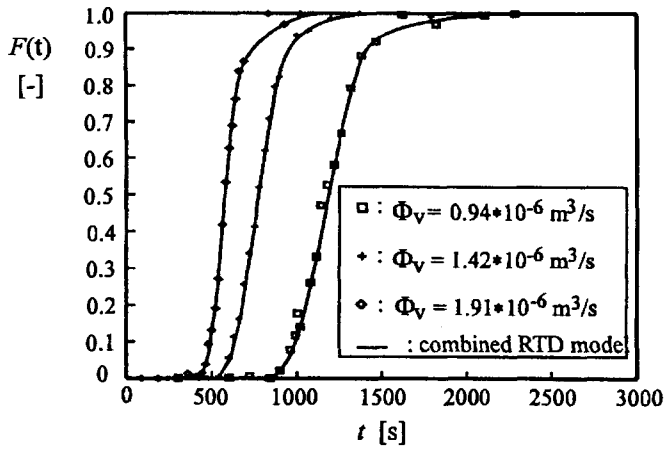


Fig. 5. Experimentally determined cumulative residence time functions for $m_{starch} = 0.28$ at T = 343.15 K and results obtained from fitting the experimental data with a residence time distribution model.

Heat transfer

The heat transfer to the wall was measured for starch pastes of various strengths, all as a function of the Peclet number for heat, based on the tube diameter:

$$N_{Pe_{h,t}} = N_{Re} N_{Pr} = \frac{\rho c_p v d_t}{\lambda}$$

with $N_{Re} = \rho v d_t / \xi$

$N_{Pr} = c_p \xi / \lambda$

See Fig. 6 for results. All data could be accurately described by:

$$N_{Nu} = 1.87 \left(N_{Pe_{h,t}} \right)^{0.44}$$

This relation nicely agrees with that of Kalbitz and Bohnet [11], whereas the relation of Sulzer [6] appears to be less accurate.

Conversion and selectivity

A set of 9 reaction experiments were carried out with:

$0.15 < m_{starch} < 0.3$ [-]

$250 < c_{NaOH} < 500$ [mol/m^3]

$343 < T < 365$ K

$300 < r < 1200$ s

The observed conversions of propylene oxide conversion toward hydroxy propylated starch are shown in Figs. 7 and 8, respectively, both in relation to theoretical values predicted from a reaction engineering model presented elsewhere [7]. Depend-

ing on the values of the operating parameters listed above a practically complete conversion appears to be possible with proven selectivities as high as 80 %. Indeed, this compares favourable, relative to a typical selectivity reported for a classical slurry batch process of 60 % [12].

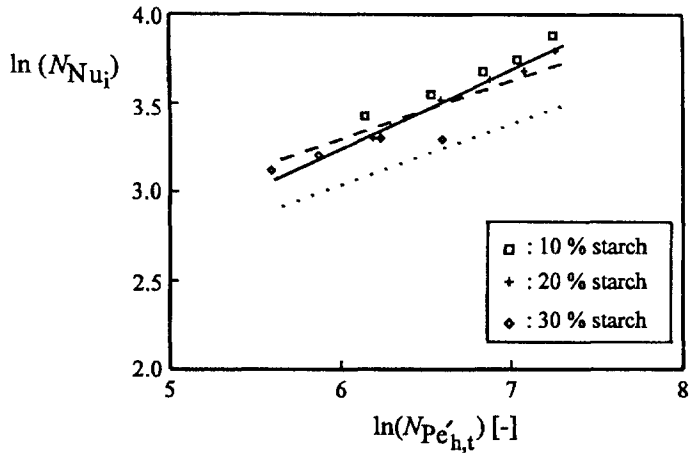


Fig. 6. Experimental data and literature relations for $\ln(N_{Nu})$ as a function of $\ln(N_{péh,t})$ [9]. – : our relation; -- : Kalbitz and Bohnet [11]; . . . . : Sulzer, [6].

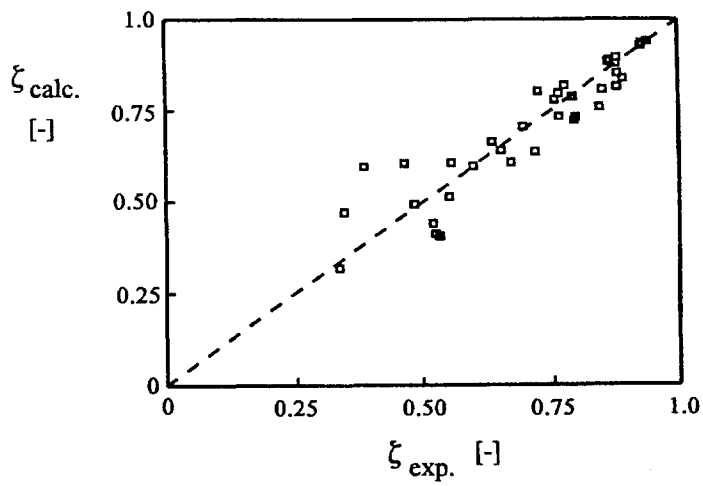


Fig. 7. Parity plot of experimentally determined and calculated propylene oxide conversions.

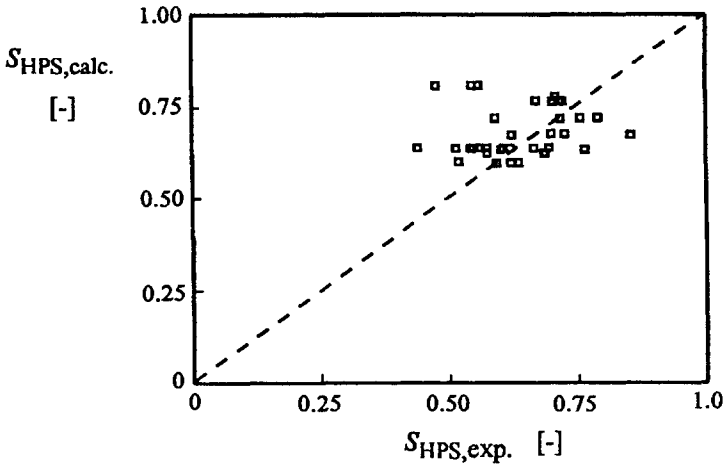


Fig. 8. Parity plot of experimentally determined and calculated selectivities toward hydroxypropyl starch.

Scale-up

Based on the experimental data of the pilot reactor we made a preliminary design for a plant having an annual production capacity of 3500 tons dry hydroxy propyl starch.

We considered both adiabatic operation with SMX mixers and isothermal operation with SMR mixers. It turned out that no significant gain in selectivity could be realised by applying isothermal operation. Hence we propose adiabatic operation. Figure 9 and 10 show the calculated required reactor volume and propylene oxide

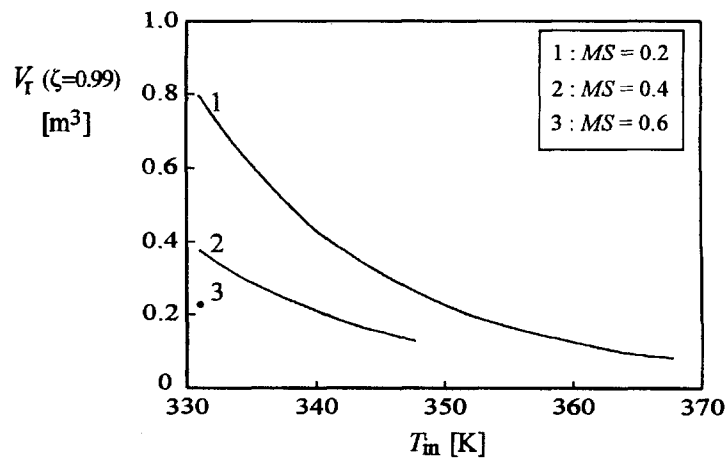


Fig. 9. Required reactor volume for 99 % propylene oxide conversion as a function of reactor inlet temperature (adiabatic operation, $m_{starch} = 0.45$).

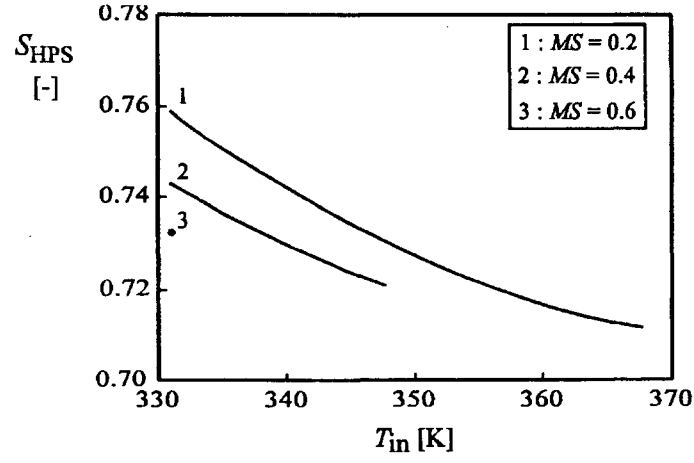


Fig. 10. Overall reaction selectivity as a function of reactor inlet temperature (adiabatic operation, m = 0.45).

selectivity, respectively, both on the basis of 99 % PO conversion as a function of degree of Molar Substitution (MS) and reactor inlet temperature. Even at a relatively low inlet temperature of 330 K the required reactor volume remains substantially below 1 m^3 and selectivities expectedly will be above 72 %.

The stirred Vibrating Fluidized Bed Reactor

In order to retain its easy flow and handling properties and to avoid excessive drying costs, it is attractive to modify starch while saving its granular structure wherever possible. Low substituted starch derivatives (MS < 0.1) are usually manufactured by modifying starch in aqueous suspensions. However, this has some disadvantages such as a low selectivity of the process and a low reaction rate due to a limited hydroxide concentration and reaction temperature (typically below 55°C), both to prevent gelatinization of the suspended starch particles, see Kuipers [13]. Because the granule gelatinization temperature lowers with increasing MS, there is a maximum MS in aqueous slurry reactions where above the granular form of the starch gets lost.

If the substrate is a gas, such as in hydroxyethylation or hydroxypropylation, a gas-solid modification process seems attractive because it does not suffer from these disadvantages. The low moisture content of (semi) dry starch might favour the desired reaction relative to the hydrolysis of the alkeneoxide while the reactivity might be increased by application of higher temperatures than are possible in the conventional slurry process. An additional, potentially very interesting, advantage of the gas-solid hydroxyalkylation of starch possibly is the extension to products with a higher degree of substitution without loss of the granule structure. An economic evaluation of the two processes showed that the estimated production costs can be lower for the novel

gas-solid process relative to the slurry process (Van Warners [14]). However, for sufficient reactivity and diffusivity, the starch granules must contain some water, typically between 15 and 25 wt % d.b. With increasing moisture content, starch becomes increasingly cohesive thus tuming from a so-called A into a C-powder. An emperical measure for powder cohesiviness is the so-called Hausner Ratio (HR) which is the ratio between the lowest and the highest density of a powder as measured by a standardized technique. Fig. 11 shows our experimental results, indicating starch of the desired moisture percentage to be at the transition from A into C powder.

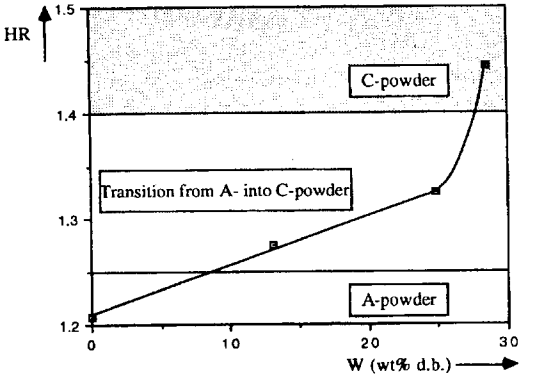


Fig. 11. Influence of moisture content W of potato starch on its Hausner ratio, HR, at T= 293 K.

AC and C powders are known to be too sticky to be processed into conventional gas-solid contactors such as a fixed bed, a moving bed or a gas-solid trickle flow reactor.

Indeed, in these reactors a poor gas-solid contact is obtained due to particle agglomeration. This results in an inferior product quality [13].

Based on ρ_p = 1500 kg m^{-3} and R_p = 25 μm, dry potato starch can be classified as an A-powder which is very suitable for dense phase fluidization as is confirmed by a patent (Thompson [15]).

In contrast, semi dry potato starch of W 10 wt % d.b. cannot be fluidized by aeration (Stigter [16]). Apparently, such a low W is already sufficient to get channelling, which is a characteristic for a C-powder. So, starch changes from an A- to a C-powder with increasing W from 0 to 10 wt % d.b.. Fluidization of cohesive powders can often be improved by the use of mechanical stirrers, vibrators or by the addition of sub-micron particles. In most chemical reactions, such as the hydroxyethylation of-granular potato starch, addition of sub-micron silica is not desired because of both difficulties in processing and purity requirements of the product.

Therefore, we focused on the effects of stirring and/or vibration of an aerated bed of potato starch.

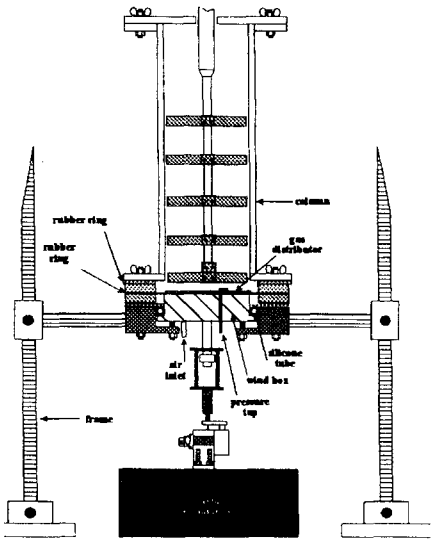


Fig. 12. Scheme of the aerated stirred vibrating fluidized bed.

Experimental

The experimental set-up is shown in Fig. 12. The bed has an inner diameter of 29 cm and a height of 1.35 m. The gas distributor, consisting of porous stainless steel, could be brought in vibration with variable frequency ($0 < f < 90$ s^{-1}) and amplitude $0 < x_0 < 1.5$ mm. Stirring of the bed with a various number of blades $1 \leq n \leq 15$ was possible at various rates $0.8 < N < 4.2$ rev/s. Always the relative humidity of the gas fed to the bed was in equilibrium with the moisture content of the starch in the bed.

The aerated bed without stirring and vibration

Kuipers [13] showed that even an aerated bed of relatively dry potato starch of W = 6 wt % d.b. cannot be fluidized without mechanical energy input. Initially, the pressure drop across the fixed bed increases proportionally with increasing air velocity. With increasing air rate, large horizontal cracks appear in the bed. The latter tends to lift as a plug (see Fig. 13a). At higher air velocities stable channels are formed in the static bed. Due to channelling and cracking, the behaviour of the bed is very unstable which means that no reproducible results are obtained after repetition of the experiment. The

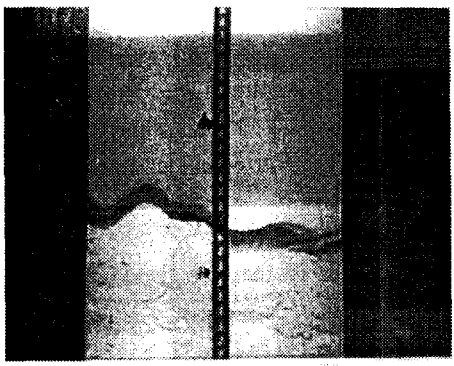


Fig. 13a. Channelling in an aerated bed of potato starch. W = 10.2 wt % d.b., H_0 = 0.76 m.

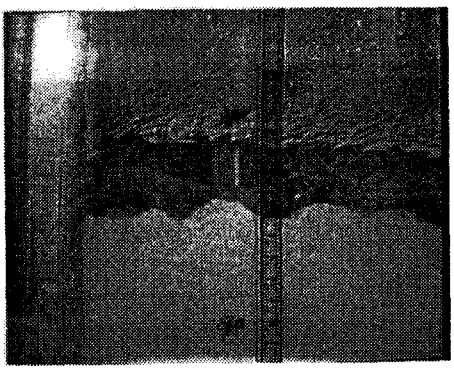


Fig. 13b. Agglomerates and flocks at the bed surface of an aerated and stirred bed of potato starch. W=10.2 wt %, H_0=0.76 m, δ_0 = 124 mm and N = 1.67 rps.

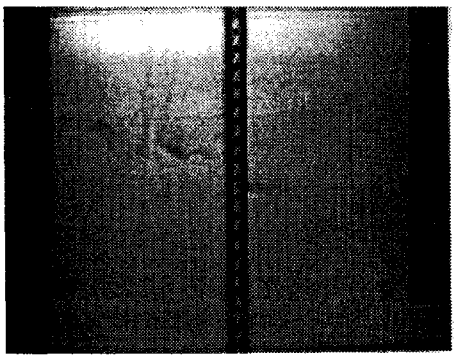


Fig. 13c. Channelling at 10 cm above the gas distributor in an aerated and vibrated bed of potato starch. W = 13.1 wt % d.b., H_0 = 0.76, x_0 = 0.83 mm and f = 30 s^{-1} (G = 3).

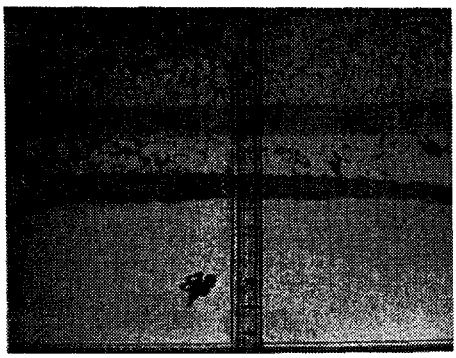


Fig. 13d. Elimination of channels and agglomerates (flocks) even at the surface in a stirred vibrating fluidized bed of potato starch. W=13.1 wt % d.b., H_0 = 0.74 m, δ_0 = 124 mm, N = 1.67 rps, x_0 = 0.83 mm (G = 3) and f = 30 s^{-1}.

gas-solid contact in such a bed is poor and inhomogeneous. Therefore, a conventional fluidized bed is not a suitable reactor for C-powders such as semi-dry granular starch.

The same conclusion follows from the Fluidisation Index (FI = ΔpA/Mg) which should be one for good fluidization. Fig. 14 shows both the instability of the bed and the poor fluidisation (FI < 0.9) for a conventional fluid bed of relatively dry starch of W = 10 wt % d.b. With increasing humidity fluidizability further deteriorates.

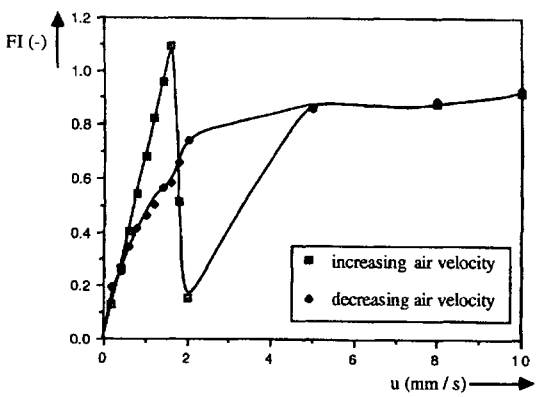


Fig. 14. Fluidisation index FI of potato starch without vibration and stirring versus gas velocity. W = 10.2 wt % d.b. and H_0 = 0.76 m.

The stirred fluidized bed

Stirring the bed with a single stirrer has a positive effect on fluidisation only if the stirrer is close to the bottom plate. (see Fig. 15). Applying multiple stirrers gives a further improvement (see fig. 16). The optimal blade distance and stirring speed appeared to depend on the moisture content. For 10 wt % d.b. moist a maximal value of FI can be realized with an interblade distance $\delta_b \leq 16$ cm and $N \geq 1.7$ rps.

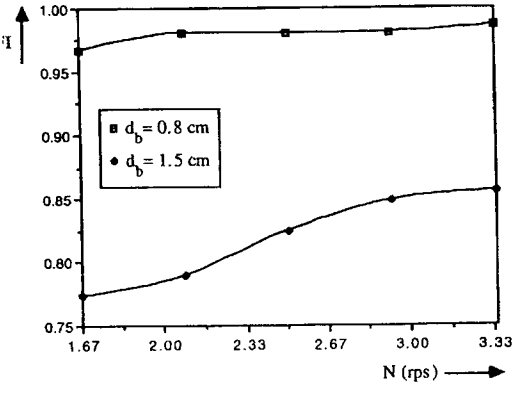


Fig. 15. Effect of stirrer position on the fluidization index FI of a stirred bed of potato starch as a function of stirrer speed N for a single stirrer. $H_0 = 0.09$ m, W = 10.2 wt % d.b., $u/u_{mf} \approx 2.9$.

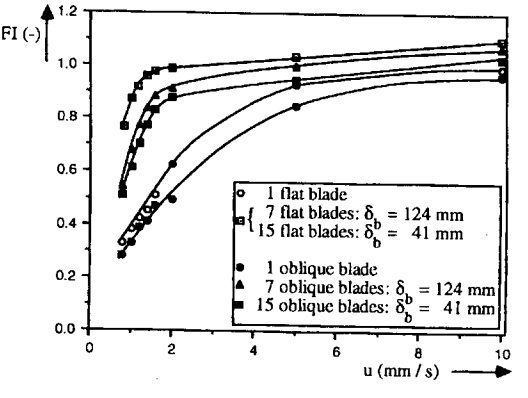


Fig. 16. Influence of interblade distance and stirrer design on FI of a stirred aerated bed of starch. $H_0 = 0.76$ m. W = 10.2 wt % d.b. and N = 1.67 rps.

Starch of higher moisture content asks for a slightly lower optimal interblade distance (e.g. 12 cm for W = 22 wt % d.b.) though no higher optimal stirring speed is required (see Fig.17a). With increasing stirring speed, bed expansion, and thus bed porosity (ε) increases and as a result the torque per stirrer blade (T') decreases, see Fig. 17. By applying the optimal stirring conditions mentioned above, it appeared possible

to eliminate any channeling in the bed. However, stirring alone is not effective in destroying large starch particle agglomerates which remain present in the fluid bed. This can be nicely seen in Fig.13b showing large agglomerates at the bed surface, resulting in a "mountaneous landscape" where a flat surface is preferred.

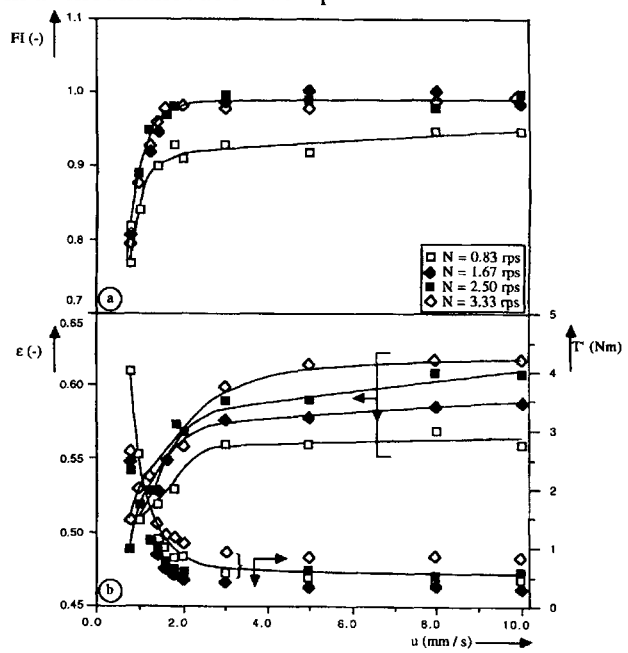


Fig. 17. Fluidisation of a stirred bed of starch of W = 22.4 wt % d.b., δ_b = 124 mm and H_0 = 0.84 m.
a) FI as a function of u for various N,
b) ε and T' as a function of u for various N.

Agglomerate, or flock formation is undesired because it may result in inhomogeneous derivatisation due to insufficient gas-solid contact in the center of the flocks.

Therefore, stirring alone is not sufficient to get at good fluidisation at the scale of the particles.

The vibrating fluidized bed

Bed vibration is characterized by frequency (f) and amplitude (x_0), often combined in a dimensionesless acceleration intensity G:

$$G = \omega^2 x_0/g$$

with $\omega = 2\pi f$ rad/s.

In our experiments G and f varied as follows: $1 \leq G \leq 8$; $30 \leq f \leq 90$ s^{-1} with starch of W = 13 wt % d.b.

By vibration of an aerated bed of potato starch, the channels in the lower part (about 0.1 m) of the bed are eliminated only. The vibration is not sufficient to eliminate channeling in the entire bed. As a result, a relatively low bed expansion is obtained. A minimum amplitude and frequency must be exceeded to balance the weight of the bed by the pressure drop over the bed. Nevertheless, at gas velocities

above 2 mm·s^{-1} always cracks and channels appeared in the upper part of bed (see Fig. 13c), independent of the values of G and f. The channels cause part of the gas to by-pass the bed, thereby reducing the gas-solid contact. However, vibration with sufficient intensity causes the agglomerates to break down, even in the upper part of a bed of H = 0.76 m.

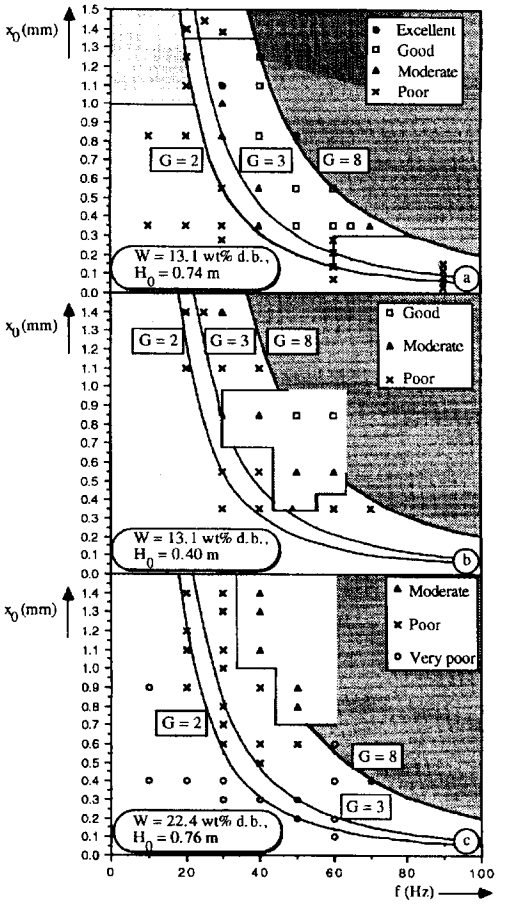


Fig. 18. Fluidisation quality chart showing the amplitude x_o and frequency f range for which both channels and agglomerates are eliminated in an aerated stirred vibrating fluidized bed of starch with u = 2mm·s^{-1}, δ_b = 124 mm and N = 1.67 rps.
a) W = 13.1 wt % d.b., H_0 = 0.74 m.
b) W = 13.1 wt % d.b., H_0 = 0.40 m.
c) W = 22.4 wt % d.b., H_0 = 0.76 m.

The stirred vibrating fluidized bed

Simultaneous vibration and stirring of an aerated bed of starch was investigated to determine what, if any, advantages can be gained this way. To guarantee elimination of channels in a bed of 0.74 m (W = 13.2 wt % d.b.), flat stirrer blades were used with δ_b = 124 mm. Experiments were performed at frequencies of 30, 60 and 90 s^{-1} whereas

G ranged from 1 to 8. The vibration intensity appears to be too low at G = 1 to break down the starch agglomerates at the surface of the bed. At G = 5 these flocks are still present for f = 90 s^{-1} but not at 60 s^{-1}. A further decrease of f at G = 5 is accompanied by the creation of slugs at $u > u_{mf} \approx 1.7$ mm/s. At G = 3 the agglomerates disappear at 60 s^{-1} only. These results show that not only G but also f influences the quality of fluidisation.

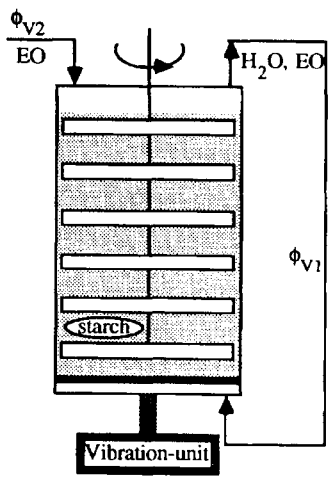


Fig. 19. Gas-solid hydroxyethylation of potato starch in a stirred vibrating fluidized bed.

Fig. 18 shows the results obtained in form of a fluidisation quality chart for three moisture contents and $u/u_{mf} = 1.1$. In the white region, moderate to good fluidisation is realized. The higher the moisture content of the starch the higher the minimal G value is for which good fluidisation is obtained.

Here, moderate, to good means a fluidisation nearly to completely free of both channels and agglomerates. An example is shown in Fig. 13d. The picture shows a completely flat surface, indicating complete break-up of agglomerates whereas no channels are visible. As soon as the vibration is stopped the agglomerates return. No measurements at G > 8 were performed due to (power) limitations in the vibrating system.

The lowest energy consumption at a particular G is obtained with the highest x_0. Therefore the upper left corner of the white regions is the preferred mode of operation.

Gas-solid hydroxyethylation of potato starch in a stirred vibrating fluidized bed

In this section a preliminary design is presented of a stirred vibrating fluidized bed reactor for producing 1 ton/hr Hydroxy-Ethylated Starch HES) of Molar Substitution of 0.1 by reaction with ethyleneoxide. In this system the same 4 reactions occur as discussed above for propylene oxide. The design was based on the reaction kinetics reported by Kuipers et al [17].

Figure 19 schematically shows a potential set-up of a stirred vibrating fluidized bed reactor for the batch-wise hydroxyethylation of potato starch. A volumetric recycle flow ϕ_{V1} is used to fluidize the starch. For fluidization the superficial gas velocity u must be minimally 0.002 $m \cdot s^{-1}$, resulting in $u/u_{mf} \geq 1.2$. For a bed already having the desired moisture content, only EO has to be added to the reactor provided no vent is applied. The pressure in the reactor is kept constant by a time-dependent feed rate ϕ_{V2}

Table 1

Optimal operation parameters for hydroxyethylation of semi-dry potato starch in a stirred vibrating fluidized bed reactor (H_{bed} =1 m) for a desired MS of 0.1 with a capacity of 1000 kg hydroxyethyl starch/h.

	Unalkalized potato starch	Alkalized potato starch
c_{NaOH} (mmol/mol AGU)	0	40
T (K)	368	333
W (wt % d.b.)	9.9	14.2
p_{EO} (kPa)	1190.5	495.1
p_{H_2O} (kPa)	32	5
S_{HES} (-)	> 0.48	0.72
t (ks)	1.2	0.9
D_b (m)	0.84	0.73
ϕ_{vl} (m^3s^{-1})	0.0011	0.00084

of pure EO which balances the EO conversion rate. So, the concentration of EO remains constant in the gas phase of the reactor.

Based on obtaining an optimal selectivity with respect to HES, the result is shown in Table 1 for both unalkalized (= not catalysed) and alkalized (= catalysed) starch. For further details, see [17]. Due to the higher temperatures possible in the dry process, hydroxyethylation seems possible without addition of hydroxide.

For superior selectivity however, also relative to the classical slurry process, pre-

Table 2

Advantages of the gas-solid process relative to the classical slurry process

Variable	Gas-solid hydroxyethylation	Classical slurry process
Temperature, T	T > 323K is advantageous for high S and reactivity. Diffusion limitation occurs at too high T, pending on c_{NaOH}, W and a_{EO}.	T < 323K due to gelatinization of the starch. This results in relatively long reaction times.
Selectivity S_{HES}	$S_{HES} \approx 0.72$ for ungelatinized alkalized starch.	< 0.62 [14]
Na_2SO_4 addition	No Na_2SO_4 has to be added	Na_2SO_4 is added to reduce gelatinization
Molar Substitution	MS of 0.5 possible without loosing the granular structure.	MS < 0.1. Higher MS results in purification problems due to loss of the granular structure.
Residence time, t	0.9 ks	58 ks

alkalining the starch is helpful. Spectacular too is the expected reduction in residence time, and thus in reactor volume, required for the gas-solid process. Table 2 compares the two processes. Here, the slurry data were taken from van Warners [14]. If our calculations can be proven in a pilot plant, the dry process will have a bright future.

Conclusions

Two novel continuous processes for the chemical derivatisation of starch have been developed. A gas-solid fluidized bed process for modifications with gaseous substrates and a gel process in a static mixer reactor for handling concentrated pastes. Based on results obtained with hydroxyethylation (dry-process) and hydroxypropylation (gel-process) it can be concluded that both processes are very attractive relative to the classical slurry process due to improved reaction selectivity, much shorter residence times and superior controlability and safety.

Acknowledgement

Financial support of AVEBE, The Netherlands is acknowledged.

Notation

c_{NaOH} = mmol NaOH/mol AGU
$c_{p,fl}$ = specific heat of a fluid [J/kg K]
d_i = inside tube diameter [m]
d_{sm} = diameter of a static mixer element [m]
d_e = diameter of the bed [m]
$D_{e,z}$ = effective axial mass dispersion coefficient [m^2/s]
f = frequency of sinusoidal vibration [s^{-1}]
4f = fanning friction factor [-]
F(t) = cumulative residence time distribution function [-)
FI = fluidization index, FI = $\Delta pA/(Mg)$ [-]
G = dimensionless vibrational acceleration: G = $(\omega^2 x_0/g)$ [-]
g = gravitational constant: g = 9.81 [ms^{-2}]
H_0 = settled bed height [m]
l_{sm} = length of a static mixer element [m]
L = length [m]
m_{starch} = mass fraction starch in reaction mixture [-]
M = mass of bed [kg]
MS = molar substitution [-]
n = number of stirrers in the bed [-]
N = stirring speed, [rev s^{-1}]

$N_{Nu_i} = \frac{\alpha_{i,l} d_i}{\lambda_{fl}}$, Nusselt number [-]

$N_{Nu,av} = \frac{\alpha w d_i}{\lambda}$, = average Nusselt number [-]

$N_{Pe} = \frac{vL}{D_{e,z}}$, Péclet number for axial dispersed flow region [-]

$N_{Pe_{h,l}} = N_{Re} N_{Pr} = \frac{\rho_{fl} c_{p,fl} v d_i}{\lambda_{fl}}$, Peclet number for heat based on the tube diameter [-]

$N_{Pr} = \frac{c_{p,fl} \eta_{fl}}{\lambda_{fl}}$, Prandtl number [-]

$N_{Re} = \frac{\rho v d_i}{\eta}$ = Reynolds number [-]

p_{EO} = partial pressure of ethylene oxide [Pa]
p_{H_2O} = partial pressure of water [Pa]
S_{HES}, = selectivity of the reaction toward hydroxyethyl and hydroxypropyl starch, S_{HPS} respectively
t = time [s]
T = temperature [K]
T' = torque per stirrer blade, [kg m^2 s^{-2}]
u = superficial gas velocity [m s^{-1}]
u_{mf} = minimum fluidization velocity [m s^{-1}]
v = superficial fluid velocity [m/s]
V_r = reactor volume [m^3]
W = moisture content of the starch [wt % d.b]
x_0 = amplitude of sinusoidal vibration [m]
$\alpha_{i,l}$ = inside wall heat transfer coefficient [W/m^2 K]
α_w = wall heat transfer coefficient [W/m^2 K]
δ_b = interblade distance [-]
Δp = pressure drop over the bed [N m^2]
ε = porosity of the bed [-]
ξ_{PO} = conversion of propylene oxide [-]
λ = thermal conductivity [W/m K]
η = viscosity [Pa s]
ρ = density [kg/m^3]
σ_0 = standard deviation at inlet of mixer [-]
σ_{mix} = standard deviation due to incomplete mixing [-]
Φ_v = volumetric flow rate [m^3/s]
ω = angular frequency of sinusoidal vibration: $\omega = 2\pi f$ [rad s^{-1}]

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NOWE REAKTORY O PRACY CIĄGŁEJ DO CHEMICZNEJ MODYFIKACJI SKROBI ZAPEWNIAJĄCE LEPSZĄ SELEKTYWNOŚĆ

Streszczenie

Wprowadzono dwa nowe ciągłe procesy do chemicznej derywatyzacji skrobi. Są to: proces w gazowo-stałym złożu fluidalnym do modyfikacji z udziałem substratów gazowych i proces żelowy w statystycznym reaktorze z mieszadłem do otrzymywania stężonych past. Na podstawie wyników w hydroksyetylowaniu (proces suchy) i hydroksypropylowaniu (proces żelowy) dochodzi się do wniosku, że oba procesy są korzystniejsze od odpowiednich klasycznych procesów prowadzonych z zawiesinami pod względem selektywności, krótszego czasu przebiegu, bezpieczeństwa i doskonałej kontroli ich przebiegu.

H. RUCK

THE NEW ORGANOSOLV PULPS – WILL THEY OUTRIVAL STARCH AS AN INDUSTRIAL RAW MATERIAL?

Abstract

Although the separation of cellulose from lignin by solubilization of the latter via sulphur derivatives makes the production of cellulose pulp fibres independent of external energy there are strong arguments against continuation of this technology. Thus, achievements of the sulphur free isolation of pulp from wood is reviewed.

Economic factors as well as regional availability of wood lead to an outlook that new cellulose can outrival starch in the European Union. In other countries starch lends itself to symbiosis with cellulose and lignin yielding combinations of carbohydrate products at price levels competitive with petrochemistry.

P.P.

In these days enthusiasts of Richard Wagner celebrate the 120 anniversary of the first performance of the "Dawn of Gods" in Bayreuth. In Cracow we certainly can remember a more recent birthday of one of the many renowned compository contributions of our local celebrity Christoph Penderecki whom I could admire as a conductor a few months ago in my home -town Wuppertal. But of more actual interest to members of our trade surely is the "Dawn of Sulphur" as the "Leitmotiv" for the next decade when environmental protection problems will outrival probably even such burning questions as providing jobs for the "run of the mill" work force in the whole of Europe.

Let me come now to the "gist of the core":

Wherever arguments are exchanged nowadays on how to preserve the world in which we live the topic of renewable resources is focused by keen interest. Within the total biomass assimilated in the billion tonnages range every season by help of our benevolent atomic reactor called "sun" two classes of substances are of prime importance:

carbohydrates (around 75 %) and lignins (about 20 % of total biomass)

University of Wuppertal/FRG

both making up about 95 % of all organic matter, besides the 2 % each of lipids and proteins as the backbones of life.

Among the carbohydrates cellulose accounts with about 45 % for almost half of nature, complemented by another 20–25 % of hemicelluloses and a smaller share of perhaps 2–5 % of starch. The latter one is also the prominent staple in the nutrition of men, whilst ruminants and selected mammals rely on hemicellulose and to a lesser extent on cellulose as their daily diet. The growth of heterotrophic animals including mankind, however, rests solely on the proteins of their plant and vegetable food /feed for autotrophic plants alone are capable to synthesize amino acids.

The latter require the availability of nitrogen as ammonia or nitrate produced either by thunderstorms rendering nitrogen into oxides transferred to arable land by rain or as ammonia by symbiosis with micro-organisms associated with the roots of plants or trees. As global balances teach lightnings alone sustain the amino acid supply for about 2.8 billion people whilst the larger part of the surplus populations satisfies its nitrogen demands primarily by the fertilizer industry burning fossils for the conversion of nitrogen to ammonia or nitric acid. Fortunately an unknown but nevertheless essential fraction of converted aerobic nitrogen is furnished by the activity of lignin decomposites in the soil. The latter originate from the lignin transferred to the soil by dead or plowed under plants together with the total floral matter (cf. fig. 1). Various micro-organisms care for the conversion of bioorganic material proceeding most slowly for lignins from trees, less slowly for gramineous lignins and almost instantaneously for all cellulosics, lipids and proteins.

The most important feature of such partially digested lignins for the maintenance of human life is elucidated by their capability to incorporate aerobic nitrogen in such a manner that zymogenic micro-organisms can convert N_2 into NH_3, Nitrates or even heterocyclic aromatics with the latter capable to swell bentonites or other planar silicates into sandwich structures supporting moisture exchange in seedbeds. Recombination of lignin fractions with converted proteins etc. finally end up in the formation of humus maintaining a varied microflora living in symbiosis with the floral matter cultivated – including starch bearing crops.

One should memorize that the relation of C : N in living plants is enhanced from 40 : 1 to impressive 10 : 1 in the humified soil with lignin as the crucial constituent. Hence, lignin is primarily responsible to allow mankind the harvesting of food in gross tonnages so that mass populations in cities can be nourished at all.

In this context it is astonishing to note that well humified soils are capable to convert aerobic nitrogen into nitrates or ammonia in amounts up to 300 kg/ha signalizing the considerable importance of this nitrogen source [1a]. In comparison nitrogen quantities by fertilizer addition rarely exceed 50 kg/ha in spite of having been tripled since 1930. Incidentally, the application of lignin for the restauration of contaminated

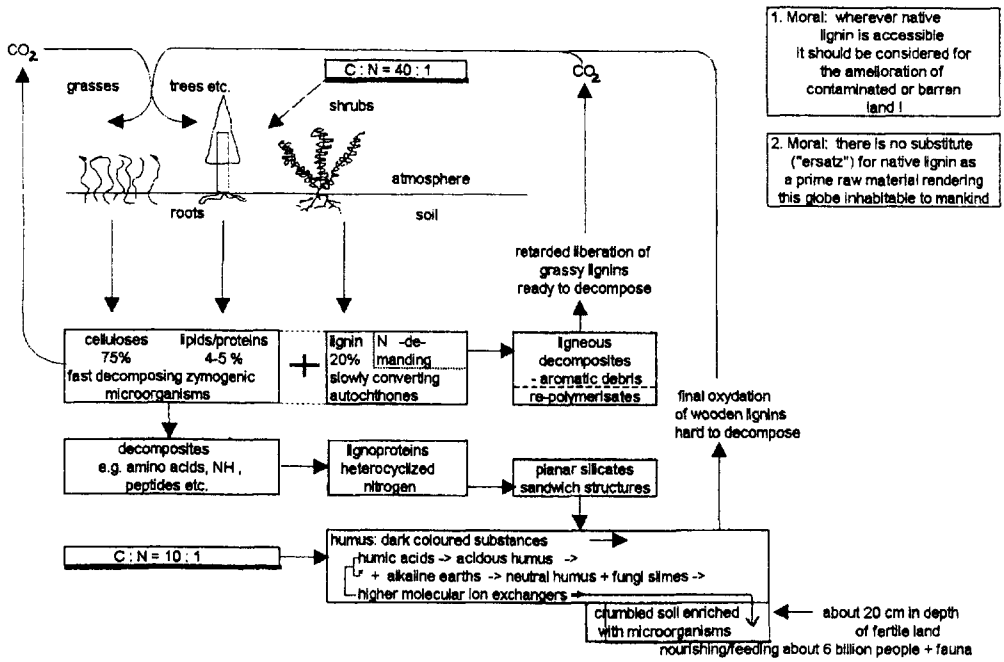


Fig. 1. The importance of lignin for the humification of soil.

soils was recently recommended by Fischer [1b] particularly after ammonolysis. Such "nitrogenized lignins" have already proven their usefulness as long-term-fertilizer furnishing nitrogen to the crop for several consecutive seasons preventing simultaneously wash out of nitrogen compounds to the underground water. – Certainly, Justus von Liebig, Haber, Bosch and Ostwald were not aware of the role of lignin and is decomposites for the assimilation of nitrogen essential for the synthesis of protein. Perhaps our ancestors surmised such contingencies for they harvested their fields only every third year after plowing under the weeds and grasses grown in the preceding two seasons – containing about 15 % gramineous lignin!

Hence, the burning of lignin or its conversion to sulphur – containing soil-poisoning derivates should be abandoned as soon as possible in order to preserve this valuable prime raw material for the amelioration of available land, replacement partially wise to use it as additive to selected petrochemical plastics rendered biodegradable beyond contents of more than 40 % of native lignin.

Consequences for the pulp producers

would imply that this industry would have to change the 120 year old policy to separate celluloses from lignin by solubilization of the latter via sulphur derivates, well

known under the names sulphite and sulphate cooking processes. Both introduce sulphur in reduced or oxidized form to the backbone of the principal unit of lignin which is a phenylpropane. In any case lignin and its sulphur groups are reutilized by intricate processes implying the burning of lignin and other associated incrustations like hemicellulose to make the production of cellulose pulp fibres independent of external energy. Pulp and paper people consider this as an important argument for the benevolent environmental character of their industry relying solely on energy produced by regrowing fuel without any impetus on the global CO_2-balance! This is indeed the fact but neglects the prices for the loss of lignin as the most efficient natural fertilizer to prevent the cementation of arable land of the globe or to allow the cultivation of contaminated or barren areas. Also, the preservation of lignin in the humified layer of land devoted to agriculture and forestry would represent the first sink for carbon during a period in which the imission of fossile CO_2 has become a severe threat to our global climate.

For this and other reasons the pulp industry of the future will have to play a dominant role to conserve our planet in the actual inviting conditions simultaneously influencing the fate of human beings of the generations to come. The question is whether this key industry can suffice its responsibilities in the foreseeable future.

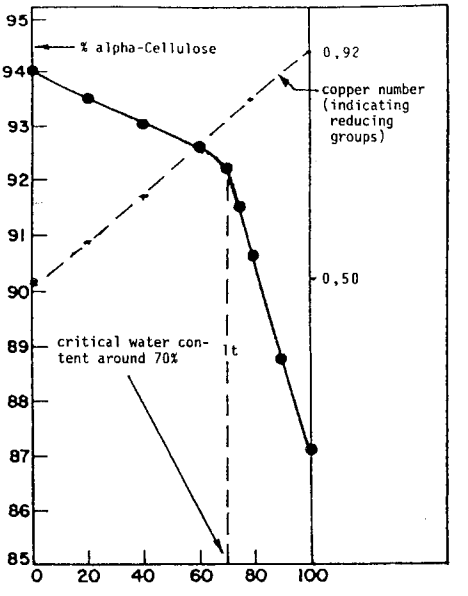
The answer is a clear "yes", for

The sulphur free isolation of pulp from wood

has been of concern to responsible researchers for about a century. As early as 1893 extraction of lignin by ethanol was tried by Scandinavian scientists, e.g. by Klason and Fagerlind. The first technological breakthrough was reported in 1932 when Th. Kleinert [1] at that time associated with the Silesion pulp industry, was granted the US-Patent 1856567 for the large – scale production of cellulose by ethanol – water cooks.

Due to circumstances Kleinert could not carry on with his interests until 1962 when he resided from the pulp and paper Institute in Montreal and became an independent college teacher (deceased 1982) directing furtheron his self-financed pertinent research projects. Several papers emerged between 62 and 76 [2] climaxed in ´71 by US-Patent 3585 104 [3]. His essential findings exhibit fig. 2 + 3 demonstrating beyond doubt that

a) ethanol-water mixtures with less than 70 % water entail only negligible cellulose-losses recommending water concentrations of 55–60 % as the most suitable liquor composition to enhance the solvent capacity for hemicellulosic oligosacharides at a level of braked hydrolysis with respect to cellulose decay.
b) an ethanol-content of 42.2 weight % approaches optimum conditions with respect to remove lignin down to residues between 2 and 4 % rendering the pulp amenable to chlorine-free bleaching operations.



ALPHA - CELLULOSE CONTENT OF ETHANOL-WATER COOKED COTTON IN % OF AIR-DRIED RAW FIBER CONTAINING ORIGINALLY 95% PURE CELLULOSE.

Experimental: 4 hours cooking in liquors of varying water contents up to 100%. Obviously at a critical water content around 70% hydrolysis proceeds at a much higher rate.

Inference: The molar ratio between ethanol and water at 70% water approaches 1 : 6 (= 19% ethanol) allowing the alcohol to cover the internal surface of all elementary fibers quantitatively with the latter accomodating ca.20% of all cellulose chains.Below this threshhold hydronium ions can attack the bridge O-atoms for hydrolysation!

(Reinterpreted from T.N.Kleinert,Das Papier 30,V18(1976)

Fig. 2. The effect of water content in ethanol-water mixtures on alpha-cellulose yield of raw cotton fibres during a four hours cook at 185°C.

Optimal pH-conditions belong to the know-how of the trade governing also the residual hemicellulose content of the bleached pulp fibres.

The physico-chemical aspects of the first Kleinert patent were first dealt with by Häusermann [4] in his ETH-thesis of 1944 on the wetting tendencies of selected plant cells by various solvents as exemplified in table 1a. Following the axiom "similia similibus solventur" (virtually Ceasar's look at chemistry) it is almost self – evident that ethanol or 1-propanol come closest to the lipophilic character of lignin. Since ethanol is abundantly available both as a nutriment or industrial solvent also accessible by fermentation of hemicelluloses (a by-product of ethanol-water cooks) it is the preferable choice as a digesting agent for wood. Future research will probably add some modifiers to the Kleinert process as it happened to supercritical fluid extraction techniques (SFE) in the recent past.

Table 1a

Wetting capacity of mesophyllic cells derived from ascension velocities

Wetting capability of mesophyllic cells (dianthus barbatus) derived from capillary ascension velocities according to lucas [a] extracted and enlarged from E. Häusermann [b]		
Ascending fluid + lignin	Degree of lipophility in %	Wetting parameter : 10^6 (from wetting angles)
water	0	0.0
formic acid	28	–
acetic acid	45	–
methanol	50	24.9 ± 2.6
ethanol	66.7	31.2 ± 2.5
Lignin	70	–
1-propanol	75	42.1 ± 3.3
1-butanol	80	49.6 ± 3.5
1-hexanol	85.7	47.0 ± 4.2
1-octanol	88.9	44.1 ± 2.9
olive oil	90	42.4 ± 2.5
paraffinic oil	100	36.9 ± 0.8
Lignin according to the ALCELL-process is made up of $C_9H_{8.5}(OCH_3)_1O_{2.5}$ – units of MW = 187.5 Dalton. Only O-atoms are considered as truly hydrophilic calling for a share of 56.0 Dalton (=3.5 O-atoms). Hence, the lipophilic section amounts to 131.5 Dalton – resulting in a degree of lypophility of 70 %! (probably 3 or 4 % more because of the limied accessibility of the O-atom in the metoxy-group!).		
Result: only polar fluids can wet wood!		
Note: the plant cuticle remains undissolvable in polar fluids in spite of its amphiphilic character!		

[a] Lucas R.: Über das Zeitgesetz des kapillaren Aufstiegs von Fluiden, Kolloid-Z. 23, 15, (1918).

[b] Häusermann: Über die Benetzungsgrösse der Mesophyllinterzellularen, Diss. ETH Zürich, Ber. Scheiz. bot. Ges., 54, 541 (1944).

The impressive capillary forces active in wooden tissues are set forth in table 1b. As such they are only valid for hydrophilic surfaces e.g. channels within the cellulose matrix. Hence, capillary effects are not so pronounced within suprastructures of lignin and similar incrustations. Intermediate efficiencies can be expected in contact regions between cellulosic matter and the aromatic sections of the interspersed lignin (benzene shows a surprising affinity for water! It can be mixed with absolute ethanol!) yielding shearing forces in interfacially bordering surfaces capable to sever hydrophilic from lipophilic contact zones.

The calculated ascension pressures in table 1b have obviously some bearing on practical applications. For example it could be shown recently by Stute [9] that pressures beyond 4000 bar (= 400 MPa) provoke gelatinization in various starches subject

Table Ib

Capillary effects in wooden tissues

The cohesion of so-called „water filaments" is responsible for the transport of plant juices up to the highest branches and leaves of tall trees. The corresponding cohesional tension was assessed by 0. Renner (Beih. bot. Zbl. 25(I), 183. 1919) to amount up to 300 bar equivalent to a water column of roughly 3000 meter.

Hence, one can calculate from the following ascension potentials: $h=2\gamma/\rho \cdot r \cdot g$

Radius r	Ascension power in height of water column	Bar	Pore diameter
0.1 mm = 100 µ	14.9 cm	–	0.2 mm
10 µ	149.0 cm	–	20 µ
1000 nm = 1 µ	15 m	1.5	2 µ
100 nm	150 m	15.0	200 nm
10 nm	1500 m	150	20 nm
5 nm	3000 m	300	10 nm
10.0 Å = 1nm	15 km	1500	2 nm
5.0 Å = 0.5 nm	30 km	3000	10 Å
2.5 Å = 0.25 nm	60 km	6000	5 Å

Note: rectangular pore of diagonal diameters around 5 Å accomodate micromolecules like water ($4.5 \cdot 4.5 \cdot 1.5 = 30$ Å^3) or ammonia! Hence, both of them are capable to mercerize cotton.

To be considered: ethanol decreases the surface tension from 22.3 at 22°C down to 15.5 at 100°C – which is much less than the equivalence for water (73/dyn/cm). Hence, actual capillary forces at 185°C are only a fraction of their spreading power at room temperature. A mild compensation for this loss is provided by the vapour pressure of ethanol around 22 bar at 185°C!

Scientific breakthrough: on April 25th, 1996, R. Stute (9) reported to attendants of the Int. Starch Congress in Detmold/FRG that pressures beyond 4000 bar trigger the cold gelatinization of various starches! Obviously the intensive swelling provoking this phenomenon requires the penetration of water into pores as narrow as 1nm – or to overcome bottlenecks within the porodine system of the starch supra – structure even smaller than 1 nm!

Stute's findings corroborate that the pore size distribution assessed here for wooden tissues is also valid for starch – the biological predecessor of cellulose during the evolution of plant life.

to suprastructures comparable to those met with in wood. The explanation of this surprising phenomenon lends itself by assuming that channels within the basic hinges of the starch microfibril are narrower than 1.0 nm which prevent water to penetrate voids beyond this bottleneck which can be overcome solely by higher pressures.

It is surprising that the Kleinert pulping process stirred up only limited interest in the Northamerican industry, save the consulting firms of Eli Cowan and the Pulmac Services in Montreal combining their financial efforts to carry out autoclave trials

confirming and extending Kleinert's patent of 1971 to cover also lignin separation. Kleinert's financial resources were exhausted after the total sale of his real estate in the mid-seventies. In fact he was virtually deprived of his patent in '78 when the CP-Associates claimed a patent by Diebold et al. [5].

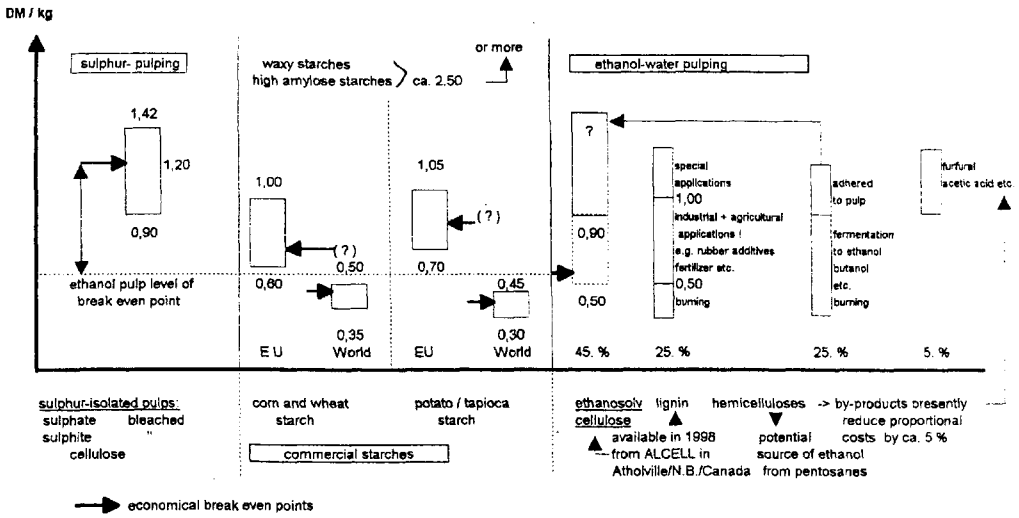


Fig. 3. Removal of residual lignin during a 60 minutes cook in a water mixture with 42.2 weight % ethanol at 185°C. Note: the concurrent loss of cellulose is negligible!

Realizing his situation Kleinert approached Bavarian authorities in 1976 who helped to finance a pilot-plant project in cooperation with MD Paper Mills in Dachau under Dr. M. Baumeister. Only four years later Baumeister reported in Baden-Baden that the Kleinert process could now be recommended for mill scale production – when he got a better position in another company. Apparently his successors saw everything from different point of view and replaced ethanol by methanol obsoleting also Kleinert's patent. But they failed to separate lignin from cellulose (as expected when comparing the wetting parameters in table 1) and required support by NaOH-extraction in a second step of their "Organocell"-process. In cooperation with a brilliant press campaign they succeeded to mobilize about 550 million marks to reconstruct the Kelheim pulp works equipped with a continous Kamyr-imitated digester to extract the lignin in a single step process applying methanol and sodium-hydroxide simultaneously. The result of taking so much risk at once was indeed shocking and agonizing : the mill was bankrupt in 1993 for the pulp was virtually unbleachable due to insufficient lignin removal and to channel formation in the 67 metre tall extractor. Another cause was perhaps the limited supply of development funds : only 15 million DM were provided to run the Munich pilot plant engineered for a ca-

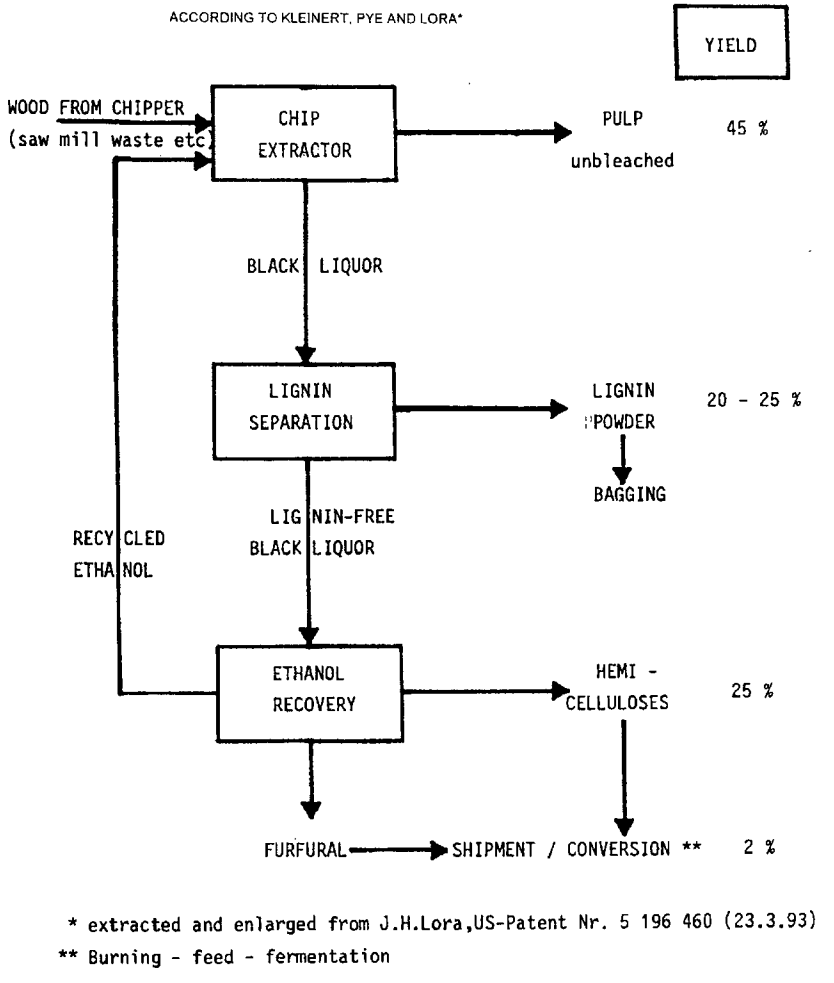


Fig. 4a. Schematic flow chart of ALCELL-ethanosolv cooking of hardwoods resulting from contributions by Kleinert, Pye and Lora.

pacity of only three tons per day. The blow-up of such facilities by a factor beyond two orders of magnitude is obviously beyond human control or assessment.

It is regrettable, however, that "Organosolv"-processes gained a bad reputation after the disaster of Kelheim – undeservedly, for the Kelheim procedure was really a "modified aquosolv-sodium-process" – for methanol is more of a methylated water than a hydroxylated methane!

A much better fate experienced the perfection of Kleinert's and Diebold's patents on the American continent, where General Electric became interested in 1983 in the ethanol-water process in their pioneering research laboratory in Valley Forge/Virg. under Dr. Pye. GE bought the mentioned CPA patent and sold their Biological Energy

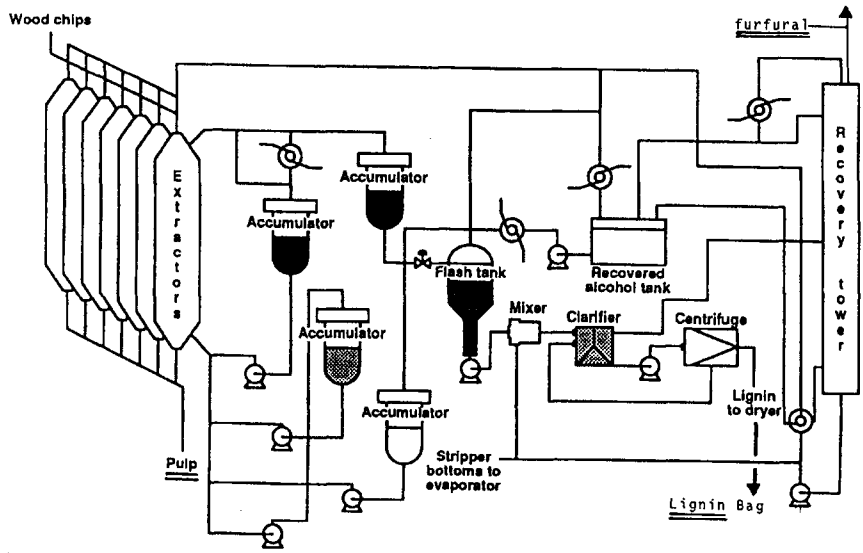


Fig. 4b. Semi – commercial flow chart of ALCELL demonstration plant in Newcastle/N.B./ Canada; Capacity per day: 10 - 15 t pulp, 5 - 6 t of native lignin.

Corporation BEC a year later to G.S. Petty's REPAP company active in pulp and paper in Canada and the U.S.

In 1988 J.H. Lora et al. [5] were granted U.S. patent No. 4764596 concerning primarily the separation of the lignin suspended in ethanol-water which turned out as the decisive key to render the Kleinert – process as economically unbeatable – denoted meanwhile as REPAP's ALCELL (alcohol and cellulose) achievement. For it is now the first time in the history of pulping that digesting of wood can be carried out without taking resort to sulphur and moreover utilizing the chips to 70 % (much more is in the offing!) by adding to the usual 45 % cellulose another 22 % of lignin as an extremely valuable native prime raw material (besides furfural, acetic acid and other by-products).

A schematic flow chart of ethanosolv cooking as perfected by Pye and Lora [5] is illustrated in fig. 4a yielding two prime raw materials (pulp and lignin) and one by-product (furfural for the petrochemical industry). As soon as sufficient external energy is available fermentation techniques converting hemicelluloses to solvents (ethanol, aceton, butanol etc.) could push up the total yield to about 95 % of wood fed to the "extractor" replacing the classical digester.

With the accumulated know-how in their hands REPAP started a pilot plant for 95 million dollars in Newcastle/N.B. with a capacity of up to 15 tons/day of pulp and more than five daily tons of native lignin; the pertinent flow chart shows fig. 4b. In the past seven years more than 6000 cooks were performed – and not a single one failed

!!! Hence, one decided to re-model an idle sulphite mill in Atholville/N.B. scheduled to operate in 1998 with a capacity of about 130,000 t.p.a. pulp and 60,000 t.p.a. of sulphur-free-ALCELL-lignin. The flow chart of this continuously operated mill exhibits fig. 4c.

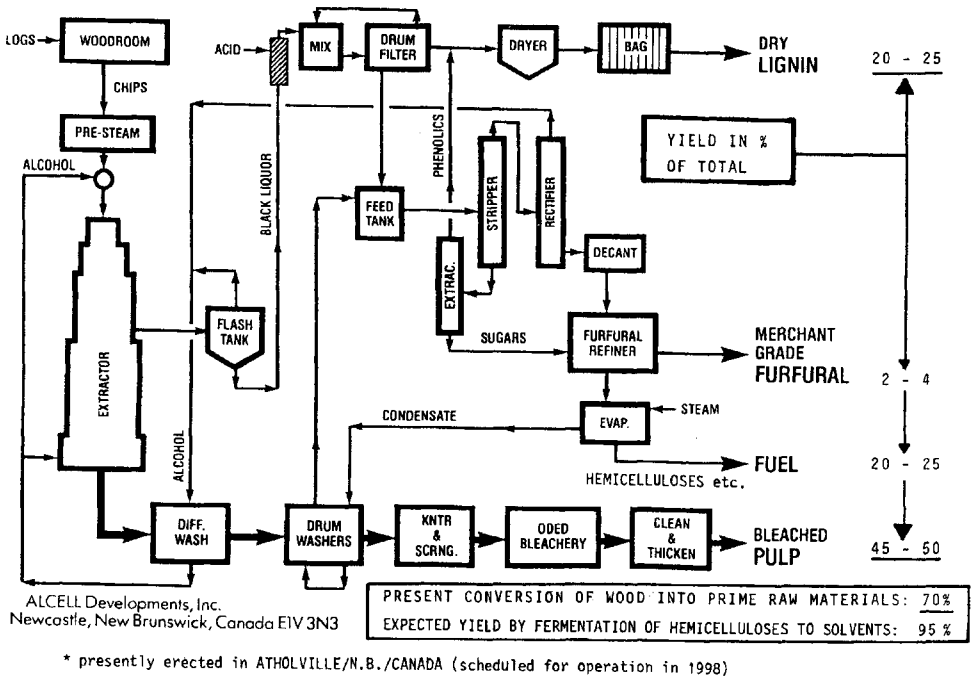
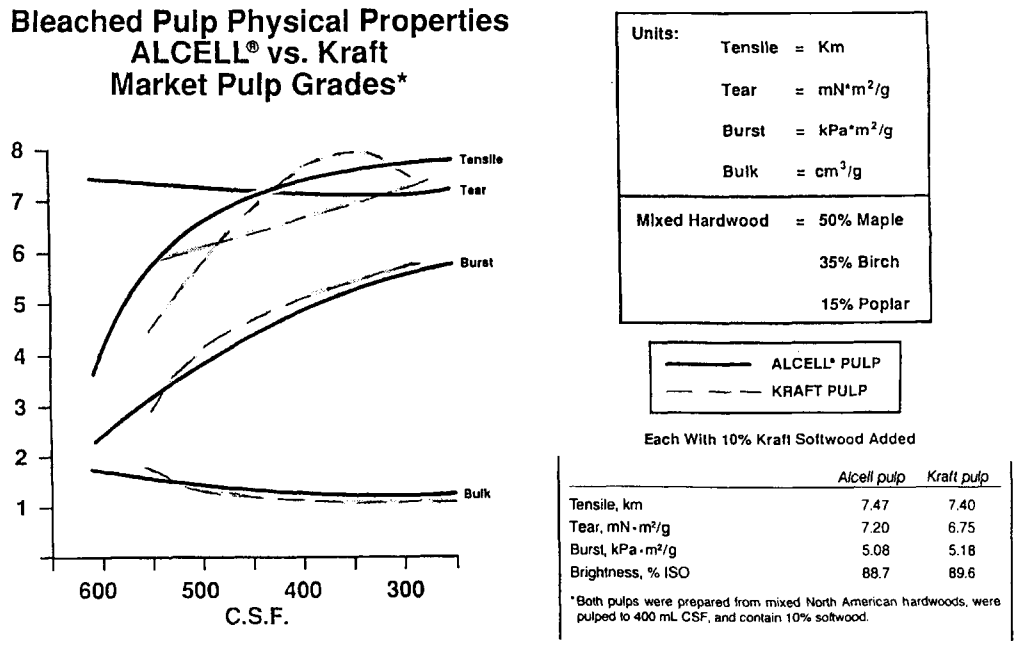


Fig. 4c. Flow chart of continuous ALCELL pulping in Atholville/N.B. Capacity per annum: 130 000 t of hardwood pulp, 60 000 t of native lignin.

This event will mark the beginning of the new age of converting trees into the prime raw materials cellulose and lignin and also ring the death-bell for the sulphate mills furnishing at present 90 % of the global pulp supply. For ALCELL-products can well compete with the qualities of kraft pulp as demonstrated by fig. 5.

As the comparison of the meaningful parameters tensile strength, tear, burst and specific volume prove quality differences between the two pulps cooked without and with sulphur respectively do not exist, for the mixture of chips was identical in both cooks. The addition of 10 % softwood considers the experience that any harvesting method for hardwood stands will always include a share of 10 % softwoods enhancing slightly the average fibre length.

Another benefit of ethanol pulping could count even more:



	Alcell pulp	*Kraft pulp*
Tensile, km	7.47	7.40
Tear, mN·m²/g	7.20	6.75
Burst, kPa·m²/g	5.08	5.18
Brightness, % ISO	88.7	89.6

*Both pulps were prepared from mixed North American hardwoods, were pulped to 400 mL CSF, and contain 10% softwood.

Fig. 5. Relevant parameters for papermakers of two pulps made from identical hardwoods mixtures cooked as ALCELL and SULPHATE market pulp grades for comparison pulped from 600 to 300 C.S.F. Facit: as Tensile, Tear, Burst and Brightness bear out after pulping to 400 ml CSF differences in quality are not discernible.

As long as kraft pulping has been applied on mill scale its emitted odours have been considered as molestations not accepted in densely populated areas as met with in middle Europe. As table 2 bears out ethanol cooking outrivals the sulphur cook by impressive lower emissions particularly with respect to malodorous reduced sulphur compounds. No doubt that true organosolv pulping will never touch the limit concentrations of any emissions imposed by environmental legislation. As to chlorinated organic substances there exists even a gap of an order of magnitude in favour of ALCELL pulp, also a factor of five with respect to the limit prescribed by authorities. Certainly environmental protection will soon become the major argument when it comes to the collection of investment capital in order to finance ethanol pulping mills of which ten are required each year to satisfy the rising demand of two million tons due to mounting consumption of paper all over the globe.

However, the most convincing argument has been money ever since mankind relied on coins when trading. A comparison of production costs bears out that the proportional expenditures to produce one ton of pulp will amount two almost 500 DM/t for sulphate pulp. Ethanol pulping will require an additional 2 or 3 % due to

Table 2

Emission of SULPHATE and sulphur-free ALCELL pulping process in relation to environmental legislation

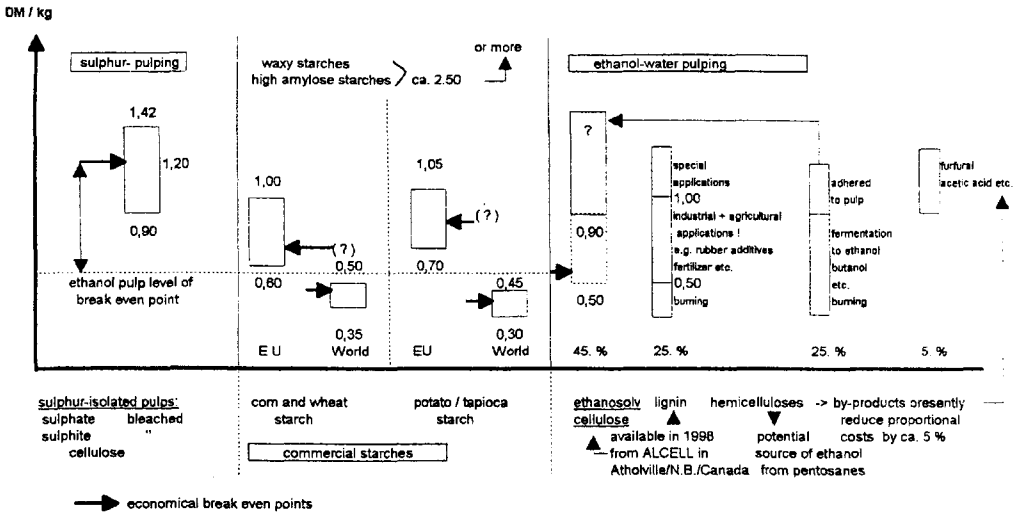
Emissions per ton of air-dry pulp for ALCELL- and sulpate cooking			
Emission into	ALCELL	SULPHATE	CANADIAN LEGISLATION
1. Air in mg/m^3 as			
a) reduced sulphur (H_2S/mercaptanes)	0	0.22	0.18
b) dust	9	15.0	13.0
2. Water in g/m^3 as			
a) suspensoids	5	10	11.3
b) BOD	5	6	7.5
c) COD	7–12	10–12	?
d) AOX	0.3–0.5	3–5	2.5
Facit: The AOX-emission of the Alcell-process is by an order of magnitude smaller than in case of the sulphate procedure! Consequently the ALCELL-technology can be considered as the cleanest pulping cooking ever invented and will meet any target of any environmental legislation anywhere!			

higher capital investment for acid-proof steel. What matters more are the sales prices for the two varieties, which perambulate within an astonishing range below and above the break-even-equator as illustrated in table 3 comparing also the band width of prices paid during the last decade for commercial starches. In view of the fact that the novel ethanosolv-pulp makes use presently of two thirds only of the total wood upgraded to commercial products, we will experience for the first time in the history of pulp making since A. Mitscherlichs first sulphite production in Kelheim, that this process will be profitable in spite of the agonizing ups and downs of pulp market prices because the break even point of the ethanol pulping will be lower than the meanest price levels ever experienced. This situation will further improve when the actual conversion of 70 % of wood thanks to the added 20–25 % of salable lignin will be further enhanced by the utilization of the 25 % hemicellulose fraction for fermentative upgrading to ethanol and other solvents.

Total utilization of the wood implies that a sufficient share for mill-based energy production by burning of wood fractions is no longer available. Hence, the process depends on external energy supplies primarily from electric power stations. Fortunately this shortcoming is outweighed multifold by the earnings on hitherto lost lignin. If nuclear energy is available the replacement of sulphur-based pulp productions by

Table 3

Price levels for standard carbohydrate commodities since 1985 compared to expected sales revenues from ALCELL prime raw materials



Note: The economical break even point for ethanosolv-pulp is highly competitive with starch prices within the EU. Outside this community the combination of ethanosolv-cellulose with starches at world market conditions could outrival even petrochemical products – a boon to the environment!

ethanosolv mills could render a serious contribution to the reduction of the climate threatening emissions of CO_2 provoked by the consumers of car and heating fuel – besides the reduced supply of nitrogen fertilizer to the soil.

There is yet another decisive advantage of ethanosolv pulping:

Because the process requires no intricate recovery boilers for black liquor etc. save the relative simple redestillation of alcohol from water such mills can be operated successfully at much lower scales than sulphur consuming units yielding already profits at capacities of only 100,000 to 150,000 t.p.a. . This extra advantage minimizes also logistics of wood supplies shortening transportation distances.

The outlook of ethanol-pulping

is even brighter then anticipated so far. For the horizon will clear up further when the considerably higher profits allow larger assignments to R & D enabling researchers to tackle all experiments forbidden so far for lack of funds. As to the novel kind of pulp cooking there is still a drawback to be overcome for the proven technique can handle

only hardwoods with an admixture of up to 15 % softwoods. Hence, the first objective will be to master also softwoods cooks "unpurified" by considerable admixtures of hardwoods. One approach in this direction was recently undertaken [7] and seemed to open up new prospects. Along this alley further trials should be envisaged to remove the residual lignin around 28 Kappa – number by physical extraction techniques – not by chemical bleaching!

The advent of a sulphur – free regenerating technique for cellulose by dissolving the undivided chain molecule in NMMNO some 15 years ago by American Enka researchers [8] and meanwhile practized by Courtaulds on a 20,000 t.p.a. scale (Tencel) suggests investigations as to the filtering off of the remnants of lignin by modern filtering techniques (accurel membranes etc.) rendering also chemical treatments superfluous. Last not least should endeavours be undertaken to marry truely dissolved cellulose with polysaccharides as filler – e.g. with micronized starch!

The dawn of sulphur remodels also the viscose industry!

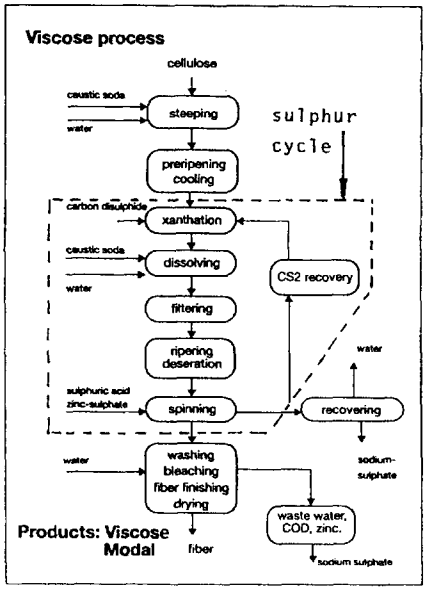
Similar simplifications with virtually comparable improvements of the environment situation are presently achieved in the regeneration of cellulose for the large scale production of man – modified textile and technical fibres. As just mentioned 20 Kt of staple fibres p.a. have conquered the market recently whilst the biggest viscose producer, the Lenzing Fibres in Upper-Austria, are now embarking on a continuos filament production close to Vienna utilizing also the Tencel experiences based on NMMNO with the latter produced by Chemische Werke Hüls in North-Rhine-Westphalia.

Fig. 6 illustrates the effects of streamlining cellulose regeneration when disposing of sulphur implying also an improvement of the quality of such rayon. The latter approaches almost polyester fibres with respect to textile parameters. With the process still in his infancy one can almost predict substantial improvements within the next decade which hopefully leads to a replacement of the many hydrophobic petrochemical fibres which by their very nature are not real textile fibres for a breathing human skin, restricting their future to technical applications. All this will make the industrialized states a little more independent from Near East oil and help bridle CO_2 emissions.

The new cellulose – can it outrival starch?

The breakthroughs in the production of sulphur free pulp will not remain without impact on the starch industry. One example is particularly striking:

During the past two decades the demand of the paper industry for starch as an additive to the stock increased steadily in order to reduce the fall-through on the wire of a paper machine by better retentions of fines and to obtain cleaner waste water particularly due to the action of added cationic starch derivatives. Actually the



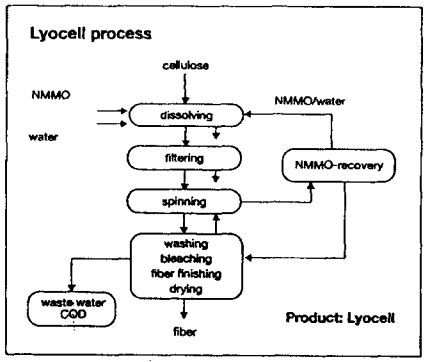


Illustration of processing steps* in the manufacture of
a) Viscose rayon and staple
b) Lyocell rayon (LENZING) and Tencel staple (COURTAULDS)

Note: sacrificing the sulphur cycle for the xanthation of cellulose streamlines the regeneration by NMMNO recoverable by 99% !

* Extracted and modified from R.Kampf and W.Schaumann,Lenzinger Berichte 75, 91 - 96(1996).

Fig. 6. The classical viscose process entailing the intermediate xanthation of cellulose was cut down to a few steps by renunciation on sulphur stages for the monomeric dissolution of cellulose in NMMNO.

consumption of starch by the paper industry exceeds a third of the total starch production allotted for upgrading in the industry, perambulating around 400 000 t of starch annually for the German paper and board production close to 15 mio tons in 1995.

The papermakers will probably be a moderate customer of starch producers in the future for two reasons:

Ethanol pulp can be cooked so that the pulp retains a predeterminable share of hemicellulose responsible for the fibre to fibre bonding governing the paper strength. Hence, hemicellulose can replace starch at lower costs!

It was recently demonstrated by Rahm [9] that the present policy to foster recycling of paper increases the CO_2 – load in the atmosphere due to the enormous energy consumption during deinking, sorting , bleaching and other efforts to maintain the required whiteness of the paper. From this point of view recycling can be recommended only for high – quality waste papers so that most of the collected waste paper (approaching 60 % in some western countries) should be better burnt - preferably right

in the paper mills where people are experts in judging paper qualities. For waste paper still has the heating value of brown coal and does not add to the CO_2 balance!

The renunciation on waste paper on a larger scale will naturally increase the demand for primary fibres. The question arises:

Can our forests supply enough wood to produce paper from virgin fibres?

A reliable forecast can be dared at present only for the forests in the Federal Republic where only 35 million m^3 are harvested annually – just half the quantity of 65 mio m^3 growing, equivalent to a growth of 2.3 % or 6.75 m^3/ha. The official figures for Poland seemingly underrate the assets of the local forest industry. Therefore, table 4 has been submitted to pertinent Polish Institutes asking for correction and actualization.

Table 4

Wood resources in Poland and Federal Republic

	Federal Republic*	Poland**
Total area of forests	9.6 mio ha	8.7 mio ha
Wood stock available per ha country total	302 m^3/ha $2.9 \cdot 10^9$ m^3	172 m^3/ha (250 m^3/ha ?) $1.5 \cdot 10^9$ m^3 ($2.4 \cdot 10^9$ m^3 ?)
Total annual growth	65 mio m^3 = 6.75 m^3 / ha } 30 mio m^3 left	36 mio m^3 = 4.13 m^3/ha (?)
Current annual harvest	35 mio m^3 } in the forest	
Current timber harvested	23 mio m^3	18.3 mio m^3
Wood allotted to pulp and paper$^{\Delta}$	9 mio m^3 (= 26 % of harvest)	3.6 mio m^3 (=10% of harvest)
Share of state forests	47 %	83 %
Share of Coniferous forests	67 %	79 %
Share of mixed woods	unknown	unknown
Waste paper recovered to paper consumed	56 %	31 % (only one deinking unit operating)
Inference: the Federal Republic could easily convert almost 25 mio m^3 presently rotting in the forests into about 5 mio t of pulp and 2.5 mio t of native lignin (to be used as filler for plastics or as natural fertilizer to increase the assimilation of nitrogen in the soil) rendering her independent from pulp imports and allowing simultaneously to reduce the share of recycling waste paper from 60 % to perhaps 25 % excluding inferior grades serving in future as fuel for paper mills. – Probably similar marginal conditions prevail also in Poland.		

$^{\Delta}$ Residual wood from saw mills etc. + slim stems etc. due to forest care/supervision

* Datae collected by Dr. Thoma, MD Papierfabriken München (1995)

** Datae extracted from Z. Fornalski, Przegląd Papierniczy Special Issue 1995/96 (assessed figutes in brackets)

The facts assure beyond doubt that the Federal Republic for instance could easily make use of the 30 mio m^3 now rotting in the forests by directing the non-timber share to ethanolsolv pulp mills converting the required 25 mio m^3 to almost 5 mio t of pulp and another 2,5 mio t of native lignin. Both are primary raw materials (and no longer a waste product as the sulphur carrying lignin presently unavoidable) and could be put to use in many industrial or agricultural applications. Their reduced sales prices would further enable both products to penetrate markets hitherto dominated by petrochemical manufacturers.

Even classical paper and board application would profit from the availability of sulphur – free wood components if the favoured recycling policy of used fibres is restricted to the upper grades. For the use of virgin fibres e.g. in board or conjugated board would soon expel many packaging items made out of plastics from the market – a boon also to environment! More virgin fibres in paper would also call for fewer chemical additives in the stock and consequently entail cleaner waste waters.

Regrettably the new cellulose will threaten market segments formerly held by starch – but only in the European Union! In the by far larger "rest of the world" starch lends itself to symbiosis with cellulose and lignin yielding combinations of carbohydrate products at price levels competitive with petrochemistry.

Unfortunately, the most research contributions offered to starch congresses anywhere restrict themselves to the improvement of market – adapted products sold in limited quantities. But what the starch industry really needs is a pioneering research effort which would finally lead to a breakthrough opening markets in the million tons range.

A prerequisite would be that we are sufficiently informed about the suprastructure of starch. In this respect my coworkers have worked on two posters concerning the role of lipids in the conversion both of starch and cellulose. I am convinced that all essential informations for the cited breakthrough are contained in the literature last not least in papers written by our predecessors. For not very far from here around 1850 the Jewish grandmother of H.F. Mark was born in Lemberg (hopefully to be renamed one day!). This multiply gifted scientists found out in 1926 working at an Institute in Berlin-Dahlem that tenacity of all polymers including carbohydrates in the form of fibres, films or foils attains only a level equivalent to solely 2 % of covalent bonds. Hence, the overwhelming contribution to the work of rupture is provided by friction during elongation.

Having this in the background of our mind we should devote more of our concern to material sciences governing the performance of solid bodies – particularly of carbohydrates. In this context I remember the work of H.Frind in the late fifties who tripled the tenacity of copper rayon just by mild modifications of the stretch – spinning conditions. Unfortunately during the second half of this century the petrochemical

industry has attracted the best talents in macromolecular chemistry. Fortunately this trend has changed for the better, as I mean, in recent years focusing public and political interest on problems like biomass conversion and on regrowing raw materials.

A steadily growing number of research institutes or government agencies concern themselves exclusively with polysaccharides problems as for instance the Carbohydrate Research Foundation in The Hague or the Non-Food Agro - Industrial Research Dissemination Network (NF-AIRID) headquartered in Newbury, 43 Kingsfisher Court in Great Britain and supported by 15 National Network Leaders in all European countries west of Finland and Greece. Financial support comes from Brussels and from governments in the respective countries. Interestingly, one such projects aims at the reduction of lignin synthesis in trees by transgenetic engineering resorting to antisense RNA. These researchers at the University of Toulouse hope to reduce in this way the amount of sulphur chemicals during pulping of trees to be felled in about 50 years (carried out under the ECLAIR programme). Other experts of AGROL in Guildford (England) were successful in adapting the Bacillus stearotherthermophilis to ferment the five – carbon sugars like xylose as the largest fraction of sugars in hemicelluloses from wood pulping also occurring in waste streams of maize syrup production. With the said bacillus active up to 75°C the production of bioethanol runs now as well as with glucose as substrate being on top also economical because hemicelluloses are cheaper than sugar or starch. Hence, it is foreseeable, that in the not too distant future wood and straw can be upgraded to cellulose, lignin and ethanol as a whole.

The latest developments in this respect will be reported during the Second European Biomass Forum in Graz in September 1996.

After all we live in a truly refreshing period in this time witnessing the uprise of carbohydrates and lignin as the coming raw material basis both for mankind and industry.

All we need is enthusiasm and perseverance to personify ourselves with our institutional and industrial research and development endeavours – and in case of success we often lack a generous person or agency providing the funds to get efforts protected in the form of patents involving fees not at the disposal of the ordinary scientist. What we need after all is a political force granting every citizen at least protection of one mental property – thereby enriching the country and all her citizens.

The USA give a good example concerning this dilemma: private persons and small companies asking for a patent pay only a few hundred dollars without obligation to pay fees when not put to use.

The Europeans should follow suit to benefit more from their own endeavours!

Thank you all for listening!

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NOWE PULPY CELULOZOWE Z PROCESÓW Z UDZIAŁEM ROZPUSZCZALNIKÓW ORGANICZNYCH – WYPRZEDZAJĄ ONE SKROBIE JAKO SUROWIEC PRZEMYSŁOWY?

Streszczenie

Chociaż oddzielanie celulozy od ligniny przez rozpuszczanie tej ostatniej poprzez jej rozpuszczanie w formie pochodnych siarkowych czyni produkcję włókien celulozowych niezależną od dodatkowej energii z zewnątrz, istnieje szereg argumentów przeciwko kontynuowaniu tej technologii. Dokonano przeglądu metod wydzielania celulozy bez pomocy związków siarkowych.

Względy ekonomiczne oraz lokalna dostępność drewna wskazują, że w Unii Europejskiej nowa celuloza wyprzedzi skrobię jako surowiec przemysłowy. W innych krajach skrobia pozostanie w symbiozie z celulozą i ligniną dając kombinowane produkty węglowodanowe o cenach konkurujących z produktami petrochemicznymi.

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PHYSICO-CHEMICAL PROPERTIES OF DEFATTED TRITICALE STARCH

Abstract

The aim of this work was to find out to what degree different defatting conditions viz,. solvents and temperature of extraction affect defatting efficiency and physico-chemical defatted starch properties. Triticale starch subjected to 10 h extraction using 75 % n-propanol at 30, 50 and 80°C, as well as mixture of chloroform:methanol:water (in volume proportion 18:6:1) at 30 and 50°C was used for investigation. Our studies confirmed the highest effectiveness of starch defatting with 75 % n-propanol at 80°C.

Introduction

Lipids in cereal starches have been divided by Morrison [8, 9] into internal starchy lipids, mainly phospholipids called also the true starch lipids, and surface lipids. A small layer of outer lipids on the surface of starch granules has a chemical composition similar to starchy lipids as it contains relatively higher amounts of monoacylic residues than non-starchy lipids and is able to form complexes with amylose [5, 8, 9]. Other outer lipids called non-starchy lipids are mainly triglycerides.

Starchy lipids by forming complexes with amylose determine many physico-chemical starch characteristics [1, 2, 3, 5, 16]. Removal of starchy lipids or a change in their chemical composition may serve therefore as a method of altering these properties [1, 5].

In the process of starch defatting surface or internal lipids can be removed. This depends on the kind of the solvent used [14, 15]. Defatting efficiency is determined by polarity of the solvent and by the temperature of extraction [12, 14]. High extraction efficiency of starchy lipids can be achieved in high temperature, by using n-propanol and water in the volumetric ratio of 3:1 [8, 12]. A standard mixture used for the removal of non-starchy lipids includes chloroform, methanol and water and is efficient even at room temperature [9, 12, 15].

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Water is an important constituent of all mixtures used for deffating as it enables starch granules to swell and makes solvent penetration within the granule more easy [8].

The objective of the work was an attempt to learn the influence of the quantity and quality of lipid removed from starch granule on the physico-chemical characteristics of defatted starch.

Material and methods

Triticale starch isolated from a winter variety Ugo served as the investigated material. The starch was defatted during 10 h extraction either with 75 % n-propanol at temperatures 30, 50 and 80°C [6], or with a mixture of chlorophorm: methanol: water (18:6:1, volumetric ratio) at 30 and 50°C. After fat extraction the solvent was evaporated and the total solids was analysed for crude fat (by dissolving in acetone) and determining sum of carbohydrates with antron reagent, as described in [7]. Defatted starch was also analysed for:

- graininess by using analyser type Analysette 22 made by Fritsch GmBH
- total phosphorus content [4]
- total protein content (N x 5,7) [13]
- the content of amylose [11]
- water binding capacity and solubility in water [13]
- gelatinization characteristic of 8.5 % starch suspension in water in rotation viscosimeter Rheotest 2 [2].

The starches were pictured by scanning electron microscope type Tesla BS-300 (magnification 900 or 1125 x).

Results and discussion

Analyses of the total solids after solvent evaporation showed that in the process of fat extraction small amounts of protein and carbohydrates were removed regardless of the type of the solvent used and extraction temperatures applied (Table 1). Differences among dry residues resulting from type of the solvent and applied temperatures can be attributed to the amounts of fat removed. The highest dry residue was found when fatty substances were extracted with 75 % n-propanol at 80°C and the residue was highest in crude fat.

Temperature of extraction with 75 % n-propanol affected total phosphorus content of defatted starches, the lowest amounts of the compound were found in starches defatted at 80°C. This proves that at 80°C phospholipids were removed from starch granules most efficiently (Table 2). Additional evidence that starch defatting at 80°C

was the most efficient provide amounts of amylose liberated from its lipids complexes from the insides of starch granules – highest at this temperature.

Compared to starting material, in starches defatted by the mixture of chlorophorm, methanol and water higher amounts of amylose were found. It seems however that the increased contents of amylose can be attributed to complexes located on the surface of the starch granules. This was concluded from the fact that starches extracted at 30 and 50°C by either of two solvents had similar contents of amylose, whereas it is well known that removal of true starchy lipids requires hot solvent to be used [12]. It seems that the removal of outer starchy lipids, having composition similar to phospholipids, as well as non-starchy lipids (triglycerides) is more efficient with the chlorophorm, methanol and water mixture than with 75 % n-propanol at 30 or 50°C.

Table 1

Results of chemical analysis performed on total solids (after evaporation of the solvent used for extraction) of Triticale starches isolated from the variety Ugo

Solvent used	Extraction temperature [°C]	Total solids [%]	Fat components [%]	Carbohydrate[x)] [%]	Protein[xx)] [%]
n-propanol	30	0.42	0.12	0.28	0.05
	50	0.54	0.20	0.34	0.07
	80	0.74	0.30	0.30	0.07
chloroform: methanol: water	30	0.39	0.11	0.16	0.06
	50	0.49	0.18	0.27	0.07

[x)] carbohydrate =dissolved in: cold water + autoclave (1 atm, temp. 121°C)
[xx)] protein in naive starch - protein in defatted starch

Table 2

The content of amylose and non-carbohydrate components in Triticale starch samples of Ugo variety

Kind of starch samples	Extraction temperature [°C]	Total phosphorus [%]	Raw protein [%]	Amylose [%]
native		0.047	0.15	26.31
defatted	30	0.038	0.11	29.63
n-propanol	50	0.032	0.09	30.09
	80	0.016	0.08	32.88
defatted	30	0.32	0.10	29.34
chloroform: methanol:water	50	0.024	0.09	30.51

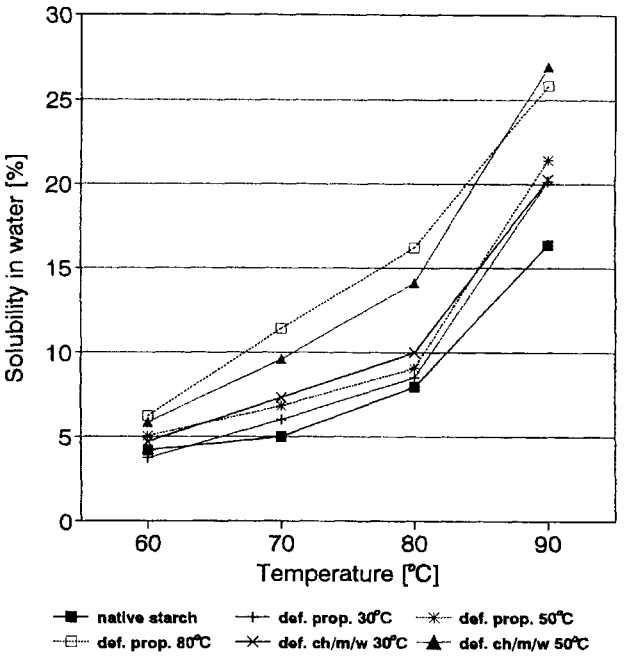

Fig.1. Solubility in water of starch samples of Ugo variety.

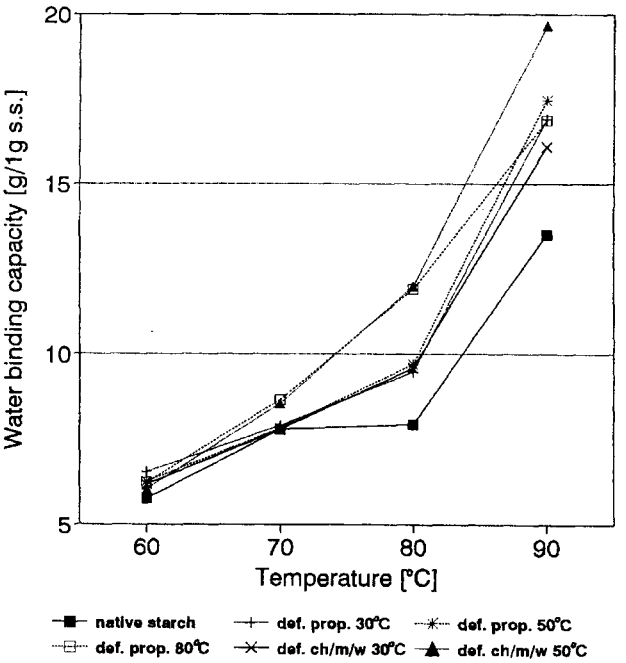

Fig.2. Water binding capasity of starch samples of Ugo variety.

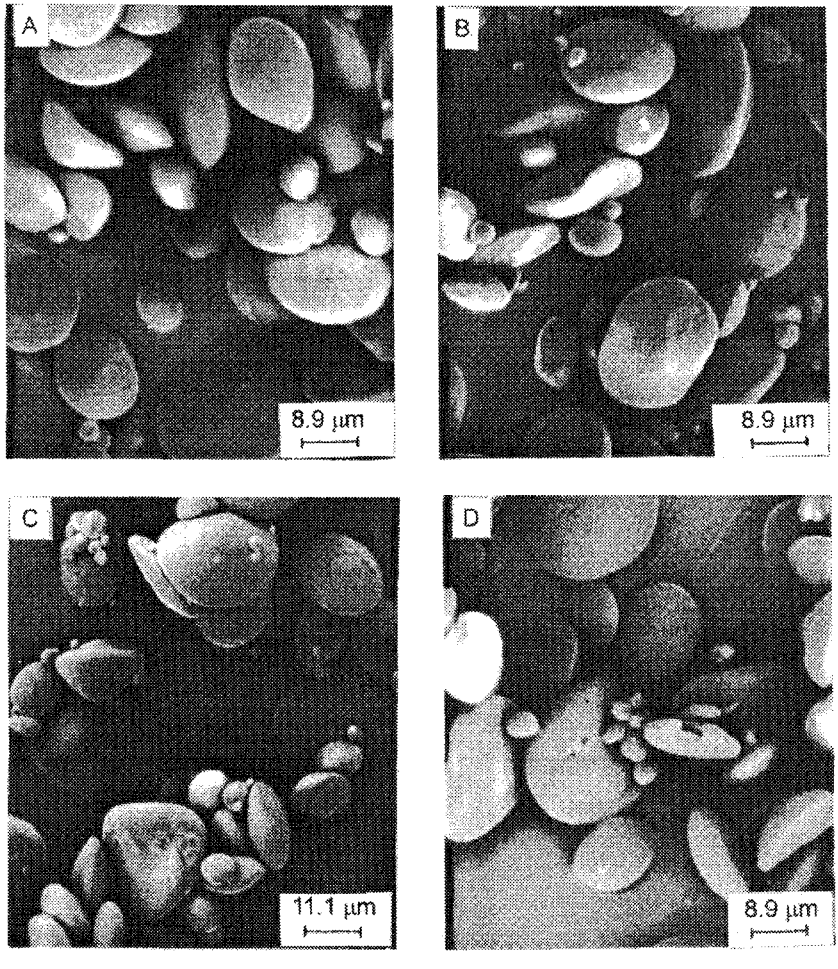

Fig.3. Scanning electron micrograph of granules of Triticale Starch native (A) and defatted in 75% n-propanol at: 30°C (B), 50°C (C) and 80°C (D).

Defatted starch had higher water binding capacity and solubility in water at temperatures 60-90°C than non-defatted starch (Figs. 1, 2). Fat removal from starch with 75 % n-propanol at 80°C caused disappearance of a two-phase character of these processes, what provides just another evidence that at the temperature lipids were released from their complexes with amylose [5, 8, 9, 16]. Lower temperatures of extraction with the same solvent, as well as the mixture of chlorophorm, methanol and water, do not destroy the amylose-lipids complexes and consequently the two-phase character of starch granules swelling is retained (Figs. 1, 2) , in spite of removing some amounts of lipids (Table 2).

Table 3

Pasting characteristic of 8.5 % water suspensions of starch samples from triticale of Ugo variety measured by means of rotation viscosimeter Rheotest 2 using rot as a measuring unit

Kind of starch samples		Pasting temperature [°C]	Maximum viscosity [J.U.]	Viscosity after cooling to 50°C [%]
native		65.0	105.7	111.0
defatted				
- n-propanol	30°C	61.0	98.3	111.3
	50°C	61.5	114.7	114.0
	80°C	61.5	115.3	118.0
- chloroform: methanol:water				
	30°C	62.0	107.3	112.0
	50°C	61.5	116.3	115.0

Table 4

Grainines of starch samples from Triticale of Ugo variety

Kind of starch samples		% starch granules < 10μm
native		11.64
deffated		
- n-propanol	30°C	10.23
	50°C	9.71
	80°C	11.18
- chloroform:methanol: water:	30°C	11.83
	50°C	11.59

Removal of the outer starchy and non-starchy fats lowered gelatinization temperatures of starches defatted by either of the solvents used (Table 3).

Extraction of lipids from complexes with amylose using 75 % n-propanol at 80°C did not cause any further decrease in gelatinization temperature. Gelati-nized defatted starch had always slightly higher maximal viscosity, and viscosity after cooling to 50°C, than gelatinized non-defatted starch. Removal of inner starchy lipids did not cause considerable changes in the values of gelatinization parameters. The influence of amylose-lipid complexes on changes in these parameters was visible only in a less pronounced two-phase character of starch gelatinization when starch was defatted with 75 % n-propanol at 80°C.

Extraction of lipids did not cause any damage to the surface of starch granules as can be seen on the pictures made by the scanning electron microscope (Fig. 3). This process did not cause any changes in graininess of defatted starches either (Table 4).

Conclusions

1. No visible changes in the appearance of defatted starch granules were observed by the electron scanning microscope.
2. Compared to native starches, defatted starches were characterized by unchanged graininess, lower contents of protein and phosphorus, higher contents of measured amylose, higher water binding capacity and solubility at 60–90°C, higher maximal viscosity of paste formed from water suspensions. Those differences were most noticeable in starches defatted with 75 % n-propanol at 80°C.
3. The research performed confirmed 75 % n-propanol at 80°C to be the most effective defatting agent.
4. Analyses done on the defatted residues showed that in addition to lipids the extraction process removes small amounts of carbohydrates and protein, no matter what the temperature and type of solvent were used.
5. Lipids inside starch granules bound in complexes with amylose were removed by 75 % n-propanol at 80°C.

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FIZYKO-CHEMICZNE WŁAŚCIWOŚCI ODTŁUSZCZONEJ SKROBI PSZENŻYTNIEJ

Streszczenie

Celem pracy było sprawdzenie w jakim stopniu różne warunki odtłuszczenia tj.: rozpuszczalniki i stosowane temperatury ekstrakcji wpływają na skuteczność odtłuszczania oraz na fizyko-chemiczne właściwości skrobi odtłuszczonej. Do badań użyto skrobię pszenżytnią, którą poddano procesowi odtłuszczania przez 10 godzinną ekstrakcję 75 % n-propanolem w temperaturach 30, 50 i 80°C lub mieszaniną chloroformu:metanolu:wody w stosunku 18:6:1 w temperaturach 30 i 50°C. Wykonane badania potwierdziły największą skuteczność w odtłuszczaniu skrobi, 75 % n-propanolu w temperaturze 80°C.

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EFFECT OF ADDITION OF OAT STARCH COMPONENT ON BREAD QUALITY AND STALING

Abstract

In model pup breads, which were baked with wheat starch and dry, vital gluten, part of the wheat starch was replaced with oat starch (5, 10, 15, 20 % of the total amount of the pup breads). It has been proven that starch fat contained in oat starch has a good effect on improving crumb structure of the model pup breads (40 g), but no such effect has been noticed while baking wheat breads (250 g) with the addition of oat starch.

Introduction

In baking technology of bread and confectionery products more and more attention is being given to a role of starch in forming structures and to its importance in creating flour baking value.

Among other compounds starch-fat complexes contribute to improving crumb structure of bread. These complexes stop retrogradation of both amylose and amylopectin. This process is regarded as one of the main factors causing deterioration of food quality. Among other things, it is responsible for bread staling [6, 10, 13, 21].

Since it is known that after baking, while cooling amylose recrystallizes, scientists think that reducing the amount of amylose in crumb can contribute to its elasticity by adding monoglicerines to dough. During baking monoglicerines become active in forming complexes with amylose causing delay in starch swelling in bread and reducing the amount of free amylose which has flowed out of starch granules [1, 10].

Starch fats also affect the degree of retrogradation. Removing lysophospholipides, which naturally occur in cereal starch, increases the degree of retrogradation. It suggests that they can have some effect on bread staling [11, 13, 14]. The above suggestions were confirmed in our reseach on defatted starches used for baking model pup breads in the starch-gluten system.

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The experiments proved that these starch fats had a crucial effect on both quality and staling of the pup breads [2].

The aim of this work is to present the effect of the starch fat, which naturally occurs in cereal starch, on quality of the pup breads. As the source of this fat we used oat starch, which is characterized by the highest content of fat substances among the studied cereal starches [17, 18].

Material and methods

In the first stage of the experiment our research material consisted of cereal starches – wheat and oat ones. The wheat starch was isolated from wheat of Emika variety by the laboratory method by using a 0.1 % solution of NaCl [5]. The oat starch was isolated by Paton's method [18] from oat of Halny variety and from commercial mixture was called the oat industial starch in the further stages of the experiment. In the investigated starches we determined the basic physicochemical properties such as: raw protein content (N x 5.7) according to [20], total phosphorus content [12] and raw fat content [20], water binding capacity, solubility in water and pasting characteristic of 8.5 % water starch pastes in a Rheotest 2 rotational viscosimeter [4].

In the second stage the research material comprised pup loaves. They were baked with the wheat starch according to the following recipe [3]: 80g starch (starch dry substance), 20 g dry vital gluten (dry substance), 8 g sugar, 3 g salt, 1.5 g dried yeast and 70 ccm of water (at 30°C). The dough yield was 170, and the mass of the bits was 40 g each.

In the pup breads we were gradually replacing the wheat starch with the oat industrial starch increasing its amount form 5 and 10 up to 15 and 20 % and once we replaced the wheat starch with 20 % of the oat starch of Halny variety.

Besides, an attempt was made to bake the wheat bread of 250 g, in which some part of the wheat flour was being replaced with the oat starch and industrial starch in the amounts of 10 % and 20 % of the total mass.

On the day of baking the volume of the loaves was measured in loose material and the indices of their quality were determined according to the standard [19]. During three days of storing up the pup breads in plastic bags in a climatic chamber at the temperatures of 23–24°C and the relative humidity of 64 % we were constantly evaluating bread staling. In our evaluation we also concentrated on crumb penetration measured by a PNR-10 penetrometer and on blue value as in indicator of amylose content in water crumb extract [15].

Results and discussion

On one hand, in comparison with the wheat starch the oat starches were characterized by a decidedly higher content of total phosphorus and raw fat, on the other hand, they had a lower water binding capacity (at 60 and 80°C) and a several times lower solubility in water at the both applied temperatures (Table 1). The analyzed oat starch of Halny variety was characterized by the lowest solubility. It is probably due to the highest content of fat substances in this starch.

Their higher pasting temperature is also connected with the higher content of fat substances in oat starches, because amylose-fat complexes reduce swelling, solubility and outflow of amylose [4, 8, 14]. The proportional rise in pasting temperature with the increase of fat content in the investigated starch confirms this theory (Table 1).

Table 1

Physicochemical properties of oat starches in comparison with wheat starch

Starch characteristic	Wheat starch of Emika variety	Oat starch	
		of Halny variety	industrial starch
Raw protein content (% d.m.)	0.18	0.13	0.21
Total phosphorus content (% d.m.)	0.044	0.071	0.076
Raw fat content (% d.m.)	0.58	1.70	1.03
Water binding capacity (g/1g d.m.)			
at 60°C	8.5	5.4	6.2
at 80°C	9.8	6.4	6.5
Starch solubility in water (% d.m.)			
at 60°C	4.2	0.8	0.6
at 80°C	8.6	0.6	0.8
Pasting temperature [°C]	74	88.5	85
Maximum viscosity [J.U.]	68	119	112
Viscosity after 20 minutes at 96°C [J.U.]	54	106	117
Viscosity after cooling to 50°C [J.U.]	106	114	122

Maximum viscosity of the industrial oat starch was almoust twice as high as that of the wheat starch. Our conclusion is confirmed by the earlier researches on that problem [17, 18].

It is probably due to a much lower oat starch granulation in comparison with wheat starch, and also to a different structure of amylose and amylopectin in oat starch. As we know from Paton's works [18[, oat amylose is characterized by a higher degree of viscisity (2.46–2.99 g/ccm) than wheat starch (2.33 g/ccm), what is attested

by the fact that it is more linear. On the other hand, a lower intrinsic viscosity of oat amylopectin in comparison with wheat one speaks for a higher degree of chain branching.

As opposed to wheat starch a slight increase of viscosity of industrial oat starch after cooling it to 50 degrees Celsius points to a weaker trend to retrogradation of this starch.

Because of these very interesting baking properties of oat starch – namely its high content of fat substances and weak trend to retrogradation – we tried to use the addition of this starch for baking the model starch pup breads.

Any of the used components did not reduce either the mass of the pup breads, or the bread yield , but on the contrary, they had a positive effect on reducing baking loss (Table 2). Taking the results presented in this table into consideration the highest addition of oat starch (15 %) and industrial oat starch (20 %) and the addition of oat starch Halny variety (20 %) seemed the most favourable.

Table 2

Effect of partial replacement of wheat starch with oat starch on selected indices of bread quality (40 g)

Sort of bread	Mass after cooling [g]	Crumb penetration		Bread yield [%]	Total backing loss [%]	Organoleptic evaluation	
		in 1 day	in 4 day			Points	Quality class
Wheat starch - 100% standard	34.7	8.7	1.6	147	13.4	38	I
Wheat starch - 95% + oat industrial starch - 5%	35.3	9.2	2.3	150	11.6	38	I
Wheat starch - 90% + oat industrial starch -10%	34.7	6.8	2.3	148	13.2	36	I
Wheat starch - 85% + oat industrial starch -15%	35.6	8.6	2.5	151	11.0	38	I
Wheat starch - 80% + oat idustrial starch - 20%	35.5	9.8	2.8	151	11.2	38	I
Wheat starch - 80% + oat starch of Halny variety - 20%	35.7	8.9	2.7	152	10.8	37	I

The presence of oat starches in the pup breads had a slight effect on reducing their volume in comparison with the standard bread. The best results were obtained using the 20 % addition of the industrial oat starch; whereas the 10 % addition of this industrial oat starch was the least favourable (fig. 1).

In general, the addition of the oat starch had a positive effect on crumb penetration, especially the 20 % addition of the industrial oat starch; the only exceptionwas the 10 % addition of this starch (fig. 2).

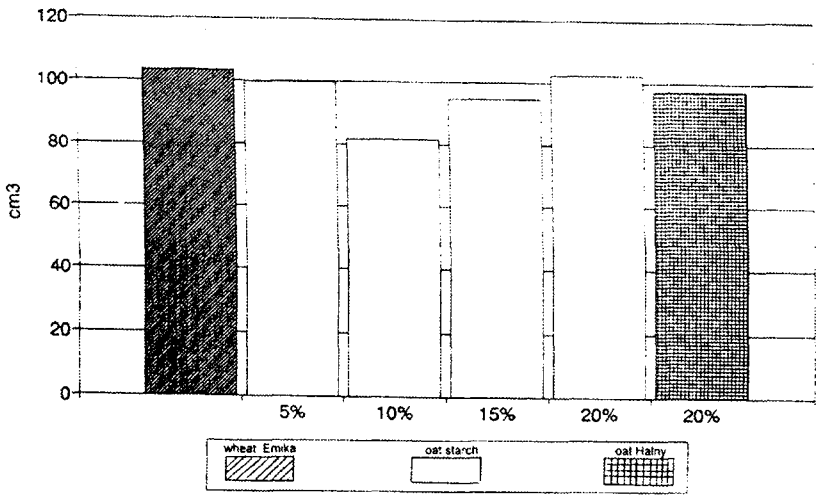

Fig. 1. Effect of various amounts of oat starches on volume of model pup breads.

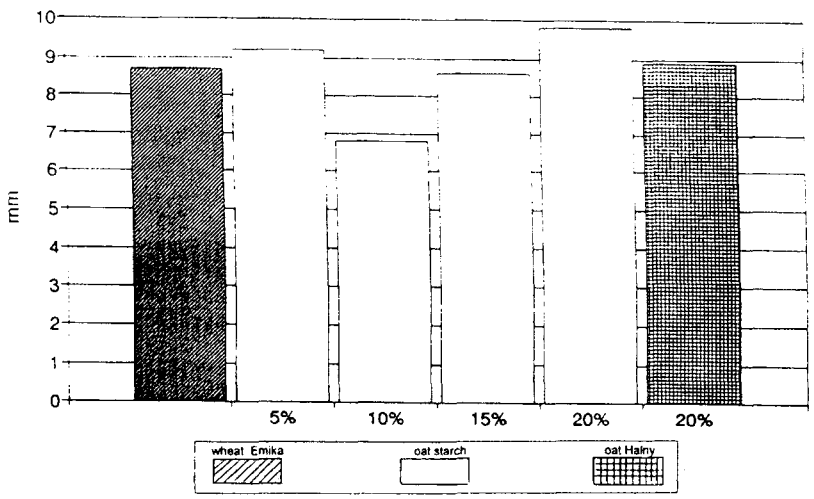

Fig. 2. Effect of various amounts of oat starches on crumb penetration of pup breads on the day of baking.

In comparison with the pup breads baked with the wheat starch, all the pup loaves containing the oat starch were characterized by a softer crumb in four days after baking (table 2, fig. 4). It seems that amylose-fat comlexes reducing swelling of oat starch

(high pasting temperature and low solubility at 80°C) stopped the outflow of amylose from this starch during baking. Additionally, some part of extrinsic monoglicerines in the oat starch could immobilize the amylose contained in the wheat starch by combining together and forming amylose-fat complexes. That is why in the crumb of the pup breads baked with the oat starch there was less amylose subject to retrogradation during their storing. This process could positively affect bread staling.

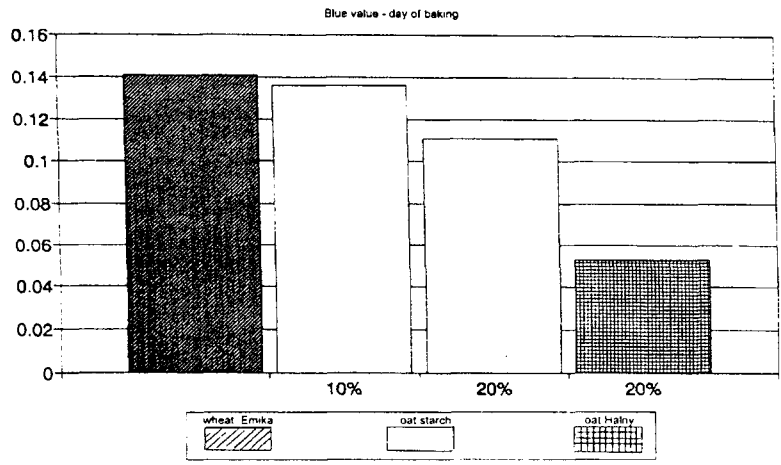

Fig. 3. Slowing down of outflow of amylose to crumb of model pup breads by fat contained in oat starch.

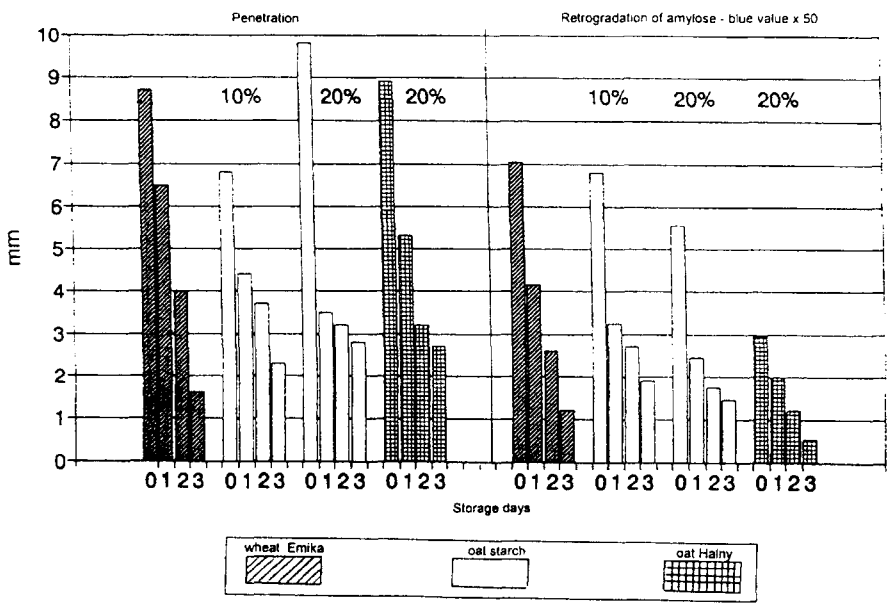

Fig. 4. Effect of various amounts of oat starches on staling of model pup breads.

Although the investigation of structural changes by means of differential scanning calorymetry (DSC) indicates that a slow retrogradation of amylopectin is responsible for bread staling, it is said , however, that concentration of the available amylose accelerates retrogradation of starch and amylopectin [22]. Hence reducing concentration of amylose and its elasticity as a result of adsortive interaction between amylose and fats allows slowing down the process og retrogradation od starch and its fractions [8].

This right assumption can further be confirmed by determination of blue value, which is an indicator of soluble amylose in crumb extract. As one can notice in fig. 3 and 4, on the very day of baking a lower content of amylose was detrmined in the crumb of the pup breads containing the oat starch as opposed to the standard bread and at the same time the decrease of the content of amylose was proportional to the increasing amount of the oat starch in the pup breads. The lowest outflow of amylose to the water crumb extract was from the bread with the 20 % addition of oat starch of Halny variety, which was characterized by the highest content of fat substances among the analyzed starches.

In each case replacing some amount of flour in the wheat bread with oat starch caused increasing baking loss and decreasing bread yield. The volume of the pup breads was also much lower, in particular of those ones containing the 20 % addition of these starches (table 3). As we know, oat starch is characterized by a small size of

Table 3

Effect of partial replacement of wheat flour with oat starch on selected indices of wheat bread quality (250 g)

Sort of bread	Mass after cooling [g]	Bread volume [cm^3]	Crumb penetration		Bread yield [%]	Total backing loss [%]	Organoleptic evaluation	
			in 1 day	in 4 day			Points	Quality class
Wheat flour - type 500 100% - standard	225	658	9.41	3.76	152	10.3	37	I
Wheat flour + oat industrial starch - 10%	220	555	10.75	2.43	150	11.7	30	II
Wheat flour + oat industrial starch - 20%	216	448	5.44	1.27	146	13.6	27	III
Wheat flour + oat starch of Halny variety - 10%	223	618	9.80	2.36	151	10.6	36	I
Wheat flour + oat starch of Halny variety - 20%	221	448	5.07	1.44	149	11.8	27	III

granules measuring (3–18μm) [17] with much worse baking properties than large ones, and probably that is why they had an unfavourable effect on quality of wheat bread, „diluting" gluten, especially in the breads with higher 20 % content of oat starch.

The lower volume of the wheat-oat pup breadsis due to a much lower crumb penetration in comparison with the standard breads.

An interesting fact is that despite the lower volume, the breads baked with the 10 % addition of industrial oat starch and oat starch were characterized by a higher crumb penetration on the day of baking. It seems however, that resistance of the small granules to pasting plays a more important role than the presence of starch fat. This phenomenon has a positive effect on plasticization of gluten. We did not notice any favourable effect of starch fat contained in oat starches on staling wheat breads.

Conclusions

1. In comparison with wheat starch in the analyzed oat starches we determined a higher content of total phosphorus and raw fat, a lower water binding capacity a several times lower solubility in water at 60°C and 80°C and a higher pasting temperature and viscosity, both its maximum value and after cooling to 50°C.
2. The addition of oat starch used for baking the model pup breads increased the degree of bread yield and improved crumb penetration. Despite a slight baling loss, the addition of oat starch contributed to high organoleptic properties.
3. The fat substancess contained in the analyzed oat starch contributed to improving crumb structure by reducing concentration of free amylose and to some extent eliminating its retrogradation. The presence of the 20 % addition of oat starches appeared to be the most effective in the model pup breads baked with wheat starch.
4. We did not notice any positive effect of the starch fat contained in the oat starches on quality and staling of the wheat breads.

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WPŁYW DODATKU SKROBI OWSIANEJ NA JAKOŚĆ I STARZENIE SIĘ CHLEBA

Streszczenie

Ze skrobi pszennej i suchego glutenu witalnego wypieczono modelowe chlebki, w których część skrobi pszennej zastępowano skrobią owsianą w ilości 5, 10, 15 i 20 %. Wykazano, że tłuszcz skrobiowy zawarty w skrobi owsianej wpłynął korzystnie na poprawę struktury miękiszu modelowych chlebków (40 g), natomiast nie wykazał takiego wpływu w przypadku dodatku skrobi owsianej do wypieku chlebów pszennych (250 g).

G. E. INGLETT, K. WARNER, R. K. NEWMAN

SOLUBLE-FIBER INGREDIENT FROM OATS: USES IN FOODS AND SOME HEALTH BENEFITS

Abstract

Soluble-fibers are considered important dietary substances for good health and disease prevention. Oat soluble fiber, β-glucan, is known to lower blood cholesterol in animals and humans. The fat substitute, Oatrim, is enzymatically derived from oats and contains the soluble β-glucan which retains its hypocholesterolemic properties. Many reduced fat and fat-free foods now use Oatrim for fat replacement. Among the many foods are low-fat meats; fat-free cheeses, and low-fat bakery products. Some sensory parameters of Oatrim usage in cookies and truffles are examined by an analytical sensory evaluation panel.

Introduction

American eating habits should be drastically changed to reduced the $950 billion in annual health-care spending according to many medical and nutritional scientists. Perhaps 10 to 20 percent of these health-care costs could be reduced if all Americans were to modify their diets based on existing knowledge of nutrition. Oatrim is a soluble-fiber containing fat substitute that is helping companies focus on foods being generated for Americans seeking a healthy life-style with increased longevity and less chronic disease.

Oatrim was discovered [6, 13] and patented [7] by the U.S. Department of Agriculture with licenses granted to Mountain Lake Manufacturing (ConAgra/A. E. Staley Manufacturing joint venture) and Quaker Oats/Rhone-Poulenc (a partnership). The process involved the α-amylase conversion of starch in the oat flour or bran to amy-

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lodextrins. The product, soluble-fiber (β-glucan) and amylodextrins from the oat flour, was referred to as oat β-glucan-amylodextrins or Oatrim. Barley and other cereal grains were used to prepare other amylodextrins containing soluble-fibers [10].

The composition of Oatrim is mainly amylodextrin and β-glucan with small quantities of lipid, protein, and minerals. Replacing fat in foods with Oatrim is possible because the combination of β-glucan and amylodextrins produce an excellent fatty textured material. It can be converted to a shortening-like gel by heating and cooling a 25 % Oatrim dispersion. The fat-like gel has one calorie per gram compared to nine calories for fat [8]. It replaces shortening in food recipes on a cup for cup substitution bases. The powder can also be used in recipes and its quantity modified based on intended function and properties desired. Both powder and gel give the sensory attributes of natural taste and fatty texture to foods as it replaces fat, lowers calories, and adds soluble-fiber to the diet. These properties make it possible for the reduced-fat foods to look-like, taste-like the traditional higher-fat foods.

Oatrim or its gel has heat stability in food applications, such as processed meats, pasteurized cheeses and baked products. Currently, Oatrim is found in a nationally distributed extra lean ground beef which is 96 % fat-free meat which has the natural taste and texture of a 80 % fat-free hamburger. A 112 gram portion has 130 calories compared with 300 calories from an equal weight of the 80 % beef. It is also used to replace fat in 97 % fat-free franks, fat-free cheeses, and various deli meats. New products containing Oatrim are appearing frequently and many major reduced-fat or fat-free products under active development which include reduced-fat meats, frozen desserts, salad dressings, sauces, gravies, soups, mayonnaise, margarine, breads, waffles, granola bars, muffins, cookies, brownies, beverages, and cakes [11]. Oatrim usage in a food item is noted on the ingredient label as hydrolyzed oat flour or hydrolyzed oat and corn flour. The ingredient is sold as TrimChoice™ by A. E. Staley or Quaker™ Oatrim by Rhone-Poulenc.

In addition to eliminating or substituting for fat in foods, Oatrim's soluble-fiber content gives a hypocholesterolemic property of lowering total blood cholesterol and LDL cholesterol levels [15]. Since dietary fat-reduction and caloric intake are considered important factors in maintaining good health, people with high blood cholesterol levels are considered to be at risk for heart disease. Oats have been recognized to be effective in lowering serum cholesterol since 1963 [1]. A recent meta analysis of several studies has shown the consistent efficacy of oats as a hypocholesterolemic agent in humans [12]. The effectiveness of oats in lowering cholesterol is dependent on consuming a sufficient quantity of oatmeal or oat bran over an extended period of time. Hence Oatrim, a purified, water-soluble nearly tasteless white powder derived from oat flour, can be easily incorporated into commonly consumed foods, such as meats,

dairy, and baked items. Its wide potential for food applications makes a sustainable consumption of oats possible.

Oatrim's blood cholesterol lowering properties were first found in chick studies by Professor Rosemary Newman [15] at the Montana State University. In the first clinical trial by humans, Oatrim received a "Two Thumbs Up for Oatrim" review in the December 1993 issue of Agricultural Research published by the USDA [14] which reports on some of its health benefits.

Materials and methods

Oatrim-5 was obtained from A. E. Staley Manufacturing Company, Decatur, IL (tradename of TrimChoice-5^{TM}). The reported composition and properties are: moisture, 4.0–8.0 %; ash, 2.0–3.0 %; fat (ether extraction), 0.5 % maximum; protein (nitrogen x 6.25), 5.0 % maximum; pH (10 % solution), 5.5–6.5; dextrose equivalent (DE), 3.0–5.0; β-glucan, 4.5–5.5; and gel strength, 10–14 mm.

Preparation and sensory evaluation of cookies

Oatmeal raisin cookies prepared from a commercial bakery recipe were selected to study shortening replacement with Oatrim. The formulation required the creaming of granulated sugar (4 lb), brown sugar (9 lb), salt (5.5 oz), baking soda (3 oz), (fine sugar Numoline or Numolina 1 lb), and margarine (9 lb). To this blend, the following ingredients were mixed in well: rolled oats (14 lb), cake flour (7 lb), regular flour (2 lb), raisins (12 lb), and eggs (2 lb and 2 oz). The yield was about 60 lb of cookies. No product development was attempted to add or avoid moisture loss during baking. Oatrim-5 and water were substituted for 50 %, 75 %, and 100 % of the margarine. Oatrim was added as a dry powder and the water was the only liquid added to complete the recipe. The formulations for cookies using margarine or Oatrim-water are shown in Table 1.

Table 1

Fat and Oatrim quantities used to prepare oat raisin cookies

Control	9 lb fat in 60 lb formula
50% Oatrim	1 lb 2 oz Oatrim-5 3 lb 6 oz water 4 lb 8 oz fat
75% Oatrim	1 lb 11 oz Oatrim-5 5 lb 1 oz water 2 lb 4 oz fat
100% Oatrim	2 lb 4 oz Oatrim-5 6 lb 12 oz water

An analytical sensory panel with 20 members trained and experienced in testing cereal-based products evaluated the oatmeal raisin cookies for the following flavor characteristics: sweetness, graininess, toasted, buttery, cardboard and the texture properties of density, cohesiveness, and moistness. Judges also rated the cookies for overall

quality. The control sample of cookies (no substitution) was rated in preliminary blind sensory tests.

Preparation and sensory evaluation of reduced-fat chocolate truffles

Chocolate truffles were selected to study replacement in their centers of whipping cream fat with Oatrim gel. To prepare a 10 lb size batch containing 25 % whipping cream, finely chopped milk chocolate (7 lb and 8 oz) was placed in a small pan; whipping cream (2 and 1/2 cups) was heated to boiling in a separate pan and poured over the chocolate. After standing 3–4 min, the mixture was stirred until smooth and glossy, cooled in a refrigerator 15 min, rounded teaspoonfuls of center were made into balls, chocolate covered, and chilled.

Oatrim-5 gel containing 30 % solids was substituted on a volume (cup-per-cup) basis for 50 % and 100 % of the whipping cream. A 50 % fat substitution required 30 % Oatrim gel (1 and 1/4 cups) and whipping cream (1 and 1/4 cups) heated as previously described. The 100 % fat substitution required the complete elimination of cream and adding only the gel (2 and 1/2 cups). Oatrim gel was prepared by blending 30 % Oatrim-5 with 70 % water and heating to boiling before allowing it to gel in a refrigerator overnight. An analytical sensory panel with 20 members evaluated the chocolate truffles for the following flavors: bitter, creamy/buttery, chocolate, sweet, and for texture properties of smoothness, chewiness, and density.

Oatrim hypocholesterolemic property in chicks

A pilot study to test the hypocholesterolemic property of Oatrim was done at Montana State University [15]. In a larger followup study to confirm earlier results, forty-eight day-old broiler chicks were fed a basal corn and casein diet containing vitamins, minerals and 0.50 % cholesterol for one week to make them hypercholesterolemic. Oatrim and other major diet components were analyzed for protein (Kjeldahl N x 6.25), fat [3], starch [4], and dietary fiber [5]. Chicks were wing-banded with identification numbers and divided into three groups of eight chicks for each diet treatment. The basal diet was continued as a control for 24 chicks, and the remainder were placed on a diet containing 38 % Oatrim. Chicks were fed diets for 14 days, then fasted overnight, weighed and blood samples were drawn. Plasma was analyzed for total cholesterol, triglyceride and HDL-cholesterol on a Kodak DT 60 analyzer (Eastman Kodak, Rochester, NY). Plasma LDL-cholesterol was calculated as described by Friedwald [2].

Results and discussion

Sensory evaluation of cookies

Results of the evaluation of fat substitution by Oatrim revealed that graininess and toasted flavor intensities were not significantly affected by the addition of Oatrim to the cookies. Cookies with 100 % Oatrim substitution for fat had significantly ($P < 0.05$) less sweet taste and buttery flavor and significantly ($P < 0.05$) more cardboard flavor than the unsubstituted sample. The texture characteristics of density and cohesiveness were not significantly changed by the addition of Oatrim; however, moistness of the cookies containing 75 % or 100 % Oatrim substitution of fat was significantly ($P < 0.05$) less than the no-fat substituted control.

Overall quality of the cookies was not significantly changed by the use of 50 % Oatrim substitution for fat; however, the quality of the cookies containing either 75 % or 100 % Oatrim substitution was lower ($P < 0.05$) than the no-fat substituted control.

Sensory evaluation of reduced-fat chocolate truffles

The 100 % substitution of Oatrim for fat only affected the creamy/buttery flavor characteristics by significantly ($P < 0.05$) decreasing its flavor compared to the 0 % and the 50 % Oatrim samples. The 50 % Oatrim substitution for fat caused no significant changes in flavor intensities compared to the no-fat substituted control.

The chewiness and density texture characteristics were not significantly affected by Oatrim substitution for fat in the truffles. The grittiness levels of samples with the 100 % and 50 % Oatrim substitutions for fat were significantly ($P < 0.05$) higher than in the no-fat substituted control.

Since some textural changes were noted in the sensory evaluation, the surfaces of the different truffle centers were examined by scanning electron microscopy (SEM). Samples of the truffle centers were mounted on aluminum stubs, coated with a layer of gold-palladium (60–40) alloy, and then examined and photographed in a JEOL model JSM 6400V scanning electron microscope. The surface areas of the truffle centers at 500 μm resolution show some differences in the surface areas. The pictures cannot be directly related to mouthfeel, but they do indicate that differences in the truffle matrix can be observed.

Oatrim provides an easy way of replacing fat and increasing soluble-fiber content in many foods, especially meats, dairy, and bakery goods where it has found considerable commercial use. This study on fat substitution by Oatrim in oatmeal raisin cookies and chocolate truffles was made to determine some of the parameters on Oatrim usage as evaluated by analytical sensory evaluation and SEM in the chocolate truffles. The study was not made as an attempt to find optimum product or conditions for producing such foods. The guidelines established in this study indicates that Oat-

rim replacement can readily be used for 50 % of the fat in oatmeal raisin cookies and chocolate truffles for shortening and whipping cream, respectively. It would be expected that greater Oatrim replacement could be made, but product formulation changes also would be necessary.

Hypocholesterolemic property of Oatrim

Recent nutritional evaluation of the hypocholesterolemic property of Oatrim powder confirmed Dr. Newman's earlier conclusions [15] of a very significant reduction in total and LDL cholesterol levels. Chicks with previously elevated blood cholesterol levels were fed Oatrim and compared with the control chicks. Chicks fed Oatrim gained significantly less body weight than the controls, and there was a significant difference in feed/gain ratio between the two groups. This is a typical response when chicks are fed high soluble-fiber diets, and is usually accompanied by a corresponding fecal excretion of fat [9]. Chicks fed Oatrim had highly significantly lower ($P < 0.0001$) total and LDL-cholesterol than controls (Table 2). The chicks that were fed Oatrim showed a 40 percent decrease in blood cholesterol compared to controls with a drop in low-density lipoprotein (LDL) cholesterol of 61 percent. There were no differences in HDL-cholesterol or triglycerides between the two groups. This experiment provides further evidence for the healthful beneficial character of Oatrim. The soluble fiber of Oatrim retains its hypocholesterolemic quality in a concentrated, bland-flavored form that can be incorporated into many foods and beverages.

Table 2

Blood plasma lipids of chicks fed Oatrim or control diets (mg/dl)

Diet	n	Plasma cholesterol			Triglycerides
		Total	HDL	LDL	
Oatrim	24	239[a]	123	99[a]	84
Control	24	400[b]	131	251[b]	87

[a,b] Columns with different superscripts are statistically different ($P < 0.0001$)

The recent review, "Two Thumbs Up for Oatrim" [14] reports on some of Oatrim health benefits in the first clinical trial by humans. Two different Oatrim products containing 1 percent or 10 percent β-glucan were investigated. Dietitians incorporated about one-half cup of each powder in various foods that were consumed by 24 volunteers with high levels of blood cholesterol. For 10 weeks the volunteers consumed Oatrim-enriched diets ranging from hamburger, cookies, and pancakes to spaghetti sauces, muffins, and fruit juices. Midway during the study, the volunteer groups were

switched to the other Oatrim-enriched diet. Both products reduced blood cholesterol levels by 8 percent with a 10 percent drop in LDL cholesterol. Additionally, their blood glucose levels decreased by 7 to 12 percent which can prove beneficial for diabetics. The largest surprise was the influence on volunteer's weight. They lost on an average of 4.5 pounds each despite a slight increase in calorie intake with no one complaining of being hungry. The future for replacing fat in the diet and reduced overall calorie intake without loss of safety appears promising for health concerned Americans.

Conclusions

A nutraceutical and nutritional evaluation of the hypocholesterolemic property of soluble-fiber containing Oatrim suggests that it could have a health benefit in foods. The hypocholesterolemic property of Oatrim in humans and chicks indicates a significant reduction in total blood cholesterol and LDL cholesterol levels. Oatrim should continue to play a larger role in healthcare and healthy life-styles with increased longevity and less chronic disease as more people realize the direct relationship of diet and health. Perhaps its greatest significance will be its long term influence on preventive heart and cancer diseases with the reduced human suffering and reduced healthcare costs. It can be used in many foods. Its applications as a fat replacer was illustrated in oatmeal raisin cookies and in making reduced-fat truffle centers.

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ROZPUSZCZALNE SKŁADNIKI BŁONNIKA Z OWSA: ZASTOSOWANIE W POKARMACH I KORZYŚCI ZDROWOTNE

Streszczenie

Rozpuszczalne składniki błonnika są ważnymi składnikami pokarmowymi zapewniającymi zdrowie i chroniącymi przed pewnymi chorobami. Rozpuszczalny błonnik owsa, β-glukan obniża poziom cholesterolu we krwi zwierząt i ludzi. Zamiennik tłuszczu Oatrim otrzymuje się enzymatycznie z owsa. Zawiera on rozpuszczalny glukan, który zachowuje swe właściwości hipocholesterolemiczne. Szereg pokarmów o obniżonej zawartości tłuszczów i zupełnie beztłuszczowych zawierają Oatrim. Wśród takiej żywności należy wymienić niskotłuszczowe mięsa, sery fermentowane i wyroby piekarnicze. Sprawdzono sensoryczne parametry Oatrimu w ciasteczkach i truflach posługując się tablicami do analitycznych ocen sensorycznych.

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NEW POSSIBILITIES IN THE STARCH EXTRUDATES INVESTIGATIONS

Abstract

Potato flour extrudates were obtained by twin screw extruder. The effects of process variables were related to the following extrudate features: expansion, density, and shearing stress. The microstructure of the extruded products was examined by mercury porosimeter. Total porosity was related to physical properties of the extrudates. Total porosity decreased when expansion of products increased. Feed moisture was the principal determinante of physical strength and affected the changes of number, size and distribution of the pores.

Introduction

It is well known that characteristics of extrudates such as texture, structure, expansion, and sensory properties are affected by many variables in the extrusion processes.

Owusu-Ansah et al, [13] revealed the textural and microstructural changes in corn starch as a function of specific extrusion variables.

They found out that such variables as feed moisture and screw speed significantly influenced breaking strength, as measured by Warner-Bratzler shear. Porosities of the extrudates increased with decreased feed moisture, parallel to an increase in expansion and a decrease in breaking strength. Texture and sensory properties of extruded products are related to the degree of their expansion [5, 8, 16].

According to Park [15], the main factor causing puffing of extrudates is the vaporization of heated water during extrusion-cooking. When gelatinized, molten starch is forced out of die. When the extrudate leaves the high pressure barrel the pressure drops rapidly, and water evaporates. Simultaneously, many small air bubbles form inside the extruded material, rendering its puffy structure. The dough cools down as it

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looses the latent heat with steam and heat transfer with the air. In the course of cooling the material becoms firmer, less plastic and solidifies.

On further cooling bubbles collapse and shrinkage of the puffed, sponge-like, structure takes place. The product looses its plasticity and elasticity. Extrudates of various expansion ratio differed in the size, number and distribution of the air pockets [6, 11].

Scanning electron microscope and appearence examination have been used to examine the microstructure of extrudates [2, 6, 18]. However, the quantitative characteristics of various pores of extrudates is difficult to perform by common laboratory methods. The study of internal porosity of biological material by mercury porosimeter is known [7, 17].

In this study the mercury porosimeter was used for examination of the microstructure of extrudates. Some relations between physical properties of potato flour extrudates and their microstructure were found.

Materials and methods

Samples

The samples of commercial potato starch were used (PN-93/A-74710). Approximate analysis gave 0.26 % of ash, 0.02 % of protein (N·6,25), 0.03 % of fat and 19.5 % of moisture.

Extrusion

Extrusion was carried out in a Polish S 9/5 industrial twin screw extruder, described by Mościcki [12]. Barrel temperature was differentiated over the length of the barrel from feed to die as follows:
sample 1 – 80-120-150-170-100, sample 2 – 80-110-140-160-100°C.

Feed moisture content was adjusted to 10.5 %, or 19.5 % (d.b.). The moisture 10.5 %, (d.b.) was achieved by air-drying of native starch.

Expansion

The expansion ratio was defined as the ratio of the diameter of the extrudate and the diameter of the die (expansion ratio = diameter of product/diameter of the opening). An average of 10 determinations was obtained.

The diameters of air-dried extrudates of each sample were measured with verrnier caliper to the nearest 0.05 mm.

Density

Ten measurements of the extrudate stick per extrusion duplicate were carried out. Density of extrudate in kg/m^3 was avaiable from Eq. (1);

$$\zeta = \frac{m}{\Pi r^2 l} \tag{1}$$

where: r - radius, l - length, m - weight

Shearing stress

A shear test was run with Instron type 4302 to determine the texture of starch extrudates. The shear stress (N/cm^2) was calculated by dividing shear by the cross-sectional area of extrudate. Averages of 10 readings were taken.

Mercury porosimetry

Estimation of the porosity with the mercury porosimetry was based on the assumption, than the liquid which does not wet the surface, cannot penetrate the pores of the surface. It is likely sofely after applying a pressure. At relatively low pressure, big pores are filled, and at higher pressure – smaller pores are available for the liquid. The external pressure is a function of the size of the pores. The size of the pores is related to the pressure, assuming the cylindrical pore model (the Washburn equation (2) [19]):

$$P = -2\delta\cos\theta/r \tag{2}$$

where: P – external pressure, r – cylinder radius, δ – mercury surface tension, Θ – wetting angle in the system : solid surface – mercury.

The radius derived from that equation, so-called equivalent radius, corresponds to the radius of the ideal capillary, because pores in natural materials have irregular shape.

The Olsen scheme can be useful for porosimetric measurements of the volume of pores and solid body:

Solid body particles	Pores inaccessible to mercury at max. porosimeter pressure	Pores filled with mercury during porosimetric analysis	Pores filled with mercury at initial pressure
m/ζ	V_b	V_{max}	V_α
V_o			

The Olson equation (3), allows to measure the volume of pores beyond the range available for the mercury porosimetry:

$$V_b = V_o - V_{max} - m/\zeta \tag{3}$$

where: V_b – pore volume below the range, V_0 – volume not taken by mercury, at the initial pressure of the porosimeter, V_{max} – volume of mercury forced at the maximum pressure, m – sample weight, ζ – sample density measured pycnometrically.

The Carlo Erba 2000 Hg intrusion porosimeter ,compled with the Carlo Erba CUT/HEC 960 computer (Carlo Erba Strumentazione, Rodano, Italy), was used in the determination of the pore radius distribution.

One gram of < 0.2 mm air-dried extrudate or whole samples were used; outgassing for 24 h was performed before measurements.

To find a link between intrusion pressure and pore radius a cylindrical shape of pores was assumed in the calculations, and a surface tension value for Hg of 0.48 N/m extrudate contact angle of 141° were used in the Laplace equation. The pressure varied from 100 kPa to 200 MPa, and corresponded to a pore radius range from 3.6 nm to 7.5 μm.

Results and discussion

Internal porosity (TP) of extrudates evaluated by the mercury porosimeter

Not too much attention was paid to the analysis of the micropores, which appear in air cell walls. Their sizes ranged from tenth parts of a microne to several microns. Their size and distribution depended on the extrusion variables and feed composition. Increased fiber content in corn feed increased the number of apertures in cell walls and reduced big air cells. Thus, the expansion ratio reduced with the fiber addition [9, 11].

In corn meal extrudates with 10-30 % of wheat bran addition, in air cell walls an abundance of spherical particles was observed [11]. According to Abdel-Aal E.-S.M. et al., after coarse grinding the extrudates turned into micro-flakes distinctly different in size, thickness, and appearance [1].

Table 1

Effect of extrusion variables on physical and textural properties of potato starch products

Sample	Barrel temp. [°C]	Feed moisture [%]	Expansion ratio	Density [kg/m^3]	Shearing stress [N/cm^2]	Total porosity [%]	
						N*	N**
1	80–170	10.50	6.10	51.94	4.03	14.42	2.87
2	80–160	19.50	1.86	365.14	301.93	4.30	3.29

N* – natural extrudate,
M** – ground extrudate.

Microstructure of natural and ground potato extrudates was analysed with the mercury porosimeter. The extrudate porosities (TP), calculated under assumption of cylindrical pore model, depended on the expansion ratio. That dependence was more straighforward for the natural extrudate samples (Tab. 1).

Grinding of the samples clearly reduced the porosityand the TP distribution making it practically independent of the expansion ratio. The TP of the natural samples ranged from about 4 % to 15 %, and these of the ground samples ranged from about 2.5 % to 3.5 %.

The link between the amount of pores and their radius is illustrated by the cumulation curve (integral) (Fig. 1) and pore size distribution curve (PSD) (Fig. 2). Fig. 2 shows the PSD curves for selected ground extrudate samples (1M and 2M). Their character is similar indicating a low porosity of the material despite of the expansion ratio. The PSD of the natural extrudate (Fig. 2; 1K and 2K) samples shows a broad

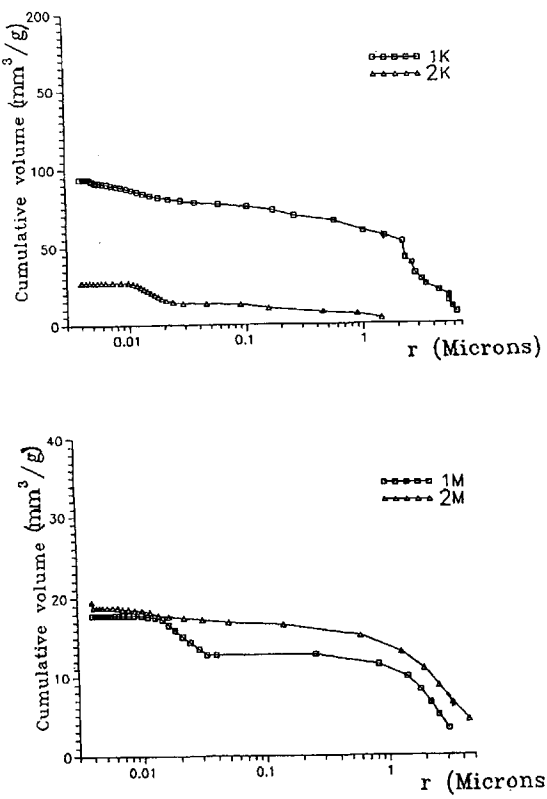

Fig. 1. Cumulative pore size distribution:
for sample 1 (expansion ratio = 6.10),
for sample 2 (expansion ratio = 1.86),
K – natural extrudate, M – ground extrudate.

distribution indicating their porous structure. In samples 1, a small number of pores with the radius ranging from 0.01 μm to 5.5 x 10^{-2} μm was observed, and this range, in sample 2, was wider (Fig. 1 sample 1K and Fig. 2 sample 2K).

The grinding of the highly expanded product maight cause pore vanishing of the range below 1 μm.

In the sample of high density (Fig. 2, sample 2) pores below 0.01 μm were observed, which to a certain degree, could constitute the reflection of the micropores in the native material.

The extrusion conditions might influence the microporosity changes in the range

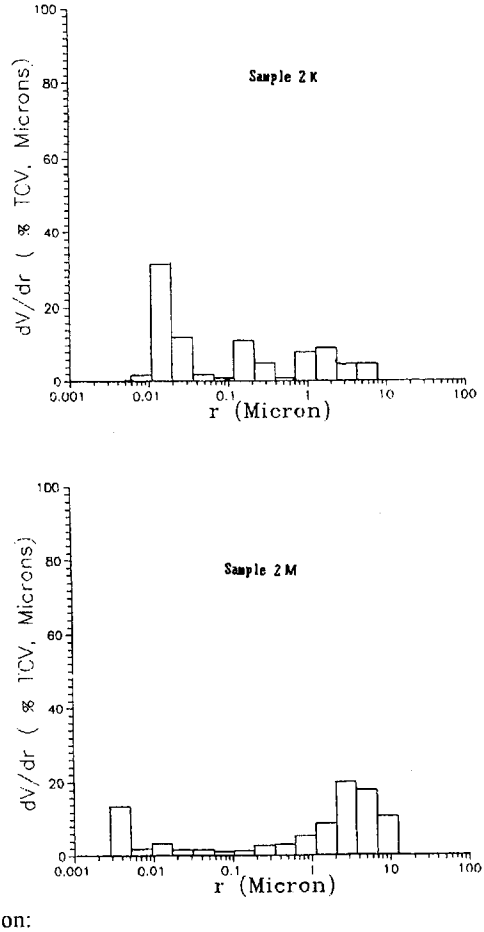

Fig. 2. Pore size distribution:
for sample 1 (expansion ratio = 6.10),
for sample 2 (expansion ratio = 1.86),
K – natural extrudate, M – ground extrudate.

from 3.6 nm to 7.5 μm. Relatively high TP values were noted in the samples of natural extrudates (14.42 %) (Fig. 1, sample 1K) , and in the most dense sample (Fig. 2 sample 2K and sample 2M), the TP (4.30 %) was close to that of the ground sample (3.29 %). In the sample with high expansion (1), usually containing big air pockets, relatively the lowest internal porosities were observed, which could due to the smooth surface of the puffed extrudates.

In the analysed sample, higher content of pores with the radius of over 1 μm, as well as small content of pores with the radius of 5.5×10^{-3} μm to 5.5×10^{-1} μm were observed.

The samples with decreasing expansion ratio, the contained the highest number of pores with the radius below 1 μm up to 0.1 μm.

Conclusion

The microstructure of the extrudate is reflected in both the expansion and the physical strength of products. The shear stress of the extruded starch products were inversely proportional to the expansion volume.

The total porosity, pore size distribution and cumulative curve were significantly lower for the ground extrudates as compared with the natural material.

Highly expanded samples showed lower internal porosity. One could can conclude that after the radial expansion, the cell walls remained smoother. In extrudates of increasing density, greater number of micropores was noted.

Extrudates were made in Departament of Food Enginering of the Agriculture University in Lublin, Poland.

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NOWE MOŻLIWOŚCI BADANIA EKSTRUDATÓW SKROBIOWYCH

Streszczenie

Ekstrudaty ziemniaczane wyprodukowano w dwuślimakowym ekstruderze. Wpływ zmiennych procesu określono w stosunku do takich cech ekstrudatów jak: ekspansja, gęstość i siły ścinania. Siły ścinania i gęstość ekstrudowanych produktów skrobiowych były odwrotnie proporcjonalne do wartości ekspansji. Mikrostrukturę produktów ekstrudowanych badano w porozymetrze rtęciowym. Ogólna porowatość (porowatość wewnętrzna) była określona w relacji do fizycznych właściwości ekstrudatów. Ogólna porowatość zmniejszała się kiedy wzrastała ekspansja produktów. Wilgotność wsadu była szczególnym determinantem fizycznej wytrzymałości i powodowała zmiany liczby, wymiarów i rozmieszczenia porów.

Rozmieszczenie wymiarów porów naturalnych próbek ekstrudatów pokazywało zróżnicowany charakter i wyróżniało ich porowatą strukturę. Względnie wysokie wartości porowatości wewnętrznej odnotowano w próbkach ekstrudatów naturalnych (14.42 %), a w próbkach o największej gęstości, porowatość wewnętrzna (4.30 %) była zbliżona do wartości próbek zmielonych (3.29 %).

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THE INFLUENCE OF THE EXTRUSION ON SOME PROPERTIES OF THE PRODUCTS FROM POTATO STARCH

Abstract

Potato starch was processed in a Polish 2S 9/5 industrial twin screw extruder to study the physical and structural modifications which occur of during exstrusion. The relations betwen the physical properties and structural modifications of starch biopolymers are reported. The study of the structure of potato extrudates by the small angle X-ray scattering was developed.

Introduction

Extrusion processing of starchy raw materials is known to cause macromolecular changes in the physical constitution of the starch granule [5, 12]. Several authors have studied the changes to define the extent of modification at a molecular level necessary understand and control the extrusion process [7, 19]. The barrel temperature and moisture content in the native provide the most versatile control expansion ratio of the extrudate [3, 4, 17].

Increased process intensity, which occurs at higher temperature, higher screw speed and lower moisture content – lead to a deeper modification. The partial or complete destruction of crystalline structure of the raw starch granule in the extruder has been demonstrated by X-ray diffraction patterns and scanning electron micrographs [2, 5, 13, 15]. The extrusion produced starches of lower pastes viscosities than the unprocesed material, and the gel permeation chromatography showed that starch biopolymers were degraded into macromolecular components [8, 19].

The formation of linear oligosaccharides has been observed on the extrusion cooking of the potato starch [14].

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Also the wide X-ray diffraction and small angle X-ray scattering methods can be used to observe the changes in the long and short range ordering in a starch granules [1, 6, 11]. Especially a reflection due to long range ordering in starch (corresponding to the distance d = 10 nm) was observed by means of the SAXS method for many native materials and for products which were differently processed. The SAXS method also allows to investigate quite amorphous materials and to obtain many parameters which can be characterized in these materials.

The relations between the extent of molecular degradation starch biopolymers and the changes in physical properties of extrudate has been studied. However, the main aim of this study was to investigate the effects of extrusion processes on potato starch products by the small angle X-ray scattering.

Materials and Methods

Samples

The samples of commercial potato starch (PN-93/A-74710) were used. Approximate analysis indicated that it contained 0.26 % of ash, 0.02 % of protein /N·6.25/, 0.03 % of fat and 19.5 % of moisture.

Extrusion

Extrusion was carried out in a Polish 2S 9/5 industrial twin srew extruder (16). Barrel temperature varied over the length of the barrel from feed to die as follows: 80-120-150-170-100, 80-110-140-160-100. Feed moisture content was adjusted to 10.5% 19.5% (d.b.). The moisture 10.5% was achieved by airdrying of native starch.

Expansion

The expansion ratio was defined as the ratio between the diameter of the extrudate and the diameter of the die (expansion ratio = diameter of product/diameter of the opening). An average of 10 estimations was utilized. The diameters of airdried extrudates from each sample were measured with verrnier caliper to the nearest 0.05 mm.

Density

Ten measurements of the extrudate stick were made per extrusion duplicate. Density of extrudate was estimated as kg/m^3 according to Eq. (1);

$$\xi = m/(\Pi r^2 l) \tag{1}$$

where: r - radius, l - length, m - weight.

Shearing stress

A shear test was carred out with Instron type 4302 to determine the texture of starch extrudates. Shearing stress (N/cm^2) was calculated by dividing shear by the cross-sectional area of extrudate (averages of 10 readings).

Macromolecular degradation of starch components was estimated by means of the gel permeation chromatography of solutions in 0.1 M K0H on Sepharose CL-2B [8].

The polysaccharide concentrations in the column fractions were determined with anthron [10].

SAXS measurements

Measurements were performed on a slit-collimated Kratky camera using a Cu anode tube as the radiation source. A scintillation counter with a nickel filter and a pulse-height analyser were used to measure the scattered intensity. The samples for the SAXS experiments were prepared as follows:

– the extrudates were ground in a coffee mill to pass through a 0.2 mm screen, then, dried in an electric dryer and placed in special 1 mm capillares. The scattering curve of a given sample was available from several subsequent runs to eliminate the background scattering (scattering of the air, empty capillary, parasitic scattering of the slits, etc.). The measurements were carried out in the range of 2Θ from 0.076 to 6.52° in 0.0076 to 0.038° intervals and counting time of 100 s. Scattering curves were presented in the intensity versus q, manner as follows:

$$q = (4\Pi/\lambda)\sin(\Theta/2) \qquad (2)$$

Geometry of SAXS camera used and other measurement conditions allowed to consider these scattering curves as slit-smeared data for a beam of infinite length. The scattering curves were polished twice i.e. prior and after subtracting the background. The calculations were carried out using modified Vonk's program [21].

Results and Discussion

The investigations of the different starch samples by means of the SAXS method were focused on measurements of the long-range ordering in starch granules (ordered distribution of polysaccharide macromolecules).

It was shown in many papers [1, 6, 11] that starch slurries of different raw materials are characterized by a distinct peak in SAXS range, which may dissapear after processing. This peak may be accompanied by a number of diffraction peaks in classical diffraction range. It is illustrated in Fig. 1 and 2, which show SAXS scattering curves for slurry of raw potato starch (curve 1) and of the potato extrudate (curve 2)

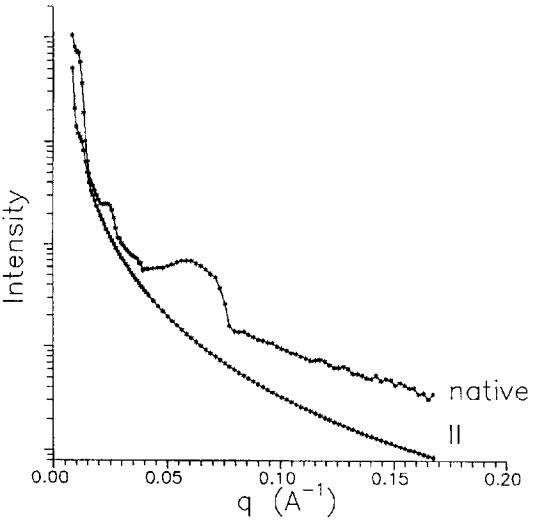

Fig. 1. SAX scattering curves for water suspension of potato starch (curve native) and potato extrudate II (curve II).

and the X-ray diffraction pattern for these materials obtained after drying the suspension, respectively. Diffraction peaks therein are due to semi-crystalline areas in starch (the peaks in the of wide angle diffraction range) and lamellar structure of semi-crystalline and amorphic areas (peak in the SAXS range).

Frequently, this semi-crystalline organization completely vanished, but SAXS method, provides to investigation of fully amorphous material. In such cases the scattered intensity is proportional to square difference betweeen electronic density of the scattered inhomogeneities and its surroudings. It depends on size and amount of these inhomogeneities. Therefore, the SAXS method is suitable for the study of inhomogeneities in condensed materials.

The characteristic of samples completed the study of starch elution (Fig. 3).

Table 1 shows treatment conditions of the extrusion process and properties of potato starch extrudates.

Results presented in Table 1 show that these samples have entirely different properties (expansion ratio, density, shearig stress) due to different extrusion conditions.

Table 1

Effect of extrusion parameters on physical and textural properties of potato starch extrudates

Sample	Barrel temp.* [°C]	Feed moisture [%]	Expansion ratio	Density [kg/m^3]	Shearing stress [N/cm^2]
I	80–170	10.50	6.10	51.94	4.03
II	80–160	19.50	1.86	365.14	301.93

* see in the text

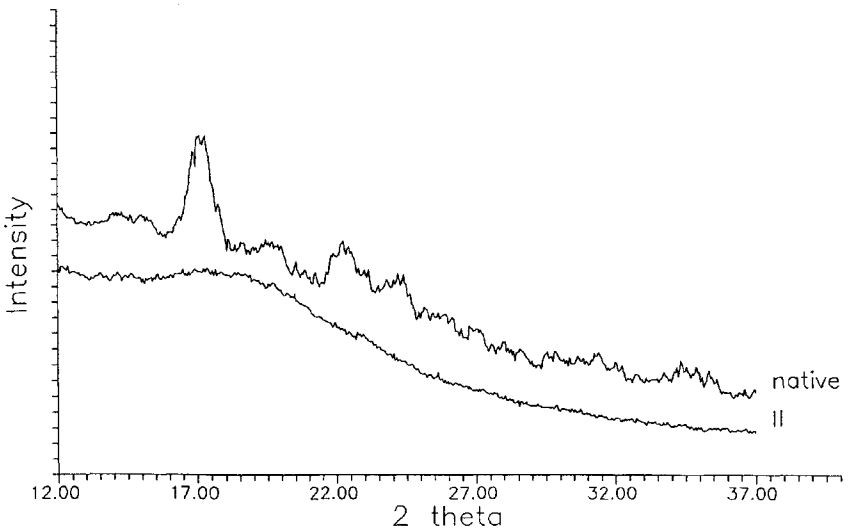

Fig. 2. X-ray diffraction pattern for native potato starch (curve native) and for potato extrudate II (curve II).

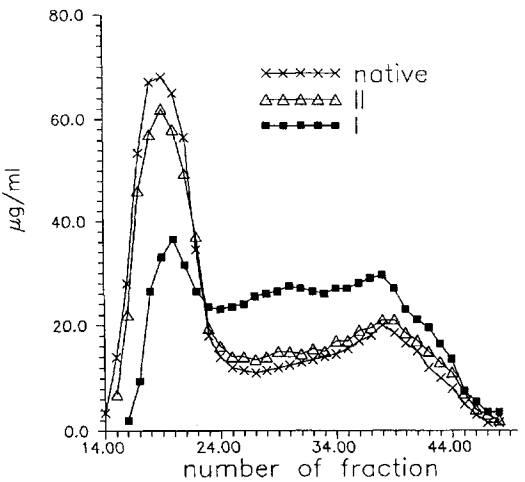

Fig. 3. Elution profiles from Sepharose CL-2B of native and extruded I (80–170°C, 10.5 % feed moisture) and II (80–160°C, 19.50 % feed moisture) potato starches.

It is generally accepted that the molecules of starch undergo degradation on extrusion [7, 9, 20]. The material eluted at the void volume from the gel permeation chromatography (Fig. 3), was amylopectin (Λ_{max}= 540–550), as identified by its reaction with iodine. On extrusion (curve 1 and 2) the contents of fraction I (amylopectin) was reduced and fraction II (amylose) was increased (tube numbers 25 and higher).

Degraded molecular products of amylopectin were eluted along with amylose forming one broad peak. The extent of this molecular transformation in extruded starch, would explain the variation of the physical properties with extrusion parameters (Tab. 1).

Fig. 1. compares the SAXS scattering curves of two samples of potato starch extrudates, properties of which are presented in Table 1. In Fig. 4. only the final part of the scattering curves are presented but sequence of the scattering curves is the same for the full measured range.

For potato starch extrudates a very interesting correlation of SAX scattering and the extrusion conditions was found (Table 1 and Fig. 4).

The expansion ratio gradually decreased from the first sample extruded more expansively to the second extruded at milder conditions.

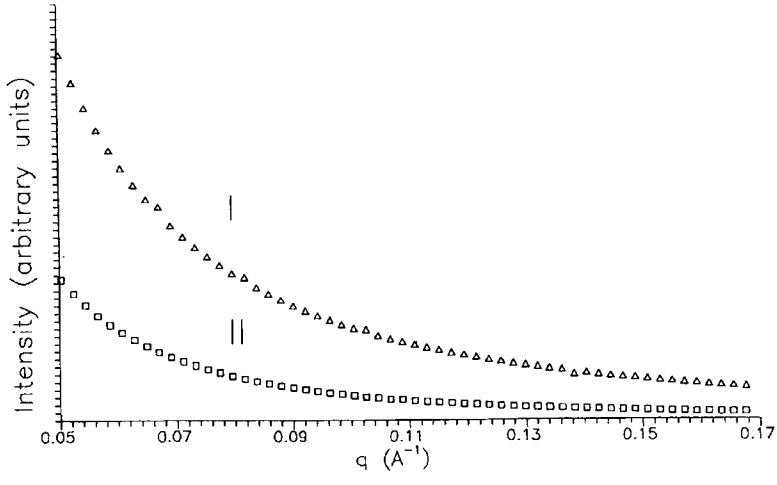

Fig. 4. The final part of the SAX scattering curves for I–II potato extrudates samples.

Investigations carried out by means of the SAXS method are presented in Fig. 4. The results show a correlation between physical properties of potato starch extrudates and intensity of the SAX scattering. The SAX scattering is the most profound for the Ip sample. This sample has the highest expansion ratio, because extrusion of the potato starch caused the formation of electronic density inhomogenieties and the more intensive process produced more inhomogeneities.

In Figs. 5, 6, the full X-ray small angle scattering curves in log-log axis are presented. The SAXS curves have very interesting pattern. In the long range of the final portion the scattering curves are almost linear. The slopes (tg α) of these parts are -2.0 and -2.6 for I and IIp lines, respectively.

Hence, question about the origin of these inhomogeneities appears.

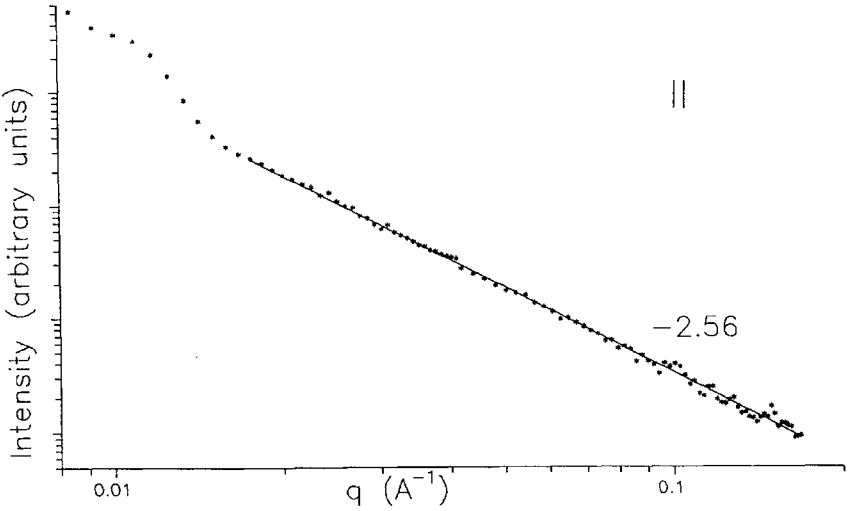

Fig. 5. SAX scattering curve (in log-log axes) for the I sample.

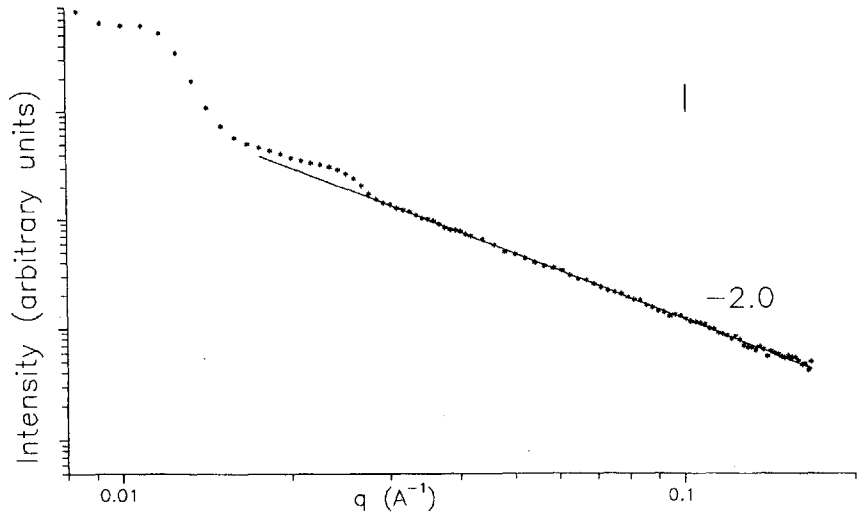

Fig. 6. SAX scattering curve (in log-log axes) for the II sample.

The SAX scattering intensity depends on the size and amount of inhomogeneities as well as on difference between eletronic density of the inhomogeneities and the surrouding. Observed intensity of the SAX scattering for four samples of potato starch is rather low. The extrusion produced the pores in the starch material but it is difficult to assume that these pores have diameter the size of which fits the range available for the

SAXS measurements (1–80 nm). When pores in a material are present and their size is within the range of SAXS measurements, the intensity of the SAX scattering is fairly high, because the electronic density of the air is very small; for example the intensity of the SAX scattering of the porous glass (with value of the specific surface about 16 m^2/g) is about 10–20 times higher than that observed for our samples. Thus, we can assume that observed SAX scattering does not originate from pores existing in the extrudates.

In the SAX scattering curves presented in Figs. 5 and 6 in the log-log system show a linearity in their long, final portions. It follows the rules for the SAXS method for a two phase system (inhomogeneities and surrounding), but for smooth-surfaced inhomogeneities slit-smeared data the slope should reach -3.

The lower slope suggests fractally rough surface of inhomogeneities [18].

Therefore, one might suggest that the inhomogeneities which were formed on extrusion originated from fluctuations and modulations of electronic density of a small part of the starch granules. **This conclusion correspond with the elution profile of extrudates; the extrusion at lower moisture levels led to an extended degradation of starch.** These fluctuations could result from a destruction of the crystalline and amorphous lamellae as well as mixing of these fragments. This suggestion needs a further proof.

Conclusions

The results obtained by the SAXS method show a link between intensity of the SAX scattering and extrudate characteristic parameters (kind of extrudate, expansion ratio, density, shearing stress) **as well as with the level of the degradation of starch biopolymers.**

The SAXS method demostrated that after the extrusion electronic density inhomogeneities are formed in the extrudates. The intensity of the SAX in the extrudate increased with the expansion ratio.

Extrudates were made in Department of Food Enginering of Agriculture University, Lublin, Poland

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WPŁYW PROCESÓW EKSTRUZJI NA NIEKTÓRE WŁAŚCIWOŚCI SKROBIOWYCH PRODUKTÓW ZIEMNIACZANYCH

Streszczenie

W skrobi ziemniaczanej, poddanej obróbce w przemysłowym dwuślimakowym Polskim ekstruderze 2S 9/5, badano fizyczne i strukturalne modyfikacje występujące w czasie procesów ekstruzji. Omówiono relacje pomiędzy fizycznymi właściwościami i strukturalną modyfikacją biopolimerów skrobi. Rozwinięto badania struktury ekstrudatów oparte na zjawisku mało kątowego rozpraszania promieni rentgenowskich (SAXS). Wpływ gotowania ekstruzyjnego, na ekspansję, gęstość i siły ścinania produktów zostały omówione w stosunku do transformacji skrobi mierzonej metodą SAXS. Wykazano bliską zależność pomiędzy intensywnością rozpraszania SAXS a charakterystyką parametrów (wskaźnika ekspansji, gęstości, sił ścinania i makromolekularnej degradacji skrobi) ekstrudatów. Wyniki otrzymane metodą SAXS wskazywały, że po procesach ekstruzji w ekstrudatach są tworzone wiele elektronowych gęstości niejednorodności; jeśli wskaźnik ekspansji jest większy wtedy intensywność rozpraszania SAXS jest również większa. Oprócz tego, metoda SAXS pozwala wnioskować o charakterze tych niejednorodności; nachylenie końcowej części krzywej rozpraszania SAXS sugeruje, że formowane niejednorodności mają fraktalną powierzchnię.

Rezultaty wskazują nowe możliwości użycia metody SAXS do badań fizycznych i teksturalnych zmian skrobiowych ekstrudatów ziemniaczanych.

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CAN STARCH BE A REAGENT IN NON-ENZYMATIC GLYCOSYLATION OF PROTEINS?

Abstract

Non-enzymatic glycosylation of proteins plays an important role in the development of the diabetic complications and the aging processes. In nature, starch is the lipid-metallo-protein complex. The starch-protein complexes may serve as the source of the amino and aldehyde groups.This investigation was carried out to test the hypothesis that the starch can be a reagent in non enzymatic glycosylation of proteins in the physiological pH. Two suspensions were incubated: (1) Potato starch in the phosphate buffer at pH 6.0, 7.0 and 8.0, at 37°C for 24 h. (2) Potato starch in the phosphate buffer a leucine solution added. Amino nitrogen and glucose were determined. The decrease in the content of the amino groups during the incubation was observed for all starch suspensions. The highest decrease of the amino groups occurred at pH 6.0, and the lowest at pH 8.0. The addition of leucine to the starch suspensions intensified a decrease in amino group amount during the incubation. The content of aldehyde groups increased mostly during the incubation at pH 6.0 and decreased at the higher pH. The liberation of aldehyde groups from the starch suspension was not influenced by the addition of leucine. The increase the aldehyde group number during the incubation proves that starch was hydrolysed. However, the increase of amino points to the Maillard reactions which can include different components of the starch complex. Finally, the results seem to confirm our hypothesis, that starch can react on the non-enzymatic glycosylation of proteins at physiological pH.

Introduction

Non enzymatic glycosylation of proteins plays an important role in the development of the diabetic complications and the aging processes [1, 2]. The starch – protein complexes may serve as the source of the amino and aldehyde groups. Then, the incubation of these complexes with amino acids, peptides, proteins or reducing sugars can lead to the Maillard components. From the theoretical and practical points of view it is interesting, whether these reactions can occur in the physiological conditions. This

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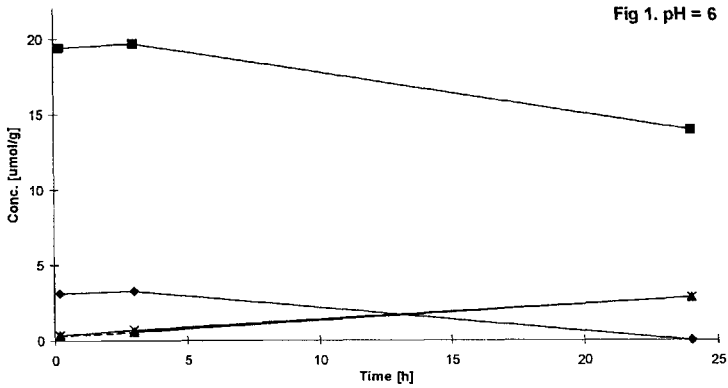
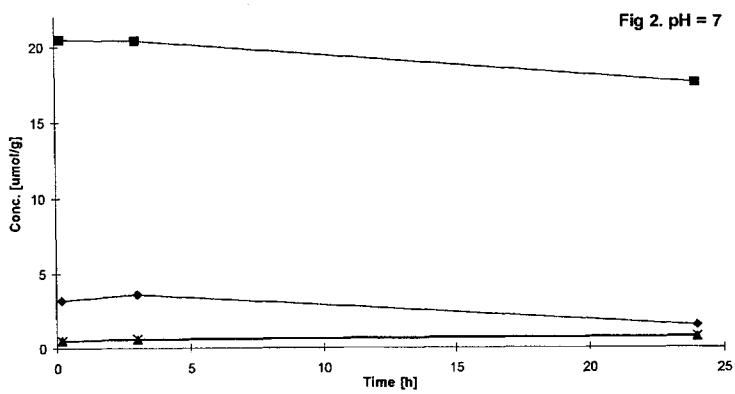
investigation was carried out to verify the hypothesis that starch can react on the non-enzymatic glycosylation of proteins at physiological pH.





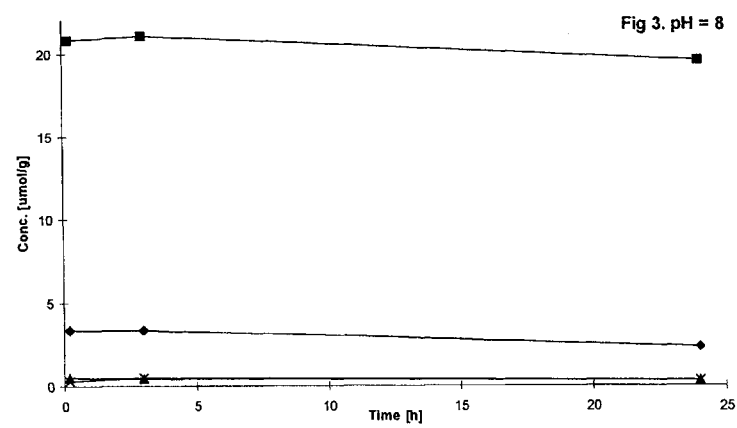


Fig. 1-3. Changes in the content of the free amino and aldehyde groups in the starch suspensions during the incubation with phosphate buffer at pH 6.0, 7.0 and 8.0, expressed in μmol/g starch. With leucine: ■ – (-NH_2), ⧓ – (-CHO), without leucine: ◆ – (-NH_2), ▲ – (-CHO).

Materials and Methods

Two suspensions were incubated on mechanical stirring: (1) Potato starch (30g) in 150 ml of the phosphate buffer (0.2 M) 15 ml water added pH 6.0, 7.0 and 8.0 were maintained at 37°C for 24 h, (2) Potato starch in 150 ml of the phosphate buffer 15 ml of leucine solution (30 mM.) added. Samples were collected at 0.6, 3 and 24 h intervals. The samples were centrifuged at 4000 g for 10 min. The supernatants were kept at -25°C followed by the determination of the amount of amino nitrogen and glucose immediately after thawing.

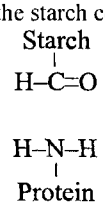
Amino nitrogen was determined by the Alvarez-Coque et.al. method [3]. An aliquot of samples (0.25 ml) was taken for analysis. The absorbance was measured at 340 nm. The calibration curve was obtained by using the leucine solutions with the concentrations ranging from 0 to 10 μmol/ml.

The concentration of glucose was determined by the Davis et.al. method [4]. The 1 ml samples were analysed. The absorbance was measured at 420 nm. The calibration curve was obtained from the glucose solutions of the concentrations ranging from 0 to 2.0 μmol/ml. The glucose solution was stabilized with 0.25 % benzoic acid.

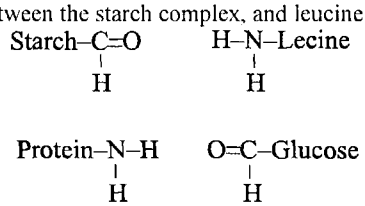
Results and Discussion

The decrease of the amino group content during the incubation was observed for all starch suspensions. The highest decrease occurred at pH 6.0, and the lowest at pH 8.0. The addition of leucine to the starch suspensions intensified the amino group ceasing on the incubation. The content of aldehydo groups increased mostly during the incubation at pH 6.0 and decreased at the higher pH. The liberation of aldehyde groups from the starch suspension was not influenced by the addition of leucine. In nature, starch is the lipid-metallo-protein complex. Thus, protein is the source of the free amino groups in this complex and the terminal glucose units deliver the aldehydo groups. The increase in the aldehydo group population during the incubation proves the starch hydrolysis. However, the increase of the amino groups points to Maillard reactions which involve various components of the starch complex, as follows:

a/ within the starch complex



b/ between the starch complex, and leucine and glucose



The highest decrease in the amino group content at pH 6.0 proves that the top reaction rate of carbonyl-amine condensation is reached in the moderately acidic medium.

General conclusion

The critical evaluation of the aldehydo groups determination suggests that glucose can partly be liberated from amylose by thermolysis. Thus, the method requires modification. However, the results seem to confirm our hypothesis, that starch can react on non-enzymatic glycosylation of proteins at physiological pH.

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CZY SKROBIA MOŻE BYĆ REAGENTEM W NIEENZYMATYCZNEJ GLIKOZYLACJI BIAŁEK?

Streszczenie

Nieenzymatyczna glikozylacja białek odgrywa ważną rolę w komplikacjach zdrowotnych u diabetyków i w procesach starzenia ludzi. Skrobia jest de facto kompleksem lipo-metalo-proteino-polisacharydowym. Fakt ten stał się powodem postawienia hipotezy, że może ona być reagentem w reakcji nieenzymatycznej glikozylacji białek przy fizjologicznych wartościach pH. Inkubację skrobi ziemniaczanej z dodatkiem i bez dodatku leucyny prowadzono przy pH 6.0, 7.0 i 8.0. Badano zmianę zawartości wolnych grup aminowych i aldehydowych w czasie inkubacji. Obniżenie zawartości grup aminowych występowało przy wszystkich wartościach pH, z tym że największe było przy pH 6.0. Podobnie przy wszystkich wartościach pH następował wzrost wolnych grup aldehydowych. Na podstawie uzyskanych wyników stwierdzono, że przy wszystkich wartościach pH skrobia ulegała częściowej hydrolizie. Natomiast wolne grupy aminowe pochodzące od białka związanego ze skrobią lub od leucyny wchodziły w reakcje Maillarda. Generalnie uznano zasadność postawionej hipotezy, że skrobia może uczestniczyć w reakcjach nieenzymatycznej glikozylacji białek w fizjologicznych warunkach pH.

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