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SELECTED PROPERTIES OF POTATO STARCH SUBJECTED TO MULTIPLE PHYSICAL AND CHEMICAL MODIFICATIONS

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The research was aimed at producing, under various conditions, a preparation of potato starch complexed with monoacylglycerol, subjecting it to retrogradation and next acetylation as well as at determining the effect of production method on properties of the modified preparations obtained. The preparations were determined for: thermal characteristics of gelatinization, characteristics of paste formation with Brabender viscosimeter, flow curves of 5% pastes at a temperature of 50°C, and dynamics of saccharification with amyloglucosidase. Multiple modification of starch evoked considerable changes in its properties, and the direction and extent of those changes appeared to depend on the type of modification applied. Considerable reduction of the susceptibility to amyloglucosidase activity (by *ca*. 40%) as well as a lack of significant differences in rheological properties of the prepared pastes predispose the acetylated preparations of starch complexed with monoacylglycerol and the retrograded one as a food additives with thickening and prebiotic properties.

INTRODUCTION

Multiple modification enables obtaining starch with a considerably changed structure and, consequently, preparations with novel properties.

Starch complexing with lipids consists in the turning of straight chains of gelatinized starch into helices around the chain of a fatty acid. Complexes formed this way are thermostable [Tufvesson *et al.*, 2001] and demonstrate diminished susceptibility to the activity of amylases [Nebesny *et al.,* 2005]. Properties of those complexes depend on the length of starch and lipid chains, type of lipid and temperature of complex formation [Tufvesson & Eliasson, 2000]. The starch-lipid complexes occur naturally in cereal starches containing phospholipids.

Retrogradation is a process defined as the linking of starch chains into ordered structures crystalline in character [Eerlingen & Delcour, 1995]. It proceeds especially readily at a temperature of *ca*. 0°C or over 100° C. Properties of the retrograded starch are determined, among other things, by the origin of starch [Sandhu & Singh, 2007], conditions of the retrogradation process [Leszczyński 2004] or the presence of other compounds [Cui & Oates, 1999; Funami *et al*., 2005; Prokopowicz, 1995]. Retrograded starch is classified as one of the forms of resistant starch (RS 3) [Englyst *et al*., 1992].

Acetylated starch with a low degree of substitution is obtained on the industrial scale by means of esterification with acetic acid anhydride in alkaline medium. It forms pastes and gels characterised by increased viscosity and owing to this it is used as a thickening or texture-forming agent. Both the degree and site of substitution with acetyl groups depend, among other things, on the origin of starch [Singh *et al*., 2004] as well as conditions of acetylation [Golachowski, 2003; Wang & Wang, 2002]. Acetylated starch is characterized by reduced susceptibility to the activity of amylases [Coma *et al*., 2006].

A combination of all the discussed so diversified modifications, having a common result of increasing resistance to the activity of amylases, may lead to obtaining a modified preparation with novel properties, most of all with increased resistance to amylases.

The research was aimed at producing, under various conditions, a preparation of potato starch complexed with monoacylglycerol, subjecting it to retrogradation and next acetylation as well as at determining the effect of production method on properties of the modified preparations obtained.

MATERIALS AND METHODS

Production of preparations. Starch preparations were obtained according to Scheme 1 (symbols used further in the paper were given in brackets). Native starch was first used to prepare 10% paste which was then subjected (or not subjected) to hydrolysis with Promozyme pullulanase (Genecor). Next, Dimodan (a mixture of distilled monoglycerides obtained from hydrogenated rapeseed oil, Danisco) dissolved in ethanol was added at a ratio of 1:5 in respect of starch to the paste heated to a temperature of 90°C. A portion of paste was kept for 1 h in an autoclave at a temperature of 121°C. The pastes were left at a temperature of 20°C till the next day, and then their 5-kg portions were frozen for 3 days at a tem-

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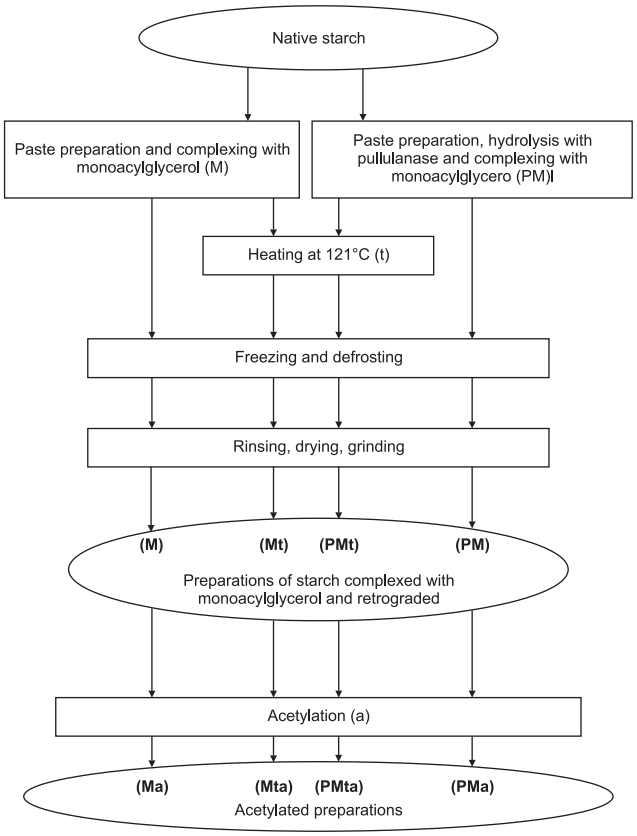

SCHEME 1. Production procedure of starch preparations complexed with monoacylglycerol, retrograded and acetylated.

perature of -20°C and defrosted for two days at a temperature of 20°C. The precipitated starch with spongy structure was rinsed three times with distilled water and next with ethyl alcohol at a temperature of 55°C. Having filtered the alcohol, the preparation was dried at a temperature of 35°C, ground and sieved through a screen with mesh size of 400 μm.

The process of acetylation was run analogously to the method using in Polish starch enterprises [Mężyński, 1972] using – due to considerable swelling of preparations of retrograded starch – threefold higher volume of water. A 200-g portion of starch was transferred into the reactor's vessels and supplemented with distilled water to a total mass of 1230 g. Next, at constant stirring and pH adjustment to a range of 8-9 (by means of a 3% solution of NaOH), 26.2 mL of acetic acid anhydride were added dropwise. Having added the whole volume of anhydride, the mixture was mixed, left for 15 min and acidified with a 10% solution of HCl (pH 5.2-5.6). Acetylated starch was rinsed with distilled water to remove residues of reagents, next dried at a temperature not exceeding 35°C, comminuted and sieved through a screen with mesh size of 400 μm.

Analyses. The obtained preparations of starch complexed with monoacylglycerol and retrograded (M, Mt, PM, PMt) as well as their acetylated modified preparations (Ma, Mta, PMa, PMta) were determined for: the per cent of acetylation – with the titrimetric method [Golachowski 2003], and water absorption and solubility in water with a temperature of 80°C [Golachowski & Brzeski, 2001].

Further analyses were carried out to determine:

(1) thermal characteristics of gelatinization – with the use of a DSC 822 differential calorimeter (Mettler Toledo), in a temperature range of 30-200°C and heating rate of 10°C/min. The analysis was carried out in ME-29990 pressure vessels using ca. 10 mg of a weighed portion of starch and 30 μL of water and an empty reference vessel;

(2) characteristics of paste formation by 6% aqueous starch dispersions – with the use of a Brabender viscosimeter. The analysis was carried out using a measuring can (700 cmg) at a stirring rate of 75 rpm. The suspension was heated from 40°C to 94°C with a rate of 1.5°C/min, and kept at this temperature for 10 min. The paste produced in this way was cooled to 30°C (1.5°C/min) and kept at this temperature for 10 min;

(3) flow curves of 5% pastes at a temperature of 50°C – with the use of an RS 100 rotary viscosimeter (Haake), at a shear rate of 0-300 s^{-1} using coaxial cylinder (Z38) with a single clearance as a measuring element. The flow curves were described by means of two rheological models:

Ostwald de Waele's: $\tau = K \cdot \dot{\gamma}^n$ (1)

Casson's: $\tau = \tau_{0C}^{0.5} + (\eta_C \cdot \dot{\gamma})^{0.5}$ (2)

where: $\dot{\gamma}$- shear rate (s^{-1}), τ – shear stress (Pa), K – consistency coefficient ($Pa \cdot s^n$), n – flow index, τ_{0C} – yield point (Pa), and η_C – Casson's plastic viscosity (Pa·s).

(4) dynamics of saccharification with amyloglucosidase (amigase by Genecor) of the preparations after production and after gelatinization at a temperature of 100°C. The content of free glucose was determined colorimetrically using a reagent for glucose concentration assay by Biosystem that contained glucose oxidase and peroxidase. The analysis was conducted as follows: acetate buffer with pH=4.5 was added to a suspension of the starch preparation. The flask was fixed in a water bath of a shaker at a temperature of 37°C and supplemented with an enzyme solution. The concentration of enzyme was adjusted so that the complete saccharification of gelatinized natural starch occurred after 120 min of the process. Each hour, 5 mL samples of hydrolysate were collected for centrifugation. From the centrifuged sample, supernatant was collected that was next with a reagent by Biosystem, incubated at a temperature of 20°C for 15 min and measured for absorbance by means of a CECIL CE 2010 colorimeter at a wavelength of λ=500 nm. Measurements were performed against a blank sample, *i.e.* reagent with acetate buffer. The content of glucose was read out from a curve plotted as above using analytically pure glucose solutions. Saccharification was computed in respect of the theoretical content of glucose produced from the total saccharification of a weighed portion of starch. The result of hydrolysis was claimed final when three subsequent results did not differ between one another.

Results were analysed statistically using an analysis of variance at a confidence level of $p<0.05$ [Stanisz, 2001].

RESULTS AND DISCUSSION

The conducted analyses referred not to native (granular) starch, but to multiply-modified starch possessing an ability to re-formation of starch paste while heated in water.

The thermal DSC characteristics of the examined starched proves their heterogeneity (Table 1). Native starch heated with monoacylglycerol at a temperature of 90°C (preparation M) was characterised by two phase transitions with relatively high enthalpy (4.0, 3.7 J/g), the first ranging from 60 to 80°C originating from amylopectin transformation and the second one in the range of 105-130°C indicating the presence of various forms of the amylose-lipid complex (AML). Heating the starch paste with lipids leads to the formation of complexes whose properties are determined by the length of amylose chains, type of lipid and heating temperature [Gelders *et al*., 2004]. Complexes formed at a temperature of up to 60°C are soluble in water at *ca*. 100°C. The two crystalline forms of AML complexes formed at higher temperatures dissociate in a temperature range of 100-125°C [Tufvesson & Eliasson, 2000], whereas complexes with lower themorstability may transform into more thermally-stable forms at higher temperatures of heating [Biliaderis & Galloway, 1989]. Keeping the paste at a temperature of 121°C (preparation Mt) evoked a decrease in heat of phase transitions (1.1, 2.5 J/g) and an increase (by *ca*. 15°C) of the temperature of second transition. Reduction in the heat of phase transition is probably linked with thermolysis of starch proceeding under experimental conditions, whereas in increase in the temperature of the second transition – with the formation of crystalline forms of the AML complex and potential retrogradation of non-complexed amalysose characterised by a very high thermal stability [Kohyama *et al*., 2004]. Hydrolysis of branched bonds in starch with pullulanase, carried out in this study and aimed at facilitating its retrogradation, caused the occurrence of starch in three forms (preparation PM). The first form dissociating in a temperature range of 60-80°C may indicate the presence in the preparations of dextrins complexed with monoacylglycerol, the second form of starch was the AML complex with phase transition proceeding at *ca*. 100°C, and the third form was the AML complex with a crystalline structure and phase transition proceeding at a temperature of *ca*. 125°C. Keeping the paste after hydrolysis with pullulanase at an elevated temperature (preparation PMt) evoked an increase in the heat of phase transition of the first form and the formation of solely crystalline form of the AML complex, which is linked with a lack of transition at a temperature of *ca*. 100°C.

TABLE 1. The per cent of acetylation – with the titretic method and temperatures of gelatinization, enthalpy of starch preparations determined from DSC thermal characteristics.

Sample	Acetyl group (%)	Temperature (°C)			Enthalpy (J/g)
		onset	end	peak	
M	—	61.7±0.6	76.3±1.4	67.9±0.4	3.7±0.4
		105.1±1.2	130.4±1.5	116.5±2.2	4.0±0.3
Ma	3.3±0.1	59.8±0.3	75.9±0.3	65.9±0.4	2.7±0.2
		99.2±0.8	119.5±1.6	109.3±0.9	2.4±0.1
Mt	—	63.2±0.50	72.0±0.2	67.0±1.2	1.1±0.1
		122.7±0.9	140.7±0.1	131.1±2.3	2.5±0.3
Mta	3.7±0.1	49.7±0.1	73.1±0.2	59.7±1.1	5.7±0.1
		114.2±0.1	136.8±0.1	126.2±1.3	2.6±0.2
PM	—	63.2±0.6	73.7±0.6	66.7±1.0	2.0±0.3
		98.1±1.9	112.5±2.8	105.8±4.8	0.8±0.4
		120.1±1.5	133.1±6.3	123.3±4.9	0.9±0.4
PMa	3.1±0.1	63.5±2.0	74.0±0.1	66.2±0.8	1.8±0.3
		118.7±0.3	30.7±0.3	124.6±1.3	0.3±0.2
PMt	—	63.5±0.2	73.7±0.4	66.6±0.3	3.5±0.1
		117.5±0.2	133.1±0.3	125.6±1.1	0.8±0.1
PMta	2.9±0.1	60.1±0.2	74.6±0.1	65.4±0.9	2.4±0.1
		111.9±0.5	130.4±0.1	121.8±0.5	0.9±0.1

Native starch heated with monoacylglycerol at a temperature of 90°C (preparation M) or at 121°C (preparation Mt) was characterised by high susceptibility to acetylation, *i.e.* 3.3, 3.7% (Table 1). Starch preparations hydrolysed with pullulanase (PM and PMt) in the process of acetylation were esterified to a slightly smaller extent, *i.e.* 2.9, 3.1%. Such a high susceptibility to acetylation is most likely linked with the porous structure of modified starch. The effect of starch granules size, hence the surface of starch, on the degree of starch acetylation is known and described [Chen *et al.*, 2004].

Acetylation of retrograded starch preparations triggered changes in the course of thermal DSC characteristics. The heat of phase transition was changing as affected by the chemical modification applied. No dependencies could, however, be found that would elucidate direction and extent of those changes. Due to a lack of literature data on acetylation of retrograded starch, that subject should be explored in further works. Acetylation caused also a decrease in temperature of phase transition of dissociation of both retrograded amylopectin fractions and AML complexes. Some reports exist on the dependency of a decrease in the initial temperature of native starch gelatinization along with an increase in acetylation degree [Golachowski, 2003] and a decrease in temperature of heat transition of gelatinization of acetylated starch as compared to the native starch [Adebowale *et al*., 2006]. It is accepted that the increasing range of temperatures is affected by disruption of crystalline structures present in starch granules. In turn, the value of transition heat depends on damage of the structure of starch chains arranged in double helices [Singh & Singh, 2001].

Preparations M and Mt were characterised by the same water absorption and water solubility reaching *ca*. 34 g/g and 6%, respectively (Figures 1 and 2). Hydrolysis of branched bonds applied during the production of preparations caused a significant decrease in water absorption and an increase in solubility of the starch preparations examined. Yet, greater changes occurred in the case of PM preparation (10 g/g and 44%) as compared to PMt preparation (21 g/g and 31%). The effect of starch depolymerization on the discussed properties has been confirmed in a number of papers [Golachowski & Brzeski, 2001; Leszczyński, 1992]. Differences between PM preparation and PMt preparation, which in the production process was kept at a higher temperature, were probably linked with the formation of solely crystalline forms of the

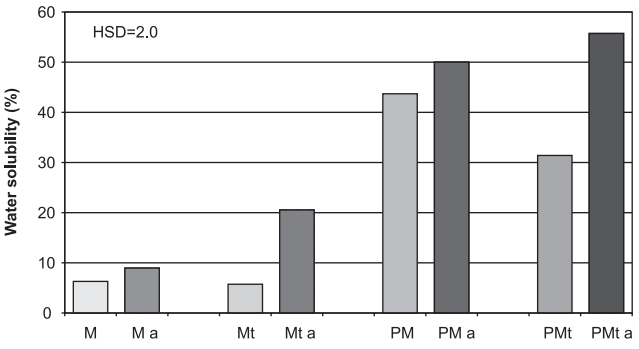

FIGURE 1. Water solubility of starch preparations determined at 80°C.

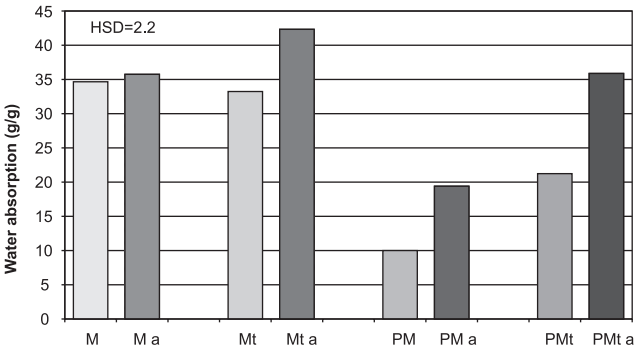

FIGURE 2. Water absorption of starch preparations determined at 80°C.

AML complex, which was described in the first part of the paper. The acetylation process evoked an increase in both water solubility and water absorption of the starch preparations. An exception was Ma preparation which did not demonstrate any significant differences in behaviour in water as compared to the preparation not modified chemically. It should be emphasized that the process of acetylation affected to a greater extent preparations subjected to hydrolysis with pullulanase than those not subjected to that process during production procedure. The PMa and PMta preparations increased their water absorption to *ca*. 20 and 36 g/g, and solubility to *ca*. 50 and 56%, respectively. Acetylated starch with a low degree of substitution used in the food industry is characterised by increased solubility in water, water absorption and viscosity of pastes formed [Golachowski, 1998].

During heating in an aqueous solution native starch first swells, then forms a paste, and after cooling forms gel characterised by high viscosity. The range of gelatinization temperatures, viscosity of paste or gel, and their resistance to shearing forces are, among other things, a measure of starch usability as a thickening or a texture-forming agent [Fortuna & Gałkowska, 2006]. Those properties are determined, among others, by the origin of starch, its granularity or modifications applied. The course of the characteristics of M starch paste formation is similar to the gelatinization characteristics of native starch with only one difference, *i.e.* after an initial significant increase of viscosity and reaching the maximum viscosity of paste formation (1325 B.U.) a rapid decline was observed in viscosity under the influence of mechanical forces (stirring) damaging the structure of paste (Figure 3). The minimal viscosity was

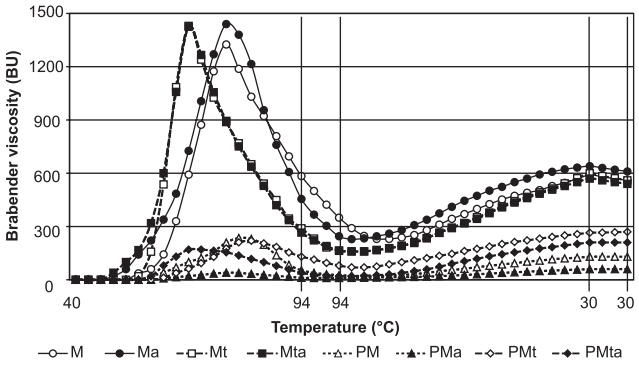

FIGURE 3. Pasting characteristics of prepared starch.

nearly 5 times lower than the maximum viscosity of the paste. During cooling there occurred a typical increase in the viscosity of a formed gel. The viscosity of paste prepared from starch M at a temperature of 30°C reached 590 B.U. The hydrolysis of 1,6 bonds, carried out in the study, caused a drastic decline in starch viscosity (preparation PM) in the entire course of characteristics. The maximum viscosity read out from the characteristics accounted for 235 B.U., whereas at a temperature of 30°C – for 130 B.U. The heating treatment at a temperature of 121°C run during the production of preparations did not evoke any significant changes in the viscosity of pastes. Only a shift of paste formation temperature was observed, which was of special significance in the case of the PMt preparation. Temperature of the maximum viscosity of paste prepared from the PMt preparation was lower by *ca*. 10°C than that of paste obtained from the PM preparation. It was not reflected in the above-described thermal DSC characteristics. This may be elucidated by a lack of correlations between gelatinization temperatures determined with various methods [Leszczyński, 1989]. Unlike in the case of native starches, the acetylation of starch preparation did not result in any significant increase in the viscosity of starch pastes. In turn, a decrease of the initial temperature of gelatinization typical of acetylated starches [Zheng *et al*., 1998] was observed in the study, which was also reflected in the determined thermal DSC characteristics.

Flow curves presented in Figure 4 divide the preparations produced into two groups demonstrating considerable differences in the rheological properties of the pastes formed. The main factor differentiating the preparations is hydrolysis

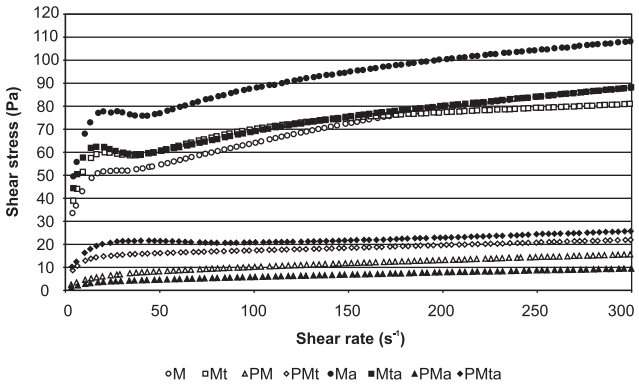

FIGURE 4. Flow curve of prepared starch paste.

of branched bonds in starch. Pastes obtained from preparations PM, PMt, PMa and PMta are characterised by several times lower values of shear stress in the entire course of flow curves plotted than the pastes obtained from preparations M, Mt, Ma and Mta. The shear stress of pastes produced from enzymatically-modified preparations at the maximum shear rate ranged from 10 to 26 Pa, whereas that of the other preparations – from 81 to 108 Pa. The highest values of shear stress were observed for the paste obtained from starch complexed with monoacylglycerol at a temperature of 90°C and subjected to the acetylation process (Ma). A characteristic shape of the first part of the curve (up to 50 s^{-1}) is linked with the accumulation of a part of energy after the first period of a very rapid increase of shear stress (up to 20 s^{-1}). An increasing shear rate (20-50 s^{-1}) is accompanied by the release of accumulated energy, which results in a temporary decrease of shear stress. All pastes characterised by higher values of shear stress behaved alike in the initial phase of flow. Parameters of the rheological models applied, used to describe the flow curves, were compiled in Table 2. The mathematical models applied in the study well described the experimental results obtained ($R^2 \geq 0.95$). The only exception was the paste obtained from the MPta preparation whose determination coefficient R^2 was lower than 0.90. The flow index n is a measure of deviation from the Newtonian flow, and the more it differs from unity, the more the viscosity is affected by shear rate [Schram, 1994]. Shearing-diluted character of the starch pastes flow is consistent with literature data [Korus *et al*., 2004]. A very low value of the n index, ranging from 0.11 to 0.40, indicates high susceptibility of the pastes examined to dilution by shearing. The pastes obtained from preparations M, Ma, Mt and Mta are characterised by a relatively high coefficient of consistency K (22-48 $Pa \cdot s^n$), being a measure of paste viscosity in the initial phase of shearing, a high yield point τ_{oc} (36-63 Pa), and low values of Casson plastic viscosity η_C (14-40 mPa·s), being a measure of paste viscosity in the final phase of shearing. Undoubtedly, this indicates a high initial resistance of the pastes analysed to flow as well as their dilution during shearing. All pastes obtained from preparations subjected to enzymatic modification during the production process (PM, PMa, PMt, PMta) were characterised by a low viscosity, which was indicated by low values of K and η_C coefficients.

TABLE 2. Rheological parameters of prepared starch.

Sample	Model of Oswald de Waele			Model of Casson		
	K ($Pa \cdot s^n$)	n (-)	R^2	τ_{oc} (Pa)	η_C (mPa·s)	R^2
M	22.26	0.24	0.99	36.40	40	0.99
Ma	48.10	0.14	0.95	63.11	20	0.97
Mt	37.55	0.14	0.98	50.31	14	0.96
Mta	34.62	0.12	0.95	47.48	20	0.98
PM	1.88	0.37	0.99	3.89	13	0.99
PMa	0.93	0.40	0.97	2.03	9	0.99
PMt	8.28	0.17	0.98	11.73	5	0.97
PMta	12.85	0.11	0.89	16.31	3	0.87
HSD	3.74	0.05	-	2.46	1	-

Figures 5 and 6 depict the dynamics of starch preparations saccharification with amyloglucosidase. The preparation of starch complexed with monoacylglycerol and subjected to retrogradation was characterised by *ca*. 90% saccharification that was reached after 3 h of hydrolysis (Figure 5). Similar effects of enzymatic activity were observed in all preparations not modified chemically. Their maximum saccharification ranged from 85 to 90%, and time of saccharification reached 2-3 h. The only exception was the PMt preparation which as early as after one hour was saccharified in 86%. The only factor that affected, to a significant extent, the susceptibility of preparations to enzymatic degradation was acetylation of starch. All chemically-modified preparations increased their resistance to amyloglucosidase by *ca*. 30%, whereas the time of reaching maximum saccharification was observed to elongate by one hour on average. Hydrolysis of gelatinized starch (Figure 6) proceeded much faster. After 1-2 h all non-acetylated preparations were subject to the maximal saccharification, which increased by 2-5% as compared to the non-gelatinized starch. Acetylated preparations were saccharified in 63%, and the period of hydrolysis reached 3 h. The only exception was the PMta preparation which was subject to saccharification in a shorter period of time and its susceptibility to enzymatic degradation was higher by *ca*. 9%. Resistance of retrograded starch to amylases accounts for *ca*. 12%, whereas that of the acetylated starch – for as little as a few per cents [Thanh *et al*., 2007]. This, the resistance (*ca*. 40%) of a starch preparation complexed with monoacylglycerol, retrograded and acetylated is not a simple sum of resistance values resulting from various modifications. Elucidation of that fact, however, requires further investigations.

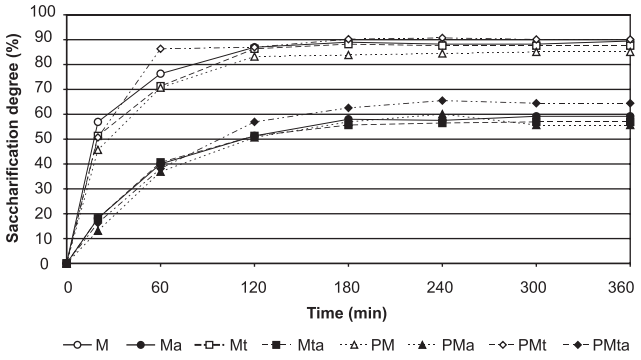

FIGURE 5. The dynamics of starch preparations saccharified with amyloglucosidase.

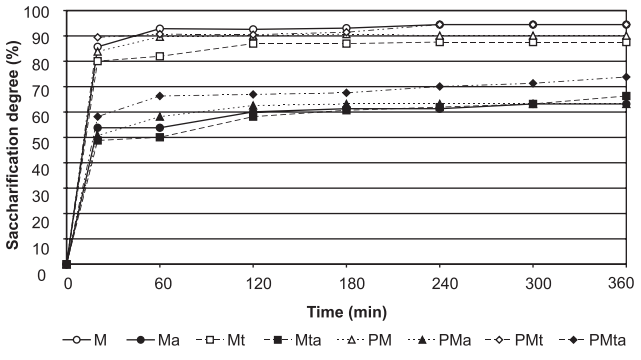

FIGURE 6. The dynamics of gelatinized starch preparations saccharified with amyloglucosidase.

CONCLUSIONS

1. The method of preparing complexed starch with monoacylglycerol was observed to affect the properties of the resultant starch preparations, and the tendency and extent of changes depended on the type of modification applied.

2. Hydrolysis of starch with pullulanase caused a reduction in water absorption of preparations, viscosity of prepared pastes and values of shear stress in the entire course of flow curves as well as in susceptibility to acetylation. It evoked an increase in the initial temperature of pastes formation and an increase of water solubility of the produced starch preparations. Modification of with pullulanase had no significant effect on the susceptibility of the preparations to the activity of amyloglucosidase.

3. Keeping the starch paste with monoacylgylcerol at a temperature of 121°C caused the formation of crystalline forms of the starch-lipid complex. That treatment resulted in decreased solubility and increased water absorption of the preparation produced from starch subjected to hydrolysis with pullulanase before complexing with monoacylglycerol, yet it did not affect those characteristics in the case of the preparation not subjected to the process of hydrolysis. Heating had no significant effect on the rheological properties of pastes nor on the susceptibility of preparations to the activity of amyloglucosidase.

4. Acetylation of starch preparations increased their water absorption and solubility in water and diminished the initial temperature of pasting. A considerable reduction in the susceptibility to amyloglucosidase activity (by *ca*. 40%) and a lack of significant changes in the rheological properties of the prepared pastes predispose the acetylated preparations of starch complexed with monoacylglycerol and retrograded as a food additive with thickening and prebiotic properties.

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WYBRANE WŁAŚCIWOŚCI SKROBI ZIEMNIACZANEJ PODDANEJ WIELOKROTNEJ MODYFIKACJI FIZYCZNEJ I CHEMICZNEJ

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Celem pracy było wytworzenie w różnych warunkach preparatu skrobi ziemniaczanej kompleksowanej z monoacyloglicerolem, poddanie jej retrogradacji, a następnie acetylacji oraz określenie wybranych właściwości powstałych modyfikatów. Wyznaczono charakterystykę termiczną kleikowania DSC, charakterystykę tworzenia kleiku za pomocą wiskozymetru Brabendera, krzywe płynięcia kleików w temperaturze 50 °C, dynamikę scukrzania amyloglukozydazą. Wielokrotna modyfikacja skrobi powodowała znaczne zmiany jej właściwości, a kierunek i wielkość tych zmian zależała od rodzaju przeprowadzanej modyfikacji. Acetylowane preparaty skrobi kompleksowanej z monoacyloglicerolem i retrogradowanej charakteryzowały się znaczną odpornością na działanie amylaz (ok. 40%) oraz właściwościami umożliwiającymi branie udziału w kształtowaniu cech reologicznych czy teksturotwórczych produktów w których skład będą wchodziły.

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EFFECT OF FREEZING AND FROZEN STORAGE ON FATTY ACID PROFILE OF CALVES' MEAT

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Key words: calves, meat, freezing, fatty acids, linseed, rapeseed, fish oil

The aim of the study was to determine the effect of frozen storage of veal on the composition and proportion of fatty acids in meat fat. Thirty bull calves, divided into 6 equal groups aged from 7 to 90 days, were fed concentrates differing in fat sources. After slaughter the meat samples from the thoracic muscle (fresh and after 3-month frozen storage) were analysed for the level of fatty acids. The composition of fatty acids in meat fat was determined by the type of dietary fat. Analysis of frozen meat samples showed a decrease in $C_{16:1}$ (by approx. 15%) and a higher ($p \leq 0.05$) sum of all fatty acids compared to fresh meat. There was a tendency towards increased PUFA ($C_{18:1}$ and $C_{18:2}$ n-6), EPA and DHA in meat fat after frozen storage.

INTRODUCTION

The health-promoting value of meat depends largely on the fat content and fatty acid composition. Due to the high level of saturated fatty acids, ruminant meat is considered to be the main factor behind several diseases of modern civilization such as obesity, atherosclerosis or cancer [Jimenez-Colmenero *et al*., 2001]. Many recent studies have shown that the appropriate feeding of animals can modify the composition of meat fat towards increasing the level of polyunsaturated fatty acids (PUFA) [Scollan *et al*., 2001; Raes *et al.*, 2003; Cooper *et al.*, 2004]. Feeding oilseeds rich in linoleic acid or fish oil rich in long-chain fatty acids EPA and DHA increases the level of n-3 PUFA in meat and improves the n-6/n-3 acid ratio [Strzetelski *et al*., 2003; Rule *et al*., 1994; Zymon *et al*., 2005]. The composition of fatty acids in meat fat affects not only the palatability and dietetic value, but also the storageability of meat. Freezing is the most natural method of preparing meat for long storage, enabling its full nutritive value to be preserved. During freezing and frozen storage, meat is subject to certain physicochemical qualitative changes, the type and extent of which depend largely on the method and rate of freezing [Petrowić *et al*., 1993]. Most of these changes, including changes in consistency, changes in colour or meat weight loss are associated with the formation of ice crystals. Ice formation results in protein denaturation, leading to lower water binding capacity [Boles & Swan, 1996]. During frozen storage, lipids are subject to some changes, mainly autooxidative and hydrolytic changes. The rate and extent of fat autooxidation depends, among others, on the degree of fatty acid saturation, oxygen exposure, and storage time and temperature [Tomás & Aòón, 1990]. Previous studies were primarily concerned with the effect of the freezing process on the physicochemical and organoleptic properties of meat, mainly buffalo meat and pork, less frequently beef [Berry & Leddy, 1989; Ngapo *et al*., 1999; Kandeepan & Biswas, 2005; Sen & Sharma, 2004]. Studies concerning the effect of freezing on fat composition and quality were conducted mainly with fish [Fernandez-Reiriz *et al*., 1995; Ortiz & Bello, 1992]. The aim of the study was to determine the effect of frozen storage of veal on the composition and proportion of fatty acids in meat fat.

MATERIALS AND METHODS

A total of 30 Polish Holstein-Friesian bull calves were randomly assigned based on the analogue principle to 6 groups (5 animals per group). Bulls were kept on perforated wooden floor in cages equipped with drinkers and feed troughs. Animals were fed individually according to IZ-INRA standards [2001]. Milk replacer, in which dried whey and soy protein concentrate were the main sources of protein, was diluted by dissolving 167 g of the powder in 1 L of water and fed from 7 to 56 days of age. Concentrates were fed throughout the experiment (7 to 90 days). The diets contained (%): ground barley (40.5-55.5), ground wheat (13-30.5), wheat bran (6-13), soybean meal (12.5-15.5), mineral-vitamin preparation (3), and different sources of fat in the form of linseed cv. Omega (10) or Linola (10), rapeseed cv. Spencer (10) or Contact (10), or fish oil (4). At the end of the study, 90-day-old bulls were slaughtered and after 24-h cooling of carcasses meat samples were taken from the thoracic muscle for analysis. Some samples were cold stored at 2-4°C for the next 24 h until analysis, and the other samples were sealed in plastic bags, frozen and stored at -18°C for 3 months. The meat samples were analysed before and after frozen storage for the level of higher fatty acids. Extraction of the total lipids was done us-

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TABLE 1. Fatty acid proportion in vegetable oils and fish oil (g/100 g fatty acids).

Fatty acid	Linseed Omega	Linseed Linola	Rapeseed Spencer	Rapeseed Contact	Fish oil
$C_{14:0}$	0.00	0.13	0.00	0.00	6.92
$C_{16:0}$	8.69	7.52	4.75	4.28	22.51
$C_{18:0}$	3.83	3.18	1.72	1.57	2.33
$C_{18:1}$	18.20	13.00	56.87	67.78	25.23
$C_{18:2}$ n-6	26.76	72.90	30.14	13.61	5.18
$C_{18:3}$ n-3	41.87	2.84	3.71	9.63	3.41
$C_{20:5}$ n-3 EPA	0.00	0.04	0.00	0.03	7.87
$C_{22:6}$ n-3 DHA	0.00	0.06	0.00	0.00	12.91
SFA	12.99	11.04	7.49	6.93	32.39
PUFA n-6	26.76	72.90	30.14	13.61	5.28
PUFA n-3	41.87	2.98	3.71	9.66	25.06

SFA – Saturated Fatty Acids; PUFA – Polyunsaturated Fatty Acids

ing chloroform/methanol (2/1; v/v) according to the method of Folsch *et al*. [1957]. The lipids were transmethylated using NaOH/MeOH followed by BF_3/MeOH. The fatty acid methyl esters were analysed by gas chromatography using a Varian 3400 gas chromatograph (column SUPELCOWAX 10, 30 m; 0.53 mm ID; 1.0 μm; temp. program. 50-280°C; injector 200(C; detector 260(C; helium as the carrier gas – 6 mL/min). The results were analysed statistically by two-way analysis of variance using the SAS package [2000].

RESULTS

The fat sources used in the experiment differed in the profile of fatty acids (Table 1).

Linseed cv. Omega was characterized by the highest proportion of linolenic acid, whereas the fat of linseed cv. Linola had the highest concentration of linoleic acid. Rapeseed cv. Spencer and Contact contained the highest proportion of oleic acid, which accounted for over half of all the acids. Fish oil was characterized by the highest proportion of n-3 PUFA, in particular EPA and DHA.

The composition of fatty acids in veal fat varied according to experimental group (Table 2). In all the groups, there was a tendency towards a lower level of saturated fatty acids (SFA) in relation to the control group, although the differences were not significant ($p>0.05$). A significant decrease ($p\leq0.05$) in the level of palmitic acid $C_{16:0}$, in relation to the control group, was found when feeding linseed cv. Omega, and in the level of stearic acid $C_{18:0}$ when calves received fish oil. Linseed cv. Omega, characterized by a 48% proportion of linolenic acid $C_{18:3}$ n-3, increased approx. 4-fold ($p\leq0.05$) the level of this acid in meat fat in relation to the other groups. Feeding fish oil caused a several-fold increase ($p\leq0.05$) in the level of long-chain n-3 fatty acids in meat fat, including eicosapentaenoic (EPA), docosapentaenoic (DPA) and docosahexaenoic acids (DHA). Feeding calves with linseed cv. Omega, rapeseed cv. Contact, and especially fish oil caused a marked decrease ($p\leq0.05$) in the n-6/n-3 ratio in relation to the control group.

After three months of frozen storage of meat, there was a significant decrease ($p\leq0.05$) in the level of $C_{16:1}$, and an

TABLE 2. Composition of fatty acids in veal fat (% of total fatty acids; n=60).

Fatty acid	Storage time (in months) n=30		p	Groups* n=10						p	SE	Interaction
	0	3		C	LO	LL	RS	RC	FO			
$C_{14:0}$	1.10	1.15	0.7	1.16	1.21	0.96	1.06	1.13	1.22	0.8	0.06	-
$C_{16:0}$	17.10	17.09	0.9	18.21ab	15.86b	16.61ab	16.47ab	16.86ab	18.58a	0.02	0.27	-
$C_{16:1}$	2.27a	1.93b	0.02	2.06	2.39	1.93	1.81	2.21	2.19	0.2	0.08	-
$C_{18:0}$	12.95	12.86	0.7	13.50ab	12.69b	12.77b	14.08b	13.14ab	11.27c	<0.01	0.17	-
$C_{18:1}$	28.39	28.85	0.7	29.28	29.38	28.50	27.61	30.29	26.64	0.5	0.55	-
$C_{18:2}$n-6	19.41	20.01	0.5	18.91	19.60	22.47	20.58	18.78	17.93	0.1	0.51	-
$C_{18:3}$n-3	1.12	1.00	0.3	0.56b	2.95a	0.59b	0.60b	1.03b	0.64b	<0.01	0.12	-
CLA	0.12	0.11	0.4	0.09	0.09	0.10	0.11	0.13	0.14	0.2	0.01	-
$C_{20:5}$n-3	0.88	1.01	0.5	0.35b	0.63b	0.33b	0.62b	0.59b	3.15a	<0.01	0.16	-
$C_{22:5}$n-3	1.08	1.15	0.4	0.82b	1.19b	0.93b	1.01b	1.11b	1.64a	<0.01	0.05	-
$C_{22:6}$n-3	0.36	0.41	0.4	0.18b	0.28b	0.21b	0.19b	0.37b	1.08a	<0.01	0.05	-
Sum	95.67a	96.14b	0.04	95.91	95.94	96.21	95.87	95.77	95.75	0.9	0.11	-
Others	4.33	3.86	0.4	4.09	4.06	3.79	4.13	4.23	4.25	0.9	0.10	-
SFA	32.67	32.54	0.8	34.70	31.07	31.66	33.02	32.54	32.64	0.08	0.36	-
PUFA n-3	3.81	3.90	0.8	2.00c	5.15b	2.13c	2.66c	3.39c	7.79a	<0.01	0.33	**
PUFA n-6	27.21	27.81	0.7	26.48	26.88	31.04	29.59	25.98	25.11	0.2	0.76	-
n-6/n-3	9.44	9.83	0.5	13.25a	5.41c	14.66a	12.06a	8.58b	3.87c	<0.01	0.60	-

* Concentrates for groups: C – control; LO – with linseed Omega; LL – with linseed Linola; RS – with rapeseed Spencer; RC – with rapeseed Contact; FO – with fish oil; $p>0.05$ – non-significant differences; a, b, c – $p\leq0.05$