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SELECTED PHYSICOCHEMICAL PROPERTIES OF FATTY ACID ESTERS WITH MONO- AND DISACCHARIDES

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In the reported research, the reaction of glucose esterification with oleic acid was run with the use of a commercial preparation of lipase originated from Candida Antarctica yeast – Novozymes 435 – as a catalyst. Selected properties of the resultant ester were investigated in comparison with properties of glucose and its complex with oleic acid. Results obtained were compared with properties of a commercial preparation of saccharose stearate, a pure disaccharide and the obtained complex of saccharose with stearic acid.

A biotechnological method was used to synthesize glucose oleinate with a degree of substitution reaching DS=0.35. The synthesis proceeded at a low temperature (60°C) at atmospheric pressure without using solvents toxic to humans. Simultaneously, a similar reaction was carried out without the use of enzyme, which enabled obtaining a complex of glucose with fatty acid. The achieved reaction products were characterised by properties different from those of a pure substrate – glucose. In addition, the character of those changes was similar as in the case of saccharose and its fatty derivatives. The ester of glucose and that of saccharose were characterised by lower heats of phase transitions than pure saccharides and their complexes with lipids. The complex of glucose with oleic acid showed high heat of phase transition and high temperature of phase transition as compared to pure glucose and its ester. Saccharose stearate reached lower values of the heat of phase transition and temperature of phase transition in respect of a pure disaccharide and its mixture with stearic acid. Solubility of glucose oleinate, in contrast to that of the other substances examined, did not increase along with increasing temperature.

INTRODUCTION

Saccharides, apart from proteins and lipids, constitute a widely distributed in nature, heterogenous group of polyhydroxyl aldehydes and ketones. In nature there occur not only saccharides in their natural form but also derivatives with amine groups, as well as esterified, oxidized and reduced or glycolipids. The most common monosaccharides are hexoses, including glucose – referred to as grape sugar. Chemical properties of glucose result from the presence of a carbonyl group and hydroxyl groups in its molecule. As polyhydroxyl compounds, glucose and saccharose readily undergo reactions of esterification. Glucose – as a monosaccharide – contains 5, whereas saccharose – 8 free hydroxyl groups. Reactions of mono-, di- and oligosaccharides with higher fatty acids lead to the synthesis of esters with properties of surface-active compounds, being of great significance in the food, pharmaceutical and chemical industry [Ganske *et al*., 2005; Sikorski, 2000]. Esters of fatty acids and saccharides are compounds that display emulsifying and stabilizing properties [Cao *et al*., 1999; Lortie, 1997; Otto *et al*., 1998; Tarahomjoo & Alemzadeh, 2003; Tsitsimpikou *et al*., 1997; Ward *et al*., 1997; Watanabe *et al*., 2000; Yan *et al*., 1999]. Depending on the number of introduced acyl groups it is possible to obtain products differing substantially in physicochemical properties from the same substrates. Those various properties are described by a coefficient of hydrophilic-lipophilic balance (HLB) which for saccharide esters attains values ranging from 1 to 18 [Antczak *et al*., 2004]. The method of chemical synthesis of esters of saccharides and fatty acids has been first described by Osipow *et al*. in 1956 [Kołakowski *et al*., 2005]. Those reactions are run in a medium of polar organic solvents often detrimental to human health, including pyridine or dimethyl sulfoxide. In addition, they proceed at elevated temperature, which results in the formation of colorants contaminating the product. Under conditions of chemical synthesis the process usually proceeds non-specifically. Esterification with fatty acids affects both first-order and secondary hydroxyl groups of saccharides. For this reason, the end product is usually a mixture of mono- and polyesters.

Nowadays, in a number of research laboratories intensive works are underway into enzymatic synthesis of esters of saccharides and fatty acids. Particular attention is paid in those studies to lipases which in the near future should replace chemical catalysts so far applied on a commercial scale. The production of sugar esters with the use of biocatalysts is characterised by a variety of advantages as compared to chemical processes. It proceeds in the medium of harmless apolar solvents or in a two-phase system: water–solvent, in a temperature range of 30–60°C. A product of that reaction is a compound with a specified chemical structure, devoid of colour contaminants.

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A biological function of lipases is catalysis of ester bonds hydrolysis in triacylglycerols [Adach & Kobayashi, 2005; Tianwei *et al*., 2004]. This is a reversible reaction, which is due to the fact that a difference in free energy linked with a change in the course of the reaction is small and reaches *ca*. 16 kJ/mol. By changing the reaction medium into that poor in water or by removing it completely, by means of an organic solvent, it is possible to induce a change in reaction's course towards esterification [Antczak *et al*., 2004; Coulon *et al*., 1998; Gulati *et al.*, 1999; Lortie, 1997; Watanabe *et al*., 2000].

Since investigations carried out these days are mainly focused on methods of obtaining esters of saccharides and fatty acids, lesser attention is devoted to a possibility of their application in various branches of industry. Determination of some physical properties of that type of compounds is likely to facilitate taking a decision on their specific applications.

The paper elaborates on the synthesis of glucose oleinate with biotechnological method using lipase – an enzyme originating from *Candida antarctica* yeast, as well as on a comparison of selected properties of the obtained glucose ester with those of pure glucose and a glucose-oleic acid complex. Diversity in the physical properties of glucose derivatives was referred to a comparison of the properties of a commercial preparation of saccharose stearate, pure saccharose and a saccharose-stearic acid complex. Investigations were also aimed at tentative determination of possibilities of applying such products in industry.

MATERIAL AND METHODS

Material. The experimental material were: a commercial preparation of extracellular lipase originating from *Candida antarctica* and immobilized on acryl resin (Novozymes A/S 435, Sigma); crystalline glucose produced in 2005 by „Przedsiębiorstwo Przemysłu Spożywczego S.A." in Łomża, Poland; analytically pure saccharose provided by Chempur co. from Pikary Śląskie, Poland; oleic acid (Fluka Chemika); stearic acid (POCh S.A. Gliwice, Poland); and saccharose ester E 473 (Sisterna).

Enzymatic esterification of glucose with oleic acid [Otto *et al*., 1998]. Crystalline glucose was dried in a vacuum dryer. A reaction mixture was prepared that contained equimolar (0.12 mol) amounts of saccharide and fatty acid, *i.e.* 22.0 g of glucose and 34.5 g of oleic acid. A 5-g portion of immobilized enzyme Novozymes A/S was added to the mixture. Tert-butanol in the amount of 2-fold equivalent of the sum of substrates masses was used as a reaction medium. In order to absorb water generated as a by-product of esterification use was made of a molecular sieve (0.4 nm) in the amount of 20% of the sum of substrates masses. The reaction was carried out for 14 day in an oil bath at 60°C under constant stirring (250 rpm). Afterwards the product was extracted with 200 mL of acetone with a temperature of 50°C within 1 h and next filtrated. Precipitate from the filter was dried at a room temperature and stored in an air-tight container.

Synthesis of complexes (mixtures) of saccharides and fatty acids. Mixtures of saccharides and fatty acids were prepared in 300-mL conical flasks as follows: 0.1 mol of glucose and 0.1 mol of oleic acid as well as 0.05 mol of saccharose and the same amount of stearic acid.

The flasks were sealed with a stopper and fixed for 10 days in an incubator at 70°C. Next, 100 mL of acetone were added to a mixture of glucose and oleic acid and the mixture was shaken for 1.5 h at a temperature of 50°C. Extraction of the saccharose and stearic acid mixture was carried out with 100 mL of acetone under a reflux condenser, for 4 h, at a temperature of 50°C. Next, the mixture was filtrated and the precipitate from filters was dried at a room temperature and stored in air-tight containers in a refrigerator.

Determination of thermal characteristics of the substances examined using a Toledo model 822^e differential scanning calorimeter (DSC) by Mettler. *Ca*. 10-mg portions of glucose, saccharose, a glucose complex, a saccharose complex, saccharose stearate and glucose oleinate were weighed into aluminum vials. Having sealed the vials, the samples were successively fixed in a DSC furnace for 5 min at 25°C. Measurement was performed during heating to 250°C at a rate of 6°C/min. Determinations were carried out for: total and specific heat of phase transitions, initial and final temperatures of phase transitions as well as temperatures of extrapolated peak centres.

Determination of solubility of esters and complexes of saccharides and fatty acids at a temperature of 30°C and 80°C [Richter *et al*., 1968]. In 300-mL conical flasks there were prepared 250 mL of 1% solutions (on dry matter basis) of complexes and esters of saccharides and fatty acids. Samples were kept for 30 min at 30°C or at 80°C at constant stirring. Next the samples were cooled and loss of matter was supplemented. Contents of flasks were poured into 4 tared centrifugal vials (50 g into each), centrifuged for 30 min at 21.000 ×g, at a temperature of 20°C, using a Biofuge 28RS centrifuge by Heraeus Sepetach. Supernatant was collected from the centrifugal vials and determined for dry matter content. Next, solubility of the examined substances was determined using the following formula:

$$R=(a/b)\cdot 100\ (\%)$$

where: R – solubility (%), a – dry matter of supernatant (g), and b – dry matter of solution (g).

Determination of a degree of glucose substitution with oleic acid. The obtained ester of glucose and oleic acid was analysed using ^{1}HNMR. The degree of substitution was determined as a ratio of 1/3 surface area of a signal of terminal group CH_3 of oleic acid at 0.86 ppm to 1/9 surface area of proton signals of a glucose ring in a range of 3.3-3.9 ppm.

Statistical analysis. Results obtained were evaluated with one-way analysis of variance. Homogenous groups were determined with Duncan's test at a significance level of $\alpha=0.05$ using STATISTICA ver. 7.1 software.

RESULTS AND DISCUSSION

Based on the ^{1}HNMR analysis, it was observed that the activity of lipase used in the study resulted in the formation of glucose and oleic acid ester. The degree of glucose substitution with oleic acid determined with the ^{1}HNMR method reached 0.35. It means that on average every third glucose ring was esterified with fatty acid. Tsitsimpikou *et al*. [1997] examined lipases from *Candida rugosa* and *Candida antarctica* for their usability in the synthesis of saccharide esters. They applied glucose, fructose, mannose and arabinose as saccharide substrates, whereas lauric acid (C_{12}) – as a hydrophobic substrate. In the synthesis of all esters the highest activity was observed for lipase originating from *Candida antarctica*. Both enzymes displayed higher activity in the presence of hexoses as saccharide substrates. In their study, elevation of reaction temperature from 30°C to 50°C caused a 2-fold increase in glucose conversion, both in the case of reaction catalysed by lipase from *Candida antarctica* as well as with that from *Candida rugosa*. Based on the cited data, in our study in the reaction of glucose esterification catalysed by lipase use was made of an enzyme originating from *Candida antarctica* yeast and the process was carried out at a temperature of 60°C fro 14 days. The reaction mixture contained equimolar amounts of saccharide and oleic acid, whereas tert-butanol was applied as a reaction medium. Solvents used as a reaction medium in the synthesis of saccharide esters should be characterised by a high capacity for solubilization of substrates, thus enabling their esterification. In the case of the production of esters to be used as food additives of key significance is solvent admission for use in the food industry [Cao *et al*., 1999; Ganske & Bornscheuer, 2005; Tarahomjoo & Alemzadeh, 2003; Yan *et al*., 1999]. Advisability of every single selection of organic reaction medium at a change of substrates and, at least, the form of an enzymatic preparation has been confirmed in a study by Degna & Zimmermann [Chen *et al*., 2005]. In carrying out synthesis of glucose myristate, catalysed by immobilized lipase of *Candida antarctica*, they examined a number of solvents, yet they observed that the reaction proceeded with the highest efficiency in tert-butanol.

Apart from the nature of solvent, the efficiency of enzymatic synthesis of saccharides and fatty acids esters is determined, to a significant extent, by the content of water in medium which inhibits the course of the process. Thereby, it was necessary to apply an effective method of removing water – a by-product of the esterification reaction – from the medium. While stipulating reaction conditions in our study, we decided to solve that problem by using in the reaction dried glucose as well as by direct addition of a molecular sieve (0.4 nm) at the amount of 20% of the total mass of substrates to the reaction mixture. Literature lacks data on enzymatic synthesis of glucose and oleic acid ester, especially with so high degree of substitution as that obtained in our study. Properties of the ester obtained were compared with those of crystalline glucose, its complex with oleic acid obtained without the use of enzymes as well as with properties of a commercial preparation of saccharose and fatty acid ester.

Total heat and specific heat of phase transitions of glucose and its fatty derivatives analysed by means of DSC were statistically significantly different (Table 1). Substitution of one hydroxyl group in statistical every third glucose molecule with a residue of oleic acid evoked a 1.8-fold increase in the initial and final melting point, as compared to unprocessed glucose. Perhaps it was due to the fact that disruption of an ester bond requires higher energy input as compared to energy expenditure linked with damage of the crystalline structure of glucose before esterification. Even more distinct elevation of temperature range of phase transition in respect of crystalline glucose was reported in the case of its complex. A twofold increase in the temperature of the onset and the end of transition of the complex as compared to glucose is likely to indicate that in the course of 10-day equimolar interaction between glucose and oleic acid in the mixture at 70°C there proceeded chemical reactions with an unsaturated bond of omega-9 acid and hydroxyl group of glucose. Literature data point also to other examples of changes in thermal properties of glucose as a result of its modification, *e.g.* shift of initial temperature of phase transition from 75°C for a monosaccharide – glucose – to 120°C for isoglucose syrup with moisture content of 24% [Coulon *et al*., 1998].

Different tendency of changes was observed in the case of saccharose and its derivatives. In a group of fatty derivatives of that disaccharide a decrease was observed in the initial and final temperature of phase transition as compared to disaccharide before the reaction (Table 2). The initial temperature phase transition of the complex was lower by 5.5°C and the final one by 8°C than thermal parameters of saccharose. More distinct differences were observed in comparison to saccharose stearate. Both the initial and final temperature of ester transition were subject to a 3-fold decrease in respect of saccharose. Such a tendency of changes amongst parameters of thermal char-

TABLE 1. Specific heat (Q_w), initial temperature (t_p), final temperature (t_k) and temperature of extrapolated peak centre (t_e) of the phase transition of glucose and its fatty derivatives.

Substance	Q_w (J/g)	t_p (°C)	t_k (°C)	t_e (°C)
Crystalline glucose	-53.07*	74.65*	81.58*	79.12*
Glucose oleinate	-37.52**	131.74**	145.13**	139.05**
Glucose complex	-130.32***	147.86***	154.52***	150.96***
NIR	4.47	1.16	0.46	0.57

Asterisks (*) denote homogenous groups (significance level α=0.05)

TABLE 2. Specific heat (Q_w), initial temperature (t_p), final temperature (t_k) and temperature of extrapolated peak centre (t_e) of the phase transition of saccharose and its fatty derivatives.

Substance	Q_w (J/g)	t_p (°C)	t_k (°C)	t_e (°C)
Saccharose	-102.53*	189.93*	195.44*	192.65*
Saccharose stearate	-45.42**	52.04**	60.39**	55.85**
Saccharose complex	-107.14*	174.35***	187.37***	180.16***
NIR	5.74	1.19	1.17	1.32

Asterisks (*) denote homogenous groups (significance level α=0.05)

TABLE 3. Solubility of fatty derivatives of saccharose and glucose at 30 and 80°C.

Substance	Solubility at 30°C (g/g)	NIR	Solubility at 80°C (g/g)	NIR
Saccharose stearate	17.66*	0.26	99.83*	0.12
Saccharose complex	99.76**		99.87*	
Glucose oleinate	58.27*	0.20	62.95*	0.11
Glucose complex	99.84**		99.88**	

Asterisks (*) denote homogenous groups (significance level $\alpha=0.05$)

acteristics is typical of derivatives of that disaccharide, *e.g.* melting point of sorbitol accounts for 101.7°C, that of isomalt for 142°C, and that of mannitol for 169°C [Borde & Cesaro, 2001].

Table 3 provides a comparative analysis of solubility at 30°C between the examined esters and complexes of the saccharides analysed. Glucose oleinate was characterised by over 3-fold higher solubility at 30°C as compared to saccharose stearate (58.27% and 17.66%, respectively). In turn, differences in solubility at 30°C between the complex of glucose and saccharose (99.84% *vs*. 99.76%) were statistically insignificant.

In the case of a complex system of saccharides with fatty acids, the solubility at 30°C was considerably higher than that of the analogous esters of those substances. Those differences were more tangible in the case of saccharose derivatives. Solubility at 30°C of a saccharose complex exceeded that of saccharose stearate 5.6 times, whereas in the group of glucose derivatives it was 1.7 times higher in the case of the complex.

Solubility of saccharose ester at a temperature of 80°C reached 99.83%, and that of glucose ester – 62.95% (Table 3). In turn, solubility of complexes of sacchorse and glucose with fatty acids at 80°C was similar to the solubility of monosaccharides in the natural form.

Fatty derivatives of saccharose and the complex of glucose with fatty acid in a solubility range at 80°C did not demonstrate statistical differences. Only solubility of glucose stearate was by 37% lower than that of the other substances.

CONCLUSIONS

1. It is possible to synthesize glucose oleinate (DS=0.35) with a biotechnological method using a biocatalyst of esterification reaction – extracellular lipase from *Candida antarctica*.

2. Esters of saccharides and fatty acids were characterised by a lower heat of phase transitions than pure saccharides. In contrast, complex systems of saccharides with fatty acids displayed higher heat of phase transition as compared to pure saccharides. Saccharose stearate was characterised by lower values of heat and temperatures of phase transition in respect of pure disaccharide and the complex with stearic acid.

3. Solubility of glucose oleinate, in contrast to that of the other substances examined, did not increase along with elevated temperature.

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NIEKTÓRE WŁAŚCIWOŚCI FIZYCZNE ESTRÓW KWASÓW TŁUSZCZOWYCH Z MONO- I DISACHARYDAMI

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W niniejszej pracy przeprowadzono reakcję estryfikacji glukozy kwasem oleinowym z wykorzystaniem jako katalizatora handlowego preparatu lipazy pochodzącej z drożdży *Candida antarctica* – Novozymes 435. Badano wybrane właściwości otrzymanego estru w porównaniu z właściwościami glukozy oraz jej kompleksu z kwasem oleinowym. Uzyskane wyniki porównano z własnościami handlowego preparatu stearynianu sacharozy, czystego dwucukru i uzyskanego kompleksu sacharozy z kwasem stearynowym.

Metodą biotechnologiczną zsyntetyzowano oleinian glukozy o stopniu podstawienia DS = 0,35. Synteza przebiegała w niskiej temperaturze (60°C) przy ciśnieniu atmosferycznym bez użycia toksycznych dla człowieka rozpuszczalników. Równocześnie przeprowadzono podobną reakcję bez użycia enzymu dzięki czemu uzyskano kompleks glukozy i kwasu tłuszczowego. Uzyskane produkty reakcji charakteryzowały się odmiennymi właściwościami od czystego substratu – glukozy. Jednocześnie charakter tych zmian był podobny, jak w przypadku sacharozy i jej pochodnych tłuszczowych. Ester glukozy i ester sacharozy odznaczały się niższymi ciepłami przemian fazowych niż czyste cukry i ich kompleksy z tłuszczami. Kompleks glukozy z kwasem oleinowym cechował się wysokim ciepłem przemiany fazowej oraz wysokimi temperaturami przemiany fazowej w porównaniu do czystej glukozy i jej estru. Stearynian sacharozy osiągnął mniejsze wartości ciepła przemiany fazowej i temperatur przemiany fazowej w odniesieniu do czystego dwucukru i jego mieszaniny z kwasem stearynowym. Rozpuszczalność oleinianu glukozy, w przeciwieństwie do rozpuszczalności pozostałych badanych substancji, nie zwiększała się wraz ze wzrostem temperatury.