MAŁGORZATA WRONKOWSKA, MARIA SORAL-ŚMIETANA

PEA STARCH AS A SOURCE OF PHYSICALLY MODIFIED PREPARATION WITH POTENTIAL HEALTH-PROMOTING ACTIVITY

Abstract

Industrially isolated pea starch was physically processed using: gelatinization, autoclaving and cooling cycles, and dehydration by spray-drying. The experimental pea starch preparation, with crystallographic pattern of the B-type had 30% d.m. of resistant starch as the RS_2/RS_3-type and showed a hydrophilic-hydrophobic character. During *in vitro* studies the sorption of some biologically active components was recognised. Specially, the sorption of deoxycholic or taurocholic acids and ions of toxic metals as lead and cadmium was noteworthy. Also, the sorption of cholesterol was observed. Physically modified pea starch preparation is suggested as additive to 'functional foods' and as agent preventing the colon diseases.

Introduction

By the end of '90s, searching for functional foods, having health-promoting functions, has attracted the attention. The digestion process in the human gastrointestinal tract can be controlled through modification of diet. The diet components affect the composition and metabolic functions of intestinal microflora. Thus, one of the most promising areas for the development of functional foods lies in modification of the activity of the gastrointestinal tract by the use of probiotics, prebiotics and synbiotics, which are already used as food ingredients.

Food saccharides, are an important source of energy. The results for population studies carried out in fourteen countries showed that complex saccharides and degradation products of starch can decrease the risk of civilisation diseases [3]. On the basis of these studies, it has been found that the frequency of colon cancer is negatively correlated (r = -0.76) with starch intake. Monosaccharides, disaccharides and starch are

M. Soral-Śmietana, M. Wronkowska, Polish Academy of Sciences, Institute of Animal Reproduction and Food Research, Division of Food Science, Olsztyn, Poland, ul. Tuwima 10, 10-747 Olsztyn.

defined as 'digestible' carbohydrates because they are digested and absorbed in the human small intestine, but dietary fibre and resistant starch, are named 'indigestible' since they are not digested by the intestinal endogenous enzymes. All poorly digestible saccharides, which are not absorbed in the small intestine, reach the large intestine and can be fermented there by the colonic microflora. A decrease in the pH of the intestine modifies the composition and character of existing microflora, what is strongly correlated with the bile acid concentration in faeces [11]. In recent years, 'resistant starch' i.e. starch resistant to the hydrolytic activity of the human gastrointestinal enzymes is of great interest for nutritionists and physiologists. According to Björck and Asp [2], resistant starch may exert potentially beneficial influence on the human digestive tract by: lowering the pH in the colon, generating the short-chain fatty acids in the colon, increasing glucose tolerance, lowering the blood lipid level. Resistant starch is an inert component in the small intestine but in the large intestine, it can have the prebiotic function as a potential energy source for colonic microflora.

Legume seeds are generally considered as a good source of starch with beneficial features arising from remarkably high levels of slow-released and resistant starch fractions. Food processing modifies the chemical nature of starch, by the way of hydration and disruption of the organized granule structure.

The objective of the study was to indicate the possibility of application of pea starch in the form of physically-modified preparation, having the resistant starch and sorptive activity towards some biological compounds.

Materials

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

Experiment

The starch preparation was obtained from granular commercial pea starch. This preparation was made in a laboratory scale using some physical processes. The suspen-

sion of pea starch in distilled water (1:3.5) was autoclaved (121°C/1 h) and then cooled at 4°C/12 h. The autoclaving/cooling cycles were performed three times. The retrograded gel was homogenized with distilled water and spray-dried at 130°C inlet temperature and 50°C outlet temperature.

Methods

Chemical components: nitrogen was determined by Kjeldahl method and ash was determined after mineralisation in muflon oven at 700°C according to standard chemical methods [1]. Starch content was analysed according to ICC Standard No. 123, by hydrochloric acid dissolution [8].

Functional properties: water holding capacity (WHC) and oil sorption were assessed according to Soral-Śmietana et al. [12].

The resistant starch analysis was carried out using the Champ's procedure (the A-method) [4]. The sample (100 mg) was incubated with 500 U porcine pancreatic alpha-amylase at 37°C for 16 h. The products of hydrolysis were extracted with 80% ethanol and the extracts were discarded. Undigested material was dissolved in 3 ml of 2 M KOH and hydrolysed with amyloglucosidase (20 U) at 65°C for 90 min. Free glucose was analysed using the oxidase-peroxidase glucose test, measuring the absorbance at 500 nm in 1 cm cuvette.

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

Estimation of the sorption properties towards metal ions: Pb^{2+}, Cd^{2+}, was performed by the electrochemical method (polarography DPP ASV), within the potentials of -1550 to -160 mV. The rate of the potential change was 10mV/s. Before the measurement, oxygen was removed from the samples by 10-min perfusion with argon. The measurement was performed at 36°C and lasted 20 h. Samples (100 mg /10 ml) were placed in buffered solutions of pH 6.4 and 2.2. Sorption was rapid and completed after 20 min; being irreversible after 20 h.

The cholesterol sorption was measured by *in vitro* analysis. The sample (100 mg) was combined with an emulsion (lecithin, sodium salt of deoxycholic acid and cholesterol) prepared in 0.1 M phosphate buffer of pH 6.8 (2 ml). The 1-h incubation was

carried out on shaking at 37°C. The kinetics of the cholesterol sorption by 20 μl emulsion was analysed in 10-minute intervals using reagent kits. The results were expressed as per cent of cholesterol sorption by the sample at each time.

The sorption of bile acids (cholic, deoxycholic, taurocholic) was measured by the *in vitro* analysis. The sample (100 mg) was treated with solution of each bile acid (10 ml). The solutions were prepared in 0.1 M phosphate buffer pH 7.6 for each bile acid in 2 μM/ml concentration. The samples and blanks were incubated at 37°C for 30 minutes. Centrifugation was carried out at 2000 x g for 5 min. The sample (50 μl) was treated on agitation with 70% sulfuric acid (5 ml) and freshly prepared solution of furan-2-aldehyde (2.3 g/l) (1 ml). Absorbance was measured at 510 nm after 80 minutes. The results were expressed as per cent of the bile acid sorption.

The scanning electron microscope (SEM) micrographs were obtained after spraying the dry starch preparation with gold in a JEE 400 vacuum evaporator and visualised in JSM 5200 at the acceleration of 10 KeV.

Results and discussion

The chemical compositions of native pea starch and pea starch preparation are presented in Table 1. The microstructure (SEM) of native pea starch, the source of investigated preparation is presented in Fig. 1, and the structure of pea starch preparation is presented in Figs. 2 a-b.

Table 1

Chemical composition of native pea starch and its starch preparation[1].

Sample	Starch [% d.m.]	Nitrogen [%d.m.]	Ash [%d.m.]	RS content	
				of sample [% d.m.]	of total starch [%]
Native pea starch	99.5 ±1.25	0.19 ±0.02	0.12 ±0.01	42.6 ±2.9	42.8 ±1.9
Pea starch preparation	84.9 ±1.87	0.13 ±0.02	0.30 ±0.02	29.8 ±1.9	35.0 ±2.2

[1] Values given are means of four replications ± standard deviation

The SEM-pictures of the preparation subjected to autoclaving and spray-drying were characteristic of starch gel particles dehydrated in the flow of the drying air. Different size and shape of characteristic collapses in the central part in large particles could be observed. These new structures are typical of dehydrated starch gels of different origin [10, 14, 15]. The process of rapid dehydration of colloidal suspension of such organic polymers as proteins of plant and animal origin has been previously observed with similar results for microstructure [6, 7, 9, 13]. Thus, the microstructure

image after spray-drying of colloidal organic polymer suspensions is characteristic rather of the process than dried material.

Fig. 1. SEM-electronogram of native pea starch.

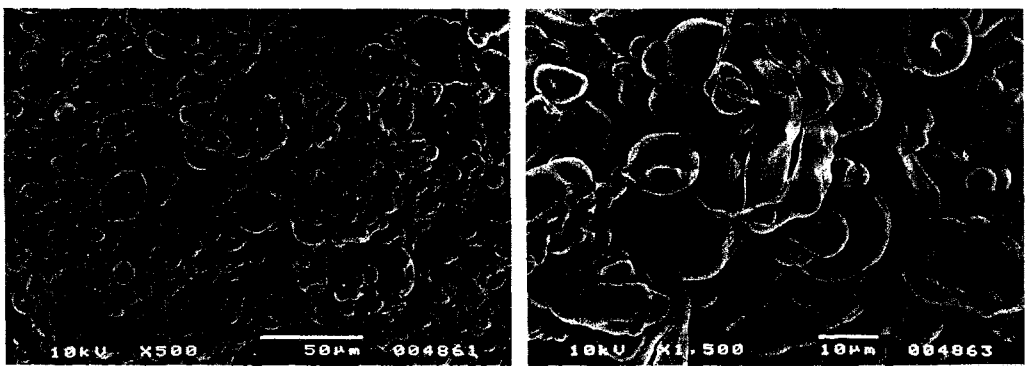

Fig. 2 a, b. SEM-electronograms of pea starch preparation.

The chemical analysis of pea starch (Table 1) and SEM-pictures (Fig. 1), clearly indicate high purity of the material. Additionally, it should be stated that the physical processes of gelling, retrogradation and dehydration used in the study caused that the resistant starch content in the experimental preparation was about 30% (Table 1). The pea preparation had crystallographic pattern of type B, as measured by X-ray diffraction [G. Lewandowicz, non published data]. On the basis of the kinetics of 24-h hydrolysis with pancreatic alpha-amylase, it was stated that the preparation has the properties similar to these of very resistant starch. The degree of hydrolysis within 6 to 24-h was insignificant (Fig. 3). It was interesting to observe the effect of the physical processes used on the affinity of the pea starch preparation towards water and oil (Table 2).

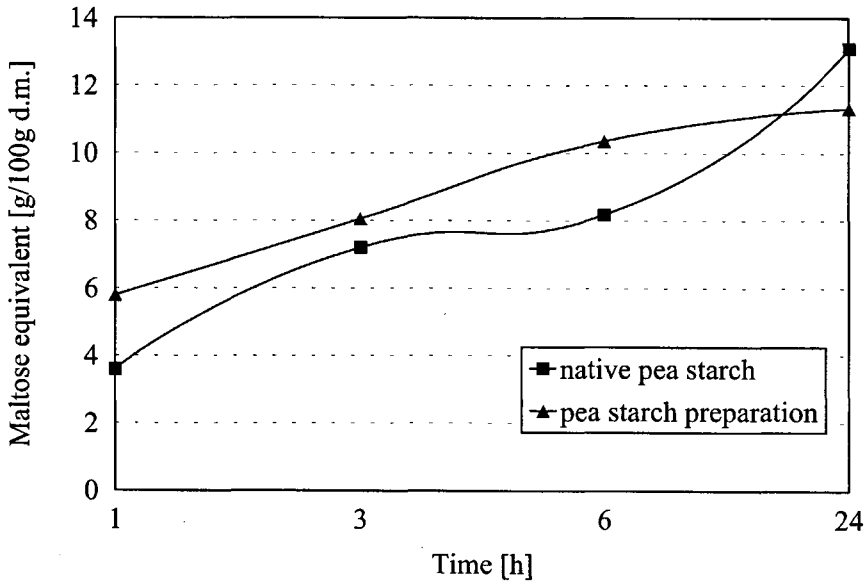

Fig. 3. The kinetics of hydrolysis of the native pea starch and its starch preparation by pancreatic alpha-amylase.

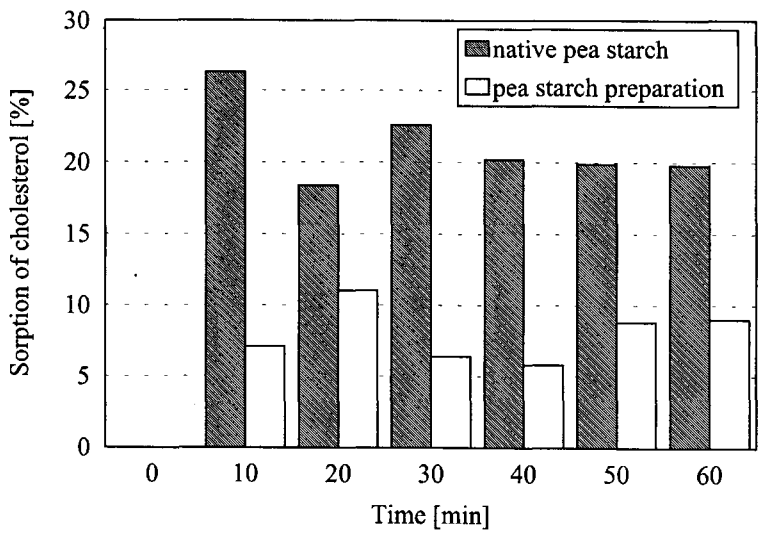

Fig. 4. Sorption of cholesterol by native pea starch and its starch preparation.

Table 2

Some functional properties of native pea starch and its starch preparation[1].

Sample	Water holding capacity [g water/g d.m. sample]	Oil sorption [g oil/g d.m. sample]
Native pea starch	0.96 ±0.02	1.50 ±0.01
Pea starch preparation	1.07 ±0.03	1.74 ±0.01

[1] Values given are means of four replications ± standard deviation

Assessment of the functional properties revealed that the starch preparation increased slightly the ability for binding water and oil. Taking into consideration the microstructure of pea preparation, it can be suggested that this ability is affected by the size and surface of the particles as well as by the chemical nature of the preparation. However, comparing the sorption of water and oil, the starch preparation seems to have the hydrophilic-hydrophobic character but with increased number of hydrophobic domains. This phenomenon has already been described, when the analysis of the possibility of forming complexes between physically-modified starches and nutrients was undertaken [5].

In the present study, experimental preparation of pea starch containing about 30 % RS was investigated considering its sorption properties towards cholesterol, bile acids and selected metals.

The cholesterol sorption ability of the experimental pea starch preparation was poorer than that of native starch (Fig. 4). However, the maximum cholesterol sorption for the starch preparation reached about 11 % after 20 min of reaction, while the native starch reached the maximum already after 10 min with about 26% sorption of this substance.

Sorption of bile acids by the tested preparation was determined on the basis of interaction with the following acids: cholic, deoxycholic and taurocholic (Tab. 3). The starch preparation had high sorptive ability towards deoxycholic and taurocholic acid, as compared to native pea starch. Special attention should be paid to the affinity of the pea starch preparation to these acids, since they are the secondary bile acids being the degradation substrates of primary bile acid. They have been shown to be involved, as a promoting agents, in the adenoma-carcinoma sequence of colorectal cancer [3]. Therefore, significant affinity of our experimental preparation to secondary bile acids may be important in prevention of the large intestine diseases.

Table 3

Sorption of the bile acids by native pea starch and its starch preparation[1].

Sample	Cholic acid [%]	Deoxycholic acid [%]	Taurocholic acid [%]
Native pea starch	12.79 ±4.0	4.30 ±2.0	3.83 ±1.5
Pea starch preparation	9.35 ±3.2	46.32 ±5.5	9.94 ±3.4

[1] Values given are means of four replications ± standard deviation

Table 4

Complexing of lead and cadmium by native pea starch and its starch preparation (μg/100 mg of sample).

Sample	Pb^{2+}		Cd^{2+}	
		r^2		r^2
Native pea starch	16.0	0.91	0.32	0.89
Pea starch preparation	22.0	0.88	0.45	0.87

r^2- regression coefficient

In the study, complexing of lead and cadmium as the antagonists of such important bioelements as magnesium and zinc was also investigated. The amounts of complexed Pb^{+2} and Cd^{+2} are presented in Table 4. In the case of the pea starch, higher sorption ability towards both ions was clearly seen. There are some unproven assumptions suggesting that both these ions occur in minimum concentrations in living organism where they, probably, play a positive physiological function [6]. On the other hand, lead and cadium ions are regarded toxic and the sorptive properties of the examined pea preparation towards these ions can also be used in health prophylaxis.

Conclusions

1. It should be emphasised that the physical modification of pea starch produced the preparation with the resistant starch content reaching 30%.
2. The preparation of pea starch, containing average amount of indigestible starch, has the affinity to such secondary bile acids such as deoxycholic and taurocholic acids, and to lead and cadmium ions.
3. The properties of pea starch preparation may suggest its use as additive to 'functional foods' to promote health and prevent the colon diseases.

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SKROBIA GROCHOWA JAKO ŹRÓDŁO FIZYCZNIE MODYFIKOWANEGO PREPARATU O POTENCJALNEJ AKTYWNOŚCI PROZDROWOTNEJ

Streszczenie

Izolowaną przemysłowo skrobię grochu poddano procesom fizycznym takim, jak: kleikowanie, cykle autoklawowania, cykle chłodzenia oraz odwodnieniu poprzez suszenie rozpyłowe. Eksperymentalny preparat skrobi grochowej, o strukturze krystalograficznej typu B, wykazujący charakter hydrofilowo-hydrofobowy, zawierał 30% skrobi amylazoopornej typu RS_2/RS_3. W wyniku badań *in vitro* oznaczono właściwości sorpcyjne w stosunku do niektórych biologicznych komponentów. Odnotowano szczególną aktywność sorpcyjną w stosunku do drugorzędowych kwasów żółciowych, deoksycholowego i taurocholowego oraz jonów metali toksycznych, jak ołów i kadm. Zaobserwowano też zdolność do sorpcji cholesterolu. Mając na uwadze uzyskane wyniki, można sugerować otrzymanie preparatu skrobi grochu fizycznie modyfikowanej, który może znaleźć zastosowanie w 'żywności funkcjonalnej', jako składnik o znaczeniu zapobiegawczym w chorobach jelita.