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RESISTANT STARCH OF PEA ORIGIN

Abstract

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

Introduction

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

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

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

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

Material

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

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

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

Methods

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

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

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

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

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

The cholesterol sorption was measured by the *in vitro* analysis. The sample (100 mg) was combined with emulsion composed of: 1% lecithin, 1.375% sodium salt of deoxycholic acid and 0.225% cholesterol prepared in 0.1 M phosphate buffer pH 6.8. (2 ml). The 1-h incubation was carried out on shaking at 37°C. The kinetics of cholesterol sorption by 20 μl emulsion was analysed for 10-minute intervals using reagent

kits. The results were expressed as per cent of cholesterol sorption by the sample at each time interval.

The scanning electron microscope (SEM) analysis was conducted for the native pea starch, pea-RS concentrate from this starch and the pea-RS concentrate after 24-h hydrolysis by pancreatic alpha-amylase. Samples were lyophilised, then mounted on aluminium stubs with double sided adhesive tape, held in nitrogen stream to remove loosely stuck particles, coated with gold in a JEE 400 vacuum evaporator and observed in JSM 5200 microscope at 10 kV.

Results and discussion

Comparison of the chemical content of pea starch and its preparation indicated that the latter was the concentrate of resistant starch with mineral and organic nitrogen compounds (Table 1). Higher levels of ash and nitrogen compounds may result not only from the concentration of starch components but also from the contribution of commercial enzymatic preparation Termamyl. However, the level of particular components in resistant starch concentration is dependent mostly on botanical source of starch granules. This is proved by comparing the pea-RS concentrate obtained from pea starch in this study with the preparations previously obtained from wheat and potato starches [12]. If we assume the resistant starch in the pea-, wheat- and potato-preparations as the major component, then at similar amounts of RS obtainable from respective starches the smallest amount of the accompanying was found for the pea starch preparation. The content of nitrogen and mineral compounds was twice as high in wheat RS-preparation and three times higher in the potato one [12]. The crystallographic pattern in the X-ray diffractograms was untypical. It was neither of the A- nor B-type [G. Lewandowicz, unpublished data]. Comparison of microelectronograms (SEM) of native pea starch granules (Fig. 1) with the pea resistant starch concentrate (Fig. 2a, 2b) allowed to observe fine granular subunits in the structure of the preparation (Fig. 2a) as well as crystalline shells with granular subunits on the edges of the surface (Fig. 2b). This picture was similar to that described by Gallant et al. [5], who studied alpha-amylolysis of granular starch of different origin. From 24-h hydrolysis of pea-RS concentrate with pancreatic alpha-amylase more compact structure resulted together with a decay of granularity as well as appearance of clear fragments of feather-like (Fig. 3a) and filament (Fig. 3b) characters. The kinetics of amylolysis (Fig. 4) shows that the availability of native pea starch for pancreatic alpha-amylase was similar to that of native wheat starch [12]. It was confirmed also by the way the enzyme attacked the granules of pea and wheat starches [11]. On the other hand, pancreatic alpha-amylolysis of pea-RS concentrate was similar to that of native potato starch considered a reference resistant II-type starch. *In vitro* enzymatic availability allows to

assume that the pea-RS concentrate closely resembles native potato starch having large amount of RS that is highly resistant to pancreatic alpha-amylase.

Table 1

Chemical composition of native pea starch and pea resistant starch concentrate[1].

Sample	Nitrogen [% d.m.]	Ash [% d.m.]	RS content in sample [% d.m.]
Native pea starch	0.2 ±0.02	0.1 ±0.01	42.6 ±1.2
Pea-RS concentrate	1.3 ±0.04	4.8 ±0.04	69.9 ±2.4

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

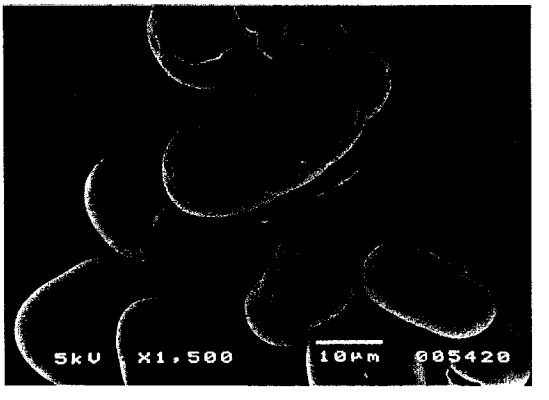

Fig. 1. SEM-microelectronogram of native pea starch.

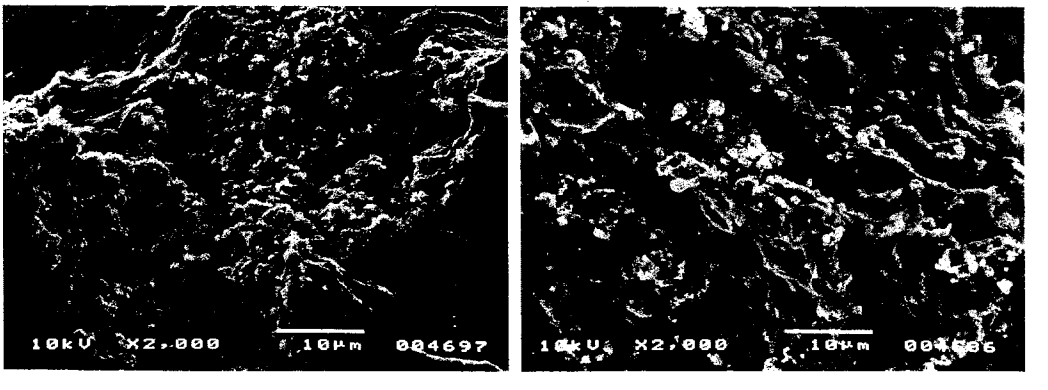

Fig. 2a; 2b SEM-microelectronograms of pea resistant starch concentrate.

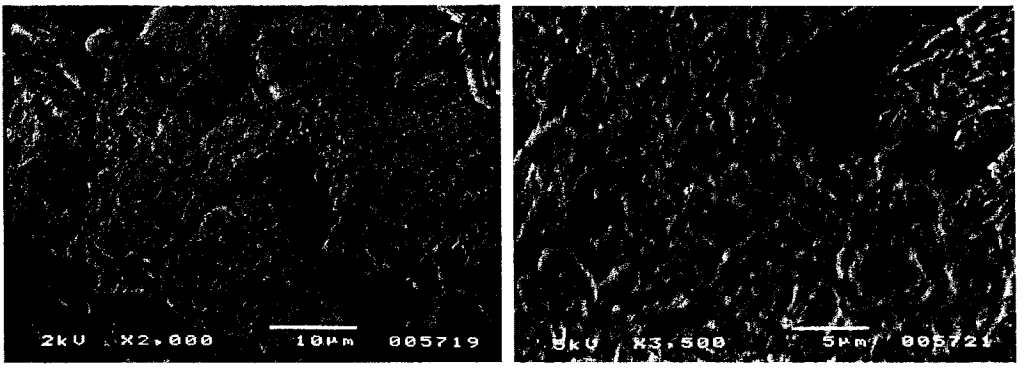

Fig. 3a; 3b SEM-microelectronograms of pea resistant starch concentrate after 24-hour hydrolysis by pancreatic alpha-amylase.

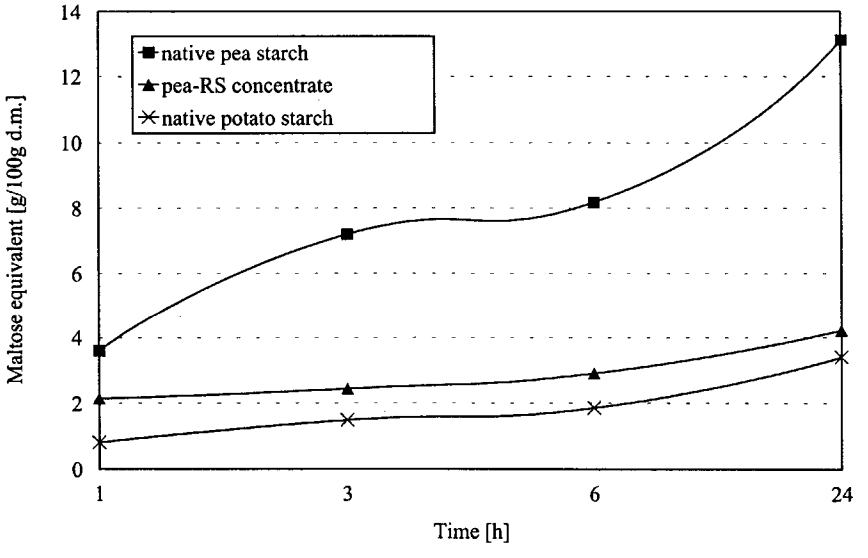

Fig. 4. The kinetics of hydrolysis of native pea starch and pea resistant starch concentrate.

Table 2

Sorption of the bile acids by native and pea resistant starch concentrate[1].

Sample	Cholic acid [%]	Deoxycholic acid [%]	Taurocholic acid [%]
Native pea starch	12.79 ±4.0	4.30 ±2.2	3.83 ±1.5
Pea-RS concentrate	0	11.62 ±3.6	0

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

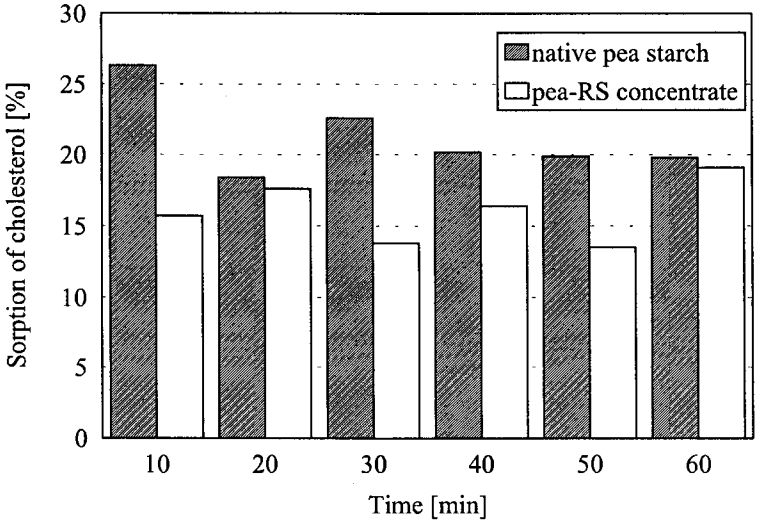

Fig. 5. Sorption of cholesterol by native pea starch and pea resistant starch concentrate.

Therefore, it was interesting to estimate the affinity of pea starch and experimental pea-RS concentrate to some hydrophobic substances such as certain bile acids and cholesterol. The results obtained for sorption of bile acids indicated considerable sorption of cholic acid by pea starch and three times waeker sorption of deoxycholic and taurocholic acids (Tab. 2). The sorption of deoxycholic acid by pea-RS concentrate was significant. The sorption of bile acids on pea-, wheat-, and potato-RS preparations is significantly different despite that the pea preparation resembled the potato-RS in this respect and in the sorption of deoxycholic acid [12]. Sorption of this acid may be of great importance in prevention of the large intestine diseases, especially in the case of patients after cholecysteomy. Present results together with our previous study [14] indicate that pea starch subjected to physical modification or physico-biochemical process has great affinity to bile acids, especially deoxycholic or taurocholic acids, which are considered as carcinogenic agents in the environment of the human intestine.

The sorption of cholesterol by native pea starch was satisfactory already after 10 min (Fig. 5). Less cholesterol was bound by the pea-RS concentrate, reaching the maximum within 1 hour. As attempted in our previous paper [8], a hypothetical model of interaction between processed starch and cholesterol is suggested here to explain the formation of specific complex with the hydrophobic tunnel domains.

Conclusions

1. Pea starch can be a good source for resistant starch concentration. Technological process with thermostable alpha-amylase provided the preparation containing up to

70% of resistant starch. It contained admixture of mineral and organic nitrogen compounds.

2. Experimental pea-RS concentrate had the affinity to secondary bile acid, deoxycholic, which considered as cancerogenic agent. Such RS-preparation has the ability for cholesterol sorption, although weaker than that of native pea starch.
3. Results of this investigations suggest that pea resistant starch concentrate may have the regulatory properties towards some hydrophobic substances. Its health-promoting properties suggest the potential use as a food component in special diets or for preventive, prophylactic, and therapeutic purposes.

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SKROBIA AMYLAZOOPORNA POCHODZENIA GROCHOWEGO

Streszczenie

Badane były możliwości uzyskania z izolowanej skrobi grochowej koncentratu skrobi amylazoopornej (RS - resistant starch) oraz określenie zdolności sorpcji niektórych substancji hydrofobowych. Stwierdzono, że skrobia grochu może być dobrym źródłem do koncentracji skrobi amylazoopornej (RS). Zastosowanie w procesie technologicznym termostabilnej alfa-amylazy umożliwia uzyskanie preparatu o zawartości ok. 70% RS. Koncentrat RS zawiera domieszkę składników mineralnych i organicznych azotowych. Wzorzec struktury krystalicznej jest nietypowy, i nie należy ani do typu A ani do typu B. Zaobserwowano, że koncentrat ten charakteryzuje się powinowactwem do kwasu żółciowego - deoksycholowego, któremu przypisuje się właściwości kancerogenne. Wykazuje też zdolność sorpcji cholesterolu, jednak nie większą niż natywna skrobia grochowa. Tak więc, grochowy koncentrat skrobi amylazoopornej (RS) może wykazywać potencjalne właściwości regulacyjne i może być użyty jako komponent do żywności w dietach specjalnych lub znaleźć zastosowanie profilaktyczno-terapeutyczne.