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PEA STARCH AS THE BASIC MATERIAL FOR PHYSICAL MODIFICATION BY ITERATED SYNERESIS

Maria Soral-Śmietana[1], Małgorzata Wronkowska[1], Grażyna Lewandowicz[2]*

[1] Division of Food Science, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland; [2] Starch and Potato Products Research Laboratory, Luboń, Poland

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In this work pea starch was modified physically acc. to the Polish patent specification P.325981 in which dehydration of pea starch solution by iterated syneresis is a dominating process. The study aimed at recongising how this modification affected the chemical composition, structure, and functional properties of pea starch and provoked formation of a starch fraction resistant to alpha-amylase. It was found that the applied modification procedure caused changes in the crystalline structure from type C to the type B of X-ray diffraction pattern accompanied by a slight increase in the relative crystallinity. It resulted in an increase in IR band at 1 016 cm^{-1} as well as the differences between relative intensity of some bands in region 1 400-400 cm^{-1}. Pea starch modification increased the ability to water sorption change to hydrophilic character, which was revealed by a decrease in the pasting temperature and an increase in viscosity. The retrograded status of both starch macromolecules, amylose and amylopectin, was also confirmed. They interpenetrated and existed as a two-phase system – parts of dense gel fragments disappeared in weak amylopectin-amylose gels. A quantitative decrease in the resistant starch content was accompanied by the change of type acc. to nutritional classification, which could promote the potential biological activity and utilisation. The physical modification process applied in this study can be recommended as a good method for improving the usability of pea starch for food applications.

INTRODUCTION

Under our climatic conditions, the seeds of grain legumes, mainly pea and bean, constitute a rich source of protein, starch, dietary fibre, and mineral components [Soral-Śmietana *et al.*, 2002]. They are being investigated for their potential in providing quality protein into the human food chain and improving human health (by plasma cholesterol reduction, heart disease and cancer prevention *etc*.) [McIntosh & Wang, 1995]. A comparative analysis of yield potential of field pea in England and France carried out in the years 1981-1998 indicated that France had regulary higher trends in the yield of pea, which was however lower than that of wheat [Biarnes-Dumoulin & Lecoeur, 1998]. Yet, in recent years, the consumption of leguminous seeds has been decreasing fairly all over the world, including Europe, which has been rising a considerbale anxiety among nutritionists. In 1996, the average European consumption of pulses was 2.5 kg per capita whereas it was 5.4 kg in Asia, 8.4 kg in Africa, and 10.0 kg in Souht America and 14.1 in Central America [Champ, 2001]. The major cause of elimination of those products from a diet is their relatively low sensory attractiveness as well as gastric disturbances faced by numerous consumers. Apart from saccharose, the main saccharides of grain legume seeds include oligosaccharides of the raffinose family. As the human gastrointestinal tract lacks an endoenzyme, α-galactosidases are not hydrolysed and their metabolism proceeds only in the large intestine through enzymes of the microflora existing therein. The production of metabolic gases is dyscomfortable to a consumer and therefore many valuable components are eliminated from a diet.

Starch of grain legume seeds is an interesting polysaccharide macrocomponent. It has been applied as a raw material for the production of amylose, sugars, and dextrins [Bogracheva *et al.*, 1998] or for non-food uses, *e.g.* production of paper, adhesives, thermoplastics [Colonna *et al.*, 1995]. Due to the possibility of genetic modifications [Hedley, 2001], pea starch may contain a higher amount of amylose fraction. It has been recognised in Europe as high amylose (*ca.* 60-85% amylose) pea starch and was searched toward non-food products [van Soest *et al.*, 2002]. These authors suggested that for packaging applications high amylose pea starch seems to be an interesting material for the potential production of starch-based bioplastics.

From the nutritional point of view, starch of pulses is characterised by a low water sorption into starch granules. The content of macromolecule – amylose – in starch of leguminous seeds, *i.e.* faba bean, often exceeds 35% [Soral-Śmietana & Dziuba, 1995]. The occurrence of a considerable natural concentration of resistant starch to alpha-amylase hydrolysis has been shown in bean seeds [Soral-Śmietana *et al.*, 2002] and in pea starch, which indicates potential health-promoting activity [Wronkowska & Soral-Śmietana, 2000]. The industrially-isolated pea

*Author's address for correspondence: Maria Soral-Śmietana, Institute of Animal Reproduction and Food Research, ul. Tuwima 10, 10-747 Olsztyn, Poland; tel.: (48 89) 523 46 51; fax: (48 89) 524 01 24; e-mail: soral@pan.olsztyn.pl

starch (normal yellow smooth pea) seems to be a good material for the concentration of the resistant starch amount [Soral-Śmietana & Wronkowska, 2000].

The objective of this investigation was to study how the physical modification processes performed acc. to the Polish patent specification P.325981 [Lewandowicz *et al.*, 1998a], in which dehydration of pea starch gel by iterated syneresis is a domianting process, affect the chemical components and structure or functional properties of pea starch and provoke formation of a starch fraction resistant to alpha-amylase.

MATERIAL AND METHODS

Material. The pea starch "Nastar" (Cosucra, Belgium) was the basic raw material for technological procedures and analytical research. The physical modification processes followed the Polish patent specification P.325981 [Lewandowicz *et al.*, 1998a]. The pea starch was solubilised in water at a temperature of 90°C for 4 h and then left at room temperature for 24 h. The starch gel obtained was frozen below -4°C and then warmed up to room temperature. The liquid exuded during syneresis was removed from solid phase. The freezing and warming processes were repeated to obtain the products of moisture content below 20%. The product was ground to the particle size smaller than 400 nm.

Methods. Total protein (N x 6.25) and mineral compounds were determined acc. AOAC [1990, 979.09 923.03]. The resistant starch analysis was carried out using the method acc. Champ *et al.* [1999], as starch fraction was not hydrolysed by pancreatic alpha–amylase (Sigma, nr A-3176, 500000 units). The amylose content was determined acc. to Morrison and Laignelet [1983]. Starch was dissolved in urea-dimethylsulphoxide and aliquots of the solution were used for the colorimertic determination of amylose as polyiodine complex at λ=635nm. The scanning electron microscope (SEM) micrographs were obtained after sprying the starch preparations with gold and visualised in JSM 5200 microscope at the acceleration of 10 KeV. The X-ray diffractometry was carried out with a TUR 62 Carl Zeiss X-ray diffractometer under the following conditions: X-ray tube CuK (Ni filter); voltage 30 kV; current 15 mA; scanning from θ=2° to 18°. The FTIR spectra were recorded in solid state with a FTIR Bruker IFS 113v spectrometer, under the following conditions: KBr pellet (1.5 mg of sample/200 mg KBr), and resolution 2 cm^{-1}. The functional properties were determined following the procedure described by Soral-Śmietana *et al.* [1998]. Gelatinisation was monitored using a Brabender viscograph procedure of 8% water dispersion (heating/cooling (25-95°C) at the rate of 1.5°C/min; thermostating for 30 min). Light microscopy preparations were obtained as follows: native pea starch and experimental preparation were examined under the initial pasting/gelatinisation temperature acc. to Brabender viscosity curves (71°C and 58°C, respectively) and both samples at 90°C. They were kept at those temperatures for 15 min; smear was made and stained with iodine (0.33% iodine in 0.67% potassium iodine) for 2 min, and observed in a light microscope Olympus BX60.

RESULTS AND DISCUSSION

The main process applied in this study consisted in solubilisation of pea starch in water and next graduate dehydration of starch gel by iterated syneresis, which was prior studied on starches from potato, tapioca, corn, and wheat [Lewandowicz & Soral-Śmietana, 2004]. It is believed that during heating to 100°C the starch granules disrupt and form phase-separated mixtures of amylose and amylopectin. Under low temperature or no shear condition, the system is probably bicontinuous in amylose and amylopectin [Englyst & Hudson, 1997]. In the gelatinisation or pasting process, firstly the linear fraction of starch granules - amylose - present in the amorphous regions is susceptible to swelling and hydrogen bond disruption [Camire *et al.*, 1990]. Our results pointed out that the physical modification procedure applied resulted in a small increase in the content of minor constituents of pea starch granules, *i.e.* ash and protein (Table 1). Some differences were also observed in the amount of linear starch fraction – amylose – upon completion of the technological procedure. The granular structure of native pea starch with shape and size characteristic of legume starch (Figure 1a) was changed to a new, quite different structure (Figure 1b). It pointed to the interpenetration of both macromolecular fractions of pea starch - amylose and amylopectin. These two pea starch fractions formed a new semi-crystalline structure of different type as compared to raw material (Figure 2). Native pea starch revealed C-type of X-ray diffraction pattern whereas modified one – the B-type. X-ray diffraction studies proved also that the modified starch showed significant crystallinity (Figure 2). These results confirmed our previous studies on potato, wheat, tapioca, corn starches, showing that all the modified starches obtained by the same procedure [Lewandowicz *et al.*, 1998b] reveal the B-type of crystalline structure irrespective of the type of X-ray diffraction pattern of raw materials [Lewandowicz & Soral-Śmietana, 2004]. The most important difference lies in the differences in relative crystallinity. Modified starches obtained from potato, wheat, corn, and especially tapioca starches reveal a lower relative crystallinity than the native ones. In the case of pea starch, relative crystallinity of pea starch preparation seems to be a bit higher than that of native material (Figure 2).

TABLE 1. Proximate chemical composition (% d.m.) of pea starch before and after modification.

Pea starch:	Ash	Total protein [N x 6.25]	Amylose	Resistant starch
Native	0.10±0.05	0.57±0.08	36.11±1.42	31.53 ±1.60
Preparation	0.40±0.03	0.86±0.09	32.25±2.50	16. 45 ±1.59

Changes in the crystalline structure proved by X-ray diffraction studies could be also observed on infrared spectra. The differences in polysaccharides conformation in solution as well as in the solid state yield differences in the region of the 1 400-800 cm^{-1} in FTIR spectra [Belton *et al.*, 1986]. The absorbance ratio of the deconvoluted peaks at 1 047 and 1 032 cm^{-1} was found by Rindlav *et al.* [1997] to be sensitive to the degree of crystallinity of potato starch films. However, most bands arise from highly coupled C-O and C-C vibrations and precise assignment is difficult [Wilson

& Belton, 1988]. The most remarkable bands are 1 019 cm^{-1} and 1 016 cm^{-1} that rise during retrogradation of waxy corn and wheat starches [Wilson *et al.*, 1987]. In the case of native potato starch, the bands located at 1 047 cm^{-1}, 1 018 cm^{-1}, and 994 cm^{-1} are reported as the most characteristic in this region [van Soest *et al.*, 1994]. According to these authors, gelatinised starch shows intense broad band at 1 022 cm^{-1}, and three bands at 1 053 cm^{-1}, 1 022 cm^{-1}, and 1 000 cm^{-1} after retrogradation. Our investigation showed that the most remarkable band, rising during modification of pea starch, occurred at 1 016 cm^{-1} (Figure 3). It should be also pointed out that the differences between relative intensity of some bands could be observed not only in the region 1 400-800 cm^{-1} but also in a wider range up to 400 cm^{-1}. This observation could be probably recognised as a typical phenomenon often observed in the so-called "fingerprint" region of infrared spectra.

Table 2. Water binding capacity (WBC) and oil absorption (OA) of pea starch before and after modification.

Pea starch	WBC [g water/g d.m. of sample]	OA [g oil/g d.m. of sample]
Native	0.69±0.04	1.16±0.11
Preparation	2.73±0.04	1.48±0.08

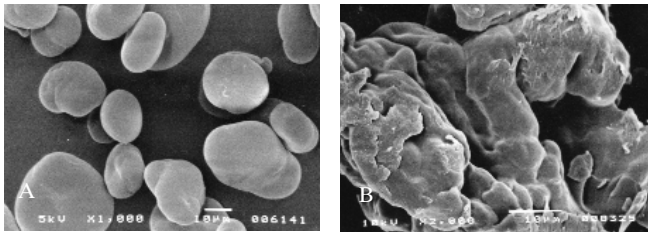

FIGURE1. Microstructure of native pea starch (A) and its preparation (B).

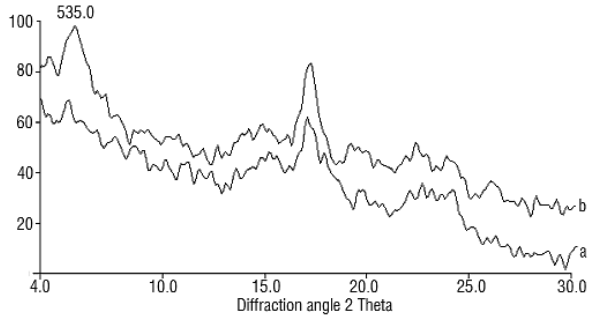

FIGURE 2. X-ray diffraction pattern of pea starch: (a) native, (b) modified.

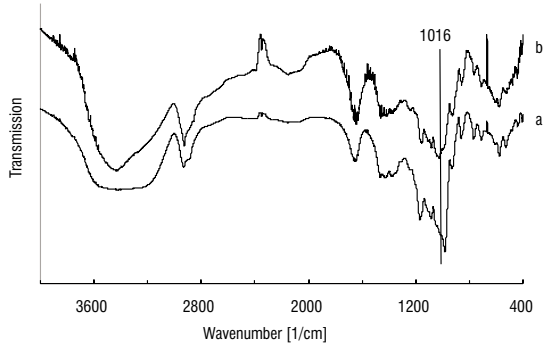

FIGURE 3. FTIR spectra of native and modified pea starch: (a) native, (b) modified.

These significant changes in the starch structure affected substantially the functional properties, especially ability to water at the room temperature (Table 2). It was also observed that an increase in hydrophilic character influenced water-pea starch interaction upon heating. Native pea starch developed typical restricted type of swelling characteristic typical of legume starches, whereas starch preparation revealed lower pasting temperatures as well as higher values of viscosity (Figure 4). The explanation of these phenomena could be found in light microscopy investigations. At the initial pasting/gelatinisation temperature, native pea starch showed certain deformation of granules, especially of the large ones (Figure 5a). Some granules in which amylose fraction migrated near the surface could be also observed. All of them existed in the medium of the dispersion of leaching amylose, which made blue complex with iodine. At the initial pasting temperature, the experimental preparation could be observed as an irregular, density fraction mixed with weak gel which generally could not form the blue complex with iodine (Figure 6a). A comparative analysis of preparations obtained at a temperature of 90°C indicated that the native pea starch formed mainly suspension of deformated granules in the fraction of free leaching amylose (Figure 5b). We suggest that the amylose phase released from native pea starch granules at 90°C tends toward some fibrylation of this macromolecule. At the same temperature, the modified pea starch existed in the form of irregular fragments dispersed in medium coloured as amylopectin-like gel with occassional traces of blue amylose complex (Figure 6b). These observations confirmed the

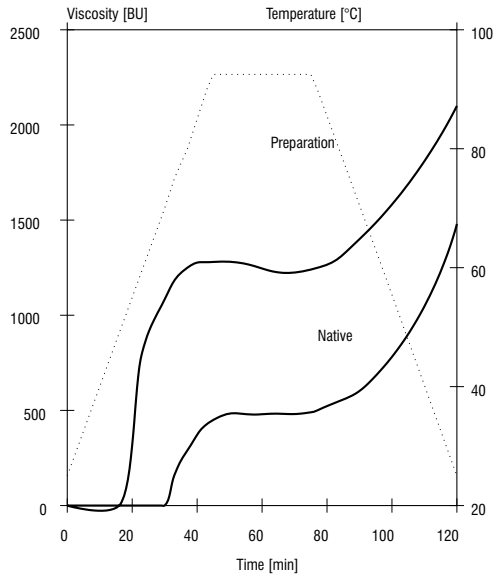

FIGURE 4. Brabender viscosity curves of native and modified pea starch.

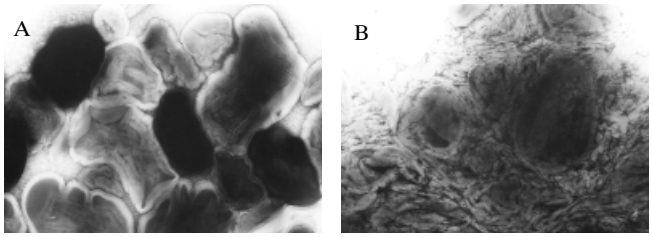

FIGURE 5. Light microscopy of native pea starch: (A) at 71°C; (B) at 90°C.

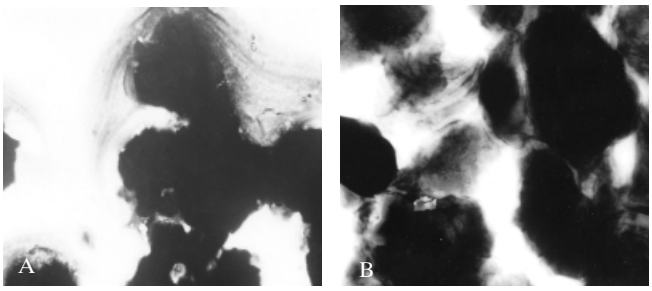

FIGURE 6. Light microscopy of modified pea starch: (a) at 58°C; (b) at 90°C.

retrograded status of both starch macromolecules, amylose and amylopectin, which interpenetrated and existed as a two-phase system – parts of dense gel fragments dispersed in weak amylopectin-amylose gels.

Pea starch as other leguminous starches displayed a C-type of crystallinity [Ring *et al.*, 1988] and according to this type of structure it is believed to be more resistant to hydrolysis than the starches characterised by an A-type [Englyst *et al.*, 1992]. Our work confirmed a higher resistance of native pea starch to alpha-amylase (Table 1), compared to the native wheat starch characterised by an A-type of crystallinity [Soral-Śmietana *et al.*, 2003]. Our previous works on resistant starch in wheat, potato, pea starch preparations obtained upon the same technological procedure indicate that both native starches and these preparations follow the decreasing order of resistant starch content: potato> >pea>wheat [Lewandowicz *et al.*, 1998b; Soral-Śmietana *et al.*, 2003]. It should be mentioned that the modification procedure applied resulted in a decrease in the resistant starch content in pea preparation, compared to native pea starch (Table 1). However, that decrease is accompanied by the change of the type of resistant starch from type RS II to RS III according to nutritional classification [Englyst *et al.*, 1992]. Taking into consideration current definition of dietary fibre: "*The edible parts of plants or analogues of carbohydrates that are resistant to digestion and absorption in the small intestine, which complete or partial fermentation in the large intestine"* [American Association of Cereal Chemist, 2001 – acc. Gray, 2003], it includes also resistant starch. Consequently our results showed that resistant starch fraction in the experimental pea starch preparation could be used in the food technologies. It could also play the role of "soluble" part bringing about viscosity and formation of gels in the intestine. The part of "insoluble" resistant starch could be rapidly fermented in the large intestine as a substrate for the microflora existing therein. Bearing in mind the results of our previous study, we may suggest that the growth of certain *Bifidobacterium* strains is stimulated by starches of different botanical origin and their preparations as observed in the *in vitro* method [Soral-Śmietana *et al.*, 2004]. The pea starch preparation after physical modification caused by different dehydration of starch gel by spry drying indicated its potential health promoting activity [Wronkowska & Soral-Śmietana, 2000; Wronkowska *et al.*, 2002].

CONCLUSIONS

The physical modification process applied in this study could be a good method for improving the usability of pea starch for food applications as it results in certain physico-chemical or structural conformations of starch granules:

1. a small increase in the minor constituentss as well as the content of total protein and mineral compounds;
2. change from the C-type to B-type accompanied by a slight increase in relative crystallinity acc. to the X-ray diffraction pattern;
3. an increase in the FTIR spectrum occurring at 1016 cm^{-1} as well as the differences between relative intensity of some bands in the region 1400-400 cm^{-1};
4. an increase in the ability to water absorption caused by forming the hydrophilic domains, which was confirmed by a decrease in pasting temperature and an increase in the viscosity;
5. confirmation of the retrograded status of both starch macromolecules, amylose and amylopectin, which interpenetrated and existed as a two-phase system – parts of dense gel fragments dispersed in weak amylopectin-amylose gels.
6. a quantitative decrease in the resistant starch content accompanied by the change of type of the resistant starch (according to nutritional classification), which can promote the potential biological activity and utilization of pea starch.

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REFERENCES

1. AOAC, Official Methods of Analysis of the Association of Official Analytical Chemists. 15th ed., 1990, Arlington, Virginia, USA
2. Belton P.S., Wilson R.H., Chenery D.H., Interaction of group-1 cations with iota-carrageenans and kappa-carrageenans studied by Fourier-transform infrared spectroscopy. Int. J. Biol. Macromol., 1986, 8, 247–251
3. Biarnes-Dumoulin V., Lecoeur J., Yield potential of field pea in England: comparison with yields in France. Grain Legumes, 1998, 22, 24–25.
4. Bogracheva T., Stubbs B., Morris V., Ring S., Wang T., Hedley C., Structure-function relationships of pea starches associated with food and non-food uses, 1998, *In:* Proceedings of the 3rd European Conference on Grain Legumes, Valladolid, 14–19 November 1998, pp. 56–57.
5. Camire M.E., Camire A., Krumhar K., Chemical and nutritional changes in foods during extrusion. Food Sci. Nutr., 1990, 29, 35–57.
6. Champ M., Martin L., Noah L., Gratas M., Analytical methods for resistant starch, 1999, *In:* Complex Carbohydrates in Foods. (eds. S. Sungsoo Cho, L. Prosky, M. Dreher), Marcel Dekker Inc., New York, pp. 169–187.
7. Champ M., Benefits of pulses in human diet, 2001, *In:* Proceedings of the 4th European Conference on Grain Legumes, Cracow, 8–12 July 2001, pp. 109–113.
8. Colonna P., Lourdin D., Della Valle G., Buleon A.,

Importance of amylose in non food uses of pea starches for thermoplastic materials, 1995, *In:* Proceedings of the 2nd European Conference on Grain Legumes, Copenhagen, 9–13 July 1995, pp. 354–355.
9. Englyst H.N., Kingman S.M., Cummings J.H., Classification and measurement of nutritionally important starch fractions. Eur. J. Clin. Nutr., 1992, 46 (suppl. 2), 33–50
10. Englyst H.N., Hudson G.J., Starch and health, 1997, *In:* Starch, Structure and Functionality. (eds. Frazier P.J., Donald A.M., Richmond P.). The Royal Society of Chemistry. Cambridge, UK, pp. 9–21.
11. Gray J., 2003, Carbohydrates: Nutritional and Health Aspects. ILSI Press, Brussels, Belgium.
12. Hedley C.L., Strategies for manipulating grain legume carbohydrates. 2001, *In:* Carbohydrates in Grain Legume Seeds: Improving Nutritional Quality and Agronomic Characteristics. (ed. C.L. Hedley). CABI Publishing, Oxon, UK, pp. 233–238.
13. Lewandowicz G., Soral-Śmietana M., Fornal J., 1998a, Sposób otrzymywania spożywczego preparatu skrobiowego o podwyższonej oporności na enzymy amylolityczne (The way of receiving alimentary starch preparation with increased resistance to amylolytic enzymes), Polish Pat. Spec. No P.325 981 (in Polish)
14. Lewandowicz G., Soral-Śmietana M., Fornal J., New resistant starch preparations - physicochemical properties and structure. Żywność, Technologia, Jakość, 1998 b, 4 (17), supl., 164–172.
15. Lewandowicz G., Soral-Śmietana, Starch modification by iterated syneresis, Carbohydr. Polym., 2004 (submitted).
16. McIntosh G.H., Wang Y.H.A., Protein quality and cholesterol lowering capacity of four grain legumes (peas, chickpeas, lentils and faba beans), 1995, *In:* Proceedings of the 2nd European Conference on Grain Legumes, Copenhagen, 9–13 July 1995, pp. 336–337.
17. Morrison W.R., Laignelet B., An improved colorimetric procedure for determining apparent and total amylose in cereal and other starches. J. Cereal Sci., 1983, 1, 9–20.
18. Rindlav A., Hulleman S.D.H., Gatenholm P., Formation of starch films with varying crystallinity. Carbohydr. Polym., 1997, 34, 25–30.
19. Ring S.G., Gee J.M., Whittam M., Orford P., Johnson I.T., Resistant starch: its chemical form in foodstuffs and effect on digestibility *in vitro*. Food Chem., 1988, 28, 97–109.
20. Soral-Śmietana M., Dziuba Z., Changes in fraction structure of faba bean starch, 1995, *In:* Proceedings of the 2nd European Conference on Grain Legumes, Copenhagen, 9–13 July 1995, pp. 402–403.
21. Soral-Śmietana M., Świgoń A., Amarowicz R., Sijtsma L., Chemical composition, microstructure and physicochemical characteristics of two commercial pea protein isolates. Pol. J. Food Nutr. Sci., 1998, 7/48, 2, 193–200.
22. Soral-Śmietana M., Wronkowska M., Resistant starch of pea origin. Żywność, 2000, 2 (23) supl., 204–211.
23. Soral-Śmietana M., Krupa U., Markiewicz K., White bean varieties – a source of elements, dietary fibre and resistant starch, Pol. J. Food Nutr. Sci., 2002, 11/52, SI 1, 17–24.
24. Soral-Śmietana M., Wronkowska M., Bielecka M., Biedrzycka E., Ocicka K., Physically modified starches - utilisation of resistant starch by bifidobacteria (*in vitro*), (in press).
25. van Soest J.J.G., Lewin D., Dumont H., Kappen F.H.J., Pea: An interesting crop for packaging applications. 2002, *In:* Plant Biopolymer Science: Food and Non-Food Applications. (eds. D.Renard, G. Della Valle, Y. Popineau). The Royal Society of Chemistry, Cambridge, UK, pp. 267–274.
26. van Soest J.J.G., de Wit D., Tourois H., Retrogradation of potato starch as studied by Fourier transform infrared spectroscopy. Starch/Stärke, 1994, 46, 453–457.
27. Wilson, R.H., Belton P.S., A Fourier-transform infrared study of wheat starch gels. Carbohydr. Res., 1988, 180, 339–344.
28. Wilson R.H., Kalichevsky M.T., Ring S.G., Belton P.S., A Fourier-transform infrared study of the gelation and retrogaradation of waxy-maize starch. Carbohydr. Res., 1987, 166, 162–165.
29. Wronkowska M., Soral-Śmietana M., Pea starch as a source of physically modified preparation with potential health-promoting activity. Żywność, 2000, 7/23 (2) Suppl., 226–235.
30. Wronkowska M., Soral-Śmietana M., Bielecka M., Biedrzycka E., Physically modified wheat or potato starches, their physico-chemical properties and metabolism by bifidobacteria. Żywność, 2002, 9/33 (4) Suppl., 74–83.