Changes in the Macrocomponents and Microstructure of White Bean Seeds Upon Mild Hydrothermal Treatment

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Abstract

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The mild hydrothermal treatment (water bath at 40°C/24 h) of three Polish bean varieties (*Phaseolus vulgaris* and *Phaseolus coccineus*) with different sizes of seeds: the small-seed variety Raba, the medium-seed variety Aura, the large-seed variety Eureka, had a significant effect on the microstructure of the cross-sections of bean cotyledons. The first 3 hours were decisive with almost all physical and chemical parameters investigated in the study. After this time, the highest water binding or water holding capacities were observed. The tendency to a distinct decrease in the resistant starch fraction was observed especially in the large and medium bean seeds (*Phaseolus* sp.), based on the comparison before and after the processing. It seems that the too high dietary fibre content determined in the study resulted from exceptional sorption properties while the especially high increase in the insoluble fraction of dietary fibre (IDF) was observed during this mild hydrothermal treatment. This suggests the possibility of the occurrence of hydrophilic domains in dietary fibre. Although it cannot be explained explicitly, distinct differences were found in both fractions of dietary fibre in the varieties examined. The results obtained indicate that the conditions of the mild hydrothermal treatment can affect the nutritional and non-nutritional components of bean seeds which play an important role in the human gastrointestinal tract as both food products and diet components.

Keywords: bean seeds; mild hydrothermal treatment; proteins; total starch; resistant starch fraction; dietary fibre; microstructure

Legume seeds, including beans, have recently received increasing attention in human nutrition due to the positive effects on the physiology of the gastrointestinal tract, as well as on the metabolism of glucose and lipids (Pirman *et al.* 2001). The bean varieties cultivated under Polish climate conditions are rich in nutritional components, especially proteins and complex carbohydrates, but also in valuable non-nutritional components such as resistant starch and dietary fibre (Soral-Śmietana *et al.* 2002). These chemical components, present in bean seeds, can play an important role in the prevention of metabolic diseases (diabetes, intestine inflammation, colon necrosis), and lower the risk of heart diseases. The intake of dietary fibre (DF), a non-homogenous substance, in Western countries is currently estimated to be 10–25 g per person per day, however, in view of physiological effects, the nutritionists recommend the intake of about 35 g per person per day (Thebaudin *et al.* 1997). The components of DF can be generally classified as soluble or insoluble, based on their reaction with water (Pilch 1987). Similarly to DF, the resistant starch fraction (RS) possesses beneficial properties, e.g. the stimulation of the growth of favourable intestinal bacterial strains (Soral-Śmietana *et al.* 2005), thus causing simultaneously the increase of short chain fatty acids (SCFA) production during the fermentation process in the colon (Bird *et al.* 2000). The amount and quality of SCFA depend on the botanical origin of starches, the technological treatment, (Wronkowska & Soral-Śmietana 2004) and the time of reaction with microflora (Soral-Śmietana *et al.* 2001a). SCFA perform important functions in the liver and muscles, especially butyric acid which is the source of energy necessary for the proliferation of epithelium cells. However, RS intake is not standardised, but according to some literature data, the intake of RS ranged widely from 3.5–8.5 g per day in many countries during 1992–1997 (Soral-Śmietana 2000). Although indigestible, resistant starch and dietary fibre are active components of our diet because of their importance in maintaining the functional integrity of the gastric tract (Davidson & McDonald 1998).

Although bean seeds are a good source of proteins, starch, dietary fibre, water soluble vitamins, and minerals, a drastic decrease in their consumption is observed (Champ 2001). In general, bean seeds are consumed after technological treatments leading to changes in the internal arrangement of cotyledons and to modifications of the main biopolymer properties. Thermal food treatment may lead to desirable changes in the food components such as protein coagulation, starch swelling, texture softening and formation of aroma components. However, the conditions applied may evoke some undesirable modifications like a loss of vitamins and minerals, the formation of indigestible aggregates of biopolymers, and the changes in their conformation (Ohlsson & Bengtsson 2002). The heat-moisture process and the annealing process influence different alternations within the starch structure (Jacobs & Delcour 1998; Collado & Corke 1999), but the distinctive criterion which distinguishes both treatments is the amount of water. The shortage of water indicates that the process runs under heat-moisture treatment conditions. The interpretation of Brabender viscosity, sorption isotherms, endotherm curves (DSC) and X-ray patterns suggests that the heat-moisture treatment results in the alternation from B-type to A-type structures, whereas in the case of the annealing processes, the alternation of properties can be explained by a modification of the binding forces between the crystallites and the amorphous matrix (Stute 1992). Annealing is a soft hydrothermal treatment at an excess (> 60% w/w) or mean (40–55% w/w) water content below the temperature of gelatinisation (Jacobs & Delcour 1998), which results the physical reorganisation of starch granules. Annealing leads to an elevation of the starch gelatinisation temperature and the narrowing of the gelatinisation range (Tester & Debon 2000) without damage to starch granules with respect to size, shape, and birefringence (Stute 1992). In most cases, annealing is seen as a negative modification of the starch properties, e.g. as after-harvest damage, when corn or wheat has to be dried after harvest (Münzing & Bolling 1989), but it can also modify the starch properties in a positive way, e.g. by increasing viscosity.

The aim of this study was to determine if and how the application of the mild hydrothermal treatment evokes changes in the macrocomponents of bean seeds with respect to proteins, total starch, resistant starch fraction and dietary fibre status, and to determine its influence on the microstructure of whole bean seeds (*Phaseolus vulgaris* and *Phaseolus coccineus*).

MATERIAL AND METHODS

Mild hydrothermal treatment. Whole seeds of three bean varieties with different cotyledon sizes served as the experimental materials. They were subjected to a mild hydrothermal treatment. They were soaked in distilled water at a ratio of 3:1 (v/v) at 40°C for 24 hours. To prevent microbial contamination, sodium azide (0.02%) was added to the water bath used in this experiment. The same parameters were analysed during the experiment after 3 h, 6 h, 24 h of the treatment.

Material. Three Polish white bean varieties *(Phaseolus vulgaris* and *Phaseolus coccineus)* were examined: the small-seed variety Raba, the medium-seed variety Aura, and the large-seed variety Eureka (Table 1). These bean seeds were industrially dried up to the preservation moisture and conditioned at temperature 6°C for one month. Whole dry seeds were milled to obtain 100% fraction containing particles smaller than 0.4 mm, or whole wet seeds were disintegrated using a varing blender and mortar.

Methods. The moisture content (130°C/1 h) as well as the contents of total starch and proteins were analysed according to the standard methods

Table 1. Physical properties of bean seeds (*Phaseolus* sp.)

Physical properties		Bean seeds		
		Raba	Aura	Eureka
Thousand seed mass (g)		172.96	505.08	908.42
Average dimension (mm)	thickness	6.09 ± 0.58	6.78 ± 0.39	7.96 ± 0.66
	length	10.64 ± 0.73	14.58 ± 0.68	18.41 ± 1.26
	width	7.50 ± 0.46	7.78 ± 0.44	12.62 ± 0.65

(AOAC 1975, 1990). The water absorption of bean seeds was determined in distilled water (3:1 v/v) at 40°C after 3, 6 and 24 hours. The resistant starch fraction (RS) content was analysed according to Champ *et al.* (1999). The contents of soluble (SDF) and insoluble (IDF) fractions of dietary fibre were determined by the enzymatic-gravimetric method according to Asp *et al.* (1983). The microstructure of non-processed bean seeds and those subjected to 3- or 24-h soft hydrothermal processing were analysed under a scanning electron microscope (SEM). Wet samples were prepared after immediate fixing according to Kalab (1981). At the end, they were dried at the critical point in a Balzers Union drier according to Dziuba *et al.* (1994). Dry samples were fixed to aluminium stubs and coated with gold in a JEE 400 vacuum evaporator. The images were analysed under a JSM 5200 microscope at 10 or 15 kV.

RESULTS AND DISCUSSION

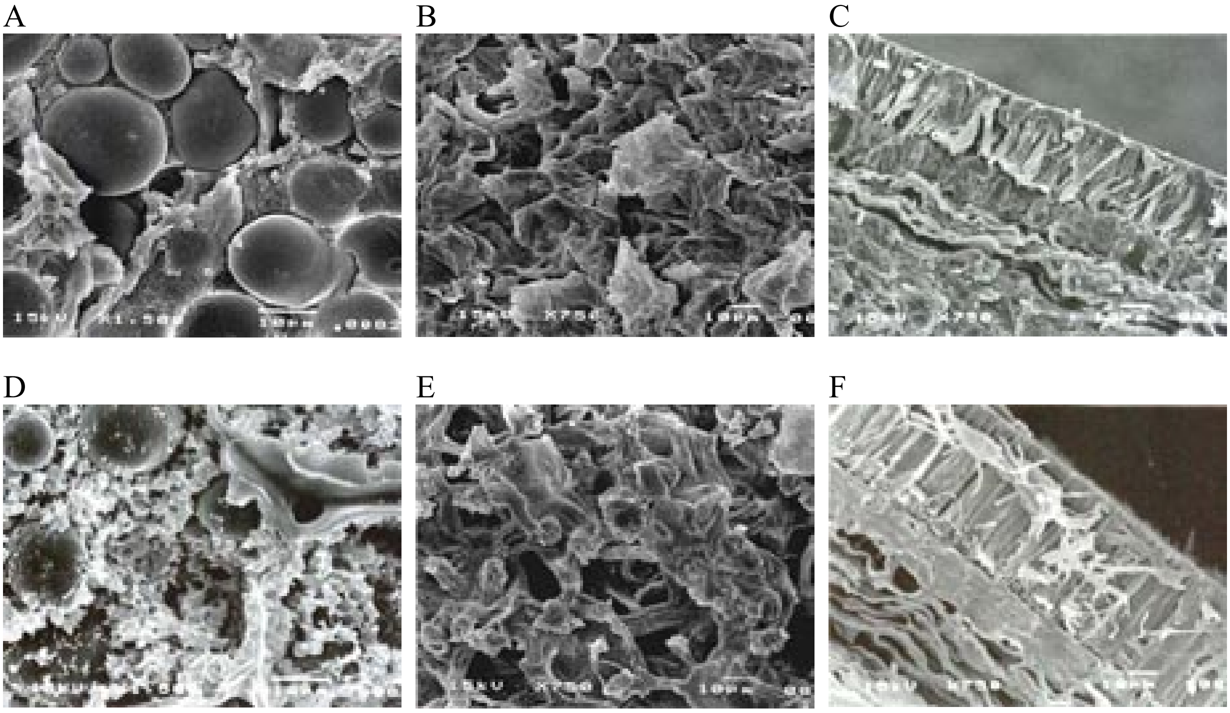
Whole bean seeds of *Phaseolus* sp. varieties (Raba, Aura, Eureka) characterised by different sizes and thousand seed weights (Table 1) were subjected to the experimental mild hydrothermal treatment. The influence of low temperature and soaking time on the changes in bean seed macrocomponents and the microstructure of the processed seeds were determined after three different time intervals (3, 6 and 24 h). These results were compared with those obtained with non-processed bean seeds. The observation of 24-h physical process treatment indicated that in the first hours of soaking bean seeds showed different water absorption, dependent on the variety (Table 2). The first 3 h of mild hydrothermal processing were most important as they caused noticeable changes. The small-seed var. Raba absorbed maximum water during this period of time and maintained its level constant over the entire experimental period. Two other varieties, medium-seed Aura and large-seed Eureka, bound and accumulated water gradually, along with the progress in the process. However, slight differences were observed between the results obtained in the 6th and 24th-h of this hydrothermal treatment with the large-seed var. Eureka. Water absorption and migration depended on the size and shape of bean seeds (Table 2), and the moisture content of all bean seeds analysed at this time increased to about 50%. Therefore, after the process about 50% of seed mass was free water present in macro- and microcapillaries, both in the seed coat and in the internal structure of cotyledones. The results of our previous research (Soral-Śmietana *et al.* 2002) indicated that the differences in water absorption of bean seeds, noted between particular varieties, could be a consequence of the seed size, different morphology of the seed coat or cell wall structure, and depend mainly on the aerial space and the concentration of cells in the cross-section of cotyledons. From the physicochemical point of view, the biopolymers of the seed cotyledons followed in this experiment, proteins and starch, show water binding capacity affecting the hydrophilic character of the material analysed. Proteins are characterised by a higher ability to absorb water than is starch (Amado 1994); seeds of var. Raba contained the highest amount of water of all varieties investigated in this study (Table 2). In this case, the chemical components of seeds, especially proteins and starch, contributed more to water absorption, but taking into consideration the small dimensions of the seeds, the water diffusion and/or migration inside could be faster and stabilised after 3 hours.

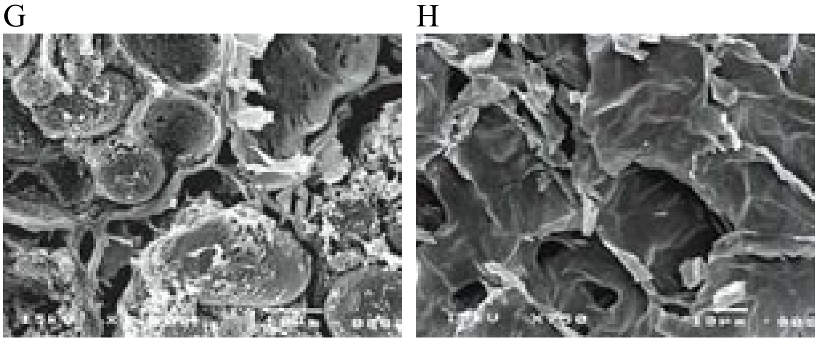
The microstructure of bean seeds during (3 h) and after the completion of the mild hydrothermal treatment was analysed under a scanning electron microscope, based on the example of small- and

Table 2. Water sorption and changes of starch and protein content of bean seeds (*Phaseolus* sp.) during 24-h mild hydrothermal treatment

Variety		Time of hydrothermal treatment (h)			
		0	3	6	24
Raba	Water absorption (g_{water}/g d.m.$_{seeds}$)	–	1.06 (± 0.09)	1.11 (± 0.13)	1.12 (± 0.06)
	Moisture (%)	9.35 (± 0.23)	52.50 (± 1.56)	56.74 (± 0.07)	58.41 (± 0.68)
	Total starch (% d.m.)	52.9 (± 0.6)	49.9 (± 0.3)	49.0 (± 0.8)	50.2 (± 1.9)
	Total protein (% d.m.)	21.6 (± 0.2)	22.9 (± 0.1)	21.8 (± 0.4)	21.7 (± 0.9)
Aura	Water absorption (g_{water}/g d.m.$_{seeds}$)	–	0.74 (± 0.03)	1.06 (± 0.13)	1.07 (± 0.05)
	Moisture (%)	12.82 (± 0.31)	51.82 (± 1.67)	55.23 (± 1.34)	57.59 (± 0.14)
	Total starch (% d.m.)	56.5 (± 1.7)	49.2 (± 0.2)	52.0 (± 1.9)	52.2 (± 2.8)
	Total protein (% d.m.)	17.8 (± 1.0)	24.3 (± 0.6)	24.2 (± 0.6)	24.2 (± 0.5)
Eureka	Water absorption (g_{water}/g d.m.$_{seeds}$)	–	0.60 (± 0.03)	1.03 (± 0.11)	1.36 (± 0.14)
	Moisture (%)	11.03 (± 0.05)	50.26 (± 0.54)	55.89 (± 0.16)	61.89 (± 0.22)
	Total starch (% d.m.)	45.0 (± 0.4)	45.4 (± 1.8)	44.9 (± 0.2)	44.8 (± 2.8)
	Total protein (% d.m.)	17.2 (± 0.2)	20.5 (± 0.4)	21.1 (± 0.5)	22.0 (± 0.1)

large-bean seeds varieties Raba and Eureka, in comparison with non-processed bean seeds (Figures 1 and 2). The microphotographs showed gradual changes after the first 3 hours of the treatment (Figures 1D–F and 2D–E). The mild hydrothermal treatment resulted in visible progressive swelling of the structure of the internal cell walls. In addition, a distinct water absorption effect was noted in the seed biopolymers when water migrated into the cotyledons. The protein matrix of var. Raba became looser, thus increasing the diameters of individual protein bodies (Figures 1A, 1D). The protein matrix structure of var. Eureka was quite different, with closely connected starch granules (Figure 2A, 2D). Water penetration through the cotyledon structure influenced protein matrix aggregation and, at the further stage of processing, caused permanent deformations and even partial denaturation of protein, which resulted in a loose arrangement of some starch granules in the cotyledon cells after 24 h of this mild water treatment at 40°C (Figures 1G, 2F). It was suggested that water is the decisive factor in the protein denaturation process (Sheard *et al.* 1986; Philips *et al.* 1988). Pilosof *et al.* (1982) indicated that a greater reduction of nitrogen solubility occurred when samples were heated at a higher moisture content. In the present study concerning the hydrothermal treatment, the mean moisture content of bean seeds was > 50% (Table 2). Such a high level of moisture, combined with temperature, does not create extreme conditions, but may induce protein aggregation and probably some denaturation as observed on the SEM cross-sections of bean seed cotyledons after 24-h processing (Figures 1 and 2). A strong effect of the process on the protein denaturation was observed by Sheard *et al.* (1986) and Philips *et al.* (1988) when the water content was higher than 20%. However, mild treatment conditions and duration had a negligible influence on the total protein content (Table 2). According to some literature data (Sheard *et al.* 1986; Yamasaki & Ikebe 1992), the effect of heating on proteins depends also on other components and carbohydrate-protein interactions. Carbohydrates protect proteins against heat denaturation due to their hydrophobic interactions. The conditions of the processing used as presented in this paper can be compared with the annealing conditions with respect to temperature and the water amount (Tester & Debon 2000). According to some authors (Hoover & Vasanthan 1994a), annealing has no effect on starch granule dimensions and shape, although early microscopic works indicated that wheat starch granule dimensions increase after annealing. The SEM images of the bean seed



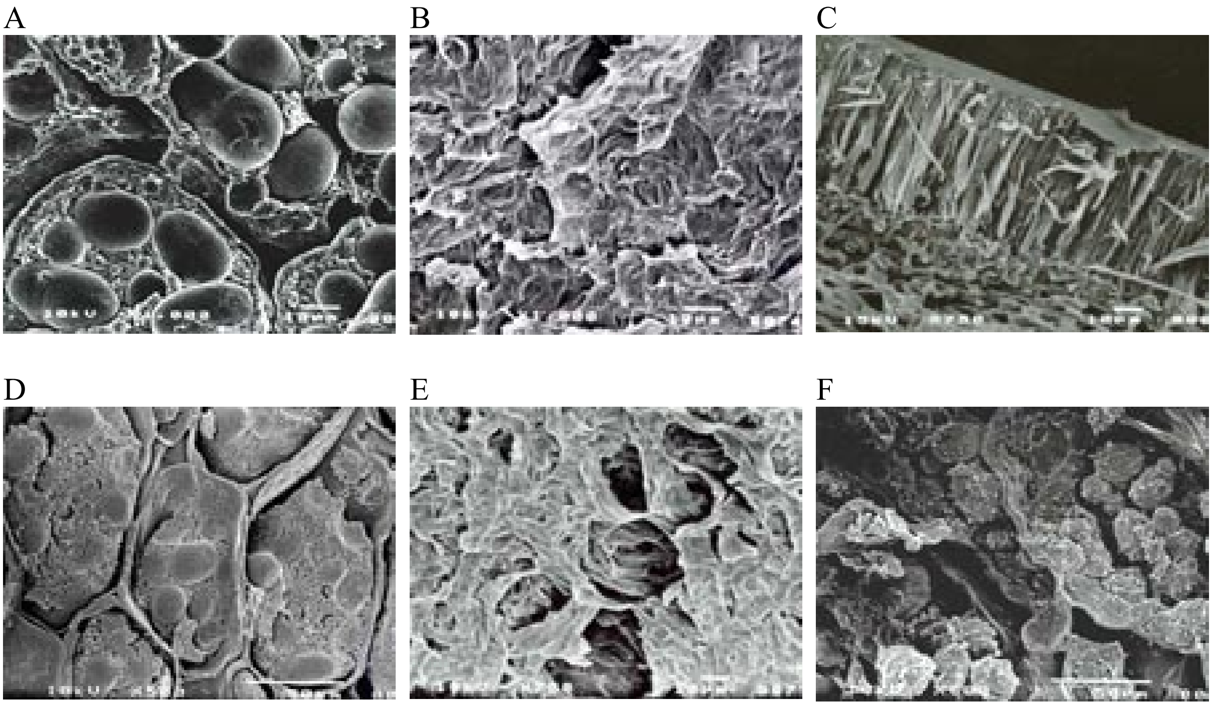


Before processing: A – cross-section of cotyledon; B – seed coat inner surface; C – seed coat cross-section
After 3 h of process: D – cross-section of cotyledon; E – seed coat inner surface; F – seed coat cross-section. After 24 h of process: G – cross-section of cotyledon; H – seed coat inner surface

Figure 1. The SEM-microstructure of small bean seeds Raba var. (*Phaseolus vulgaris*) upon mild hydrothermal treatment

cotyledons analysed in this investigation do not display significant changes in the size of bean starch granules, however, small differences in size cannot be accurately quantified due to the swelling effect associated with the process applied. Investigating the heat-moisture influence on starches of different origin, Hoover and Vasanthan (1994b) obtained similar results, claiming that those conditions did not alter the size or shape of starch granules, but oat starch granules were less compactly packed after the heat treatment. The results of this work indicated some influence of the mild hydrothermal treatment on the cell structure of native bean seed cotyledon (Figures 1 and 2). It was visible as the initial compact aggregation became less regular. In some cases, the prolapse of starch granules from the cotyledon matrix structure was noted. However, the mild processing applied in this experiment did not cause any significant quantitative changes in the total starch content of bean seeds (Table 2).

The conditions of the hydrothermal treatment, temperature and soaking time, have a significant influence on the resistant starch status manifested as a considerable decrease of the resistant starch content (Figure 3). This phenomenon can be explained as a consequence of decreasing compactness and an easier access of enzymes to starch granules, resulting probably from the increase of the surface of the solid phase of starch, as well as water migration or diffusion into cotyledons by external and internal microporosity. It may



Before processing: A – cross-section of cotyledon; B – seed coat inner surface; C – seed coat cross-section
After 3 h of process: D – cross-section of cotyledon; E – seed coat inner surface
After 24 h of process: F – cross-section of cotyledon

Figure 2. The SEM-microstructure of large bean seeds Eureka var. (*Phaseolus coccineus*) upon mild hydrothermal treatment

facilitate the leaching of some starch granules because the binding forces between the crystal and amorphous matrixes can be modified. Dry legume seeds contain different amounts of resistant starch, depending on the seed size (Soral-Śmietana *et al.* 2002), and the results obtained can be compared with the previous results concerning native pea starch or its physically modified preparations (Soral-Śmietana *et al.* 2003). The enzymatic process requires, among others, the adsorption of enzymes onto the solid phase of substrates and diffusion of enzyme molecules into the solid phase (Colonna *et al.* 1992); thus a mild hydrothermal treatment could improve the reaction progress. Our previous study on the changes in the microstructure during a 24-h *in vitro* experiment with pancreatic α-amylase confirmed the susceptibility of pea starch to multiple activity of this enzyme on legume starch: (1) radial direction to the inside of a granule; (2) equatorial along the main axis; (3) needle-like holes within the hilum area (Soral-Śmietana *et al.* 2001b). The impact of the processing used was visible especially during the first 3 hours when the resistant starch content decreased suddenly to $1/10^{th}$ in var. Aura and to $1/18^{th}$ in var. Eureka (Figure 3). Such dynamic changes were no longer observed at further stages of the experiment, when the resistant starch content was decreasing slowly. These results suggest that the status of the resistant starch fraction in bean seeds is of the RS_1 or RS_2 type. The decrease in RS content could be connected with water absorption into the compact starch granules enclosed in the cotyledon matrix, which was conducive to the availability of amylolytic enzymes. Still, none of the known *in vitro* methods of analysis for the resistant starch content enables to detect the whole amount of RS, as defined by Asp (1992), especially "the products of starch degradation". Indeed, RS analysed *in vivo* seems to consist of three fractions: oligosaccharides (including glucose), crystallities (linear chains of α-glucans), and long chains which are fragments of starch granules (Champ 1995). The team of INRA researchers (Faisant *et al.* 1993a, b; Champ 1995) who performed *in vivo* RS trials, reported that in bean flakes the

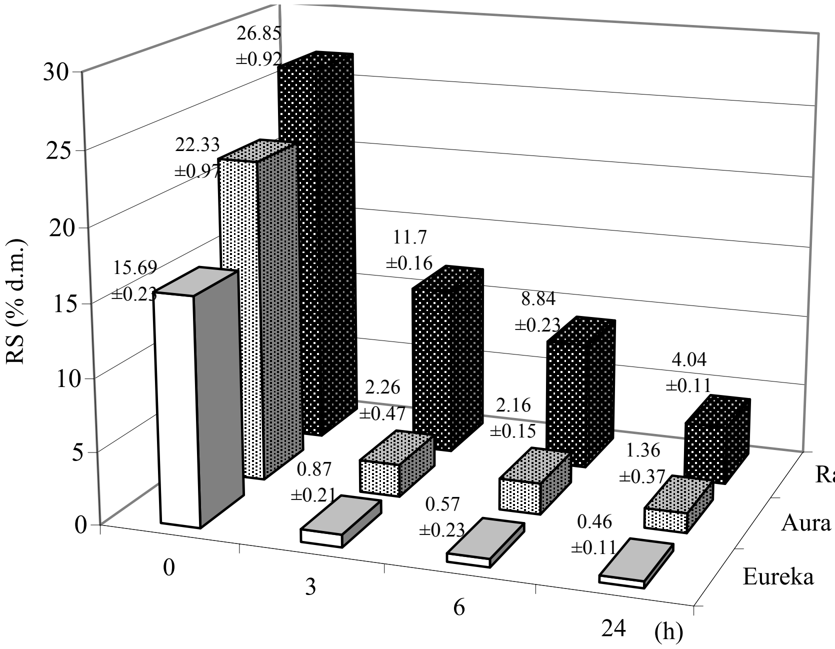
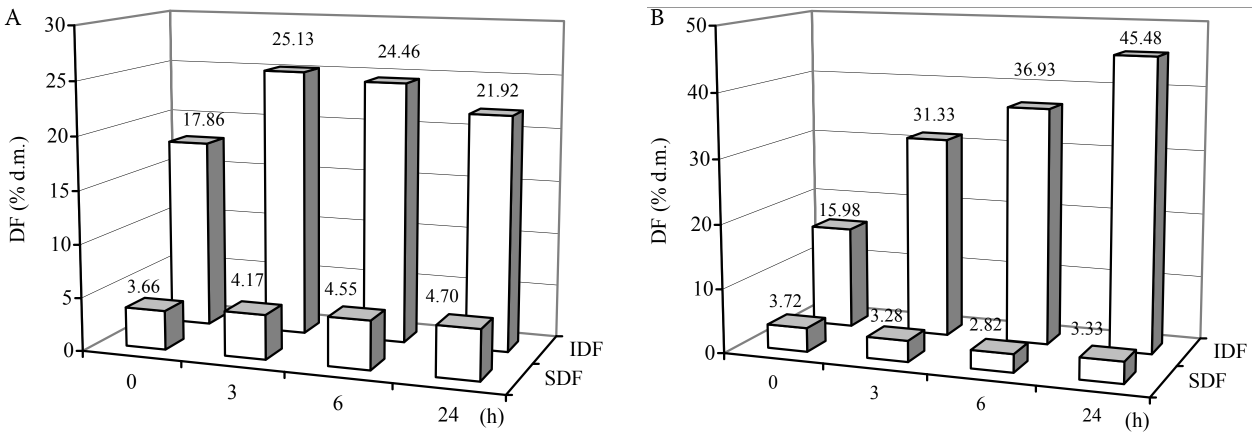
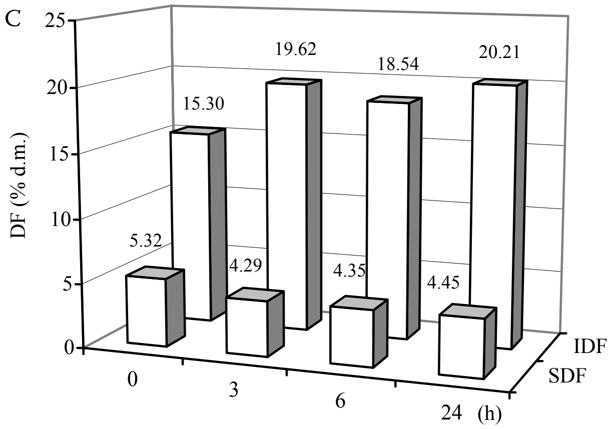


Figure 3. The changes in the resistant starch fraction of bean seeds (*Phaseolus* sp.) during 24-h mild hydrothermal treatment

molecule size distribution is as follows: $DP_n \leq 5$ (13%), $DP_n \sim 15–38$ (53%), $DP_n > 100$ (34%). Thus, about 66% of the total amount of the resistant starch is characterised by small-size molecules, such as oligosaccharides (including glucose) or dextrins, which cannot be determined by *in vitro* methods. This was probably the case during the mild hydrothermal experiment used (Figure 3).

The characteristics of dietary fibre (DF) in relation to its affinity to water were analysed by the





A – var. Raba; B – var. Aura; C – var. Eureka

Figure 4. The insoluble (IDF) and soluble (SDF) fractions of dietary fibre in bean seeds (*Phaseolus* sp.) during 24-h mild hydrothermal treatment

enzymatic-gravimetric method, comparing processed and non-processed bean seeds (Figure 4). The water-insoluble (IDF) and water-soluble (SDF) fractions of dietary fibre were analysed in bean seeds during the mild hydrothermal treatment. The results obtained with the non-processed seeds showed that the insoluble to soluble DF ratio was 5:1 in small or medium bean seeds and 3:1 in large seeds. We observed a visible effect of the conditions of processing on the DF content during the first 3 hours of water migration into the cotyledons, especially in the case of the insoluble dietary fraction (Figure 4). Assuming some methodological errors, the results obtained showed intensive water absorption into the porous structure of IDF, visible especially in the seed coat of var. Raba (Figure 1C, 1F) and in the internal structure of the seed coat surface of the bean varieties (Figures 1B, 1E, 1H and 2B, 2E). This can be explained by strong physicochemical interactions of the multiple IDF fraction, and interactions between those compounds and water during the 24-h reaction. It may also be related to cellulose and non-polysaccharide compounds – lignins, which impregnate cellulose fibres in DF arrangement. As a result of drying, which follows the previous modification conditions, cellulose fibres collapse and close the capillaries, fixing the absorbed water. However, lignins as highly cross-linked, three-dimensional structures based on phenylpropane units, chemically linked to hemicellulose in the plant cell wall, may show strong affinity to water. In the present research, the swelling cell wall structure in varieties Raba or Aura were especially visible in SEM images (Figures 1A, 1D, 1G), and significant quantitative changes in DF were observed (Figure 4). During 24-h mild hydrothermal treatment, some amount of soluble fraction (SDF) migrated to the solvent, which was observed with medium (var. Aura) and large (var. Eureka) bean seeds. In the small-seed bean variety (Raba), a slight growth and stabilisation of SDF were observed after 3 hours of the process, which can have resulted from limited water absorption during 3 to 6 hours of the process (Table 2). According to Amado (1994), "conformation of polysaccharides, which is determined by monomers and the type of glycosidic linkages, plays an important role in the solubility as well as the water binding properties of dietary fibre. Helical conformations show certain tendencies to form inclusion compounds with hydrophilic and hydrophobic low molecular weight substances, ribbon shaped polysaccharides (such as cellulose) can be stabilized by hydrogen bonds to form micelles and/or associates."

In view of the applied conditions of the mild hydrothermal treatment (temperature 40°C), the results obtained may be helpful in the recognition and explanation of the same process in the gastrointestinal tract. The results of this study, as an analysis of transformations of bean seed macrocomponents upon the mild experimental treatment, are relevant to both the pre-processing of legume seeds on a food technological scale and the process of preparing meals at home.

CONCLUSIONS

Mild hydrothermal treatment (water bath at 40°C/24 h) of bean seeds (*Phaseolus* sp.) can change the microstructure of their cotyledons and some components of bean seeds independently of bean seed size.

Water absorption induces physicochemical effects, such as certain structural consequences in biopolymers, proteins and starch, and a distinct decrease in the resistant starch fraction, especially during the first three hours of the processing. The mild hydrothermal treatment applied caused also changes in both dietary fibre fractions, generally, the increase of insoluble fraction, however, the changes in both fractions were more distinct in small-seed bean varieties (*Phaseolus* sp.).

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