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CHANGES OF FLATULENCE-CAUSING SUGARS IN LEGUME PROTEIN SAMPLES BY HIGH HYDROSTATIC PRESSURE

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ABSTRACT

Changes of flatulence-causing sugars in protein samples of bean, lupine and pea were examined. Protein samples were treated by high hydrostatic pressure (300 and 600 MPa, 30 min, 20°C) and by heat (100°C, 30 min). The content of raffinose family sugars were determined by HPLC, gas production was evaluated by *in vitro* method. Gas production decreased in the treated samples and was not significantly correlated with the amount of raffinose family sugars. The content of sugars increased after pressure and heat treatment. Such tendency might be resulted of bonds protein-saccharide disruption.

Key words: Flatulence- causing sugars, raffinose family sugars, legume proteins

INTRODUCTION

Legumes are known to contain certain oligosaccharides, especially raffinose, stachyose, and verbascose, which are contributory factors to the flatulence problem. Oligosaccharides of the raffinose family are not digested due to the lack of alpha-1,6-galactosidase in the intestinal mucosa. Consequently, these saccharides are not absorbed into the blood and are metabolized by the biota (mainly *Clostridia*) of the lower intestinal track resulting in production of large amounts of carbon dioxide and hydrogen. The absence of alpha-1,6-galactosidase capable of hydrolyzing the alpha-1,6-galactosidic linkage leads to accumulation of these saccharides in the lower intestine

and undergo anaerobic fermentation by bacteria, especially *Clostridia* [24, 25, 26]. The latter process is accompanied by an emission of gases causing a great discomfort to many consumers.

Studies on removal or reduction of raffinose family sugars have been mostly on genetic selection [22, 24], fermentation [9, 31], ultrafiltration [21]. Thermal process also reduce their content [1, 10, 14, 28, 31].

High hydrostatic pressure technology is new alternative food processing method to heating and food technologists are more and more interested in this process [4, 23, 29]. Only a few papers are available on the effect of high hydrostatic pressure on saccharides. Monosaccharides do not change at pressures of 100 – 1000 MPa [27]. An attention is made mainly on polysaccharides because of their very important role in the texture of food [6, 7, 8, 12]. There are no information on galactosugars.

This study was undertaken to evaluate the effect of high hydrostatic pressure on galactosaccharide content and on potential flatulence caused by these sugars present in protein samples prepared from bean, pea and lupine. Moreover, the effect of heat treatment was also examined. A microorganism involved in gas forming in the intestine *Clostridium perfringens* was used to estimate the volume of produced gases.

MATERIALS AND METHODS

MATERIALS. Dry seeds of bean *Phaseolus vulgaris*, pea *Pisum sativum* and lupine *Lupinus albus* were obtained from Polish Plant Breeding Stations. Dry legume seeds were dehulled, ground and sieved (100 mesh). Protein samples were prepared from legume flours according to the procedure described previously [16]. Flour was extracted with NaOH (pH 9.2). Proteins were precipitated at their points of minimum solubility (pH 4.2-4.7) by addition of HCl. The precipitates were adjusted to pH 7.0 and lyophilized. The protein content of bean, pea and lupine protein samples were 78.6, 86.3 and 84.5 % d.m., respectively.

Lyophilized protein samples in the solid state (not solubilized in water) were processed in a pressure apparatus LCP-20 by method of Jurczak and Gryko [15]. Samples were pressured at 20°C for 30 min at 300 and 600 MPa. The heat-treated (100°C, 30 min) samples were also examined.

DETERMINATION OF OLIGOSACCHARIDES BY HPLC [19]. The samples of flour and protein samples were extracted with aqueous alcohols (50%) at 86-90^{o}C. Soluble proteins were precipitated by adding lead acetate (10%). After centrifugation at 10000 g for 10 min supernatants were filtered through Supelco (Sep-Pack, 0.45 micrometers). The identification of individual sugars was performed by means of HPLC technique. A Shimadzu LC6A apparatus was equipped with refractive index detector (RID-6A) and CR6A recording integrator. The column Lichrosorb NH_2 (250x4 mm) for carbohydrate analysis was used. The mobile phase was acetonitrile: water 60 : 40. The flow rate was 1 ml/min. Sample volume was 20 microliters. The standards of sucrose was obtained from MERCK (Germany), raffinose from LOBA (Austria), stachyose from SIGMA (USA) and verbascose from Nestle (Switzerland).

FLATULENCE DETERMINATION BY *IN VITRO* METHOD [26]. Water suspension was prepared from pressurized and heated samples (about 0.1 g dry matter/ml solution). The used strain *Clostridium perfringens 546* was obtained from the Polish State Institute of Hygiene in Warsaw (Poland). The test strain was activated by transferring of pure culture from Robertson medium to thioglycollate medium [3]. The culture was incubated in 37^{o}C for 16 h and again inoculated on thioglycollate medium under the same conditions. The bacteria cell numbers in inoculum was determined in hemacytometer (Thom) and adjusted to the same amount in all repetitions. The amount of gas produced during incubation of medium containing protein samples with *Clostridium perfringens 546* was determined in syringes (20 ml volume). The gas volume was measured after 2, 4, 6, 21 and 24 h by following the movement of the plunger in the syringe. Simultaneously the untreated (control) protein sample was examined.

STATISTICAL ANALYSIS. All results of experiments were done in triplicate. One-way variation analysis was applied to the data using Statgraphics Statistical Graphic System Software ver. 5.0. Correlation coefficients were calculated by least square method [5].

RESULTS AND DISCUSSION

UNTREATED SAMPLES (CONTROL). Table 1 shows the contents of total sugars and of the raffinose family saccharides at flour and control protein samples (untreated by high pressure and temperature). The percentage of alpha-galactosides at bean flour was lower (47%) than that of pea and lupine flours (73-71%). Besides a great decrease of raffinose family saccharides in protein samples, the percentage of total sugars content was almost the same (1-4% decrease).

Table 1. Content (percent dry weight) of saccharides in flour and protein samples of bean, lupin and pea determined by HPLC

Sample	Raffinose family sugars	Sucrose	Total saccharides
Bean			
flour	2.54	2.87	5.41
protein sample (untreated)	0.17	0.23	0.40
Lupin			
flour	7.86	2.84	10.70
protein sample (untreated)	0.13	0.05	0.18
Pea			
flour	6.68	2.69	9.37
protein sample (untreated)	0.31	0.14	0.45

PRESSURE- AND HEAT-TREATED SAMPLES. Table 2 and Figure 1 show the changes of oligosaccharides in protein samples treated by high pressure and by temperature. Sucrose and stachyose were predominant in the bean and lupine control protein samples, the pea control sample also contain significant amounts of verbascose. Raffinose was present in all samples in smaller content than that of stachyose. The predominant found in this study for individual sugars is comparable to values found by other groups [10, 20, 33].

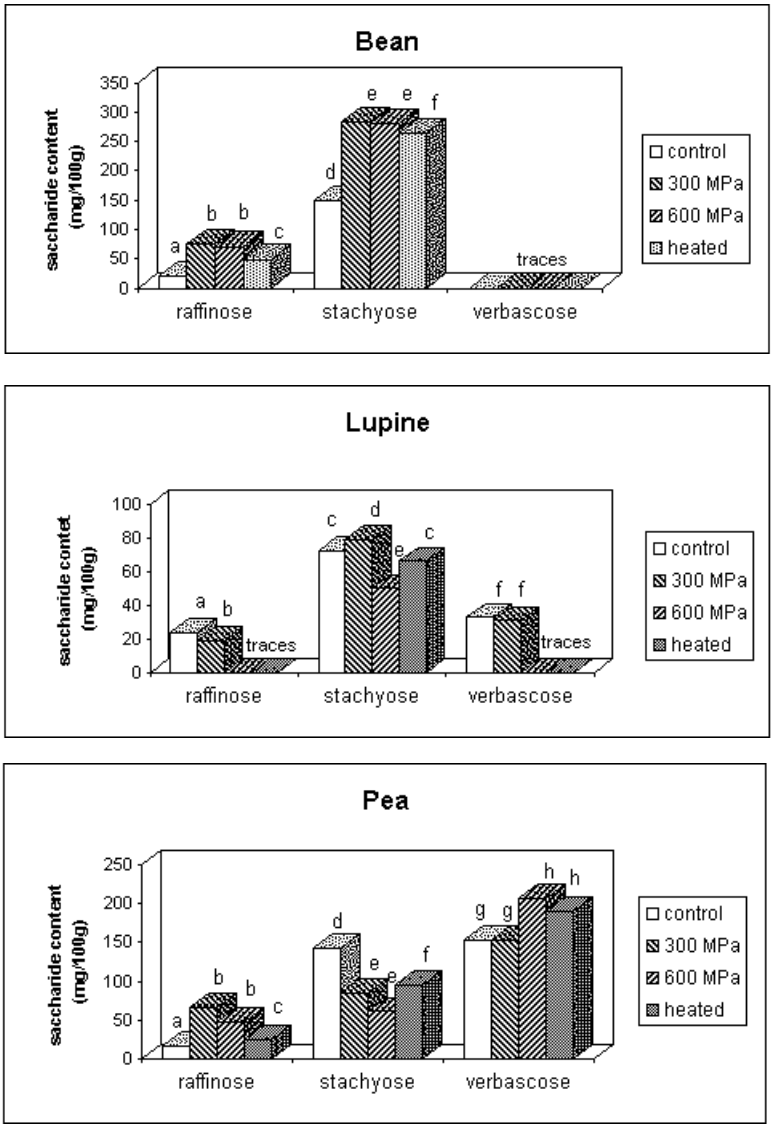
Table 2. The influence of high pressure on the percentage content of oligosaccharides in protein samples

Protein sample	Oligosaccharides (%)			
	sucrose	raffinose	stachyose	verbascose
Bean				
control	57.2	5.3	37.5	nd*
300 MPa	40.2	12.5	47.3	nd
600 MPa	46.6	10.8	42.6	nd
heated	38.9	7.1	39.0	nd
Lupin				
control	28.7	13.3	39.8	18.2
300 MPa	32.5	10.3	41.2	16.0
600 MPa	55.3	nd	44.6	nd
heated	41.2	nd	58.8	nd
Pea				
control	31.0	3.8	31.5	33.7
300 MPa	28.3	15.8	19.8	36.1
600 MPa	32.5	10.1	13.1	44.3
heated	29.5	5.7	21.6	43.2

*Not detected

High pressure caused a statistically significant changes of raffinose and stachyose content in relation to the control samples (Figure 1). The raffinose and stachyose content increased in bean samples. Pea samples treated by high pressure were richer in raffinose and werbascose, but stachyose content decreased after pressuring. The content of these sugars in lupin was dependent on pressure dose. Similar tendencies were observed after heat treatment, however the changes appeared in smaller extent. The rise of galactosugar content can be associated with better extraction ability from studied material after pressure treatment. The interpretation of this phenomenon should based on the changes of interaction protein-sugar induced by high pressure. In the literature there is any publications on this topic. Based on Imeson and co-workers [13] study, concerned interaction polysaccharides and proteins, it can be stated that the major forces responsible for such interactions are electrostatics in nature. High pressure acts on individual chemical bonds in different manner. Hydrogen bonds are favoured and stabilised, while aromatic and covalent bonds stay intact [11].

Fig. 1. Changes of oligosacharydes in protein samples treated by high pressure and temperature. Values with different superscripts (a, b..) are significantly different at p<=0.05



Large molecules or cell structures are destroyed by high pressure, while small molecules, not having a secondary or tertiary structure, remain unaffected. It can be supposed that the changes observed in oligosaccharides present in protein samples are dependent on protein denaturation precesses. Such changes were observed earlier in our investigations [17, 18]. We established that high pressure denaturation influenced on the protein structure in another manner than thermal process. That can be the reason of the differences in the studied saccharide levels.

Flatulence Effect. Gas volumes formed during incubation of mediums containing protein samples with *Clostridium perfringens 546* were estimated after 2, 4, 6, 21 and 24 hours. The data in Tables 3, 4 and 5 show that the volume of gas formed in suspension of protein samples after 21 hours was the same as after 24 hours. It means that fermentation process has been finished. The results for pressurized and heat-treated samples were compared with that for control ones. Gas production by protein samples treated by pressure was significantly lower than by the control sample. Earlier studies by Nowak [25] suggested that water extracts of pea and soy had compounds which alternated the growth and gas production by *Clostridia.* Nowak and Steinkraus [26] also stated that among oligosaccharides stachyose had the biggest influence on such processes.

Table 3. Production of gas (ml) by *Clostridium perfringens* in suspension of bean protein samples after different fermentation time

Protein sample	Fermentation time (h)				
	2	4	6	21	24
control	0.46[a]	1.28[a]	1.69[a]	1.75[a]	1.75[a]
300 MPa	0.35[b]	0.87[b]	1.33[b]	1.42[b]	1.42[b]
600 MPa	0.35[b]	1.05[c]	1.48[b]	1.91[c]	1.91[c]
heated	0.46[a]	1.05[c]	1.40[b]	1.75[a]	1.75[a]

The same superscript in the same column means no significant differences (alpha ≤ 0.05)

Table 4. Production of gas (ml) by *Clostridium perfringens* in suspension of lupin protein samples after different fermentation time

Protein sample	Fermentation time (h)				
	2	4	6	21	24
control	0.46[a]	1.46[a]	1.75[a]	2.10[a]	2.10[a]
300 MPa	0.52[b]	1.05[b]	1.40[b]	1.75[b]	1.75[b]
600 MPa	0.35[c]	0.87[c]	1.22[c]	1.57[b]	1.57[b]
heated	0.35[c]	1.22[d]	1.40[b]	1.75[b]	1.75[b]

The same superscript in the same column means no significant differences (alpha ≤ 0.05)

The process of modification of bean protein samples differentiated the ability of gas production, especially after 6 and 24 hours of fermentation. Smaller amounts of gas were observed in the samples of bean protein modified by high pressure although the differencies were not always statistically significant (Table 3). Similar results were obtained for pea and lupin protein samples (Tables 4 and 5). It was noticed that the pea protein samples treated by 600 MPa had significantly lower gas production after 4 hours of fermentation and lupin protein samples after 2, 4 and 6 hours. Observation made up to 6 hours, time corresponding with legume digestion, showed that volume of produced gas by pressurized samples was statistically significantly lower (level of confidence 0.05) as compare to the control. From our investigation it can be presumed that pressurization as well as heating decrease gas production by protein samples.

Table 5. Production of gas (ml) by *Clostridium perfringens* in suspension of pea protein samples after different fermentation time

Protein sample	Fermentation time (h)				
	2	4	6	21	24
control	0.43[a]	1.23[a]	1.79[a]	1.75[a]	2.27[a]
300 MPa	0.17[b]	1.02[b]	1.40[b]	1.42[b]	1.92[bc]
600 MPa	0.17[b]	0.31[c]	1.34[b]	1.91[c]	2.10[ac]
heated	0.09[c]	0.44[d]	1.14[b]	1.75[a]	1.66[b]

The same superscript in the same column means no significant differences (alpha ≤ 0.05)

CORRELATION OF SUGARS WITH GAS PRODUCTION. The results were analyzed statistically to find the relationship between oligosaccharide content and gas production. The correlation coefficients were -0.21, 0.33 and 0.72 for bean, pea and lupine, respectively. The significant correlation between gas production and content of individual sugars (raffinose, stachyose or verbascose) also was not found. This means that relationship pointed out in present study is not significant in spite of data found in literature showed close relation of discomfort (deleterious effect) when legume are ingested by man and raffinose family sugars.

Although the galactosaccharide content in the pressure and heat treated samples increased in comparison with the untreated sample it was found that during 6 and 24 h fermentation of most of protein samples by *Clostridium perfringens* production of gas was smaller than that of the control. It is then evident that pressurization has positive effect on compounds causing discomfort. These results confirm that gas forming during legume digestion is also connected with other compounds, not only with galactosaccharides. Some investigators had attributed the flatulence to the oligosaccharides while others related it also to another unidentified chemical components [2, 30, 32, 34].

In conclusion, it was stated that the increase of the raffinose and stachyose content was in pressurized protein sample of bean higher than in heated sample. Similar tendency was observed for raffinose in pea and stachyose in lupine. Growth of raffinose family sugars is likely related to better extractability as a consequence of disruption of bonds protein-saccharide. This increase is only apparent, connected with analytical method and higher extractability of sugars from examined materials, but not with real content of these compounds.

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