



RAFFINOSE-SERIES OLIGOSACCHARIDES IN SOYBEAN PRODUCTS*

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Soybean foods forming a substantial part of Asian diet have still more expanded into European diet. Raffinose-series oligosaccharides (RSO) are important constituents of soya beans and they can be found also in soybean products. These oligosaccharides can be considered potentially prebiotic for their capability of influencing the composition of the host's intestinal microbiota. The aim of the present paper was to determine the oligosaccharide content in various soybean products. Enzymatic assay has been used for the determination of oligosaccharides. RSO have been found in all tested samples and their content varied from 0.66 g per 100 g in soybean beverage to 5.59 g per 100 g in first clear soybean flour. Generally, the highest content of RSO has been detected in soybean flour in the average amount of 4.83 g per 100 g. There was no statistically significant difference observed in the amount of oligosaccharides in all four types of soybean flour ($P < 0.01$). Considerably high amounts of RSO have been found in sweet soybean bars and textured soy protein. Foods as soybean flour and soybean bar 'Sójový suk' seem to be effective natural sources of prebiotic oligosaccharides for humans.

soya; saccharides; legumes; stachyose; intestinal microbiota



doi: 10.1515/sab-2015-0019

Received for publication on August 15, 2014

Accepted for publication on January 27, 2015

INTRODUCTION

Soya beans (*Glycine max*) are a legume crop and excellent food and feed source all over the world. Dried soya beans contain approximately 40–45% of protein, 20% of total lipids, 30–35% of carbohydrates including 20% of total dietary fibre and around 5% of ash (Berk, 1992). Various types of carbohydrates present in soya beans are described in Table 1.

Soya beans are a rich source of oligosaccharides, namely stachyose, raffinose, and verbascose. Raffinose is a trisaccharide containing galactose linked by α -(1-6) bond to the glucose unit of sucrose, stachyose and verbascose are further elongations by α -(1-6) linked galactose unit bonded to tetrasaccharide and pentasaccharide, respectively. These oligosaccharides are called raffinose-series oligosaccharides (RSO), raffinose family oligosaccharides, galactosyl-sucrose oligosaccharides or α -galactosides. Other reported major sugar of soya beans is sucrose, with lower amounts

of monosaccharides: fructose, rhamnose, and arabinose. Significant levels of glucose occur only in immature seeds (van der Riet et al., 1989).

A characteristic α -galactosidic bond between sucrose and galactose is very important as humans do not possess the α -galactosidase enzyme that is necessary for hydrolyzing the bond typical in these oligosaccharides, so that they cannot be digested when consumed. Intact oligosaccharides reach the colon, where they are fermented by microorganisms that contain α -galactosidase (Liu, 1997).

Soya beans are potential prebiotics (Robertson, 2007), but there are different views on the effects of soybean oligosaccharides on human intestinal microbiota. Bounnik et al. (2004) observed significant increase of population of bifidobacteria *in vivo* by soybean oligosaccharides. Similar results were obtained both *in vitro* and *in vivo* in a previous study published by Hayakawa et al. (1990) and in *in vitro* study by Saito et al. (1992). Rada et al. (2008) tested effects

* Supported by the Grant Agency of the Czech University of Life Sciences Prague (CIGA) (Project No. 20132023 and Project No. 20142013).

Table 1. Classification of carbohydrates present in soybeans (Berk, 1992)

Total carbohydrates	30 - 35 %
• Soluble sugars	10 %
- Sucrose	5 %
- Stachyose	4 %
- Raffinose	1 %
• Insoluble fibre (cellulose, hemicelluloses, pectic polysaccharides)	25 %
• Starch	< 1 %

of raffinose and stachyose on infant bifidobacteria and clostridia *in vitro* and found that both oligosaccharides stimulated the growth of both bifidobacteria and clostridia, which raises the question regarding the selectivity of these oligosaccharides. Suarez et al. (1999) observed increased gas production in healthy volunteers when consuming conventional soybean flour which also supports the doubts about the selectivity taking into consideration that bifidobacteria are not gas-producing bacteria (Biavati, Mattarelli, 2012). Other study also concluded that low molecular constituents of soya bean are responsible for flatulence in men (Steggerda et al., 1966). On the other hand, Bounhnik et al (2004) did not observe increased symptoms of intestinal discomfort in volunteers when consuming soybean oligosaccharides.

A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health (Roberfruid, 2007). Although some authors do not consider RSO as prebiotic (Gibson et al., 2004; Roberfruid, 2007), there are strong suggestions that they are able to promote bifidobacteria at the expense of other intestinal bacteria and thus to promote the host's health (Saito et al., 1992; Mitsuoka, 1996).

Oligosaccharides can be removed from soya beans by various methods. Soaking is the easiest, but also most ineffective way to reduce oligosaccharides in whole grains with around 25% reduction. By the combination of soaking, dehulling, washing, and cooking, more than half of total oligosaccharides can be removed (Egounlety, Aworh, 2003). Bianchi et al. (1983) recommended cooking instead of soaking as a more useful method for reduction of RSO. Enzymatic degradation by α -galactosidase can be also used to reduce RSO from soybean products (Kotiguda et al., 2007) as well as aqueous ethyl alcohol extraction (Singh et al., 2008). Low oligosaccharide meal from genetically modified soya beans represents another way of reduction of RSO in the diet (Parsons et al., 2000).

Soya beans are primarily cultivated for edible oil and animal feeding. Considering high protein content,

soya beans are potentially rich source of protein for human nutrition. There is a variety of products made from soya beans nowadays. Many soy products are traditionally used in eastern Asia as an important part of the diet (Berk, 1992), a great nutritional potential of soybeans has led to extension of soybean products to western world. A soy beverage, sometimes incorrectly called soymilk, is one of such products. It is a water extract of soya beans nutritionally comparable to bovine milk, but not of the same quality as it has a slightly different amino-acid pattern, significantly lower micronutrients content, and it lacks cholesterol and cobalamin in comparison to bovine milk. Moreover, it contains typical soybean compounds as phytic acid, saponins, phytoestrogens, and oligosaccharides (Dostálková, 2003). Textured soy protein or textured vegetable protein was analyzed within this study. It has often been used as a meat extender or a meat analogue (Berk, 1992).

The aim of the present study was to determine the amount of RSO in various kinds of soybean flour and several soybean products and to evaluate possible prebiotic effect on human intestinal microbiota.

MATERIAL AND METHODS

Soybean products selected for measurement of RSO content are shown in Table 2. The products were purchased from local Czech manufacturers.

Measurements were performed by commercial assay kit for determination of galactosyl-sucrose oligosaccharides (raffinose, stachyose, verbascose), K-RAFGL (Megazyme International Ireland Ltd., Bray, Ireland). The principle of the assay is that RSO are hydrolyzed to D-galactose and sucrose using α -galactosidase. Subsequently, sucrose is hydrolyzed to D-glucose and D-fructose using invertase. D-glucose is then determined using glucose oxidase/peroxidase reagent. The method does not distinguish between raffinose, stachyose, and verbascose, but rather, measures these as a group. Since one mole of each of the raffinose-series oligosaccharides contains one mole of D-glucose, the concentrations are presented on a molar basis. Free sucrose and D-glucose in sample extracts are determined concurrently in duplicates not containing α -galactosidase. With samples used in this assay, we considered stachyose as the main oligosaccharide contained in the samples. Therefore the amount of RSO is expressed as a stachyose equivalent. After the incubation, the colour change appears. All samples were measured spectrophotometrically with 510 nm absorbance by Infinite 200PRO microplate reader (Tecan Group Ltd., Männendorf, Switzerland).

STATISTICA (Version 12, 2013) software was used to perform one-way ANOVA analysis (Duncan test) with multiple range comparison at a confidence

Table 2. RSO content of soybean products.

Product	Manufacturer	mMol/100 g ¹	g/100 g ^{1,2}
Soybean flour – reference sample	-	7.44 ± 1.80	4.95 ± 1.20
Organic first clear soybean flour	Natural Jihlava, CZ	6.37 ± 0.66	4.24 ± 0.44
First clear soybean flour	Paleta, Lipnice, CZ	8.38 ± 2.20	5.59 ± 1.47
All-purpose soybean flour	Paleta, Lipnice, CZ	6.80 ± 1.22	4.53 ± 0.82
Soybean beverage ‘Zajíc’	Morgador, Otrokovice, CZ	0.99 ± 0.40	0.66 ± 0.27
Sweet soybean bar ‘Sójový suk’	Unimex, Prague, CZ	4.17 ± 0.80	2.78 ± 0.54
Sweet soybean bar ‘Sójový řez’	Altis, Kolín, CZ	3.25 ± 0.32	2.16 ± 0.15
Soybean bar with coconut flavor ‘Margot’	Nestlé, Prague, CZ	2.45 ± 0.50	1.63 ± 0.33
Textured soy protein ‘Sojové vločky’	Natural Food, Prague, CZ	5.03 ± 1.03	3.36 ± 0.69
Soya creamer	Morgador, Otrokovice, CZ	2.73 ± 0.49	1.82 ± 0.33

¹All values are means of three measurements ± standard deviation.

²Values are calculations of stachyose equivalent.

level of 99% for the differences among various types of soybean flour and among sweet soybean bars.

RESULTS

All soybean products contained detectable amounts of RSO. The largest amount of RSO was measured in first clear soybean flour. Other types of soybean flour contained similar amounts of oligosaccharides as in first clear soybean flour. Average amount of oligosaccharides in all kinds of soybean flour was 4.83 g per 100 g. These values correlate with those recorded by Karr-Lilienthal et al. (2005) and Choct et al. (2010) indicating that concentration of stachyose in soybean flour is 2–5% of dry matter (DM) and concentration of raffinose is 0.5–2% of DM. Textured soy protein contained the highest amount of oligosaccharides among non-flour samples. Two of soybean bars contained more than 2 g of RSO and soybean bar with coconut flavour contained around 1.5 g of RSO. Almost 2 g of RSO was found in soya creamer. Soybean beverage contained the lowest amount of RSO. No statistically significant difference ($P < 0.01$) in the amount of oligosaccharides present in all kinds of soybean flour and in both sweet soybean bars was observed. All results are shown in Table 2.

DISCUSSION

Concentrations of oligosaccharides determined in the four samples of soybean flour corresponded with those given in literature (Karr-Lilienthal et al., 2005) despite quite great standard deviations in some samples, probably due to nature of the method which is adequate for tentative determination of RSO

(Vinjamoori et al., 2004). On the other hand, enzymatic methods are highly specific.

There are no limits and regulations for prebiotics or oligosaccharides in food whatsoever, so it is difficult to compare our results with official data. The exception is the Commission Directive of the European Union on infant formulae and follow-on formulae that recommends maximum content of 0.8 g per 100 ml of oligosaccharides in a combination of 90% of galactooligosaccharides and 10% of long chain fructooligosaccharides (Commission Directive 2006/141/EC).

Moreover, RSO can be included either in the category of carbohydrates or fibre in the product composition data sheet. It is recommended to consume 2.5–10 g of inulin type prebiotics per day to increase population of bifidobacteria in gut (Kelly, 2009). Bounik et al. (2004) reported that all dosages from 2.5 to 10 g of RSO per day increased the number of bifidobacteria in human gut. Considering these data, consumption of three slices (150 g) of soya bread per day is suitable for increasing the number of gut bifidobacteria as soya bread can contain up to 40% of soybean flour with no deterioration of sensory quality (Kopáčová, 2002; Řezáčová, 2010). Two pieces (100 g) of soybean bar ‘Sójový suk’ per day can also stimulate growth of bifidobacteria in human intestinal tract.

Textured soy protein also contains detectable amount of RSO and, moreover, it does not contain large amounts of monosaccharides and disaccharides unlike the sweet bars. However, it does not contain a sufficient amount of RSO per serving (up to 50 g) to stimulate the bacterial population of gut and thus cannot be considered as a potential prebiotic, the same as the soybean bars ‘Sójový řez’ and ‘Margot’ and soya creamer, because its recommended serving is only 5 g per cup of coffee. The lowest amount of

RSO, which has been found in soybean beverage, can be attributed to deliberate action during the processing. Recommended quantity of soybean beverage per serving is 25 g of powder into 250 ml of water. This amount is not enough for influencing of gut bacteria population and thus the soybean beverage cannot be considered as prebiotic.

The issue of prebiotic effect of RSO still needs to be resolved when we take into account that there are different opinions on prebiotic effects of RSO (S u a r e z et al., 1999; B o u h n i k et al., 2004; G i b s o n et al., 2004).

CONCLUSION

Raffinose-series oligosaccharides are substantial components of soybean foods which may beneficially affect the host's health by influencing the composition of microbiota. We have determined amounts of raffinose-series oligosaccharides in 9 products used in human nutrition and it can be concluded that consumption of sweet soybean bar 'Só jov ý s u k' and pastry containing sufficient amount of soybean flour can influence the host's intestinal microbiota due to perceptible amount of RSO.

REFERENCES

- Berk Z (1992): Technology of production of edible flours and protein products from soybeans. FAO Agricultural Services Bulletin, 97. Food and Agriculture Organization of the United Nations, Rome.
- Bianchi MLP, Silva HC, Campos MAP (1983): Effect of several treatments on the oligosaccharide content of a Brazilian soybean variety (*Glycine max*. (L.) Merrill). Journal of Agricultural and Food Chemistry, 31, 1363–1364. doi: 10.1021/jf00120a054.
- Biavati B, Mattarelli P (2012): Bifidobacterium. In: Goodfellow M, Busse HJ, Trujillo ME, Suzuki K, Ludwig W, Whitman WB (eds): Bergey's manual of systematic bacteriology. Volume 5. Springer, New York, 171–206.
- Bouhnik Y, Raskine L, Simoneau G, Vicaut E, Neut C, Flourié B, Brouns F, Bornet FR (2004): The capacity of nondigestible carbohydrates to stimulate fecal bifidobacteria in healthy humans: a double-blind, randomized, placebo-controlled, parallel-group, dose-response relation study. American Journal of Clinical Nutrition, 80, 1658–1664.
- Choct M, Dersjant-Li Y, McLeish J, Peisker M (2010): Soy oligosaccharides and soluble non-starch polysaccharides: a review of digestion, nutritive and antinutritive effects in pigs and poultry. Asian-Australasian Journal of Animal Sciences, 23, 1386–1398. doi: 10.5713/ajas.2010.90222.
- Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC (text with EEA relevance). Official Journal of the European Union, L401/1. <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ%3AL%3A2006%3A401%3A0001%3A0033%3AEN%3APDF>. Accessed 21 May, 2014.
- Dostálová J (2003): Comparison of nutritional value of cow milk and soybean beverages. Výživa a Potraviny, 58, 2–3. (in Czech)
- Egounlety M, Aworh OC (2003): Effect of soaking, dehulling, cooking and fermentation with *Rhizopus oligosporus* on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (*Glycine max* Merr.), cowpea (*Vigna unguiculata* L. Walp) and groundbean (*Macrotyloma geocarpa* Harms). Journal of Food Engineering, 56, 249–254. doi: 10.1016/S0260-8774(02)00262-5.
- Gibson GR, Probert HM, Van Loo J, Rastall RA, Roberfroid MB (2004): Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutrition Research Reviews, 17, 259–275. doi: 10.1079/NRR200479.
- Hayakawa K, Mizutani J, Wada K, Masai T, Yoshihara I, Mitsuoka T (1990): Effects of soybean oligosaccharides on human faecal flora. Microbial Ecology in Health and Disease, 3, 293–303. doi: 10.3109/08910609009140252.
- Karr-Lilienthal LK, Kadzere CT, Grieshop CM, Fahey GC (2005): Chemical and nutritional properties of soybean carbohydrates as related to nonruminants: a review. Livestock Production Science, 97, 1–12. doi: 10.1016/j.livprodsci.2005.01.015.
- Kelly G (2009): Inulin type prebiotics: a review (Part 2). Alternative Medicine Review, 14, 36–55.
- Kopáčková O (2002): Tasty soybean bread. Prepared Foods, 171, 44. (in Czech)
- Kotiguda G, Kapnoor SS, Kulkarni D, Mulimani VH (2007): Degradation of raffinose oligosaccharides in soymilk by immobilized alpha-galactosidase of *Aspergillus oryzae*. Journal of Microbiology and Biotechnology, 17, 1430–1436.
- Liu KS (1997): Chemistry and nutritional value of soybean components. In: KeShun L: Soybeans: chemistry, technology and utilization. Aspen Publishers Inc., Gaithersburg, USA, 25–113.
- Mitsuoka T (1996): Intestinal flora and human health. Asia Pacific Journal of Clinical Nutrition, 5, 2–9.
- Parsons CM, Yhang Y, Araba M (2000): Nutritional evaluation of soybean meals varying in oligosaccharide content. Poultry Science, 50, 1927–1931. doi: 10.1093/ps/79.8.1127.
- Rada V, Nevoral J, Trojanová I, Tománková E, Šmehilová M, Killer J (2008): Growth of infant faecal bifidobacteria and clostridia on prebiotic oligosaccharides in *in vitro* conditions. Anaerobe, 14, 205–208. doi: 10.1016/j.anaerobe.2008.05.003.
- Řezáčková S (2010): Influence of soya flour addition to the quality of pastry. Dissertation, Tomas Bata University in Zlín. (in Czech)
- Roberfroid M (2007): Prebiotics: the concept revisited. The Journal of Nutrition, 137, 830–837.

- Saito Y, Takano T, Rowland I (1992): Effects of soybean oligosaccharides on the human gut microflora in *in vitro* culture. *Microbial Ecology in Health and Disease*, 5, 105–110. doi: 10.3109/08910609209141296.
- Singh P, Kumar R, Sabapathy SN, Bawa AS (2008): Functional and edible uses of soy protein products. *Comprehensive Reviews in Food Science and Food Safety*, 2(1), 14–28. doi: 10.1111/j.1541-4337.2007.00025.x
- Steggerda FR, Richards EA, Rackis JJ (1966): Effects of various soybean products on flatulence in the adult man. *Experimental Biology and Medicine*, 121, 1235–1239. doi: 10.3181/00379727-121-31014.
- Suarez FL, Springfield J, Furne JK, Lohrmann TT, Kerr PS, Levitt MD (1999): Gas production in human ingesting a soybean flour derived from beans naturally low in oligosaccharides. *American Journal of Clinical Nutrition*, 69, 135–139.
- van der Riet WB, Wight AW, Cilliers JLL, Datel JM (1989): Food chemical investigation of tofu and its byproduct okara. *Food Chemistry*, 34, 202. doi: 10.1016/0308-8146(89)90140-4.
- Vinjamoori DV, Byrum JR, Hayes T, Das PK (2004): Challenges and opportunities in the analysis of raffinose oligosaccharides, phytate, and glucosinolates. *Journal of Animal Science*, 82, 319–328.

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