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COMPOSITION OF CARBOHYDRATES IN FRESH WHEY AS WASTE IN TOFU PROCESSING

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Soy whey is generated as a process waste while preparing tofu from soy milk, causing environmental pollution and also representing an economic burden for the soybean processing industry. On the other hand, literature data indicate that fresh tofu whey is characterized by a high content of total proteins with all essential amino acids present, a favorable residual activity of trypsin inhibitors, and a low content of lectins. This indicates a good nutritional value of tofu whey. Therefore, its potential valorization is of importance to the soy industry. The present investigation aims to determine the carbohydrate composition of tofu whey, which was obtained in the process of preparing tofu, from six soybean genotypes, using the hydrothermal-cooking-method, with the application of the chymosin-pepsin rennet. High-performance liquid chromatography (HPLC) was used. The presence of monosaccharide arabinose (0.39-1.73%) and disaccharide sucrose (2.19-4.60%) was registered. The presence of pentose-arabinose was expected, since it can be part of glycoproteins, so it was probably separated from the protein part of the molecule, since some of the soybean proteins are glycoproteins. In addition, arabinose is part of the disaccharide vicianoze, which is part of the soybean saponins. The presence of sucrose indicates that the weakly acidic environment of the whey was not sufficient to hydrolyze this disaccharide (to glucose and fructose). The results indicate that tofu whey can be potentially useful for application as a cheap, functional, and nutritional food additive, enabling sustainable production through the recycling of waste.

Keywords: tofu whey, HPLC, arabinose, sucrose, sustainable production

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INTRODUCTION

The most commonly used soy food products in the world are soy milk and tofu. In the process of soy milk production, two by-products are also produced: soy soaking water whey (which is separated after soaking soybeans) and okara (the solid part of soybean seeds that remains after milk extraction). Further processing of soy milk into soy cheese - tofu produces another by-product/waste, which is tofu whey. Tofu whey is a pale yellowish liquid that is separated after pressing tofu, which has a specific aroma/taste.

These by-products in the tofu production process represent a serious problem in terms of waste disposal. They represent organic waste that is very suitable for the development of many microorganisms. Today, more and more attention is paid to finding opportunities for the further use of this waste according to the principles of the circular economy and sustainable waste treatment [1-3].

In our previous research, we characterized soy milk and tofu produced using the hydrothermal cooking method (HTC) using chymosin-pepsin rennet [4]. In addition, we investigated the characteristics of soaking water whey [5] and okara [6-8] and their potential for use.

Also, in our previous work, we examined the major storage protein composition and the activity of bioactive proteins of fresh tofu whey [9]. Our research results, as well as literature data, indicate that fresh tofu whey is characterized by significant nutritional value. It has been found that tofu whey contains a high content of total proteins (22.67-28.00%), with a wide spectrum of amino acids, with all essential amino acids present (phenylalanine 7.23 mg/L, histidine 4.27 mg/L, isoleucine 4.40 mg/L, leucine 6.12 mg/L, lysine 8.49 mg/L, methionine 2.77 mg/L, tryptophan 12.84 mg/L, valine 5.26 mg/L, threonine 5.39 mg/L) [9, 10]. It was found that basic 7S globulin is the dominant fraction of the 7S protein fraction of tofu whey, followed by β-conglycinin and γ-conglycinin [9]. The presence of isoflavones (50.84 mg of total isoflavones/L; with dominant genistin 25.99 mg/L and daidzin 17.66 mg/L), as well as the presence of organic acids (4.77 g of total organic acids/L), was registered in tofu whey [10].

In our earlier research for the preparation of tofu, the hydrothermal cooking method (HTC) using chymosin-pepsin rennet as a coagulant [4] was used, and the research results indicated that the applied heat treatment significantly reduced the activity of proteins with potential antinutritional properties, such as trypsin inhibitors and lectins in the tofu whey. These phytochemicals can have adverse antinutritional effects after consuming a raw soybean meal (e.g., a disorder of vitamin and amino acid metabolism, adverse changes in the digestive tract, pancreatic hypertrophy and hyperplasia, and disturbance of mineral composition in the body) [11]. That is why the activity of trypsin inhibitors must be reduced. But on the other hand, they are high in sulfur-containing amino acids (with which soy proteins are in deficit), [12] so that their presence is desirable. To improve the nutritional quality of soy foods, trypsin inhibitors are generally inactivated by heat treatment [13-16], with the aim of balancing their content and activity (to preserve content and reduce activity). Namely, residual activity of trypsin inhibitors (1.95-3.76%) and a low content of lectins (5.04-5.48% of total extracted proteins) were found, as well as low urease index activity of the fresh tofu whey, which indicates that the heat HTC treatment was adequate to inactivate antinutritional factors [9]. So, this very low residual TIA of tofu whey suggested that tofu whey obtained by hydrothermal cooking could be applied in human consumption, because it features <20% of soybean trypsin inhibitor activity. Friedman and Brandon [17] reported that the commercial soy food can retain at most 20% of the activity of trypsin inhibitors, without any unwanted consequences on the body. In addition, literature data indicate that, given that trypsin inhibitors are more thermostable than lectins, achieving a satisfactory level of residual trypsin inhibitory activity leads to a satisfactory level of lectin content [17].

Since the application of the HTC process (short time/high temperature/under pressure) is significantly different from the traditional method of obtaining tofu (heating soybeans without pressure and using acids, glucono-δ-lactone, or salts ($MgCl_2$, $MgSO_4$, $CaCl_2$ and $CaSO_4$) as the coagulating agents), it can be expected that the characteristics of the obtained products and by-products will also differ after applying different processing conditions. The aim of this study was to determine the carbohydrate composition of tofu whey, which was obtained in the process of preparing tofu, from six soybean genotypes, using the hydrothermal-cooking-method, with the application of the chymosin-pepsin rennet. Namely, we believe that the knowledge of the characteristics of fresh tofu whey, which has not yet been studied, will help to better understand the possible utilization of this by-product obtained using the HTC process. The results obtained in this study complement our previous work on the nutritional and non-nutritive characteristics of products and by-products obtained by the HTC process [3-9, 18-21], so the results of these studies can potentially provide an opportunity for the production of soy-food (soy milk and tofu) according to the principles of sustainable production and circular economy.

MATERIALS AND METHODS

Materials

For tofu preparation, six commercial soybean genotypes were used: Novosađanka, Balkan and Krajina (selected by the Institute of Field and Vegetable Crops, Novi Sad, Serbia), Lana, Nena and ZPS-015 (selected by the Maize Research Institute Zemun Polje, Belgrade, Serbia), Commercial chymosin-pepsin rennet was used as the coagulating agent (Rennet workshop, Idealka," Novo Selo, Serbia).

Tofu processing and separation of tofu whey

Tofu was made using the method described by Stanojević et al [4], using a hydrothermal cooking method that includes short time/high temperature/under pressure, by applying the chymosin-pepsin as the coagulating agent. Briefly, soybeans (soybeans/water ratio = 1/6) were soaked in water for 14 h, at 5–7 ºC. Soaked soybeans were cooked by the hot-grind method with a steam injection system - 8 min/at 110 ºC/1.8 bar, (SoyaCow VS 30/40, model SM-30, Russia). The slurry was filtered to separate soy milk and okara, and then soy milk was cooled at room temperature, and commercial chymosin-pepsin rennet was added (10 mL rennet/L soy milk). The tofu curd was pressed mechanically (35 min), and the obtained fresh tofu whey samples were stored at 4 ºC before further analysis.

High-performance liquid chromatography (HPLC)

The characterization of carbohydrates using the HPLC technique was performed on a Waters chromatographic device (Waters Chomatography Div., Millipore, Milford, USA), which consists of a solvent and sample delivery system - Waters 600E System Controller and a detector - Waters 410 Differential Refractometer, on a column - Carbohydrate Analysis (Waters Chomatography Div., Millipore, Milford, USA) for the analysis of monosaccharides and disaccharides, while for the analysis of oligosaccharides (raffinose and stachyose) used Shugar-PAKTM - column of the same manufacturer. As a protective column, a GuardPakTM and RadialPakTM pre-column with ResolveTM Silica filling from the same manufacturer was used. As a mobile phase, a solution of acetonitrile/water in the ratio 83/17 (v/v) was used, for monosaccharides, and for polysaccharides, a solution of acetonitrile/water in a ratio of 65/35 (v/v). The column was calibrated by passing the mobile phase, with a gradual increase in the flow rate of 0.5 - 2 mL/min over a period of 5 hours.

In order to characterize monosaccharides and disaccharides, the standard solution contained: monosaccharides: xylose, arabinose, mannose, glucose, fructose; disaccharides: sucrose, maltose (Sigma, USA). The standards were dissolved in redistilled water, so that the standard mixture contained each of the listed monosaccharides and disaccharides at a concentration of 0.1%. Before application, they were filtered on a 0.25 μm filter (Nucleopore, Corp., Pleasanton, CA, USA). 10 μl of the standard solution was applied to the column and eluted for 25 minutes, at a flow rate of 2 mL/min and a temperature of 30ºC.

The examined samples were mixed with 95% ethanol in the ratio sample: ethanol = 1:5 v/v; 1 hour, in a water bath (T=50ºC), and then filtered (Whatman 42) while washing the precipitate (2 times) with 95% ethanol (supernatant:ethanol=1:1 v/v). After that, the samples were evaporated to dryness under vacuum (Devarot, Type-D-3, Elektromedicina, Ljubljana, Slovenia), then dissolved in an ultrasonic bath (Sonic, Italy) with redistilled water (initial sample: redest.H_2O=1:4 v/v). The extract was then filtered (Whatman 4) and frozen until use. Before injection into the HPLC device, all samples were filtered on a 0.25 μm filter (Nucleopore, Corp., Pleasanton, CA, USA) and applied to the column in the amount of 20μl. Chromatography was performed under identical conditions for samples and standards: flow rate - 2ml/min, at 30ºC, 25 min.

Statistical analysis

The experiments were performed in triplicate, and all results are presented as mean values with standard deviations. The significance of the differences between the means was determined using a t-test procedure at $P<0.05$. The data were analyzed using the Statistica software version 7.0 (StatSoft Co., Tulsa, OK, USA).

RESULTS AND DISCUSSION

The presence of the monosaccharide- arabinose (0.39-1.73%; Table 1) was registered in the tofu whey obtained from all investigated soybean varieties. The presence of this pentose could be expected, considering that it very often enters into the composition of glycoproteins. So, during the production process, it was probably released from the protein part of the molecule, considering that some of the main proteins of soybeans are precisely glycoproteins. On the other hand, arabinose is included in the composition of disaccharide vicianose, which is included in the composition of saponins [22], and saponins are present in non-defatted soybean flour with about 0.6-6.2% [23,24].

Table 1. Carbohydrate composition of tofu whey obtained using the HTC procedure*

Rt (min)	8.11	17.13
genotype	arabinose	sucrose
Nena	1.27±0.01^{b}	4.60±0.20^{a}
Krajina	1.13±0.02^{c}	4.25±0.22^{b}
Balkan	1.73±0.03^{a}	3.77±0.12^{c}
Novosadjanka	0.39±0.01^{f}	2.19±0.12^{e}
ZPS-015	0.54±.0.02^{e}	3.20±0.11^{d}
Lana	0.71±0.01^{d}	3.31±0.11^{d}

*Means in the same column with different roman letters are significantly different (P<0.05).
HTC - hydrothermal cooking method. Rt (min) - retention time.

Tofu whey also contained sucrose (2.19-4.60%; Table 1). The presence of this disaccharide may also be a consequence of its relatively large participation in defatted soybean flour (4.45%; or even from 6.1% to 12.4% [25,26]). On the other hand, considering that whey is characterized by a slightly acidic medium (pH 4.02; [10]), it is evident that the acidity is not sufficient to cause sucrose hydrolysis, since it is known that it undergoes acid hydrolysis to glucose and fructose.

The results obtained indicate a greater dependence of the genotype type in terms of sucrose content than arabinose. Namely, although a statistically significant difference was registered in the arabinose content of all tested genotypes, it can still be seen that the values are relatively approximate (0.39-0.71% for the Novosađanka, ZPS-15, and Lana genotypes and 1.13-1.73% for the Nena, Krajina, and Balkan genotypes). On the other hand, the influence of the genotype type is significant in terms of sucrose content. Namely, the highest sucrose content was registered in tofu whey obtained from the Nena genotype (4.60%), while the lowest sucrose content was registered in tofu whey obtained from the Novosađanaka genotype (2.19%).

The Novosađanka genotype was selected as high-protein (58.02% total protein, [4]) so that the high total protein content may be a reason for the lower carbohydrate content in products obtained from soybeans of the Novosađanka genotype. Namely, Hymowitz et al. [27] evaluated 60 soybean lines for protein content, total sugar, and individual sugar content. Their results suggested that total sugar content and individual sugar content are negatively correlated with protein content. These results indicate that the soybean genotype may have an impact on the carbohydrate composition of tofu whey.

Also, the presence of only these two sugars in tofu whey may be due to the significant retention of carbohydrates in okara as well as the conditions of the applied HTC procedure for preparing tofu, which differ significantly from the conditions of traditional soybean processing. Namely, the okara obtained after the same conditions of HTC processing contains 7.02-8.21% carbohydrates [7]. In addition, using the traditional method of obtaining soy milk and calcium sulfate as a coagulant in obtaining tofu, Chua and colleagues [10] registered: fructose 0.4 g/L, glucose 0.14 g/L, sucrose

111.72 g/L, and raffinose 0.30 g/L in tofu whey. Such results indicate that, in addition to the soybean genotype, the processing method has a significant effect on the characteristics of tofu whey. The results obtained in this study complement our previous work on examining the characteristics of soybean products and by-products obtained using the HTC process and indicate the specificity of the applied technological process.

CONCLUSION

Carbohydrate composition of tofu whey obtained from all tested soybean genotypes, using the HTC procedure, was characterized by the presence of monosaccharide arabinose and disaccharide sucrose. This result could be expected considering the carbohydrate composition of okara and the specificity of the applied technological process. In addition to other favorable characteristics of the composition of tofu whey (protein content and composition, as well as biologically active components), it can be assumed that tofu whey can potentially be used as a nutritional additive. Its distribution can reduce the production of organic waste in soybean processing.

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Izvod

SASTAV UGLJENIH HIDRATA U SVEŽOJ SURUTCI DOBIJENOJ KAO OTPAD U PRERADI TOFU-a

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Sojina surutka nastaje kao otpad iz procesa pripreme tofua od sojinog mleka, što uzrokuje zagađenje životne sredine i predstavlja ekonomski teret za proces prerade soje. S druge strane, literaturni podaci ukazuju da se sveža tofu surutka karakteriše visokim sadržajem ukupnih proteina sa prisutnim svim esencijalnim aminokiselinama, povoljnom rezidualnom aktivnošću inhibitora tripsina i niskim sadržajem lektina. Ovo ukazuje na dobru nutritivnu vrednost tofu surutke. Stoga je njena potencijalna valorizacija važna za industriju prerade soje. Cilj ovog istraživanja je da se utvrdi sastav ugljenih hidrata tofu surutke, koja je dobijena u procesu pripreme tofua, od šest genotipova soje, primenom metode hidrotermčkog kuvanja, uz primenu himozin-pepsinskog sirila. Korišćena je visokoefikasna tečna hromatografija (HPLC). Registrovano je prisustvo monosaharida arabinoze (0,39-1,73%) i disaharida saharoze (2,19-4,60%). Prisustvo pentoze - arabinoze bilo je očekivano, s obzirom da može biti deo glikoproteina, pa je verovatno odvojena od proteinskog dela molekula, s obzirom da su neki od sojinih proteina glikoproteini. Pored toga, arabinoza je deo disaharida vicinoze, koji je deo sojinih saponina. Prisustvo saharoze ukazuje na to da slabo kisela sredina surutke nije bila dovoljna da hidrolizuje ovaj disaharid (do glukoze i fruktoze). Rezultati ukazuju da tofu surutka može biti potencijalno korisna za primenu kao jeftin, funkcionalni i nutritivni aditiv u pripremi hrane i da može omogućiti održivu proizvodnju kroz reciklažu otpada.

Ključne reči: tofu surutka, HPLC, arabinoza, saharoza, održiva proizvodnja