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THE IMPACT OF ASPARAGINASE ON TEXTURAL PROPERTIES OF WHOLEGRAIN CEREAL BISCUITS ENRICHED WITH SEA BUCKTHORN POMACE

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ABSTRACT

Cereal biscuits are popular snacks of daily usage. Their nutritional value is enhanced if they are produced from wholegrain flour of nutritionally rich cereals and/or contain valuable natural sources of health promoting compounds. In this study, wholegrain flour from wheat, rye, and triticale (hybrid of wheat and rye) were used as a basis for biscuit production and dried sea buckthorn (*Hippophae rhamnoides* L.) pomace as an additive for enhancement of their nutritional value. Besides nutritional benefits, sea buckthorn pomace addition increased hardness up to 37% and fracturability up to 30%, and moreover, an undesirable acrylamide formation was increased twice in case of wheat and triticale biscuits, and up to 4.5 times in rye biscuits. For diminishing the acrylamide formation, the asparaginase treatment of wet sea buckthorn pomace was applied which resulted in a substantial decrease of acrylamide (30 – 60%) in final biscuits. This intervention was examined in terms of the impact on textural properties of cereal biscuits with sea buckthorn pomace and with enzymatically treated sea buckthorn pomace. Despite expectations of negligible impact on quality properties of biscuits, texture analysis showed significant differences between cereal biscuits containing untreated and enzymatically treated sea buckthorn pomace reflected in a decrease of hardness and fracturability to original values in controls, at least. However, these alterations cannot be fully attributed to an enzymatic treatment of sea buckthorn pomace since the procedure of asparaginase application, including the adjustment of pH to neutral values, affected the properties of this additive. Descriptive sensory analysis and consumer acceptance test revealed significant preferences of triticale and rye biscuits with enzymatically treated sea buckthorn pomace addition.

Keywords: cereal biscuits, sea buckthorn, acrylamide, asparaginase, texture, hardness, fracturability, preference score

INTRODUCTION

Cereal biscuits are popular snacks among consumers of any ages, as well as there are a preferred matrix for investigation of many interventions improving their nutritional values. Due to higher amount of bioactive compounds (dietary fibre, proteins, amino acids, minerals, antioxidants etc.) wholegrain flour is frequently used for innovative health-promoting cereal products (**McKevith, 2004**). Moreover, their nutritional values can be improved also by addition of various plant-based ingredients or selected plant-derived bioactive compounds which is well described in scientific literature **(Borczak et al., 2022; Quiles, Campbell, Struck, Rohm, & Hernando, 2018**). Among them, sea buckthorn (*Hippophae rhamnoides* L.) is exceptionally valuable due to its rich and balanced composition of polyphenols, flavonoids, carotenoids, antioxidants, vitamins, fibre, unsaturated fatty acids, essential amino acids, macro and micro elements, etc. **(Bal, Meda, Naik, & Satya, 2011; Ciesarová et al., 2020; Dienaitė et al., 2020; Gâtlan & Gutt, 2021; Janotková et al., 2021**). Sea buckthorn pomace left over from juice production is still full of valuable bioactive substances, but due to its astringent taste, it is rarely used and consumed separately (**Ciesarová et al., 2020**). For this reason, it is a good option to use it in such matrices that provide dominant sensory attributes to food products to mask the undesirable aspects of sea buckthorn taste and aroma (**Janotková et al., 2021**). On the other hand, sea buckthorn has a huge potential to produce undesirable acrylamide which is classified as probably carcinogenic compound (**IARC, 1994**). Different cereals have different dispositions to produce acrylamide based on their amino acid profile (**Ciesarová et al., 2021**). There are many available tools how to reduce acrylamide, but usually they require adjustment of processing conditions, selection of raw materials, or addition of other ingredients which can alter the expected quality properties of final products (**Palermo et al., 2016**). However, enzymatic treatment with asparaginase prevents from acrylamide formation by conversion of the main precursor amino acid asparagine to other amino acid aspartic acid which does not enter reactions leading to formation of acrylamide. The proclaimed advantage of asparaginase usage is any or negligible impact on quality properties of final products (**Gazi, Göncüoğlu Taş, Görgülü, & Gökmen, 2023**). These properties are expressed by visual characteristics, especially color, appearance, volume, spread ratio etc., and characteristics defined impressions during and after eating. Texture, defined as the sensory manifestation of food structure and the way in which this structure reacts to the forces applied, represents the junction of all the mechanical, geometric and superficial attributes of a product, sensed through mechanical, tactile, visual and hearing receptors (**Szczesniak, 2002**). Moreover, texture can be related to the deformation, disintegration and flow of the food when a force is applied **(Bourne, 2002**). Texture characteristics of singular products can be perceived with positive or negative impressions which is revealed by consumer acceptance test. This approach was used for determination of the impact of enzymatic treatment of sea buckthorn pomace which was used as nutritionally valuable additive to the wholegrain biscuits from wheat, triticale and rye on textural properties and overall consumer acceptability of improved products.

MATERIAL AND METHODS

Chemicals and biological material

Commercial wholegrain flours from common wheat (*Triticum aestivum*) and rye (*Secale cereale*), as well as flour obtained by milling grains of triticale (×*Triticosecale*) variety 'Odisej', were used as the basic ingredients in the production of biscuits. Triticale grain was milled using Quadrumat® Senior roller mill (Brabender, Germany), and the yield of refined flour was in the range of 66-68%. Refined flour was combined with bran in ratio 70:30 to reconstitute wholegrain triticale flour.
Crystal sugar (Sunoko, Serbia), vanilla sugar (Dr Oetker, Germany), soybean lecithin (Sojaprotein, Serbia) and table salt (So Produkt, Serbia) were bought at a local market. Vegetable fat HF with melting temperature of 36-38 °C (Puratos, Belgium) was used as a shortening.
Sea buckthorn (*Hippophae rhamnoides* L.) berries were obtained from the crop of PD Tvrdošovce farm (Slovakia) harvested in 2020. The berries were collected on twigs, frozen at -21°C, then plucked from the branches.

Procedure of enzymatic treatment of sea buckthorn pomace

Sea buckthorn berries were washed and grinded into mash by a hand mixer (Electro-Pulver EP8, Grâce-Hollogne, Belgium) at 13,000 rpm for 2 min. The mash was pressed and filtered manually through gauze to separate juice and pomace. A part of sea buckthorn pomace (SBP) was dried in a drying machine (Memmert UF260, Schwabach, Germany) at 55 °C for 24 hours, then grinded (Grindomix GM200, Retsch, Haan, Germany) at 5000 rpm for 15 s for obtaining untreated SBP powder. The rest of sea buckthorn mash was buffered from pH 3.4 to 6.8 by adding sodium hydro carbonate under continuous manual stirring. The pH-neutral mash was pressed and filtered as previously to separate a pH-neutral juice and a pH-neutral SBP which was dried following the same drying procedure as untreated SBP. Commercially produced asparaginase Acrylaway®L (Novozymes, Denmark) complimentary provided by the producer was applied into the pH-neutral wet mash in a dosage of 5 mL of enzyme (3500 ASN/mL) per kg of mash. Incubation of enzyme was performed in a stainless-steel container (Kitchen Aid, Artisan Series, Michigan, U.S.A.) with low stirring for 60 min at laboratory temperature (20 °C). After the enzymatic treatment of sea buckthorn mash, the procedure of pressing, filtration and drying was the same. This exact procedure of fruit and vegetable enzyme treatment in general was applied for industrial right protection and registered at the Office of Industrial Properties of the Slovak Republic as a utility model No 9572 **(Ciesarová, Kukurová, & Jelemenská, 2022)**. Untreated SBP powder and enzymatically treated pH-neutral SBP powder, were used for biscuits enrichment.

Procedure of biscuits production

The first step in the procedure of biscuits production (**Belović et al., 2020**) started with the mixing of fatty components (shortening and soybean lecithin) with sugar and vanilla sugar in a planetary mixer (Conti, model PL16 5B, Bussolengo, Italy). Afterwards, all powdered components were added (flour, SBP powder, sodium bicarbonate and salt). When homogenous mixture was obtained, water was added to reach the desired dough hydration degree. The obtained dough was stretched using a dough sheeter (MAC PAN, model MK 600, Thiene, Italy) to a thickness of 7 mm. Biscuits were shaped using a round mould with a diameter of 45 mm and baked in a pre-heated oven at 180°C for 14 min (MIWE Michael Wenz GmbH, model Gusto, Arnstein, Germany).

Determination of acrylamide

Acrylamide in biscuits was determined using LC-MS/MS technique after extraction to 0.2 mM acetic acid and pre-extraction to ethyl acetate according to **(Ciesarova, Kukurova, Bednarikova, & Morales, 2009)**. A HPLC system 1200 series (Agilent Technologies, Santa Clara, California, USA) coupled to an Agilent 6460 Triple Quad detector with an ESI interface. The analytical separation was performed on Atlantis dC18 (100 x 3 mm, 3 µm) column (Waters, Milford, MA, USA) using an isocratic mixture methanol:acetic acid:deionized water (5:1:500, v/v/v) at a flow rate of 0.4 mL/min at 25 °C. Data acquisition was performed using multiple reaction monitoring (MRM) with transition for acrylamide: 72 → 55 and acrylamide-D3: 75 → 58. The quantification of acrylamide was calculated with a calibration curve of the standard compound in the range from 5 to 200 µg/L. Time of analysis was 11 min; retention time of acrylamide and acrylamide-D3 was 2.0 min. LOD of the applied procedure was 10 µg/L; LOQ was 15 µg/L.

Texture analysis

TA.XT Plus Texture Analyser (Stable Micro Systems, Godalming, UK) was used to determine the textural properties of biscuits The instrument was equipped with a 30 kg load cell and a 3-Point Bending Rig (HDP/3BS) for biscuit/cookies snap. Instrumental settings were taken from the sample project BIS4/3PB included in the software package (Texture Exponent Software TEE32, version 6.1.6.0., Stable Micro Systems, Godalming, UK). Measure force in compression mode was used with return to start option, pre-test speed 1.0 mm/s, test speed 3.0 mm/s, post-test speed 10.0 mm/s, distance 5mm, auto trigger force and auto tare mode in TA settings. Hardness and fracturability values were measured in each test. The hardness was determined from the maximum peak force (N), fracturability was determined from the distance (mm) at which the biscuit breaks. Hardness and fracturability of biscuits were calculated using the Texture Exponent Software TEE32. Texture characteristics were determined after 6-months storage of biscuits in freezer at -18°C. Before analysis, 5 pieces of each sample were allowed to thaw at room temperature for 1 hour. Typical plot of hardness and fracturability is shown in Figure 1.

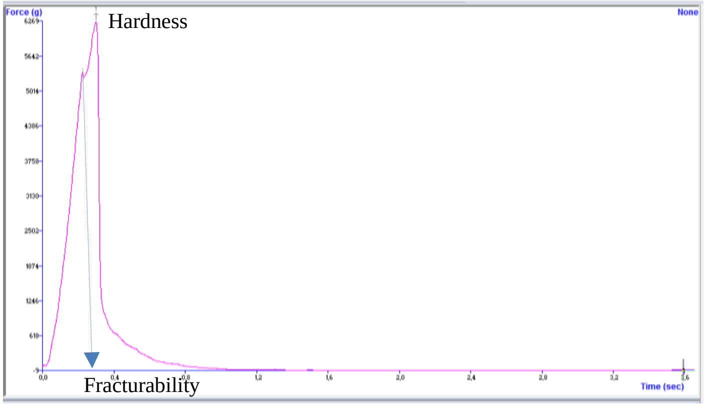

Figure 1 Typical plot of textural analysis of hardness and fracturability of biscuits

Sensory analysis

Sensory acceptability is one of the basic quality criteria in food and snacks. The acceptability test was carried out by a preference ranking test (**ISO, 2013**). It was performed by a trained sensory panel consisting of 15 women aged 32 – 62, employed at the National Agricultural and Food Centre, Food Research Institute in Bratislava. Due to the ongoing COVID-19 pandemic, the sensory assessment was adapted to a distance form according to **Dankwa, Aisala, Kayitesi, and de Kock (2021)**. The trained panelists were provided with half biscuits on a white plate in a random order and instructed to cleanse their palate with cold, tap water before tasting each sample. Panelists were asked to order biscuits samples from the worst to the best in three sessions evaluated separately wheat, rye and triticale biscuits. The order was scored from 1 to 3 points. The final score of each biscuit sample was given by counting the points.

Statistical analysis

Software Statistica 14.0.0.15 (Tibco Inc., USA, 2020, https://www.tibco.com/products/data-science) was used for statistical analysis. One-way analysis of variance (ANOVA) and Tukey's honestly significant differences (HSD) test were used to determine the significant differences ($p < 0.05$).

RESULTS AND DISCUSSION

Generally, the texture characteristics of biscuits are strongly dependent on ingredients and processing conditions thus changes in hardness and fracturability are legitimately expected.

The texture of the snacks was usually described using the attributes of hardness, crispness, adhesiveness, fracturability and chewiness **(Paula & Conti-Silva, 2014)**. These are the most used descriptors in studies evaluating the texture of extruded snacks **(Ding, Ainsworth, Plunkett, Tucker, & Marson, 2006; Yuliani, Torley, D'Arcy, Nicholson, & Bhandari, 2006)**. Furthermore, while hardness relates to the "force applied by the molar teeth to compress the food", fracturability relates to the "ability to break food into pieces when it is bitten using the incisors" (**Paula & Conti-Silva, 2014)**. Thus, different forces applied by the teeth were evaluated. A similar investigation was conducted by **Varela, Salvador, and Fiszman (2009)** to evaluate the crispness of extruded snacks **.**

Instrumental analysis of hardness and fracturability was usually done by a texturometer which simulates human description of texture attributes. Using a texturometer, hardness is given by the peak load of the initial compression. Peak hardness tends to occur at the maximum compression depth. The peak load of the first chew compression is a primary attribute of all foods. The calculation for hardness if simple and just involves logging the peak load value of the first chew.

Fracturability is a parameter for brittle foods such as cereals, chips and crackers **(Swackhamer & Bornhorst, 2019)**. Fracturability is defined as the distance traveled by a probe until the break point (the first significant peak). Higher values of fracturability in mm indicate that food is less brittle, i.e. longer application of the force is needed to cause the break of the food product. Hardness and fracturability in biscuits are closely related to water content, individual ingredients, the type of chemical bonds in complex molecules such as proteins and starch and their ability to bind and retain water (**Torbica, Mocko Blažek, Belović, & Janić Hajnal, 2019**). Although the protein composition and starch grain size is different in various cereals, in this study, the observed differences of hardness between wheat (W-SBP0) and triticale (T-SBP0) control biscuits (+25%) and between wheat (W-SBP0) and rye (R-SBP0) control biscuits (–5%) were non-significant ($p < 0.05$) (**Figure 2**). Comparing hardness of triticale and rye control biscuits, the triticale ones manifested 1.3 times higher hardness value than rye ones. Fracturability is a parameter which is more sensitive to dimension, dryness, and water activity of biscuits, thus the values of fracturability of wheat, triticale and rye biscuits varied in a larger extent and the differences between wheat biscuits

(W-SBP0) on one side and triticale (T-SBP0) and rye (R-SBP0) biscuits on the other side were significant ($p < 0.05$) (**Figure 3**). Wheat biscuits (W-SBP0) showed 1.35 times higher fracturability value than triticale ones (T-SBP0) and 1.27 times higher value than rye ones (R-SBP0). The correlation analysis showed a significant positive correlation ($p < 0.05$) between hardness and fracturability of cookies ($r = 0.4452$). Therefore, it can be concluded that higher the hardness of cookies, lower the fracturability of cookies. The hardness increased in the order of wheat – rye – triticale biscuits, and the fracturability decreased in the same order.

The addition of sea buckthorn pomace powder to biscuit formulation resulted in a substantial increase of acrylamide formation (**Figure 4**). Acrylamide content was increased twice in case of wheat and triticale biscuits (76 and 103 µg/kg, resp.), and up to 4.5 times in rye biscuits (480 µg/kg). Moreover, the presence of sea buckthorn pomace powder affected the textural properties of biscuits. The 10% substitution of wholegrain flours by untreated sea buckthorn pomace powder resulted in an increase of hardness and fracturability in all three kinds of biscuits (W-SBP1, T-SBP1, R-SBP1). The highest impact in both parameters was observed in triticale biscuits (T-SBP1) (**Figure 2 and Figure 3**). Hardness of triticale biscuits with untreated sea buckthorn pomace powder addition was 1.36 times higher, and fracturability 1.29 times higher in comparison with triticale control biscuits (T-SBP0). In case of wheat biscuits (W-SBP1), the increase of hardness was similar (up to 37%), an increase of fracturability was non-significant (6%, $p < 0.05$). Hardness and fracturability of rye biscuits with untreated sea buckthorn pomace powder addition (R-SBP1) increased significantly up to 19% and 12%, respectively. A similar impact of berry powders originated from other fruits was observed by **Molnar, Rimac Brnčić, Vujić, Gyimes, and Krisch (2015)**. The addition of black current powder resulted in an increase of hardness up to 20% and of fracturability up to 40%. Even a higher impact on both parameters was detected with the addition of josta berry powder (75% of hardness; 55 % of fracturability). It could be a result of starch composition, starch-protein interactions, higher proportion of protein than control, leading to a harder structure **(Brnčić et al., 2011; Gallagher, Kenny, & Arendt, 2005; Tiwari, Brennan, Jaganmohan, Surabi, & Alagusundaram, 2011)**.

On the other hand, if wholegrain flour was substituted by enzymatically treated sea buckthorn pomace, the values of hardness and fracturability of wheat biscuits (W-SPB3) decreased to values of wholegrain biscuits with no sea buckthorn pomace addition (W-SBP0) (**Figure 2 and Figure 3**). In triticale biscuits (T-SBP3), the hardness reached only 81% of control triticale biscuits (T-SBP0), meanwhile the fracturability was non-significantly higher (108%, $p < 0.05$). The highest decrease of hardness was observed in rye biscuits (R-SBP3), in which the hardness values were only 40% of the control biscuits (R-SBP0) (**Figure 2**). However, these biscuits were the lowest in moisture and water activity, too (data are not presented here). The fracturability of rye biscuits with enzymatically treated sea buckthorn pomace powder (R-SBP3) was similar to the control biscuits (R-SBP0), the difference was non-significant ($p < 0.05$) (**Figure 3**).

Based on the study of (**Gazi et al., 2023**), the enzymatic treatment with asparaginase was successfully applied in other cereal-based products with no detrimental effect on sensory properties. Although it seems that in our experiment, the enzymatic treatment had the visible impact on hardness and fracturability of wholegrain biscuits, it must be highlighted that also other aspects should be taken into consideration. The properties of sea buckthorn pomaces were affected by the procedure of enzymatic treatment applied in wet sea buckthorn pomace. In order to optimize conditions of enzyme activity, the pH value of wet sea buckthorn pomace was adjusted at the neutral value (pH 6.8). The details of this procedure are given in the utility model application No 9267 registered at the Industrial Property Office of the Slovak Republic (**Ciesarová et al., 2022**). Under these conditions, the conversion of asparagine to aspartic acid in sea buckthorn pomace was more than 95% (data are not presented here). Incorporation of such enzymatically treated sea buckthorn pomace as an ingredient of a biscuit recipe resulted in a significant reduction of acrylamide in enriched biscuits between 30 and 60% (**Figure 4**). In case of wheat biscuits (W-SBP3), the acrylamide value is in a level of control biscuits without any sea buckthorn pomace powder addition (W-SBP0). Acrylamide in triticale biscuits (T-SBP3) decreased up to 30% of Even in usually high-acrylamide rye biscuits, the enzymatic treatment of sea buckthorn pomace brought a decrease of acrylamide below the benchmark level established for biscuits and wafers (350 µg/kg) **("Commission Regulation (EU) 2017/2158 of 20 November 2017 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food,")**.

However, the untreated sea buckthorn pomace had a strong acidic character, the pH value was 3.4 which substantially limited the activity of enzyme. It can be supposed that this low pH value affected also rheological properties of dough for biscuits with the 10% substitution of flour by dried sea buckthorn pomace and finally, resulted in altered textural properties of final products. This is supported by the comparison of biscuits with no addition of sea buckthorn pomace (SBP0) and biscuits with untreated sea buckthorn pomace (SBP1). In case that the presence of pH neutral sea buckthorn pomace did not change the acidity of dough, the textural properties of final biscuits are more similar to control wholegrain cereal biscuits (the comparison of SBP3 and SBP0) (**Figure 2 and Figure 3**).

From the consumer point of view, textural properties are only one of aspects which contribute to overall sensory perception. For this reason, the alteration in hardness and fracturability of biscuits by the presence of acidic sea buckthorn pomace or pH-neutral enzymatically treated sea buckthorn pomace can be perceived in different ways taking into account all organoleptic properties of experimental biscuits.

Sensory analysis performed by the panel of 15 previously trained members in the preference test revealed the increasing preferences to wholegrain cereal biscuits without sea buckthorn pomace addition in the order triticale – rye – wheat (**Figure 5**). Wholegrain wheat biscuits as a typical snack were accepted in a form of plain biscuits without any other addition since they are well-recognized by consumers with expected organoleptic properties. The addition of sea buckthorn pomace in both forms increased the preference score of all cereal biscuits, especially due to the attractiveness of orange color, fruit odor, flavor and taste. The preference score of biscuits with pH-neutral enzymatically treated sea buckthorn pomace was higher in case of triticale (T-SBP3) and rye (R-SBP3) biscuits in comparison with triticale (T-SBP1) and rye (R-SBP1) biscuits with acidic sea buckthorn pomace powder addition. Since the differences in acid taste between SBP1 and SBP3 biscuits were not recognized by panelists, the increase of preferences of SBP3 biscuits is probably attributed to the substantial decrease of hardness comparing to triticale (T-SBP1) and rye (R-SBP1) biscuits with acidic sea buckthorn pomace powder (**Figure 2**). Triticale and rye biscuits with pH-neutral enzymatically treated sea buckthorn pomace powder (T-SBP3 and R-SBP3) were evaluated by better scores than related wheat biscuits (W-SBP3). The non-wheat biscuits were more attractive for consumers, probably due to their exceptionality in origin, but also with well-balanced and harmonized attributes of appearance, odor, flavor and taste with smooth, non-grainy, compact texture attributes improved by sea buckthorn pomace powder addition (**Figure 5**).

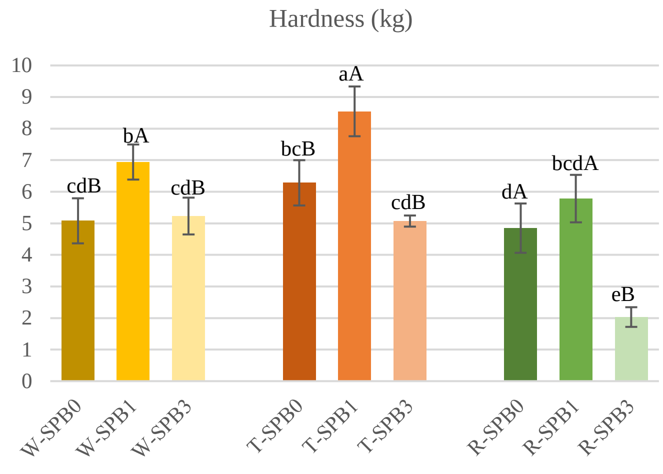

Figure 2 Hardness of wheat (W), triticale (T) and rye (R) biscuits without (SPB0) and with dried sea buckthorn pomace untreated (SBP1) and enzymatically treated (SBP3) addition. Small letters mean significant differences ($p < 0.05$) between all values. Capital letters mean significant differences ($p < 0.05$) between values of one cereal.

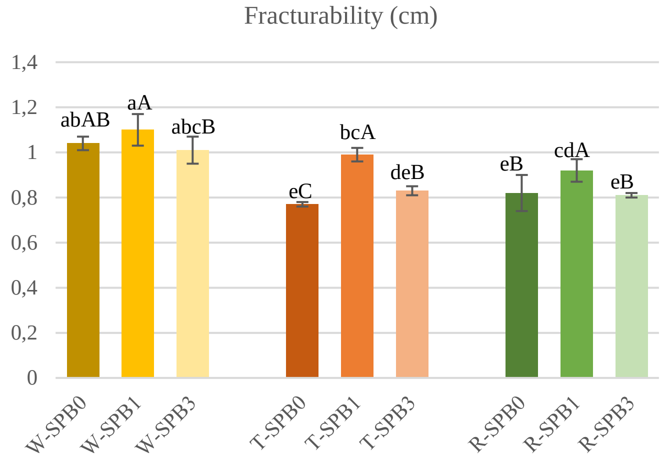

Figure 3 Fracturability of wheat (W), triticale (T) and rye (R) biscuits without (SPB0) and with dried sea buckthorn pomace untreated (SBP1) and enzymatically treated (SBP3) addition. Small letters mean significant differences ($p < 0.05$) between all values. Capital letters mean significant differences ($p < 0.05$) between values of one cereal.

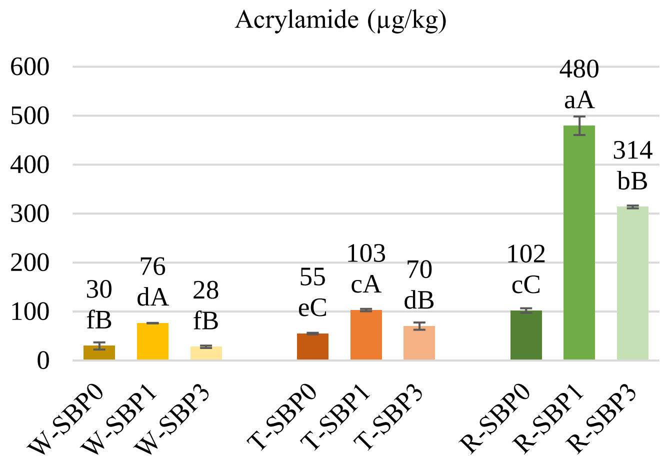

Figure 4 Acrylamide content (µg/kg) of wheat (W), triticale (T) and rye (R) biscuits without (SPB0) and with untreated (SBP1) and enzymatically treated (SBP3) dried sea buckthorn pomace addition. Small letters mean significant differences ($p < 0.05$) between all values. Capital letters mean significant differences ($p < 0.05$) between values of one cereal.

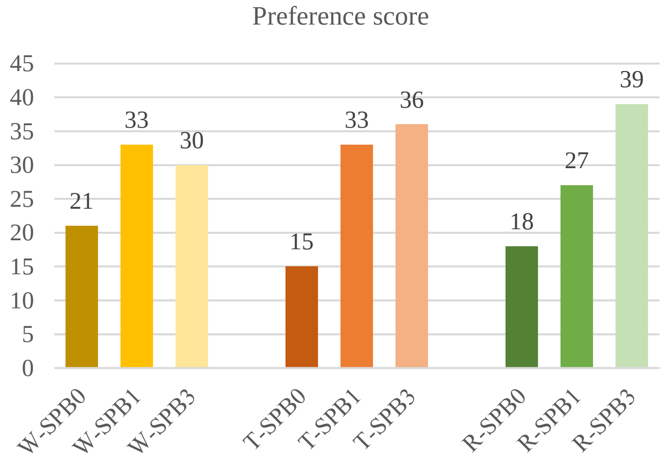

Figure 5 Preference score of wheat (W), triticale (T) and rye (R) biscuits without (SPB0) and with untreated (SBP1) and enzymatically treated (SBP3) dried sea buckthorn pomace addition.

CONCLUSION

The observations from the study give the positive feedback to the efforts to introduce nutritionally rich but rarely used cereals such as rye and triticale as well as to enrich traditional plain biscuits with exceptionally valuable sea buckthorn pomace powder which were accepted by consumers despite measurable impact of these plant materials on hardness and fracturability of biscuits. Moreover, the introduction of enzymatic treatment of sea buckthorn pomace as the highest donor of asparagine, the main precursor of acrylamide formation, positively affected the preference score of biscuits by consumers.

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