www.foodandfeed.fins.uns.ac.rs
FOOD AND FEED RESEARCH
Journal of the Institute of Food Technology – FINS
University of Novi Sad
ISSN
2217-5369
(print version ceased in 2023)
2217-5660 (online)

UDK 664.641.12:635.651+577.112]:664.661
Original research paper
DOI: 10.5937/ffr0-53247

FABA BEAN FLOUR AND PROTEIN ISOLATE AS PARTIAL SUBSTITUTES IN WHEAT-TRITICALE BREAD: TECHNOLOGICAL AND SENSORY PROPERTIES

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Abstract: The incorporation of faba bean flour (FBF) and protein isolate (FBI) into wheat-triticale flour blends is a potential promising strategy to enhance the nutritional and functional properties of bread. This study aimed to investigate the potential of these ingredients from both technological and sensory perspectives. Multiple analyses were conducted, including chemical composition, texture profile analysis, colour, specific volume and sensory properties of breads made with a blend of wheat and triticale flours (70:30). Part of the blend was substituted with either 20% faba bean flour or 7.2% faba bean protein isolate to ensure an equal proportion of faba bean protein in the final formulations.

The results showed a significant increase in protein content for both FBF and FBPI breads, with the faba bean protein isolate also increasing the ash content. Texture profile analysis (TPA) indicated that the substitutions affected bread properties by increasing hardness, gumminess, and chewiness, particularly in bread with FBI. However, cohesiveness, springiness, and resilience decreased similarly in both cases. Additionally, the specific volume decreased with the addition of faba bean flour and protein, especially in FBI bread. From the colour aspect, analysis revealed a darker hue and a more pronounced reddish nuance in the bread crust in FBF and FBI breads. Sensory evaluation indicated a slightly lower overall likeability with faba bean addition, yet the breads remained acceptable to the panellists. Detailed sensory analysis supported the TPA findings and colour differentiation, showing higher values for hardness, crust compactness, and crumbliness. Texture and colour were rated lower compared to the control sample. Moreover, both FBF and FBI breads exhibited higher overall odour intensity. Specifically, bread with the protein isolate had less uniform pores and a more pronounced flour/cereal/bran odour. In contrast, bread with faba bean flour had more uniform pores but a noticeable bitterness.

In conclusion, this study demonstrates that faba bean flour and protein isolate can be used as partial substitutes in wheat-triticale flour blends to produce bread with acceptable properties. This leaves room for future in-depth analysis and modifications to address the identified challenges.

Key words: *faba bean, protein isolate, wheat, triticale, bread*

INTRODUCTION

Bread is a staple food in many cultures worldwide, serving as a primary source of energy and nutrients for billions of people. However, traditional wheat-based bread often lacks in terms of nutritional diversity, particularly in protein content and the presence of bioactive

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compounds related to health benefits. As consumer awareness of nutrition and health continues to grow, there is an increasing demand for food products that not only satisfy basic dietary needs but also offer enhanced nutritional benefits.

This has encouraged interest in the development of novel bread formulations that incorporate alternative ingredients to improve the nutritional profile of bread without compromising its sensory properties (Fraś et al., 2016; Hoehnel et al., 2020).

One promising approach to achieve this goal is the incorporation of legume-based ingredients into bread formulations (Angioloni & Collar, 2012). Legumes, such as faba beans *(Vicia faba* L.*)*, are well-known for their high protein content, rich fibre composition, and the presence of bioactive compounds, including polyphenols and antioxidants, which have been associated with various health benefits, such as reduced risk of chronic diseases (Dhull et al., 2022; Karataş, Günay & Sayar, 2017). Faba bean, in particular, has gained attention due to its favourable nutritional profile and its potential to enhance the quality of cereal-based products.

In addition to its nutritional advantages, faba bean is also recognized for its agricultural benefits, as it is a nitrogen-fixing crop that can improve soil fertility and reduce the need for synthetic fertilizers, thereby contributing to sustainable agriculture (Jensen, Peoples & Hauggaard-Nielsen, 2010).

The use of faba bean flour and protein isolate in bread formulations presents an opportunity to create bread products that are higher in protein and fibre depending on the ingredient type, while also delivering additional health-promoting compounds. However, the incorporation of these non-standard ingredients into bread leads to several technological challenges.

The unique composition of legume flours and protein isolates can alter the rheological properties of the dough, affecting its elasticity, cohesiveness, and overall handling characteristics during bread production.

These changes in dough behaviour can, influence the final texture, volume, and appearance of the bread, which are critical factors in consumer acceptance (Bojňanská, Musilová & Vollmannová, 2021; Paraskevopoulou, Provatidou, Tsotsiou & Kiosseoglou, 2010). Moreover, the sensory characteristics of the bread, such as taste, aroma, and mouthfeel, may also be impacted by the introduction of legume ingredients, necessitating a careful balance between nutritional enhancement and sensory quality (Benayad, Taghouti, Benali, Aboussaleh & Benbrahim, 2021). Moreover, faba bean contains antinutrient factors, therefore their inclusion should be carefully considered when formulating and studying products (Rahate, Madhumita & Prabhakar, 2021).

Although the use of various legume flours in bread making is explored, there is limited knowledge regarding the specific effects of faba bean flour and protein isolate on the quality attributes of bread obtained with wheat-triticale flour blends. Triticale, a hybrid of wheat and rye, is itself a valuable cereal that combines the best qualities of its parent grains, offering higher protein content and better disease resistance than wheat, along with the adaptability and robustness of rye (Fraś et al., 2016). The combination of triticale with wheat flour provides a promising base for bread making, which, when supplemented with faba bean derivatives, could yield a nutritionally superior product.

This study aimed to investigate the potential of faba bean flour and protein isolate as partial substitutes in wheat-triticale flour blends for bread production.

Specifically, the impact of substituting a portion of the wheat-triticale flour with either 20% faba bean flour or 7.2% faba bean protein isolate on the bread's chemical composition, texture profile analysis (TPA) to evaluate changes in mechanical properties, colour measurements, specific volume assessment and sensory evaluation were explored. By examining the relationships between faba bean flour, protein isolate, and wheat-triticale flour in breadmaking, this study aims to establish a foundation for creating bread products with superior nutritional profiles.

Additionally, the findings of this study could help identify potential challenges associated with the use of these ingredients and suggest directions for further optimization to achieve an optimal balance between improved nutritional value and desirable sensory characteristics.

MATERIALS AND METHODS

Materials

Wheat flour (moisture 13.87%, protein 11.20%, carbohydrates 73.91, ash 0.52, fat 0.50%), triticale flour (moisture 12.0%, protein 14.9%, carbohydrates 71.08%, ash 0.55%, fat 1.47%), faba bean flour (moisture 10.0%, protein 32.34%, carbohydrates 52.66%, ash 3.5%, fat 1.5%), yeast and salt were purchased from a local market (Novi Sad, Serbia), while faba bean protein isolate was obtained by alkali isoelectric precipitation with protein content of 90.15% (Hadnađev et al., 2017; Hadnađev et al., 2018).

Bread production

Basic dough recipe (control bread) on 100 g wheat flour T-500 and triticale flour blend (ratio 70:30) consisted of 2% yeast and 3.5% salt. The amount of water (30 °C) was added to the flour blend to achieve final consistency of 400 BU after 5 min of mixing. Simultaneously, two additional formulations were created: one with 20% substitution of blend with faba bean flour (FBF) and another with a 7.2% addition of faba bean protein isolate (FBI). These substitution levels were specifically chosen to ensure that the final formulations contain the same amount of protein contributed by the faba bean ingredient (providing of 6.5% faba bean proteins to the flour blend in the final formulation). After mixing, dough was fermented for 60 min at 30 °C and 75% relative humidity (RH), with punch after 45 min. The fermented dough was divided into portions and placed in baking pans. After final proofing during 45 min at 30 °C and 75% relative humidity (RH), the loaves were baked at 220 °C for 20 min in a modular deck oven (MD, Macpan SNS, Thiene, Italy) until mass loss of 8 g/100g. Consequently, bread samples were removed from pans and left to cool down at room temperature for 2 h, sealed in polyethylene bags and stored at 22 °C for further bread quality evaluation. Three batches of each sample were prepared.

Chemical composition

Moisture content, protein, fat, and total sugars content of obtained breads were determined according to Association Official of Analytical Chemists (AOAC, 2000) methods 925.10, 950.36, 935.38, and according to standard AACC methods 80-68. Total starch content was determined according to ISO standards 10520:1997.

Colour, texture and volume analysis

Measurement of bread colour was performed by a Chroma Meter CR-400 (Konica Minolta Co., Ltd., Osaka, Japan. The colour of crust and crumb were measured at five points, where L* (lightness), a* (+a* = redness, -a* = greenness) and b* (+b* = yellowness, -b* = blueness) were read using a D65 light source and the observer angle of 2°. Specific bread volume was determined using a Volscan Profiler (Stable Micro Systems, Godalming, UK). All tests were carried out on 4 loaves per batch. Textural properties of breadcrumb samples were investigated by texture profile analysis (TPA) at room temperature using a TA-XT plus Texture Analyser (Stable Micro Systems, Godalming, UK) equipped with a 30-kg load cell and a P/75 (75-mm diameter) aluminium compression platen. All tests were carried out on six slices (35-mm diameter, 10-mm thickness) obtained from the centre of each loaf in a compression mode at pre-test, 1 mm/s; test and post-test speed, 5 mm/s; deformation 75%; and wait time between first and second compression cycles, 5 s, 24 h after baking. The obtained parameters were hardness, cohesiveness, springiness, chewiness, and resilience.

Sensory analysis

Sensory analysis of breads was performed by ten expert panellists from the Institute of Food Technology (University of Novi Sad, Serbia) with sensory experience in evaluation of different types of bakery products. The list of descriptors was established in several sessions. Afterwards, samples were evaluated by the panel using a 100 mm linear scale with the anchor points 0 – not perceptible and 100 – strongly perceptible. Bread samples were presented in randomized order on the plastic plates, marked with three-digit numbers. Drinking water was provided for palate cleansing after testing of each sample. Afterward, liking study was applied where participants evaluated overall liking, flavour liking, odour liking, taste liking, colour liking and texture liking for each sample on 9-point hedonic scales (1 = I don't like it at all, 9 = I like it very much).

The study was approved by the Ethics Committee of the Institute of Food Technology in

Novi Sad, University of Novi Sad, Serbia (Ref. No. 175/I/36–3).

Statistical analysis

All measurements were performed at least in triplicates if not stated differently. The mean values of replicates for analysed parameters were statistically processed using the software package XLSTAT. Analysis of variance (ANOVA) and Tukey's honest significant difference test ($p<0.05$) were used to determine the significance of differences between sample mean values.

RESULTS AND DISCUSSION

The chemical composition of obtained bread is shown in Table 1. As expected, protein content increased while total carbohydrate content decreased in both samples where part of blend was substituted (FBF and FBI). Also, there was a moisture decrease in both samples where substitutions were made. Substituting part of the flour in both cases did not affect the fat content. However, the FBI sample showed higher ash content compared to the other two samples. Additionally, both substitutions with faba bean sources led to a slight increase in energy value. Although the protein content of the substituting ingredients was considered during the formulation process, the higher protein values observed with the addition of faba bean protein isolate compared to faba bean flour can be attributed to differences in their composition. Faba bean flour contains a mix of protein, carbohydrates, fibre, and other constituents, meaning that it contributes a significant amount of non-protein components (Chan et al., 2019; Gangola et al., 2021). In contrast, faba bean protein isolate is primarily composed of protein with minimal other constituents, leading to a more substantial increase in overall protein content when used as a substitute. In accordance with Regulation (EC) No 1924/2006 on nutrition and health claims for foods, all of the analysed samples qualify to bear the claim "source of protein".

According to texture analysis (Table 2), the results indicate that substituting wheat-triticale flour with FBF and FBI significantly alters the bread's texture, making it firmer, chewier, and less cohesive and resilient. These changes are due to the high protein content and different ratio of other constituents in FBF and FBI, which in the absence of gluten-forming proteins, leads to a denser, less elastic dough structure (Hoehnel et al., 2020). Similarly to the previously mentioned explanation, the more pronounced effects observed with FBI are likely due to the composition of substituting part. A higher proportion of pure protein relative to other components leads to a stiffer and more rigid crumb structure. These findings suggest that while FBF and FBI can enhance the nutritional profile of bread, their impact on textural properties may require careful formulation adjustments to maintain the desired bread quality and consumer acceptability.

Table 1.
Proximate composition of bread (g/100g)

Sample	Moisture (%)	Proteins (%)	Fat (%)	Ash (%)	Total carbohydrates (%)	Energy (kJ/kcal)
Control	36.7 ± 0.28^{a}	9.63 ± 0.06^{b}	0.11 ± 0.04^{a}	1.68 ± 0.01^{b}	51.88 ± 0.18^{a}	1048/247
FBF	36.18 ± 0.18^{b}	12.8 ± 0.10^{a}	0.11 ± 0.01^{a}	1.66 ± 0.01^{b}	49.25 ± 0.23^{b}	1068/251
FBI	35.3 ± 0.19^{ab}	13.1 ± 0.10^{a}	0.08 ± 0.01^{a}	1.78 ± 0.04^{a}	49.74 ± 0.25^{b}	1072/252

The mean values ± standard deviation in the same column are not significantly different (p<0.05) if they are followed by the same letters in the superscript

Table 2.
Textural properties of bread samples

Sample	Hardness (g)	Springiness	Cohesiveness	Chewiness	Resilience
Control	3375 ± 761^{b}	1.12 ± 0.23^{a}	0.68 ± 0.02^{a}	2488 ± 341^{c}	0.36 ± 0.01^{a}
FBF	8826 ± 681^{a}	0.92 ± 0.01^{a}	0.55 ± 0.01^{c}	4440 ± 236^{b}	0.22 ± 0.00^{b}
FBI	10464 ± 1340^{a}	0.94 ± 0.01^{a}	0.59 ± 0.00^{b}	5835 ± 781^{a}	0.23 ± 0.01^{b}

The mean values ± standard deviation in the same column are not significantly different (p<0.05) if they are followed by the same letters in the superscript

Regarding colour measurement (Table 3), the substitution with faba bean flour and protein isolate led to a decrease in lightness (L*) and an increase in the red tone (a*) of the bread crust. This is attributed to the higher protein content, particularly amino acids, which enhanced Maillard reactions during baking. Moreover, bread prepared with faba bean flour exhibited significantly lower L* and higher a* values compared to bread made with faba bean protein isolates. This difference is likely attributed to the higher reducing sugar content in faba bean flour relative to pure faba bean protein isolate (Crépon et al., 2010; Vidal-Valverde et al., 1998). Additionally, a notable decrease in the yellowish hue (b*) was observed in the bread with faba bean flour. Conversely, the crumb colour did not undergo significant modifications, except for an increase in the yellowish tone in both samples with substituted ingredients.

The results of the specific volume analysis are shown in Table 3, which demonstrates a decrease in specific volume with the partial substitution of the flour blend. This reduction is also visible in Figure 1, where the cross-sectional view of the bread is presented. Notably, the decrease in specific volume was more pronounced in the bread containing protein isolate compared to the sample containing faba bean flour. This reduction in specific volume can primarily be attributed to gluten dilution, altered dough rheology, and increased dough density. The dilution effect, combined with possible interactions between gluten and faba bean proteins simultaneously to gluten network development and reduced dough extensibility, results in stiffer dough with diminished gas retention capacity. Consequently, the final bread product exhibits a lower specific volume. Similar behaviour of reduction in specific volume by addition of faba bean flour has been observed by Ni et al. (2020) and Coda, Varis, Verni, Rizzello and Katina (2017). To possibly overcome these challenges, implementation of sourdough fermentation and addition of hydrocolloids could be valuable approaches (Wang et al., 2018).

The sensory analysis and liking study (Figure 2) demonstrate that the substitution of wheat-triticale flour with FBF and FBI significantly impacts the sensory attributes of bread, particularly in terms of odour intensity, texture, and crumb density. During the training sessions, all

Table 3.
Bread crumb, crust colour and specific volume of bread of loafs

Parameter		Control	FBF	FBI
Crust colour	L*	65.6 ± 3.07^a	49.4 ± 2.51^c	58.8 ± 3.41^b
	a*	9.5 ± 2.08^c	15.8 ± 0.33^a	12.9 ± 1.66^b
	b*	32.2 ± 1.76^a	28.6 ± 2.14^b	34.0 ± 1.12^a
Crumb colour	L*	75.9 ± 0.69^a	74.1 ± 0.91^a	74.2 ± 3.35^a
	a*	-1.2 ± 0.1^a	-1.2 ± 0.14^a	-0.9 ± 0.25^a
	b*	17.6 ± 0.53^b	20.8 ± 0.74^a	19.7 ± 1.13^a
Specific volume (ml/g)		3.38 ± 0.06^a	3.06 ± 0.04^b	2.83 ± 0.02^c

The mean values ± standard deviation in the same row are not significantly different (P > 0.05) if they are followed by the same letters in the superscript

Figure 1. Cross section of bread

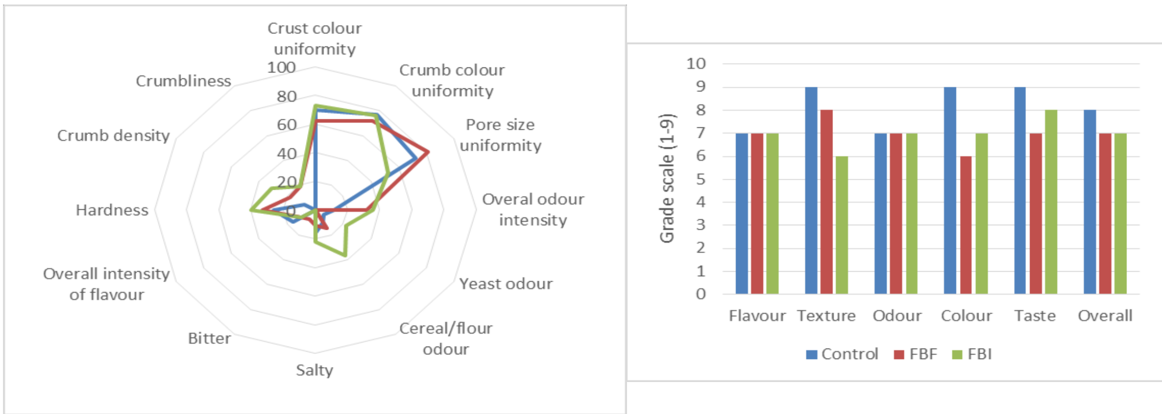

Figure 2. Sensory evaluation of bread

descriptors which were not perceptible in all samples were eliminated from the final evaluation. The more pronounced effects observed in the FBI sample, such as higher hardness, crumb density, and odour intensity, suggest that while protein isolates can enhance certain nutritional properties, they may also lead to challenges in maintaining the desired sensory qualities of bread. The FBF sample, while also affected, exhibited a more balanced profile, suggesting that faba bean flour might be a preferable substitute in applications prioritizing the preservation of texture and sensory characterristics. These findings highlight the importance of optimizing formulations to balance nutritional enhancements with sensory acceptability in bread products. The liking study results show that while the control bread was generally favoured across most attributes, the FBF and FBI samples were still considered acceptable by participants. Flavour, texture, odour, and taste ratings for the FBF sample were slightly lower than the control.

However, with a texture rating of 8 compared to control's 9, the FBF substitution had a relatively mild impact on overall consumer acceptance.

The FBI sample showed a more pronounced decrease in texture liking (6), consistent with the higher hardness and crumb density observed in the sensory analysis. However, the FBI sample did maintain a good score for taste (8) and colour (7), suggesting that despite its textural drawbacks, it had a favourable taste profile and appearance. Similarly, studies by Bojňanská et al. (2021) on faba bean incorporation in wheat-rye bread and Hoehnel et al. (2020) in wheat bread reported acceptable sensory properties of the resulting products.

CONCLUSIONS

This study demonstrates the significant impact of substituting wheat-triticale flour blend with faba bean flour (FBF) and faba bean protein isolate (FBI) on the nutritional, textural, and sensory properties of bread. The addition of faba bean derivatives led to an increase in protein content, but also introduced challenges in maintaining desirable textural and sensory qualities. FBF and FBI both resulted in harder, denser bread with reduced cohesiveness and resilience, with FBI showing more pronounced effects. Sensory analysis revealed alterations in colour, pore uniformity, and aroma intensity, with FBI contributing to a stronger yeast and cereal odour, while FBF introduced a slight bitterness. Despite these changes, both FBF and FBI resulted in bread with acceptable sensory profiles. However, further optimization is required to achieve a balance between improved nutritional profile and desirable sensory attributes. This research highlights the potential of faba bean ingredients in bread formulations, but also underscores the importance of careful formulation to address the identified challenges in texture and flavour.

AUTHOR CONTRIBUTIONS

Conceptualization, J.T. and M.H.; Methodology, T.D.H. and M.P.; Investigation, formal analysis, validation, writing-original draft preparation, N.M., J.T. and M.H.; Writing-review and editing, T.D.H. and M.P.; Supervision, J.T. and M.H.

DATA AVAILABILITY STATEMENT

Data contained within the article.

ACKNOWLEDGEMENTS

This research was funded by the Ministry of Science, Technological Development and Innovation, Republic of Serbia (Contract No. 451-03-66/2024-03/200222) and by the EU Horizon 2020, grant No. 101000847, acronym CROPDIVA.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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BRAŠNO I IZOLATI PROTEINA BOBA KAO DELIMIČNI SUPSTITUENTI U HLEBU OD MEŠAVINE PŠENICE I TRITIKALEA: TEHNOLOŠKA I SENORSKA SVOJSTVA

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Sažetak: Zamena dela mešavine pšeničnog brašna i brašna tritikalea sa brašnom od boba (FBF) i proteinskog izolata boba (FBI) je potencijalno obećavajuća strategija za poboljšanje nutritivnih i funkcionalnih svojstava hleba. Ova studija je imala za cilj da istraži potencijal ovih sastojaka iz tehnološke i senzorne perspektive. Sprovedeno je više analiza, uključujući hemijski sastav, analizu profila teksture, boju, specifičnu zapreminu i senzorna svojstva hleba napravljenog od mešavine brašna pšenice i tritikalea (70:30). Deo mešavine je zamenjen ili sa 20% brašna boba ili sa 7,2% izolata proteina boba, kako bi se obezbedio jednak udeo proteina boba u finalnim formulacijama.
Rezultati su pokazali značajno povećanje sadržaja proteina u oba uzorka hleba (FBF i FBI), pri čemu je proteinski izolat boba povećao i sadržaj pepela. Analiza profila teksture (TPA) je pokazala da su zamene uticale na svojstva hleba povećanjem tvrdoće, gumosti i žvakanja, posebno u hlebu sa proteinskim izolatom (FBI). Međutim, kohezivnost i elastičnost su se smanjile na sličan način u oba slučaja. Pored toga, specifična zapremina se smanjila dodatkom brašna i izolata proteina boba, posebno u FBI hlebu. Sa aspekta boje, analiza je otkrila tamniju nijansu i izraženiju crvenkastu nijansu u kori hleba kod oba uzorka. Senzorna procena je pokazala nešto nižu opštu dopadljivost sa dodatkom faba pasulja, ali su hlebovi ostali prihvatljivi za paneliste. Detaljna senzorna analiza pokazala je saglasnost sa prethodnim analizama teksture (TPA) i boje, pokazujući veće vrednosti za tvrdoću, kompaktnost kore i mrvljivost. Tekstura i boja su ocenjene niže u poređenju sa kontrolnim uzorkom. Štaviše, i FBF i FBI hleb su pokazali veći ukupni intenzitet mirisa. Konkretno, hleb sa izolatom proteina imao je manje ujednačene pore i izraženiji miris brašna/žitarice/mekinje. Nasuprot tome, hleb sa brašnom od faba pasulja imao je ujednačenije pore, ali primetnu gorčinu.
U zaključku, ova studija pokazuje da se brašno boba i izolat proteina boba mogu koristiti kao delimične zamene u mešavinama pšeničnog i tritikale brašna za proizvodnju hleba sa prihvatljivim svojstvima. Ovo ostavlja prostor za buduću dublju analizu i modifikacije za rešavanje identifikovanih izazova.

Ključne reči: *bob, izolati proteina, pšenica, tritikale, hleb*

Received: *06 September2024/* ***Received in revised form:*** *11 October 2024/* ***Accepted:*** *11 October 2024*

Available online: *October 2024*

This work was reported at the 5th International Congress "Food Technology, Quality and Safety (FoodTech 2024)" held in Novi Sad, Serbia, October 16-18, 2024 organized by the Institute of Food Technology in Novi Sad.