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EFFECT OF ADDITION OF FENNEL (*FOENICULUM VULGARE* L.) ON THE QUALITY OF PROTEIN BREAD

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*Fennel (*Foeniculum vulgare *L.) is an aromatic plant belonging to* Apiaceae *family widely cultivated elsewhere for its strongly flavoured leaves and seeds. Fennel seeds are of particular interest as a rich source of both vegetable and essential oils with high amounts of valuable components. However, residual cakes after oil extraction were typically considered as by-products, in the present framework, the potential added value of these cakes was studied. The aim of this study was to investigate the effect of addition of fennel cake and seeds to protein bread quality. In the current research, a single-screw extruder, which is a solvent-free technique, was used for fennel seed oil extraction. For the protein bread making, fennel seed and cake flour in concentrations from 1 to 6% were used. Moisture, colour L*a*b*, hardness, total phenolic concentration, DPPH radical scavenging activity, and nutritional value of protein bread were determined. The addition of fennel cake and seeds had significant (*$p < 0.05$*) effect on bread crumb colour and hardness attribute, whereby the bread became darker and harder in texture than the control. Moreover, higher antioxidant activity and total phenolic concentration were observed for both protein breads enriched with fennel cake and seed flour. The overall results showed that addition of fennel cake and seed had beneficial effects on phenolic concentration, antioxidant activity and quality of protein bread. This result suggests also that added value of fennel seeds oil by-products could be increased by their utilisation in bread production.*

Key words: *fennel, fennel cake, bread quality, total phenolic, radical scavenging activity.*

INTRODUCTION

Bread is the main dietary source in many countries; however, wheat bread is the most popular due to its textural and sensory properties (Ngozi, 2014). Nowadays, consumers increasingly require foods with functional properties. To meet consumer health requirements, the use of functional ingredients in bread formulations is increasingly expanding in the bakery industry as part of bread nutritional improvement (Alam *et al*., 2013).

Thus, daily consumption of whole grain bread is recommended as a substitute to refined products due to richness of bread in functional ingredients such as fibre, phytochemicals, minerals, essential amino acids and soluble vitamins (Ndife *et al*., 2013). However, more additives should be supplemented to bread formulation in order to overcome the undesirable effects of whole wheat flour on the bread quality, such as textural properties and bread volume (Karaoglu and Boz, 2013); vital wheat gluten is a wheat protein isolate used as an additive to increase dough and bread yield, also to improve mixing tolerance and bread crumb texture, while additionally promoting the protein level and hence the nutritional value of the product (Constandache, 2005; Gianno and Tzia, 2016).

Fennel (*Foeniculum vulgare* Mill.) is an aromatic plant belonging to the *Apiaceae* family, and it is considered as one of the oldest medicinal plant cultivated throughout the world (Rather *et al*., 2012). Fennel seeds have particular economic importance, as they are widely used in the pharmaceutical, food, cosmetic, and healthcare industries. Fennel seeds are also a rich source of dietary fibre, proteins, vitamins, sterols and phenolic compounds (Kooti *et al*., 2015), as well as of both fixed and essential oil with high amounts of valuable components (80% petroselinic acid and

70% trans-anethole, respectively) (Weiping and Bookang, 2011; Moghtader, 2013).

Large amounts of residual cake remain as waste products after fennel oilseed extraction. Recently there has been an increasing demand of exploitation of crops residues as a source of high-valuable molecules and antioxidant compounds, and thus these by-products could be utilized as valuable raw materials for functional food production (Saavedra *et al*., 2015). Nevertheless, in order to be competitive and capture bakery markets, an environmental friendly fennel seed extraction process should be established. The mono-screw pressing technique has been proposed as an ideal alternative to conventional techniques, as it is a solvent-free technique matching the green extraction principles by producing co-products instead of waste (Wahidu *et al*., 2014).

The aim of this study was to investigate the effect of addition of fennel cake and seed to protein bread (with added vital wheat gluten) quality.

MATERIALS AND METHODS

Fennel seed extraction. Extrusion was done by a Single-screw (Model OMEGA 20, France) press with the following parameters: a 0.75 kW motor (230 V, 5.1 A), screw length 18 cm, pitch screw 1.8 cm, internal diameter 1.4 cm, channel depth 0.5 cm, and sleeve 2.5 cm internal diameter, and equipped with a filter-pierced outlet for liquid at the end of the screw and at the surface of the nozzles. The filter section to separate extracted oil had diameter 2 mm. The feed rate and the screw rotation speed were maintained constant at 15 g min^{-1} (0.9 $kg{\cdot}h^{-1}$) and 40 rpm, respectively. The nozzle diameter used in the pressing of fennel seed was 5 mm. The nozzle/screw distance was 3 cm. The screw press was first run for 15 min without seed material, but with heating via an electrical resistance-heating ring attached around the press barrel, to raise the screw press barrel temperature to the desired value. Fennel cakes obtained as by-products by the extrusion process were used for further research.

Raw materials for protein bread preparation. Whole wheat flour (JSC Rīgas Dzirnavnieks, Latvia), wheat protein isolate Arise 5000 (GmbH Lorima, Germany), sugar (Nordzucker GmbH & Co, Germany), salt, dry yeast (S. I. Lesaffre, France) were procured from the local market of Jelgava, Latvia; while fennel seeds (*Foeniculum vulgare* Mill. var. *dulce*) were purchased from the local market of Toulouse, France.

Protein bread making technology. To determine the influence of fennel seeds and fennel cakes on protein bread quality and chemical composition, fennel seeds or fennel cakes was added at 2%, 4%, and 6% of whole wheat flour amount. All ingredients were mixed for 5 ± 1 min at a minimum speed using a dough mixer BEAR Varimixe (Wodschow & Co, Denmark). Dough samples were fermented for 25 min at 36 ± 2 °C temperature. Bread samples were then baked at 200 ± 5 °C temperature for 20 min in a rotating convection oven (Sveba Dahlen, Sweeden) and then cooled at room temperature 22 ± 2 °C for 2 h.

In this paper, the following abbreviations of the samples are used:

- C – Protein bread without fennel flour
- FS2 – protein bread with 2% of fennel seed
- FS4 – protein bread with 4% of fennel seed
- FS6 – protein bread with 6% of fennel seed
- FC2 – protein bread with 2% of fennel cake
- FC4 – protein bread with 4% of fennel cake
- FC6 – protein bread with 6% of fennel cake

Protein bread moisture. The moisture of protein bread was determined using standard method ISO 712:2009. Measurements were made in triplicate.

Protein bread crumb hardness. Protein bread hardness was tested on the day of baking, at least 2 h after baking. Hardness of experimental bread samples was measured using a TA-XT plus Texture Analyser (Stable Micro Systems Ltd., Surrey, UK) with the following parameters: probe – 25 mm diameter aluminium cylinder; test speed — 1 mm s^{-1}; trigger force — 0.049 N and distance — 4 mm to the bread slice. All values are given as average of six measurements.

Protein bread crumb colour. To measure the colour of bread samples a Colour Tec-PCM/PSM (Accuracy Microsensors Inc., USA) was used based on the CIE L*a*b* colour system: L* 0 = black, 100 = white; a* + value = red, - value = green; b* + value = yellow, - value = blue. Colour was measured at five different points within the crumb region; mean values were reported for each sample.

The total colour difference (ΔE) was defined by the Minolta equations (1, 2):

$$\Delta L = (L - L_0);\ \Delta a = (a - a_0);\ \Delta b = (b - b_0); \quad (1)$$

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (2)$$

where

L, a and b — measured values of protein bread samples with fennel seed or cake;

L_0, a_0 and b_0 — values of the protein bread (control).

Extraction of phenolic compounds from protein bread. 1 g of protein bread was extracted with ethanol / acetone / water (7/7/6 v/v/v) solution in an ultrasonic bath YJ5120-1 (Oubo Dental, USA) at 35 KHz for 10 min at 20±1 temperature. The mixture was then centrifuged in a centrifuge CM-6MT (Elmi Ltd., Latvia) at 3500^{-1} for 5 min.

Residual bread was then re-extracted with the same procedure and supernatant was combined. A triplicate extraction process was done for each sample.

Determination of total phenolic concentration (TPC). The TPC of the protein bread extract was determined by the Folin-Ciocalteu method (Singleton *et al*., 1999) with some modifications. 0.5 mL of extract was mixed with 2.5 mL Folin–Ciocalteu reagent (diluted 10 times with water); 3 min later, 2 mL sodium carbonate (Na_2CO_3) (75 $g \cdot L^{-1}$) was added and mixed. The mixture was allowed to stand for a further 30 min in the dark at room temperature, and absorbance was measured at 765 nm. The TPC was calculated from the calibration curve of Gallic acid, and the results were expressed as Gallic acid equivalents (GAE) 100 g^{-1} dry weight (DW) of the samples.

Determination of DPPH´ radical scavenging activity. Antioxidant activity of the extract was measured using the 2.2-diphenyl-1- picrylhydraziyl DPPH method (Yu *et al*., 2003) with slight modifications. A solution of DPPH was freshly prepared by dissolving 4 mg DPPH in 100 mL methanol. 0.5 of extract was added into a sample cavity containing 3.5 mL of DPPH solution. The mixture was then incubated in the dark for 30 min at room temperature. The absorbance was measured at 517 nm using a UV–VIS spectrophotometer JENWAY 6300. The radical scavenging activity was expressed as Trolox mM equivalents (TE) 100 g^{-1} dry weight (DW) of the samples.

Theoretical calculation of protein bread nutritional value. Nutritional value of protein bread was calculated using conversion factors according to EU Regulation No. 1169/2011 on the provision off food information to consumers:

- carbohydrates (except polyols) 17 KJ g^{-1} — 4 $kcal \cdot g^{-1}$;
- protein, 17 KJ g^{-1} — 4 $kcal \cdot g^{-1}$;
- fat, 37 KJ g^{-1} — 9 $kcal \cdot g^{-1}$;
- fibre, 8 KJ g^{-1} — 2 $kcal \cdot g^{-1}$.

Statistical analysis. Means and standard deviations were determined. The data were subjected to one way analysis of variance (ANOVA). Significance was defined at $p < 0.05$.

RESULTS

Protein bread moisture. Figure 1 shows the moisture (%) of tested protein bread samples. The moisture of the control sample was 44.99 ± 0.23%. The moisture ranged from 50.30 ± 0.09% (FC2) to 50.85 ± 1.15% (FC6) in samples fortified with fennel cake, and from 48.23 ± 0.92% (FS2) to 49.11 ± 0.43% (FS6) in samples fortified with fennel seed.

Protein bread hardness analysis. Hardness of protein bread with fennel seeds and cakes analysis is shown in Figure 2. Hardness of the control sample was 1.27 ± 0.13 N, while in bread fortified with fennel cake, hardness ranged from 2.31 ± 0.48 N (FC2) to 4.48 ± 0.12 N (FC6) and from 2.60 ± 0.18 N (FS2) to 3.37 ± 0.19 N (FS6) in bread fortified with fennel seed.

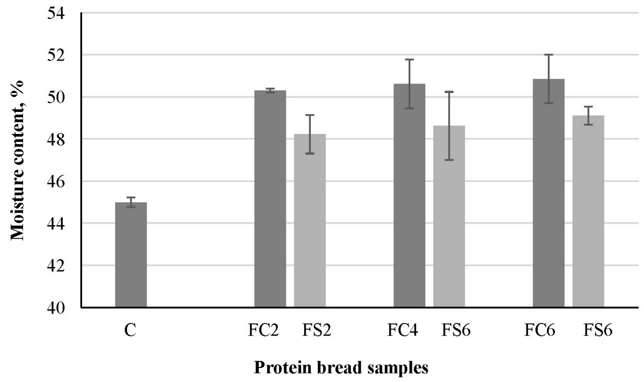

Fig. 1. Moisture of protein bread with fennel seeds and cakes.

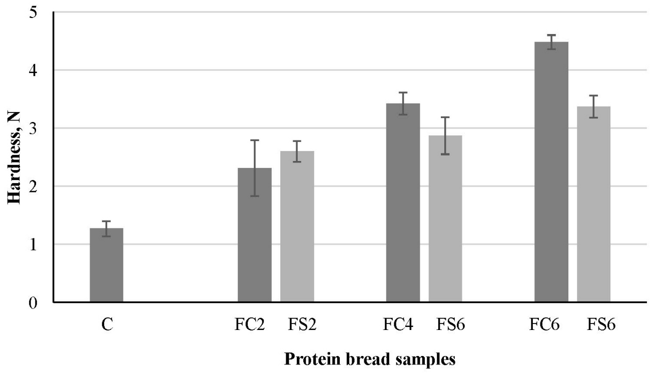

Fig. 2. Crumb hardness of protein bread with fennel seeds (FS) and fennel cakes (FC).

Table 1

THE COLOUR OF PROTEIN BREAD WITH FENNEL SEED (FS) AND FENNEL CAKE (FC)

Bread samples	L*	a*	b*
C	61.08^{a} ± 2.06	0.47^{d} ± 0.69	20.32^{a} ± 1.96
FC2	54.81^{c} ± 0.68	2.24^{cb} ± 0.76	18.07^{ab} ± 1.53
FC4	51.88^{d} ± 1.59	2.99^{ab} ± 0.66	16.78^{ab} ± 3.04
FC6	50.45^{d} ± 0.85	3.54^{a} ± 0.71	18.80^{ab} ± 2.85
FS2	52.99^{bc} ± 1.75	2.64^{cd} ± 0.27	16.42^{b} ± 0.79
FS4	50.97^{c} ± 1.03	4.50^{b} ± 0.43	17.00^{ab} ± 2.81
FS6	49.99^{d} ± 1.34	3.75^{b} ± 1.15	16.18^{b} ± 1.76

*Values marked with the same subscript letters in columns are not significantly different (*p* > 0.05). L, whiteness; a, redness; b, yellowness.

Values of measured whiteness (L), redness (a) and yellowness (b) for bread with different ratios of added fennel cake and seed flour are shown in Table 1. The values of L, a and b in the control bread were 61.08 ± 2.06, 0.47 ± 0.69, and 20.32 ± 1.96, respectively. In the case of bread fortified with fennel cake, the range in L value was from 54.81 ± 0.68 (FC2) to 50.54 ± 0.85 (FC6), a values from 2.24 ± 0.76 (FC2) to 3.54 ± 0.71 (FC6) and b values from 18.07 ± 1.53 (FC2) to 18.80 ± 2.85 (FC6); while for bread fortified with fennel seed, L values varied from 52.99 ± 1.75 (FS2) to

49.99 ± 1.34 (FS6), a values from 2.64 ± 0.27 (FS2) to 3.75 ± 1.15 (FS6) and b values from 16.42 ± 0.79 (FS2) to 16.18 ± 1.76 (FS6).

Values of total colour difference (ΔE) are shown in Figure 3. ΔE values ranged from 6.88 to 11.16 in bread with fennel cake (FC) and from 9.24 to 12.28 in bread with fennel seed (FS).

Total phenolic concentration (TPC) analysis. The total phenolic concentrations (TPC) expressed as Gallic acid equivalents (GAE) 100 g^{-1} dry weight (DW) of bread samples are given in Figure 4. TPC of the control sample was 113.73 ± 1.4 mg GAE 100 g^{-1} DW (range 148.95 ± 4.15 for FC2 to 187.49 ± 6.38 mg GAE 100 g^{-1} DW for FC6) in the bread samples with fennel cake and from 167.96 ± 3.27 (FS2) to 196.05 ± 0.01 mg GAE 100 g^{-1} DW (FS6) in bread samples with fennel seed.

Radical scavenging activity analysis. DPPH radical scavenging activity of bread samples is shown in Figure 5. The values are expressed as Trolox mM equivalents (TE) 100 g^{-1} DW of the samples. Radical scavenging activity of control bread was 6.30 0.15 mM TE 100 g^{-1} DW. DPPH radical scavenging activity had a range between 6.47 ± 0.2 and 7.68 ± 0.19 mM TE 100 g^{-1} DW in bread enriched with 2 and 6% of fennel cake, respectively, and between 7.92 ± 0.15 and 9.54 ± 0.17 mM TE 100 g^{-1} DW in bread enriched with 2 and 6% of fennel seed, respectively.

Nutritional values of protein bread. Nutritional and energy values of protein bread with fennel seed and cake were

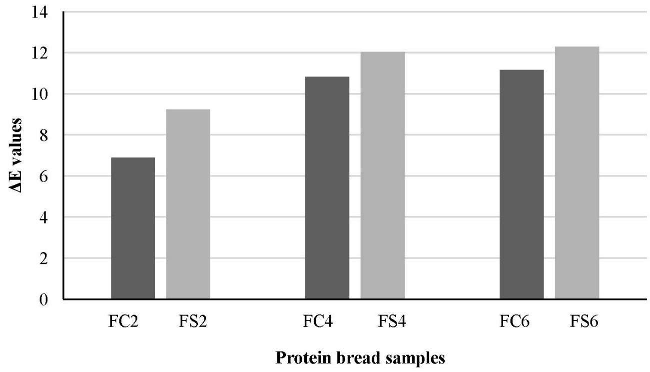

Fig. 3. Total colour difference (ΔE) of protein bread with fennel seeds (FS) and fennel cakes (FC).

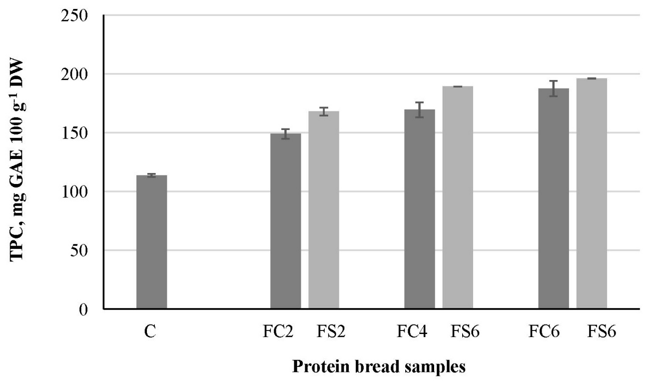

Fig. 4. Total phenolic concentration (TPC) in the protein bread samples.

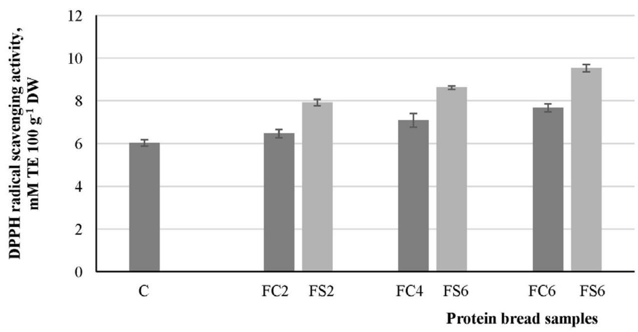

Fig. 5. DPPH radical scavenging activity of protein bread samples.

Table 2

CALCULATED NUTRITIONAL AND ENERGY VALUES OF DIFFERENT PROTEIN BREAD

Bread samples	Nutrients, g·100 g^{-1}				Energy value, 100 g^{-1}	
	Carbohydrates	Protein	Fat	Fibre	kcal	kJ
C	25.59	21.64	0.97	4.96	207.57	3614.00
FC2	25.88	21.71	1.05	5.09	213.71	3638.04
FC4	26.16	21.78	1.14	5.22	219.85	3662.08
FC6	26.45	21.85	1.23	5.35	225.57	3686.12
FS2	25.83	21.69	1.12	5.08	214.47	3643.02
FS4	26.08	21.74	1.27	5.19	221.37	3668.04
FS6	26.32	21.79	1.43	5.31	228.27	3695.06

calculated using conversion factors, and the results are illustrated in the Table 2. Composition of protein bread control was as follows: total carbohydrates — 25.59 g·100 g^{-1}, protein — 21.64 g·100 g^{-1}, fat — 0.97 g·100 g^{-1}, fibre — 4.96 g·100 g^{-1}, and energy value 3614.00 kJ / 207.57 kcal·100 g^{-1}. The obtained results indicate that all the values increased with increasing levels of fennel flour substitutions (2, 4 and 6%) except for fat concentration and energy value, which showed the reverse.

DISCUSSION

As can be seen in Figure 1, samples fortified with fennel seed flour had significantly higher ($p < 0.05$) moisture than the control sample: 44.99 ± 0.23% in the control and 49.11 ± 0.43% in the sample with 6% fennel seed flour. Moisture was significantly ($p < 0.05$) higher in bread with fennel cake flour (Fig. 1) than in the control and in bread with fennel seed flour. Several studies reported higher moisture with increased substitution level (Das *et al.,* 2013) (Olaoye and Onilude, 2011). The differences in moisture of protein bread samples might be due to inclusion of a greater amount of insoluble dietary fibre with fennel (Maneju *et al*., 2011). As a drawback, high moisture promotes microbial proliferation and thus it is linked with shorter shelf life of composite breads (Ndife *et al*., 2011).

Texture is a key quality parameter that consumers appreciate in bread. Bread crumb hardness was significantly affected ($p < 0.05$) by fennel seed and fennel cakes addition

(Fig. 2). Hardness of bread increased with increasing amount of fennel cake flour in bread. A similar trend was observed for bread with fennel seed flour, but in this case significant differences were observed only between samples FS2 and FS6 fennel seed flour. Moreover, bread enriched with fennel cake flour had higher hardness than that enriched with fennel seed flour. These findings are in agreement with similar studies (Das *et al*., 2013), which reported that fennel seed addition increased the firmness of white bread. Hardness is mainly attributed to the solid / water ratio, the effect of added fennel powder on bread viscosity and increase of moisture in the composite bread (Amir *et al*., 2013; Giannou and Tzia, 2016).

The quality of final product is also highly affected by the bread crumb colour. The results showed that all fortified samples had significantly ($p < 0.05$) lower L and b than the control, indicating darker colour (Table 1). Moreover, a values indicated that the formulas prepared with different levels of fennel were more brown then those of the control sample. This increase in a values can be attributed to the light brown colouration of fennel cakes and seed. The same trend was observed for L values of white bread fortified with fennel seed, which can be explained by the difference in the initial flour type used (Das *et al*., 2013).

Regarding the total colour difference (ΔE) (Fig. 3), bread fortified with fennel seed showed higher ΔE values than bread with fennel cake, which may be due to the darker colour of fennel seed flour, as fennel seed powder is darker than fennel cake powder.

The results given in Figure 4 showed that TPC of bread in both cases increased with increase of fennel level. When fennel substitution was increased from 0 to 6%, TPC of the bread increased from 113.73 to 187.49 and 196.05 mg GAE $^{-1}$100 g DW in bread with fennel cake and seed, respectively. Moreover, TPC of bread with fennel seed was higher than that with fennel cake at the same fennel level. Previous studies showed that oil extraction is usually accompanied with loss of some lipophilic phenolic compounds, which might explain the lower TPC of bread with defatted seeds (Yu *et al.,* 2013). However, this is not a huge lost and almost all the phenolic compounds remain in the cake, which make it a rich source of phenolics and underline its added value in bread.

DPPH radical scavenging activity was significantly higher (Fig. 5) in bread enriched with 2–6% fennel flour than in the control bread. The antioxidant activity of extract from enriched bread increased from 6.03 (control bread) to 7.67 and 9.54 mM TE 100 g^{-1} DW in bread with fennel cake and seed, respectively. The correlation coefficients between TPC and DPPH scavenging capacity for bread enriched with fennel cake and seed were 0.931 and 0.918, respectively, which indicated a good linear relationship between TPC and DPPH radical scavenging capacity. The obtained results are comparable to literature (Das *et al*., 2013), which report that increasing level of fennel seeds (up to 7% of fennel seed powder) increases antioxidant capacity of bread. Several studies showed also the negative impact of seed defatting on the antioxidant activity, which is attributed to the presence of vegetable oils, which generally are an important source of antioxidants (Brodowska *et al*., 2014).

As can be seen in the Table 3, an increase of fennel flour addition in produced protein bread leads to an increase of carbohydrate, protein and fibre concentration, and decrease of fat concentration. Moreover, bread enriched with fennel cake showed a slightly higher concentration of carbohydrate, protein and fibre than bread enriched with fennel seed, which was expected as similar results were obtained by El-Demery *et al*. (2015) in the case of bread fortified with full fat and semi-defatted flaxseed.

CONCLUSION

The obtained results showed that the addition of fennel cake flour in protein wheat bread imparted significant improvement in nutritional constituents, moisture and hardness, compared to bread enriched with fennel seed flour and to control bread. This work shows also that fortified bread is a good source of antioxidant compounds like polyphenols beneficial for human health and thus it can be recommended as functional food for the public who are really aware about their well-being. Therefore, fennel cake residues could be processed as co-product in value-added applications such as bread making.

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FENHEĻA (*FOENICULUM VULGARE* L.) IETEKME UZ OLBALTUMVIELU MAIZES KVALITĀTI

Fenhelis (*Foeniculum vulgare* L.) ir aromātisks augs, kas pieder *Apiaceae* dzimtai. Fenheļa sēklas ir bagātas ar aromātiskajām eļļām. Pēc eļļas iegūšanas pārstrādes procesā rodas spiedpaliekas, kuras iespējams izmantot citos produktos. Tāpēc pētījuma mērķis bija pētīt fenheļa eļļas spiedpalieku ietekmi uz olbaltumvielu maizes kvalitāti. Olbaltumvielu maizes gatavošanā fenheļa sēklas un fenheļa spiedpaliekas no eļļas ražošanas pievienotas no 1% līdz 6%. Maizes paraugiem analizēti sekojoši parametri — mitrums, krāsas izmaiņas, cietība, kopējais fenolu saturs un antiradikālā aktivitāte, kā arī aprēķināta maizes uzturvērtība. Fenheļa sēklu un spiedpalieku pievienošana būtiski ietekmē ($p < 0.05$) olbaltumvielu maizes mīkstuma krāsu un cietību, kā arī maizei ir paaugstināts kopējo fenolu saturs un antiradikālā aktivitāte. Iegūtie rezultāti parādīja, ka fenheļa spiedpaliekas var izmantot olbaltumvielu maizes ražošanā, palielinot maizes bioloģisko vērtību.