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Research article

Protein bread fortification with cumin and caraway seeds and by-products flour

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Abstract

This study investigated the effect of protein bread fortification with 2, 4 and 6% of cumin (*Cuminum cyminum*) and caraway (*Carum carvi*) whole seeds and by-products flour, respectively. Fortified protein bread samples were compared to control protein bread and evaluated for their sensory, color, moisture, hardness properties as well as their nutritional values. Total phenolic contents and Trolox equivalent antioxidant capacity were also analyzed. Results indicated that bread fortification shows significant effects on bread properties depending on fortification level. A higher acceptability was observed specially for bread fortified with by-products flour. Increased tendencies of color darkness, moisture content, bread hardness, nutritional values as well as total phenolic content and radical scavenging activity compared to control bread were observed as the percentage of fortification increased in both cases. The overall results showed that addition of cumin and caraway seeds and by-products flour can improve the antioxidant potential and overall quality of protein bread.

Key words: caraway, cumin, bread quality, by-products, radical scavenging activity, total phenolic.

1. Introduction

Wheat bread is very popular foodstuff in the daily diets of most population with more than 32 million tons of annual consumption in the European market only. With the increasing awareness of consumption of healthy food, production of bread from whole wheat flour is highly recommended in bakery industries. Whole wheat flour led to improvement of the nutritional values and fiber content of the final bread, while the aesthetic value and the sensory properties are negatively affected by comparison with bread made from white flour [1]. In this context, vital wheat protein appears as an adequate additive which can enhance not only the texture and the shelf life of the bread, but also a bread enriched in protein is obtained [2].

Cumin (*Cuminum cyminum* L.) and caraway (*Carum carvi* L.) belong to the Apiaceae family. Originated from Mediterranean region and India, they are widely cultivated in temperate regions and used as spices in many popular cuisines [3]. For centuries, cumin and caraway seeds have been

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germinated for food and medicinal uses owing to their high nutritional values with presence of high content of proteins, fiber, minerals, bioactive compounds, volatile and vegetable oils [4]. Nevertheless, vegetable oils extracted from cumin and caraway seeds are considered as rich source of petroselinic acid (C18:1n-12) which is a rare monounsaturated fatty acid used as a raw material in chemical and cosmetic industries. Petroselinic acid is a precursor of both lauric and adipic acids which are used for the production of detergents and surfactants and the synthesis of nylon polymer, respectively. Petroselinic acid is also an important ingredient used in skin hydrations and anti-aging formulas [5]. However, after oil extraction, the remaining cakes from cumin and caraway seeds are underutilized and generally considered as waste. Recently, there is a growing focusing on valorization of seed by-products for their potential health benefits as antioxidant and antimicrobial agents due to their richness in bioactive compounds [6].

Consumers increasingly request functional foods, taking into account their higher content in nutraceutical compounds and their direct contribution in preventing nutrition-related diseases. Therefore, supplementing of bread with nutritious additives in order to boost its physical and nutritional properties is very trendy nowadays [7]. Previous studies have focused on bread fortification with different kinds of plant seed and by-products such as pumpkin seed [8], grape seed [9], fennel seed [10] and by-products of walnut kernel and brown linseed [11]. In spite of having different health benefits, cumin and caraway seeds and by-products have not yet attracted much attention. Due to the fact that they could be regarded as functional agents to improve bread quality, this study is dedicated to investigate the effect of addition of cumin and caraway powder seeds and by-products on the sensory, rheological and biological properties of protein enriched bread.

2. Materials and Methods

2.1. Seed extraction

Extrusion was done by a Single-screw (Model OMEGA 20, France) press with the following parameters: a motor (0.75 kW, 230 V of maximal tension, 5.1 A of maximal intensity), a screw length of 18 cm, a pitch screw of 1.8 cm, with an internal diameter of 1.4 cm, a channel depth of 0.5 cm, and a sleeve of 2.5 cm of internal diameter equipped with a filter-pierced outlet for liquid at the end of the screw and at the surface of the nozzles. The filter section was of 2 mm in diameter to separate extracted oil. The feed rate and the screw rotation speed were maintained constant to 15 g min-1 (0.9 kg 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 15min 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. Cumin and caraway obtained as by-products by extrusion process were used for further research.

2.2. Raw materials for protein bread preparation

Whole wheat flour (G mbH Rigas Dzirnavnieks, Latvia), wheat protein isolate Arise 5000 (GmbH Lorima, Germany), sugar (GmbH Dan sucker), salt, dry yeast (GmbH S.I. Lesaffre, France) were procured from the local market of Jelgava, Latvia; while cumin and caraway seeds were purchased from the local market of Toulouse, France.

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2.3. Protein bread making technology

To determine the influence of cumin and caraway powder seeds and by-products on protein bread quality and chemical composition, cumin powder seeds and by-products were added at 2%, 4% and 6% of whole wheat flour amount, while caraway powder seeds and by-products were added at 2%, 4% and 6% of whole wheat flour amount (Table 1). 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 connection oven (Sveba Dahlen, Sweeden) and then cooled at room temperature 22±2°C for 2 h.

2.4. Sensory evaluation of protein bread

Bread samples were analyzed by sixty panelists of both sexes aged 18-46 years students' control and food expertise, Faculty of Food Technology, Latvia University of Agriculture. The samples were presented to the participants in identical containers labelled with randomized 3-digit numbers. An acceptance test was applied to attribute the degree of preference using a 5 point hedonic scale; the scale ranges from 1 – 5 with 1 signifying the least score (dislike very much) and 5 highest score (like very much).

2.5. Protein bread moisture content

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

2.6. Protein bread crumb hardness

Protein bread hardness test were performed on the day of baking, at least 2 h after baking. Hardness of experimental bread samples was measured using TA-XT plus Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK) with the following parameters: probe – a 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.

2.7. Protein bread crumb color

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

The total color difference (ΔE) was defined by the Minolta equations (1&2):

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

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

Where

L, a and b – measured values of protein bread samples with cumin or caraway flour;

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

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2.8. Extraction and determination 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 [12]. 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. Triplicate extraction process was done for each sample.

TPC of the protein bread extract was determined by Folin-Ciocalteu method [13] with some modifications. 0.5 mL of extract was mixed with 2.5 mL of Folin–Ciocalteu reagent (diluted 10 times with water), 3 min later, 2 mL of sodium carbonate (Na_2CO_3) (75 g 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. TPC values were 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. Measurements were made in triplicate for each extract.

2.9. Determination of trolox equivalent antioxidant capacity (TEAC)

Antioxidant activity of extracts was measured with the 2,2-diphenyl-1- picrylhydraziyl DPPH method [14] 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. Measurements were made in triplicate for each extract.

2.10. 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), 4 kcal g^{-1};
* Protein, 4 kcal g^{-1};
* Fat, 9 kcal g^{-1};
* Fibre, 2 kcal g^{-1}.

Statistical analyses

All experiments were performed in triplicate and the results were presented as the mean ± SD. The values were reported as mean. One-way ANOVA and Tukey test by pairewise at 5% probability level were used for the analyses.

3. Results and discussion

3.1. Protein bread sensory analysis

Hedonic scale was used to measure food preferences. The overall liking of protein bread samples in the present study was determined using 5-point hedonic scale (5=like extremely; 3=neither like nor dislike; 1=dislike extremely). Of the 60 participants, 30.2% were male and 69.8% female, 89.4% aged between 18 and 26 years, and 10.6% from 27 to 46 years.

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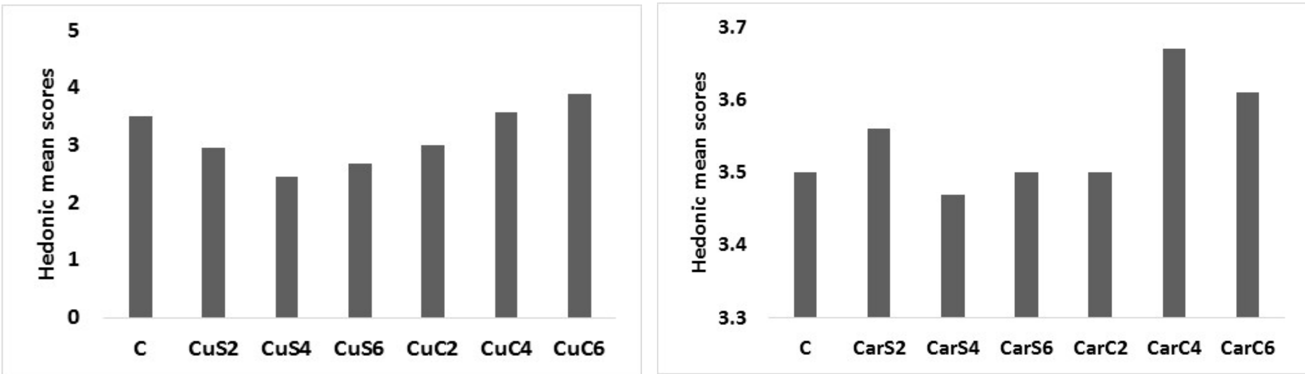
Figure 1 show the mean scores assigned to each sample containing different level of cumin or caraway substitutions as compared to the control.

Significant difference was observed in the overall acceptability of the protein bread samples fortified with cumin seeds and by-products (Figure 1). Our results showed that the scores generally decreased with increase in cumin seeds substitution when compared to control protein bread. Samples CuS4 and CuS6 had the lowest scores since they had a bitter aftertaste, as reported by several participants. Increased scores was observed with increase in cumin by-products substitution, sample CuC6 was the highest suggesting that the panel preferred the sweet taste and aroma of cumin over the control protein bread.



(a) (b)

Figure 1. Mean values for overall acceptance of protein bread samples fortified with (a) cumin and (b) caraway seeds and by-products.

No significant difference among samples fortified with caraway powder seeds and by-products. Yet, they were all accepted given that all scores were higher than 3. Several participants didn't found an impact of bread fortification with cumin and caraway flour on the overall acceptability of protein bread since they didn't have a strong influence on the final bread taste and aroma. However, sample CarC4 with 4% of caraway by-products substitution was found to be the most acceptable with the highest score (Figure 1).

Our overall results revealed that protein bread fortified with by-products flour showed more acceptability than both control bread and fortified with seeds flour as they improve the sensory properties of the samples without affecting bread's aftertaste.

3.2. Protein bread color analysis

Color is the first feature that consumers rely on for any food products acceptance. Mean protein bread color values with different levels of substitution of cumin and caraway flour along with control bread are presented in Table 1. Results showed that seeds and by-products flour addition led to significantly lower luminosity values of protein bread samples, while redness and yellowness parameters were significantly higher compared to control protein bread.

Increasing the levels from 0 to 6% of cumin seeds and by-products led to a 16 % and 7.75% of reduction in lightness (L*), respectively; a* values increased more than 11% in CuS6 and 6% in CuC6 compared to control bread. The values of b* values increased also about 11% in CuS6 and CuC6 samples compared to control bread. Similar trend was observed in the case of addition of caraway seeds and by-products flour (Table 1). Overall results showed that increasing of substitution levels is accompanied

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with increasing of L* values and decreasing of a* and b* values which led to a more yellow brown color were observed.

Table 1. Abbreviations of the samples used in the present article, crumb color analysis and total color difference (ΔE) values of protein bread fortified with cumin and caraway seeds and by-product.

Bread samples	Abbreviations	L*	a*	b*	ΔE values
C	Control	$61.08^{a}\pm2.06$	$0.47^{d}\pm0.69$	$20.32^{c}\pm1.96$	-
CuS2	2% of cumin powder seed	$55.79^{cd}\pm0.52$	$1.21^{cd}\pm0.04$	$20.56^{c}\pm0.01$	5.35
CuS4	4% of cumin powder seed	$53.77^{d}\pm0.75$	$3.59^{b}\pm0.03$	$21.19^{bc}\pm0.09$	8.01
CuS6	6% of cumin powder seed	$50.86^{e}\pm0.89$	$5.48^{a}\pm0.65$	$22.56^{ab}\pm0.46$	11.62
CuC2	2% of cumin by-product	$58.90^{ab}\pm1.18$	$0.90^{cd}\pm0.01$	$20.16^{c}\pm0.79$	2.22
CuC4	4% of cumin by-product	$57.69^{bc}\pm0.14$	$1.70^{c}\pm0.39$	$22.44^{ab}\pm0.55$	4.18
CuC6	6% of cumin by-product	$56.35^{bcd}\pm0.12$	$3.09^{b}\pm0.05$	$23.59^{a}\pm0.46$	6.13
CarS2	2% of caraway powder seed	$58.21^{b}\pm0.07$	$1.32^{d}\pm0.11$	$20.37^{d}\pm0.43^{b}$	2.04
CarS4	4% of caraway powder seed	$57.72^{b}\pm0.27$	$3.20^{b}\pm0.09$	$22.66^{c}\pm0.82^{b}$	3.84
CarS6	6% of caraway powder seed	$56.34^{b}\pm0.30$	$4.94^{a}\pm0.77$	$26.98^{a}\pm1.03^{a}$	9.31
CarC2	2% of caraway by-product	$59.03^{ab}\pm0.13$	$1.28^{d}\pm0.31$	$22.43^{bc}\pm0.24$	3.05
CarC4	4% of caraway by-product	$58.33^{b}\pm0.81$	$2.19^{c}\pm0.08$	$24.09^{bc}\pm0.54$	4.97
CarC6	6% of caraway by-product	$57.70^{b}\pm0.38$	$3.49^{b}\pm0.04$	$25.83^{ab}\pm0.58$	7.13

*values marked with the same subscript letters in columns are not significantly different ($p>0.05$).

Total color difference (ΔE) is a combination of L*, a* and b* values generally used to illustrate bread colors variation. ΔE values revealed that incorporation of cumin and caraway flour resulted in high color changing (Table 1).

Our findings are in line with those of Tarek-Tilistyak *et al*. (2015) where darker bread was obtained after addition of linseed oil-seed pressing residues [11]. Besides, a darker bread color was obtained in

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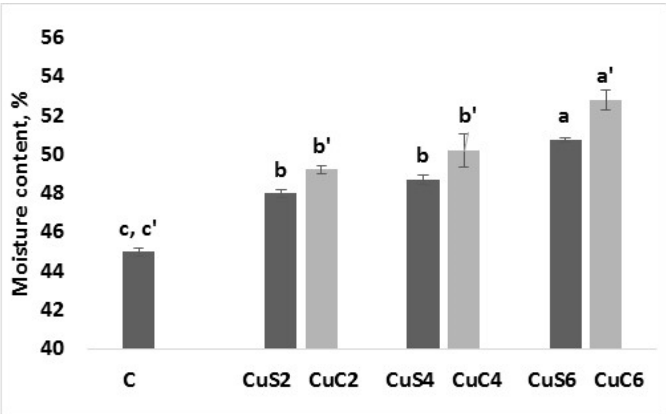
samples fortified with cumin and caraway by-products flour than bread fortified with seeds flour. The results showed also that bread samples fortified with caraway flour were browner than those fortified with cumin flour (Table 1). Colour changing can be attributed to Maillard reaction which is a browning reaction between amino acids and sugars and to the differences in moisture content between bread samples which influence also the Maillard reaction. The brown color of added cumin and caraway flour had also a great impact on the final color of bread samples resulting with darker protein bread [15].

3.3. Protein bread moisture content analysis

Moisture content is a key parameter used to determine bread shelf-stability and susceptibility to microbial infections. The proximate moisture content of protein bread fortified with cumin and caraway powder seeds and by-products are shown in Figure 2. A significant increasing of moisture content was obtained in fortified bread samples comparing to control bread.

The moisture content of protein bread increased nearly 6% and 8% in samples fortified with cumin seeds and by-products flour compared to control bread, respectively (Figure 2), and also about 8% and 10% in bread fortified with caraway seeds and by-products flour compared to control bread, respectively (Figure 2).



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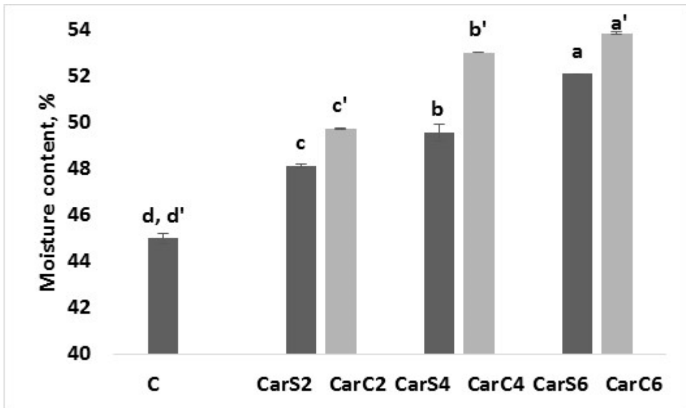

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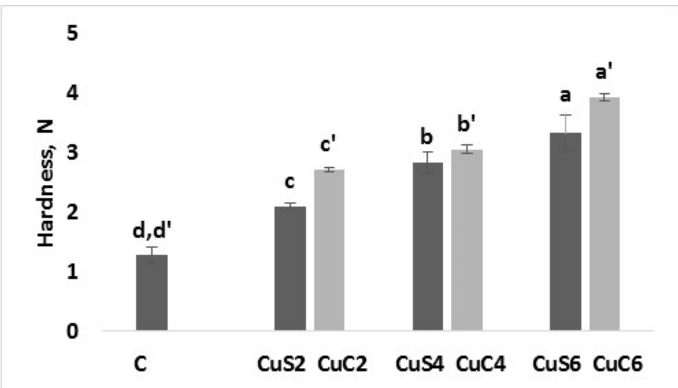

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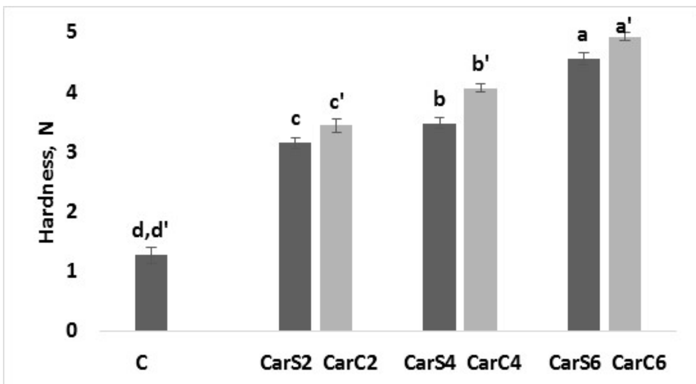

(d)

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Figure 2. Moisture content (%) and hardness (N) of protein bread fortified with cumin (a and c) and caraway (b and d) seeds and by-products.
*Column marked with the same subscript letters in each bar chart are not significantly different (p>0.05).

The overall analysis of protein bread samples revealed that addition of cumin and caraway seeds and by-products flour led to a significantly increasing of crumb moisture content, this can be attributed to the higher crumb moisture retention caused by the introduction of cumin and caraway powder; similar trend was obtained by Bansal *et al.* (2015) who studied the effect of bread fortification with soya flour blends [16]. Furthermore, moisture content of protein bread fortified with by-products flour was higher than those fortified with seeds flour which can be due to the substantial amount of protein and fiber contents as a result of defatting process. Also, protein bread with added caraway powder has higher moisture content than bread with added cumin flour. This increase in water retention was most likely due to the higher fiber content in bread fortified with caraway flour resulting by a higher water holding capacity [17].

3.4. Protein bread hardness analysis

Figure 2 list the hardness profile of analyzed protein bread samples. The hardness of protein bread crumbs were positively related to the level of fortification and a significant hardness increasing were observed. Crumb hardness increased more than 2 times in bread fortified with cumin flour (CuS6 and CuC6), and more than 3 times in bread fortified with caraway flour (CarS6 and CarC6) compared to control bread (C). These results are in agreement with the work of Das *et al.* (2013) who studied the effect of fennel fortification on the bread firmness [18].

However, hardness profile of protein bread fortified with by-products was higher than bread fortified with seeds flour. Hardness increasing might be due to the higher fiber content which is generally accompanied with restriction of gas cells expansion, resulting by a compact structure of bread [19]. Moreover, since the plasticizing effect of water in the bread, hardness increasing can be also attributed to the increasing of moisture content of protein bread samples [20].

3.5. Nutritional values of protein bread

Calculated nutrient content and energy values of protein bread samples enriched with cumin and caraway seed and by-product are given in Table 1. Generally, as the level of fortification increased in the all formulations carbohydrate, protein, fiber and fat content increased in comparison with control bread, this increasingly amount of nutrients is responsible for the observed increased energy values in all fortified bread samples compared to control bread (Table 2). However, carbohydrate, protein and fiber content was higher in bread samples fortified with by-products flour than those fortified with seeds flour while fat content was highest in bread fortified with seeds flour due to the lower fat content in initial by-products flour in both cases. This latter fact was expected as the seeds powder contains more lipids while by-products resulted from defatted seed. These results are in line with previous investigation on the effect of the addition of fully fat and defatted flaxseed flour on wheat bread [21].

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Table 2. Calculated nutritional and energy values of whole wheat, cumin and caraway seeds and of protein bread fortified with cumin and caraway seeds and by-products.

Bread samples	**Nutrients, g 100g^{-1}**				**Energy value, 100g^{-1}**
	Carbohydrates	Protein	Fiber	Fat	Kcal
Whole wheat	59.70	11.90	11.20	2.30	340
Cumin seed	44.24	17.81	10.50	22.27	375
Caraway seed	49.90	19.77	38.00	14.59	333
C	25.59	22.37	4.96	0.97	210.49
CuS2	25.77	22.4	5.01	1.2	213.50
CuS4	25.95	22.42	5.06	1.42	216.38
CuS6	26.13	22.45	5.11	1.65	219.39
CuC2	25.93	22.48	5.05	1.09	213.55
CuC4	26.27	22.58	5.14	1.22	216.66
CuC6	26.60	22.69	5.22	1.34	219.66
CarS2	25.82	22.42	5.24	1.14	213.70
CarS4	26.04	22.47	5.51	1.31	216.85
CarS6	26.26	22.52	5.78	1.48	220.00
CarC2	26.00	22.51	5.38	1.06	214.34
CarC4	26.41	22.65	5.78	1.15	218.15
CarC6	26.81	22.79	6.19	1.24	221.94

3.6. Total phenolic content (TPC) analysis

Phenolic compounds are plant secondary metabolites which act as antioxidants owing to their redox properties, consumption of food with high phenol content is highly recommended due to their health promoting effects as they are involved in preventing of many diseases such as cancers, diabetes and cardiovascular diseases [22].

The total phenolic content (TPC) of different protein bread fortified with cumin and caraway seeds and by-products are presented in Figure 3. Fortified bread samples had significantly higher TPC than of control protein bread. The TPC of bread fortified, whatever the added flour, were higher than the TPC of control bread more than 2 times (Figure 3), This increasing in TPC in all cases can be attributed to the high content of phenol in added cumin and caraway flour which agrees with previous studies such as the addition of sweet-lupines and rice bran [23], [24]. However, bread samples fortified with cumin flour showed greater phenolic content than those fortified with caraway flour which could be attributed to the highest phenolic content in cumin seed [3]. The TPC of bread fortified with by-products flour was lower than the TPC of bread fortified with seeds flour due to the process of defatting which is responsible of the loss of some lipophilic phenolic compounds [14].

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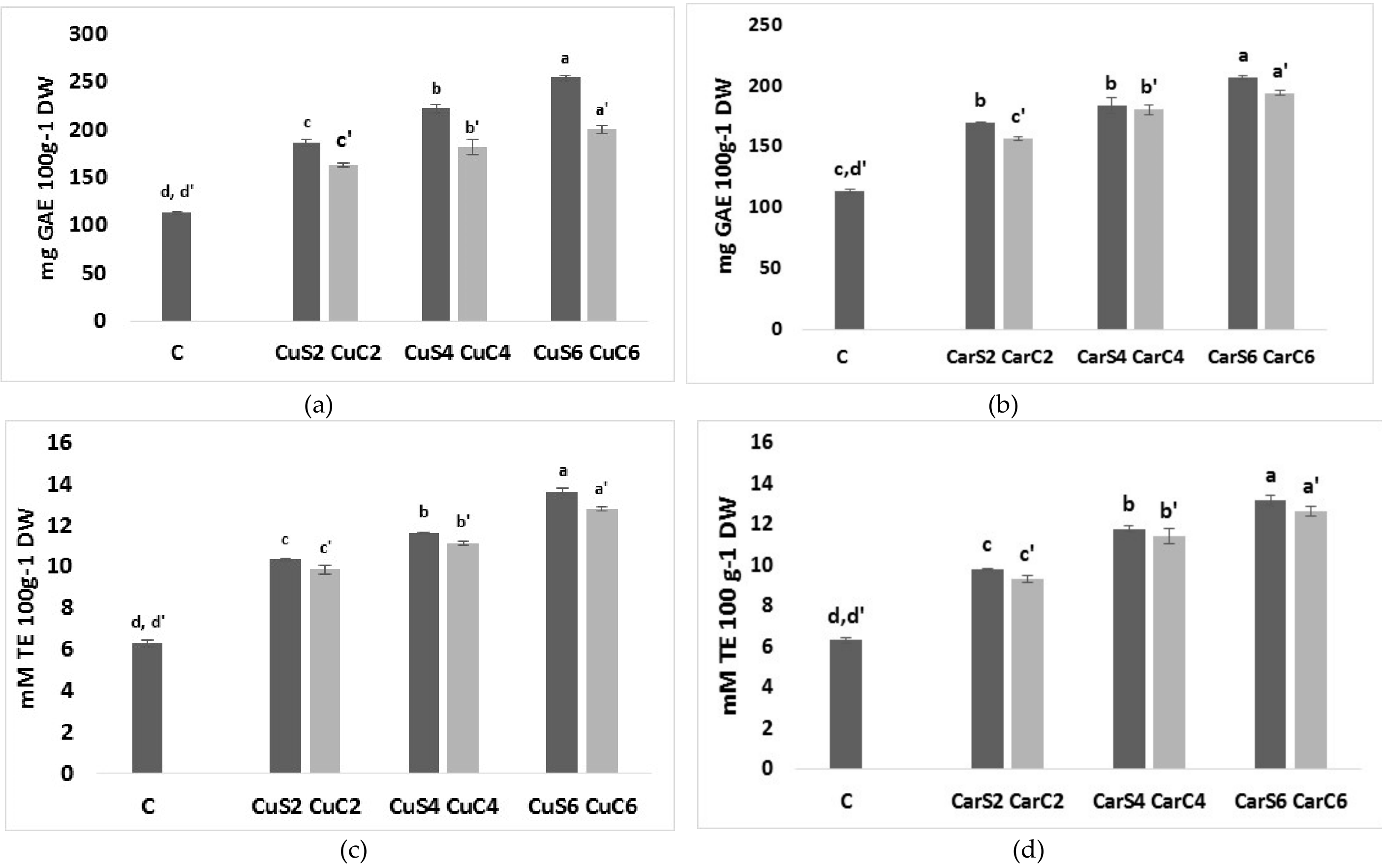

Figure 3. Total phenolic content (TPC, expressed as mg GAE 100g-1 DW), Trolox equivalent antioxidant capacity (TEAC expressed as mM TE 100g-1 DW) of protein bread fortified with cumin (a and c) and caraway (b and d) seeds and by-products

*Column marked with the same subscript letters in each bar chart are not significantly different ($p>0.05$).

3.7. Trolox equivalent antioxidant capacity (TEAC) analysis

Trolox equivalent antioxidant capacity (TEAC) assay is a rapid, simple and inexpensive method employed for determining antioxidant capacity, it measures the ability of a compound to act as free radical or hydrogen donor, and thus it is used to is widely used to evaluate antioxidant activity of foods for both lipophilic and hydrophobic antioxidants [25]. The total antioxidant activities (TEAC) of bread fortified with cumin and caraway seeds and by-products flour are shown in Figure 3. TEAC values were strictly dependent on the level of fortification and the differences between control bread and fortified bread were statistically significant.

TEAC values increased with increasing of fortification level of cumin (CuS6 and CuC6) and caraway (CarS6 and CarC6) flour about 2 times in comparison with control bread (Figure 4). Higher TEAC values means greater antioxidant activity, nonetheless, our results are in accordance with previous studies that reported the positive effect of bread fortification on its antioxidant properties [18], [26].

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The correlation coefficients (R^2) of total antioxidant activity (TEAC) and total phenolic content (TPC) of the protein bread fortified with seeds and by-products flour were 0.98 and 0.99, respectively in both cases which is in line with several previous studies [27], [28].

4. Conclusion

This study showed the positive impact of bread fortification with different levels of cumin and caraway seeds and by-products fortification on the protein bread quality and overall acceptance. Regarding the organoleptic properties, the percentage should not exceed 4% for cumin and caraway seeds flour and 6% for cumin and caraway by-products flour, respectively. This fortification was advantageous due to the increased nutritional value and higher moisture content with acceptable rheological and sensory features. However, daily intake of fibers and oils containing monounsaturated fatty acids provides many health benefits such as improving of cardiovascular health and digestion system. It could be concluded also that bread production may be an ideal alternative for the valorization of cumin and caraway residual by-products.

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