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Nutritional and sensory evaluation of wheat breads supplemented with oleic-rich sunflower seed

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Abstract

Wholegrain and refined (white) wheat breads were prepared with the addition of high-oleic sunflower seed at various levels (8%, 12%, 16% flour basis). The nutritive value of breads was determined by measuring the chemical composition, including the mineral content, the fatty acid composition (saturated, monounsaturated, polyunsaturated, linoleic and linolenic acids) and the contents of tocopherols (α-, β-, γ-, δ-). The obtained data were used to estimate the intakes of nutrients and compare them to the dietary reference intakes (DRIs). The breads made with the addition of sunflower seed were sensorially acceptable, containing significantly more tocopherols, fat, essential fatty acids, crude fibre, copper and zinc. It was estimated that wholegrain supplemented breads would contribute to the corresponding DRIs in the range 33.7–40.8% (adults) for copper and 4.7–18.4% (males), i.e. 6.4–25.3% (females) for zinc, 18.3–26.8% (males), i.e. 25.9–37.9% (females) for linoleic (omega-6) acid, 7.4–7.6% (males), i.e. 10.7–11.0% (females) for alpha-linoleic (omega-3) acid.

Keywords: Bread; Sunflower seed; Nutrients; Minerals; Tocopherols; Fatty acids; Intake

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1. Introduction

Bread and bakery products have an important role in human nutrition. Generally, wheat bread is considered to be a good source of energy and irreplaceable nutrients for the human body. This is especially true for the products made from wholegrain or high-yield flour types. Bread prepared from refined flour is nutritionally much poorer and does not adequately meet the requirements for many macro- or micro-nutrients. It has been reported that bread made from refined flour has low micronutrient content (Al-Kanhal, Al-Mohizea, Al-Othaimeen, & Akmai Khan, 1999; Isserliyska, Karadjov, & Angelov, 2001). Also, wheat protein lacks the balance of essential amino acids- lysine, threonine and valine. Therefore, there have been many on-going investigations on enhancing the nutritive value of bread to fulfill the expanding demands of modern dietary habits, considering the products' protein, mineral, vitamin and/or fibre contents. Bakery products, supplemented with various nutritious, protective and ballast substances, have been gaining popularity worldwide. Mixed grain, wholegrain breads and related products are even considered as functional foods because they are convenient vehicles for important nutrients and phytochemicals.

Various bread types enriched with combinations of whole oilseeds are being readily accepted by consumers. This interest in whole oilseeds relates to their high content of polyunsaturated fatty acids, vegetable protein, phosphorus, iron, magnesium, vitamin E, niacin, folate and phytoestrogens. For example, sunflower seeds contain around 20% protein, high levels of potassium (710 mg/100 g) and magnesium (390 mg/100 g) and are especially rich in polyunsaturated fatty acids (approximately 31.0%) in comparison with other oilseeds: soy (3.5%), peanut (13.1%), cottonseed (18.1%), flaxseed (22.4%), sesame seed

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(25.5%), and safflower seed (28.2%) (Food Standards Agency Institute of Food Research, 2002). There has recently been a trend to introduce new lines of oleic-rich oil seed plants (e.g. sunflower, canola) in order to obtain oils with reduced linoleic acid to increase the oxidative and thermal stabilities of oils, thus avoiding the use of hydrogenated oils (*trans*-fats) in the products (Smith, King, & Min, 2007). Oleic acid contents of oleic-rich sunflower oils (70–90%) are comparable with those of olive oil (70%) (McKevith, 2005). Sunflower seed contains an appreciable amount of vitamin E – 37.8 mg/100 g unlike linseed, sesame seed and soy that contain less than 3 mg/100 g while peanut is estimated to contain 10.1 mg/100 g of vitamin E (Food Standards Agency Institute of Food Research, 2002). Tocols (tocopherols and tocotrienols) that possess vitamin E activity are recognized as the most important tissue antioxidants, having a role in preventing or controlling non-specific reactions from various oxidizing species produced in normal metabolism.

The Serbian population shows high prevalence of consumption of large quantities of bread. The average per capita consumption of bread per day has been reported to be around 300 g (Škrbić, 2007). Therefore, the present investigation was undertaken to study whether supplementing wheat breads (refined or wholegrain) with high-oleic sunflower seed at three levels of supplementation (8%, 12%, and 16%, flour basis) would contribute to meeting the dietary reference intakes (DRIs) for various nutrients (minerals, tocopherols, fatty acids, proteins, fat) and thus benefit the general population. Besides the chemical composition, including macro elements, essential microelements, tocopherol content, fatty acid composition, as well as the nutritional adequacy of supplemented wheat bread, the effect of adding the sunflower seed on finished product sensory quality was also estimated.

2. Materials and methods

2.1. Raw materials

Commercially available refined wheat flour (12.99% water content, 0.51% d.b. (dry basis), ash content, 11.5% d.b. protein content, obtained from "Fidelinka" Mill Subotica, Serbia), wholemeal wheat flour (12.67% water content, 1.72% d.b. ash content, 11.8% d.b. protein content, obtained from "Fidelinka", Mill Subotica, Serbia), fresh compressed yeast (70% moisture content, obtained from "Alltech Fermin" Senta, Serbia), salt, gluten, commercial improver ("Golden Tiger" purchased from "Puratos", Belgium) and sunflower seed were the raw materials. Sunflower seed sample (5.28% water content, 55.93% oil content, 399 mg/g oleic acid content) was prepared by blending equal weights of three high-oleic hybrids. The high-oleic hybrids: NS-H-OL1, NS-H-OL2, and NS-H-2033 were bred in the Institute for Crops and Vegetables, Novi Sad, Serbia.

2.2. Preparation of breads

The bread dough formula was: flour (refined or wholegrain) (100%), compressed yeast (3% for breads made with refined flour, and 4% for breads made with wholegrain flour), salt (2%), gluten (2% for breads made with refined flour, and 4% for breads made with wholegrain flour), commercial improver (dosed as recommended by the manufacturer), water (optimum), and sunflower seed (tested at 8%, 12%, 16% levels). Percentages are based on flour weight.

Bake trials were carried out under laboratory conditions. Dough mixing, processing and baking were performed on laboratory-scale equipment. A straight dough process was used. Doughs were mixed to optimum consistency in a high-speed Diosna mixer with low speed of 85 r/min for 1 min and high speed of 120 r/min for 7 min. Final dough temperature was 30 °C. Doughs rested in bulk for a period of 45 min, and then they were hand-kneaded and left to rest for 15 min. Doughs were scaled into 340 g portions, manually rounded, rolled, put into tin pans (24.5 × 9 × 6.5 cm) to final fermentation for 75 min (for bread made with refined flour), i.e. 40 min (for bread made with wholegrain flour) at 30 °C and 80% relative humidity. Baking was done at 230 °C in a deck-type oven (Termotehnika, Zagreb) until the initial dough mass was reduced by 8%.

2.3. Bread evaluation

Bread quality attributes were evaluated 24 h after baking. Loaf weight and volume (rapeseed displacement method) were determined, as well as sensory evaluation of the bread crumb and crust. Sensory evaluation was carried out by seven trained panel assessors. Each bread sample was evaluated on the basis of the following characteristics: specific volume, penetrometer number, crumb attributes (elasticity, crumb grain structure), crust colour, shape regularity (height/diameter ratio), porosity according to Dallmann (1958) and flavour. The rating scale for elasticity ranges from 1 (unacceptable) to 5 (excellent), for crumb grain structure; it ranges from 1 (distinctively coarse) to 5 (spongy), for crumb grain uniformity; it ranges from 1 (uneven pore distribution) to 5 (even pore distribution), and crust colour from 1 (pale or burned) to 5 (deep golden). Penetrometer number was determined by a PNR6 SUR Berlin (Sommer&Runge KG) penetrometer with spherical probe (25 mm diameter) and was used to evaluate crumb resilience instrumentally. The ranking scale for porosity, according to Dallmann (1958), ranges from 1 (open grain structure) to 8 (close grain structure). Flavour was determined using a 5-point hedonic rating scale ranging from 1 (dislike extremely) to 5 (like extremely).

2.4. Chemical analyses

Protein (Official Method No. 950.36), fat (Official Method No. 935.38), crude fibre content (Official Method

No. 950.37), reducing sugar (Official Method No. 975.14), ash (Official Method No. 930.22) and water contents (Official Method No. 926.5) were determined by standard methods of analysis (AOAC 2000). Starch content was determined by hydrochloric acid dissolution according to the ICC Standard (ICC Standard No. 123/1 1994). In the calculation of the starch content of bread, the specific optical rotation for wheat starch was used (182.7 grd ml/g dm^{-1}) (Kaluđerski & Filipović, 1990).

The mineral content of breads was determined by atomic absorption spectrophotometry in previously wet acid-digested samples that is described in detail in Škrbić and Ćupić (2005).

The tocopherol content was determined by standard method ISO 9936:2006.

The fatty acid composition was determined according to standard AOAC Method No. 996.06.

2.5. Statistical analysis

All determinations were performed in triplicate. The statistical analyses were conducted using two-way ANOVA procedures. Differences in samples due to the addition of sunflower seeds were tested for statistical significance at the $p = 0.05$ level. Tukey's Honestly Significant Difference was used to differentiate between the mean values. Analyses were done with Statistica 7.1 statistical software (StatSoft Inc., Tulsa, Oklahoma).

3. Results and discussion

3.1. Sensory evaluation

Sensory evaluation of breads (Table 1) implied that, as the level of sunflower seed increased, crumb elasticity decreased, while the crumb grain structure, the crumb grain uniformity, and the crust colour were not significantly affected by the addition of sunflower seed as compared to the corresponding control sample. The addition of sunflower seed significantly decreased the crumb elasticity of white breads as compared to the control sample. The elasticity of supplemented wholegrain breads was significantly decreased at higher levels of supplementation (12%, 16%). However, the penetrometer number showed that there was a significant difference only in the elasticity between the groups of white and wholegrain breads but no significant differences within the groups, indicating that the crumb elasticity was minimally affected by the addition of sunflower seeds.

The addition of sunflower seed improved the flavour of bread samples. All the samples of wholegrain bread scored significantly higher for flavour when compared to the white control bread. Increasing substitution levels increased the flavour scores of white breads as compared to the control sample. In the case of wholegrain breads, only the taste score of bread with 16% sunflower seed differed significantly compared to the control.

Table 1
Sensory and physical characteristics of bread prepared from refined wheat flour and various levels of sunflower seed (means ± SD of three independent determinations)

	Bread samples	Specific volume, ml/g	Height/diameter ratio	Crust colour	Porosity	Crumb grain uniformity	Crumb grain structure	Crumb elasticity	Penetrometer number	Flavour
White bread	Control	5.63 ± 0.57^{a}	1.38 ± 0.11^{a}	4.40 ± 0.60^{a}	6	1.67 ± 1.15^{a}	3.33 ± 0.42^{a}	4.67 ± 0.42^{a}	125.2 ± 2.9^{a}	3.07 ± 0.12^{a}
	Bread + 8% sunflower seed	5.25 ± 0.35^{a}	$1.29 \pm 0.08^{a,b}$	4.00 ± 0.00^{a}	6	2.00 ± 1.41^{a}	3.40 ± 0.57^{a}	4.10 ± 0.14^{b}	118.6 ± 3.7^{a}	3.93 ± 0.17^{b}
	Bread + 12% sunflower seed	5.20 ± 0.16^{a}	$1.25 \pm 0.01^{b,c}$	3.80 ± 0.20^{a}	7	3.00 ± 0.00^{a}	3.33 ± 0.42^{a}	4.07 ± 0.12^{b}	118.67 ± 2.8^{a}	$4.0 \pm 0.00^{b,c}$
	Bread + 16% sunflower seed	4.97 ± 0.15^{a}	1.13 ± 0.03^{c}	4.07 ± 0.12^{a}	7	3.00 ± 0.00^{a}	3.60 ± 0.35^{a}	3.93 ± 0.12^{b}	117.7 ± 19.6^{a}	$4.13 \pm 0.12^{b,c}$
Wholegrain bread	Control	3.62 ± 0.09^{b}	0.98 ± 0.02^{d}	4.00 ± 0.00^{a}	7	2.33 ± 1.15^{a}	2.60 ± 0.35^{a}	3.00 ± 0.00^{c}	68.1 ± 14.6^{b}	$4.00 \pm 0.00^{b,c}$
	Bread + 8% sunflower seed	3.58 ± 0.11^{b}	$0.91 \pm 0.04^{d,e}$	4.00 ± 0.00^{a}	7	3.00 ± 0.00^{a}	2.50 ± 0.42^{a}	3.00 ± 0.28^{c}	63.1 ± 2.6^{b}	$4.07 \pm 0.12^{b,c}$
	Bread + 12% sunflower seed	3.26 ± 0.13^{b}	$0.87 \pm 0.03^{d,e,f}$	4.00 ± 0.00^{a}	7	2.33 ± 1.15^{a}	2.60 ± 0.35^{a}	2.20 ± 0.00^{d}	53.0 ± 7.5^{b}	$4.20 \pm 0.00^{b,c}$
	Bread + 16% sunflower seed	3.20 ± 0.05^{b}	0.82 ± 0.02^{f}	4.00 ± 0.00^{a}	7	4.33 ± 1.15^{a}	2.60 ± 0.35^{a}	2.20 ± 0.00^{d}	53.7 ± 9.6^{b}	4.40 ± 0.35^{c}

[a–f] Different superscripts in the same column indicate that means were significantly different ($p < 0.05$).

The specific volume of both refined and wholegrain breads decreased, but not significantly, with the addition of sunflower seed, by approximately 11.5%. The specific volume of wholegrain breads was significantly lower than that of white breads.

The addition of sunflower seed generally affected the shapes of white and wholegrain breads (described by height/diameter ratio) giving flatter products. These changes were less pronounced for wholegrain bread types where only the sample with the highest supplementation level differed significantly from the corresponding control.

3.2. Nutritional evaluation

3.2.1. Macronutrient content of breads

The wheat breads had 14.5% and 14.8% protein contents for refined and wholegrain breads, respectively. The protein content of breads gradually increased among the supplemented breads (Table 2) but no significant increase was observed.

The fat content of bread made with refined wheat flour was 1.7% and it was 2.0% for wholegrain bread (Table 2). The fat contents of both bread types increased significantly upon adding the sunflower seeds. This is due to the high oil content of sunflower seeds. The maximum supplementation dose increased the fat content up to 12.0% for both bread types.

The addition of sunflower seed decreased the starch content of refined and wholegrain breads because the sunflower seeds, containing less starch, replaced part of the wheat flour. In the case of white breads, the starch decrease was significant for 16% added sunflower seed. Wholegrain breads contained significantly less starch than did refined breads. There was no observed trend in the reducing sugar content of the supplemented breads.

The ash content of the bread made from refined flour was 0.92% and it increased non-significantly with the increasing supplementation levels. The same trend was observed for the wholegrain breads – the ash content increased from 2.01% for the control to 2.29% for the maximum supplementation level (Table 2). Wholegrain breads had significantly higher contents of ash than did refined breads, except white bread with 16% sunflower seed.

The content of crude fibre significantly increased with the increasing levels of sunflower seed in both refined and wholegrain bread types as compared to the corresponding control samples.

3.2.2. Total tocopherol content of breads

It is generally accepted that vitamin E (tocopherols and tocotrienols) is essential as a radical chain-braking antioxidant to protect integrity of tissues and cells and function of cell membranes (α-tocopherol being the most potent compound). However, reconsideration of the biological activity of tocopherols showed that the primary antioxidative activity depends on their structure, following the

Table 2
Chemical composition of wheat breads (means ± SD of three independent determinations)

	Bread samples	Protein (g/100 g d.b.)	Fat (g/100 g d.b.)	Starch (g/100 g d.b.)	Reducing sugar (g/100 g d.b.)	Ash (g/100 g d.b.)	Crude fibre (g/100 g d.b.)
White bread	Control	14.5 ± 1.25^{a}	1.7 ± 0.10^{a}	56.8 ± 1.08^{a}	2.4 ± 0.20^{a}	0.92 ± 0.15^{a}	0.50 ± 0.03^{a}
	Bread + 8% sunflower seed	14.4 ± 1.15^{a}	6.1 ± 0.20^{b}	54.1 ± 1.95^{a}	$2.9 \pm 0.31^{a,b}$	1.08 ± 0.24^{a}	1.44 ± 0.02^{b}
	Bread + 12% sunflower seed	14.6 ± 0.99^{a}	9.5 ± 0.30^{c}	52.5 ± 2.50^{a}	$2.2 \pm 0.15^{a,b,c}$	1.15 ± 0.18^{a}	2.33 ± 0.04^{c}
	Bread + 16% sunflower seed	14.8 ± 0.87^{a}	11.9 ± 0.20^{d}	$51.1 \pm 1.11^{a,b}$	$2.3 \pm 0.20^{a,c}$	$1.33 \pm 0.33^{a,c}$	2.36 ± 0.02^{c}
Wholegrain bread	Control	14.8 ± 0.95^{a}	2.0 ± 0.17^{a}	$45.4 \pm 4.03^{b,c}$	1.3 ± 0.06^{d}	$2.01 \pm 0.22^{b,c}$	2.39 ± 0.02^{c}
	Bread + 8% sunflower seed	15.0 ± 0.36^{a}	7.1 ± 0.53^{b}	$44.3 \pm 3.28^{b,c}$	$1.6 \pm 0.20^{a,c,d}$	$2.09 \pm 0.33^{b,c}$	2.86 ± 0.08^{c}
	Bread + 12% sunflower seed	14.9 ± 1.05^{a}	9.4 ± 0.42^{c}	42.3 ± 2.63^{c}	$1.6 \pm 0.21^{a,c,d}$	2.18 ± 0.42^{b}	3.97 ± 0.16^{d}
	Bread + 16% sunflower seed	14.9 ± 0.56^{a}	12.0 ± 0.26^{d}	40.9 ± 2.27^{c}	$1.2 \pm 0.11^{c,d}$	2.29 ± 0.37^{b}	4.60 ± 0.15^{e}

$^{a–e}$ Different superscripts in the same column indicate that means were significantly different ($p < 0.05$).

order: δ- > γ- > β- > α- (Elmadfa & Wagner, 2003; Shahidi, 2000).

The total tocopherol content of bread made with refined flour was not significantly lower than that of wholegrain bread (Table 3). The addition of 8%, 12%, and 16% (flour basis) sunflower seeds to white bread significantly increased the total tocopherol content from 0.71 mg/100 g d.b. (control) to 4.26 mg/100 g (16% level). Significant increase of total tocopherol content was also observed in supplemented wholegrain breads as compared to the control (from 0.90 mg/100 g d.b. (control) to 4.50 mg/100 g d.b. (16% level)). Increasing levels of sunflower seed raised the contents of α- and β-tocopherol in both bread types while the contents of γ- and δ-tocopherols were not significantly affected.

3.2.3. *Fatty acid composition of supplemented wheat breads*

Many studies have been carried out on the physiological and molecular mechanisms of the various fatty acids in health and disease. Oleic acid is a monounsaturated omega-9 fatty acid that is believed to lower the risk of heart attack by lowering the level of serum triacylglycerides and LDL cholesterol and increasing the HDL cholesterol (Wolfram, 2001), and recently was found to influence the breast cancer risk by dramatically cutting the levels of gene involved in the disease development (Menendez, Vellon, Colomer, & Lupu, 2005). Linoleic acid is a polyunsaturated omega-6 fatty acid that is essential for humans and is a precursor of many substances that regulate blood clotting, blood pressure, temperature, inflammation and many other functions (Wolfram, 2001). Alpha-linolenic acid is an essential omega-3 acid that is associated with hypolipidemic, antithrombotic and anti-inflammatory effects (Weber & Leaf, 1991). Today, in Western diets, there has been an enormous increase in consumption of omega-6 fatty acids, shifting the ratio of omega-6 to omega-3 from the traditional 1-2:1 (Simpopoulos, 1995) to 10-20:1 because of the recommendation to substitute saturated fats with omega-6 fatty acids to lower serum cholesterol concentrations (Report of the National Cholesterol Education Programme Expert Panel, 1988). However, numerous studies have indicated that a high intake of omega-6 fatty acids has prothrombotic, proaggregatory effects, increasing blood viscosity and vasospasm (Simpopoulos, 2001). According to the nutritionists' claim, a well balanced diet should maintain the ratio of omega-6/omega-3 fatty acids in the range 5 to 10, or lower (FAO/WHO, 1994). It is worth mentioning that the investigated oil seeds with low levels of polyunsaturated fatty acids, such as high-oleic sunflower seed, satisfy some specific needs of food processors (longer stability of product), partly protecting the needs of consumers (exclusion of *trans*-fatty acids in products) but do not achieve the ratio of omega-6/omega-3 in the range proposed by nutritionists.

Analysis of fatty acid composition of breads showed that polyunsaturated fatty acids prevailed in all bread types, which is consistent with the findings of Kashlan, Hassan, Srivastava, and Mohanna (1992). The inclusion of sunflower seed in bread formulation significantly increased the content of fatty acids, especially the content of monounsaturated fatty acids due to the high content of oleic acid in sunflower seeds (Table 3). There was a significant increase in linoleic (omega-6) acid content for all supplementation levels for both bread types. Linolenic (omega-3) acid content gradually rose with the addition of sunflower seeds but to a smaller, non-significant extent. Upon analyzing the ratio of omega-6/omega-3 fatty acid, it was revealed that the addition of sunflower seed elevated the ratio proposed by nutritionists.

3.2.4. *Macro and microelement contents of breads*

Bread made with refined wheat flour had 1.66 mg/100 g of iron, 0.96 mg/100 g of manganese, 0.13 mg/100 g of copper, 0.39 mg/100 g of zinc, 245 mg/100 g of potassium, 759 mg/100 g of sodium, 66.6 mg/100 g of calcium and 15.4 mg/100 g of magnesium (Table 4). Bread made with wholegrain wheat flour had significantly higher contents of all macro and microelements than had its refined counterpart: 5.25 mg/100 g of iron, 3.15 mg/100 g of manganese, 0.38 mg/100 g of copper, 2.21 mg/100 g of zinc, 755 mg/100 g of potassium, 773 mg/100 g of sodium, 141 mg/100 g of calcium and 96.3 mg/100 g of magnesium (Table 4). The addition of sunflower seed did not significantly increase the contents of iron and manganese of the breads. Significant differences between the contents of these minerals were found only between the groups of refined and wholegrain breads. The copper content increased with the addition of sunflower, but not significantly, except for the highest dose (16% flour basis) for white breads, i.e. 12% and 16% levels for wholegrain breads, when compared to the corresponding controls. Sunflower seed supplemented breads exhibited significantly higher contents of zinc at all levels of supplementations. The potassium content did not vary significantly within the groups with the increasing sunflower seed levels. Supplemented refined breads exhibited a significantly lower content of sodium than did the control. Calcium content of refined supplemented bread with maximum supplementation level was significantly decreased, whereas all supplementation levels significantly decreased the content of calcium for wholegrain breads. There was no significant variation in the magnesium content of the refined breads at different levels of supplementation. Wholegrain breads made with sunflower seed had significantly lower magnesium contents than had the control sample.

3.2.5. *Nutritional adequacy of breads*

3.2.5.1. General. To estimate the nutritional adequacy of breads supplemented with sunflower seeds, the contribution of daily per capita portion of bread to the intake of macro and micronutrients was calculated and compared to the dietary reference intakes (DRIs) derived for male and female adults (30–50 years of age) (NRC, 2001).

Table 3
Tocopherol content and fatty acid composition of breads (means ± SD of three independent determinations)

Bread samples		Tocopherols					Fatty acids					
		α-Tocopherol (mg/100 g d.b.)	β-Tocopherol (mg/100 g d.b.)	γ-Tocopherol (mg/100 g d.b.)	δ-Tocopherol (mg/100 g d.b.)	Total tocopherols (mg/100 g d.b.)	Saturated (C14:0, C16:0, C18:0, C20:0, C24:0) (g/100 g d.b.)	Monounsaturated (C18:1, C 20:1, C22:1) (g/100 g d.b.)	Polyunsaturated (C18:2, C18:3), (g/100 g d.b.)	Linoleic (n-6) (mg/100 g d.b.)	Alpha-Linolenic (n-3) (mg/100 g d.b.)	Ratio (n-6)/(n-3)
White bread	Control	0.39 ± 0.02^{a}	0.16 ± 0.01^{a}	$0.12 \pm 0.01^{a,b}$	0.04 ± 0.00^{a}	0.71^{a}	0.19 ± 0.01^{a}	0.19 ± 0.01^{a}	0.56 ± 0.01^{a}	509 ± 10.0^{a}	47.7 ± 3.5^{a}	
	Bread + 8% sunflower seed	1.40 ± 0.13^{b}	1.11 ± 0.08^{b}	$0.12 \pm 0.00^{a,b}$	0.04 ± 0.00^{a}	2.67^{b}	0.44 ± 0.00^{b}	2.98 ± 0.02^{b}	1.37 ± 0.01^{b}	1312 ± 15.0^{b}	49.6 ± 4.5^{a}	10.7
	Bread + 12% sunflower seed	1.89 ± 0.16^{c}	1.54 ± 0.10^{c}	$0.12 \pm 0.01^{a,b}$	0.04 ± 0.00^{a}	$3.59^{b,c}$	0.57 ± 0.01^{c}	4.27 ± 0.05^{b}	1.74 ± 0.00^{c}	1688 ± 14.0^{c}	50.8 ± 3.2^{a}	26.4
	Bread + 16% sunflower seed	$2.24 \pm 0.15^{d,e}$	1.87 ± 0.13^{d}	0.11 ± 0.01^{a}	0.04 ± 0.00^{a}	4.26^{d}	0.66 ± 0.01^{d}	$5.31 \pm 0.03^{b,c}$	2.03 ± 0.02^{d}	1973 ± 16.1^{d}	50.1 ± 5.4^{a}	33.2
Wholegrain bread	Control	0.50 ± 0.05^{a}	0.20 ± 0.03^{a}	0.15 ± 0.01^{c}	0.05 ± 0.00^{a}	0.90^{a}	0.19 ± 0.01^{a}	0.28 ± 0.01^{a}	0.84 ± 0.00^{e}	732 ± 11.2^{e}	56.4 ± 3.8^{a}	39.4
	Bread + 8% sunflower seed	1.51 ± 0.11^{b}	1.15 ± 0.09^{b}	$0.14 \pm 0.01^{b,c}$	0.05 ± 0.00^{a}	2.85^{b}	0.45 ± 0.02^{b}	3.07 ± 0.01^{b}	1.63 ± 0.01^{f}	1528 ± 12.5^{f}	58.2 ± 4.3^{a}	13.0
	Bread + 12% sunflower seed	$2.02 \pm 0.15^{c,d}$	1.55 ± 0.10^{c}	$0.14 \pm 0.01^{b,c}$	0.05 ± 0.00^{a}	$3.76^{c,d}$	0.56 ± 0.01^{c}	4.32 ± 0.05^{b}	1.99 ± 0.01^{g}	1886 ± 14.1^{g}	58.7 ± 4.0^{a}	26.3
	Bread + 16% sunflower seed	2.38 ± 0.18^{e}	1.94 ± 0.11^{d}	$0.14 \pm 0.00^{b,c}$	0.04 ± 0.00^{a}	4.50^{d}	0.67 ± 0.00^{d}	5.48 ± 0.05^{c}	2.31 ± 0.01^{h}	2209 ± 13.9^{h}	59.0 ± 5.0^{a}	32.1

[a–e] Different superscripts in the same column indicate that means were significantly different ($p < 0.05$).

Table 4
Mineral content of supplemented breads (means ± SD of three independent determinations)

Bread samples		Mineral content (mg/100 g d.b.)							
		Fe	Mn	Cu	Zn	K	Na	Ca	Mg
White bread	Control	1.66 ± 0.26^{a}	0.96 ± 0.08^{a}	0.13 ± 0.08^{a}	0.39 ± 0.15^{a}	245 ± 13.0^{a}	759 ± 15.0^{a}	66.6 ± 0.22^{a}	15.4 ± 0.33^{a}
	White bread + 8% sunflower seed	1.79 ± 0.24^{a}	1.01 ± 0.09^{a}	0.25 ± 0.10^{a,b}	0.79 ± 0.11^{b}	263 ± 9.0^{a}	711 ± 12.0^{b}	65.3 ± 0.19^{a,b}	15.4 ± 0.46^{a}
	White bread + 12% sunflower seed	1.86 ± 0.32^{a}	1.04 ± 0.15^{a}	0.31 ± 0.07^{a,b}	0.98 ± 0.07^{b,c}	273 ± 10.0^{a}	696 ± 10.0^{b}	65.2 ± 0.21^{a,b}	15.5 ± 0.41^{a}
	White bread + 16% sunflower seed	1.86 ± 0.54^{a}	1.02 ± 0.10^{a}	0.35 ± 0.05^{b}	1.11 ± 0.05^{c}	272 ± 15.0^{a}	656 ± 14.0^{b}	62.7 ± 0.11^{b}	15.1 ± 0.23^{a}
Wholegrain bread	Control	5.25 ± 0.43^{b}	3.15 ± 0.18^{b}	0.38 ± 0.07^{b,c}	2.21 ± 0.13^{d}	755 ± 28.6^{b}	773 ± 18.1^{a,c}	141 ± 1.4^{c}	96.3 ± 0.42^{b}
	Wholegrain bread + 8% sunflower seed	5.21 ± 0.76^{b}	3.08 ± 0.20^{b}	0.50 ± 0.06^{c}	2.54 ± 0.14^{d,e}	748 ± 30.1^{b}	729 ± 24.0^{a,b,c}	137 ± 1.0^{d}	92.1 ± 0.25^{c}
	Wholegrain bread + 12% sunflower seed	5.17 ± 0.37^{b}	3.04 ± 0.15^{b}	0.55 ± 0.07^{c,d}	2.68 ± 0.10^{e}	742 ± 31.2^{b}	708 ± 15.3^{a,b}	134 ± 1.8^{d}	89.8 ± 0.34^{d}
	Wholegrain bread + 16% sunflower seed	5.10 ± 0.28^{b}	2.98 ± 0.21^{b}	0.59 ± 0.04^{d}	2.79 ± 0.17^{e}	732 ± 24.8^{b}	684 ± 24.4^{a,b}	131 ± 1.2^{e}	87.1 ± 0.18^{e}

$^{a–e}$ Different superscripts in the same column indicate that means were significantly different ($p < 0.05$).

3.2.5.2. *Intake of micronutrients.* The ratios of the average intakes of micronutrients to the respective dietary reference intakes (DRIs) taken from NRC (2001) are summarized for bread types in Table 5.

White bread meets the adult daily requirements for K, Mg, and Ca by 11.0%, 7.7 (males)–10.2% (females), and 14.1%, respectively (Table 5). The supplemented white breads would contribute to non-significant increases in K and Mg intakes while the intake of Ca insignificantly decreased.

White bread contributes to 19.5–43.8% of DRIs of iron for females and males, respectively, while supplemented breads contribute to slightly increased intakes of iron, ranging from 48.1% to 51.4% DRIs for males, i.e. from 21.4% to 22.8% of DRIs for females. High intakes of manganese are presumed by consumption of an average daily portion of white bread because of its high content in wheat (Škrbić & Čupić, 2005): 88.8% of DRIs for males and 113.5% of DRIs for females. The addition of sunflower seed to bread increases the intake of manganese from 94.5% to 98.5% of DRIs for males and from 120.7% to 125.9% of DRIs for females. A daily portion of white bread meets 31.5% of DRIs for copper and 7.4 (males)–10.2% of DRIs for zinc (females). The consumption of sunflower seed supplemented breads significantly increases the intake of copper and zinc: from 61% to 86.9% of DRIs for copper and from 15.4% (21.2%) to 22.5% (30.9%) of DRIs for zinc, for males and females. White bread portions meet only 5.5% of DRIs for α-tocopherol. The lowest sunflower seed supplementation level (8% flour basis) would increase the α-tocopherol intake 3.6-fold, meeting 20% of DRIs. The highest supplementation level (16% flour basis) would satisfy 33% of the daily requirement for α-tocopherol (Table 5).

Wholegrain breads are richer sources of macro and microelements. An average daily portion of wholegrain bread meets 32.2% of DRIs for K, 46.0-60.4% of DRIs for Mg and 28.4% of DRIs for Ca (Table 5). Sunflower seed breads contribute to slightly lower intakes of macro elements than does control bread (K, Na, Mg, Ca). Wholegrain wheat breads contribute to very high intakes of Fe, Mn, and Cu. Our data show that wholegrain bread represent 132% (58.5%) of DRIs for Fe, 275% (351%) of DRIs for Mn, 85.3% of DRIs for Cu, and 40.3% (55.4%) of DRIs for zinc, for males (females), respectively. The addition of sunflower seed slightly lowers the intakes of iron and manganese while copper and zinc intakes increase when compared to the control, meeting 113–136% of DRIs for Cu, i.e. 46.8% (64.4%)–52.3% (71.9%) of DRIs for Zn, for males (females), respectively. A daily portion of white or wholegrain wheat bread represents less than 10% of DRIs for α-tocopherol, while supplementation with sunflower seed increases the intake from 20% of DRIs for the 8% level to more than 30% of DRIs for the 16% level for both refined and wholegrain bread types.

To adequately discuss the intakes of macro and microelements, their bioavailability should be addressed. It is known that the absorption of nutrients is affected by the presence of enhancers and inhibitors contained within the diet. Cereals contain phytates and other components, such as polyphenols, fibres, phosphorus that are inhibitors of non-heme iron absorption (Charlton & Bothwell, 1983). Similar influences of phytic acid on the absorption of zinc, calcium, copper, magnesium and manganese have been reported (Bohn, Davidsson, Walczyk, & Hurrel, 2004; Gargari, Mahboob, & Razavieh, 2007; Holm, Kristiansen, & Pedersen, 2002; Hurrell, 2003; Lönnerdal, 2000). Phytic acid readily forms complexes with Zn^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Ca^{2+} and Fe^{2+} in decreasing order of stability (Cheryan, 1980). It is assumed that the absorption of iron from cereal-based diets is not more than 2–5% (Krishnaswamy, 2003). Individuals with good iron status absorb 3–5% of non-heme iron from most food sources (King, 2002). Previously reported studies implied that the proportions of other minerals absorbed from food were 3–4% for manganese (Johnson, Lykken, & Korynta, 1991), 10–30% for zinc (Abdulla & Chmielnicka, 1990) and 30% for copper (Abdulla et al., 1990). Therefore, the predicted intakes of iron, copper, manganese and zinc, calculated on the basis of their contents in breads, especially wholegrain, are certainly overestimated and thus also overestimated in contribution to the DRIs. Thus, the assumed contribution of a wholegrain bread portion to DRIs of these minerals could be only in the range 2.6–6.6% (males), i.e. 1.2–2.9% (females) of DRIs for iron, 8.0–11.0% (males), i.e. 10.3–14.0% (females) of DRIs for manganese, 25.6–40.8% of DRIs for copper, and 4.0–18.4% (males), i.e. 5.5–25.3% (females) for zinc (Table 5, data in superscript). As the consequence of bran removal during milling, the contents of phytates, fibres, and polyphenols decrease, since they are mainly concentrated in outer layers of the grain. Due to this fact, the bioavailability of minerals from white bread may presumably be higher. On the other hand, minerals (especially iron and zinc) are concentrated in the husk, the aleurone and the embryo, causing their substantial loss during milling, which leads to smaller contributions of refined bread portions to corresponding DRIs for iron, manganese, copper and zinc.

There is also concern about the negative interactions between the absorption of zinc and iron. Olivares, Pizarro, and Ruz (2007) reported that the inhibitory effect of zinc on iron bioavailability depends on the molar ratio of zinc-to-iron and on the total amounts of both minerals present in the intestine. Administering higher doses of zinc (11.71 mg) inhibited iron absorption by 56% while lower doses (0.59 mg) did not have a significant effect, at the zinc-to-iron molar ratio 1:1 (Olivares, Pizarro, & Ruz, 2007). Higher molar ratios of zinc-to-iron (>3:1) were reported to inhibit iron bioavailability when administered in combined aqueous solution (Lönnerdal, 2000; Rossander-Hultén, Brune, Sandström, Lönnerdal, & Hallberg, 1991) while extremely high zinc-to-iron molar ratios 255:1 did not inhibit iron absorption (Rossander-Hultén et al., 1991). However, the same study suggested that when

Table 5
Contribution of micronutrient intakes to the relevant DRIs for consumption of an average portion (300 g) of white or wholegrain breads supplemented with sunflower seed

Micro-nutrient (g/day)	Gender	DRIs (mg/day)	Contribution to DRIs (%)[a]							
			White bread				Wholegrain bread			
			Control	Bread + 8% sunflower seed	Bread + 12% sunflower seed	Bread + 16% sunflower seed	Control	Bread + 8% sunflower seed	Bread + 12% sunflower seed	Bread + 16% sunflower seed
K	Adults	3500	11.0	12.0	12.4	12.8	32.2	32.3	32.2	32.1
Na	Adults	1500	107	102	99.4	96.8	103.3	98.6	96.3	94.0
Mg	Male	420	7.7	7.9	7.9	8.0	46.0	44.5	43.6	42.7
	Female	320	10.2	10.4	10.4	10.5	60.4	58.4	57.2	56.1
Ca	Adults	1000	14.1	14.0	14.0	13.9	28.4	27.7	27.3	26.9
Fe	Male	8	$43.8^{0.9-2.2}$	$48.1^{1.0-2.4}$	$49.8^{1.0-2.5}$	$51.4^{1.0-2.6}$	$132^{2.6-6.6}$	$132^{2.6-6.6}$	$132^{2.6-6.6}$	$131^{2.6-6.6}$
	Female	18	$19.5^{0.4-1.0}$	$21.4^{0.4-1.0}$	$22.1^{0.4-1.1}$	$22.8^{0.5-1.1}$	$58.5^{1.2-2.9}$	$58.8^{1.2-2.9}$	$58.6^{1.2-2.9}$	$58.4^{1.2-2.9}$
Mn	Male	2.3	$88.8^{2.7-3.5}$	$94.5^{2.8-3.8}$	$96.6^{2.9-3.9}$	$98.5^{3.0-3.9}$	$275^{8.2-11.0}$	$272^{8.2-10.9}$	$270^{8.1-10.8}$	$268^{8.0-10.7}$
	Female	1.8	$114^{3.4-4.5}$	$121^{3.6-4.8}$	$123^{3.7-4.9}$	$126^{3.8-5.0}$	$351^{10.5-14.0}$	$348^{10.4-13.9}$	$345^{10.3-13.8}$	$342^{10.3-13.7}$
Cu	Adults	0.9	$31.5^{9.5}$	$61.0^{18.3}$	$74.3^{22.3}$	$86.9^{26.1}$	$85.3^{25.6}$	$113^{33.7}$	$125^{37.3}$	$136^{40.8}$
Zn	Male	11	$7.4^{0.7-2.2}$	$15.4^{1.5-7.4}$	$19.0^{1.9-8.4}$	$22.5^{2.2-9.5}$	$40.3^{4.0-14.8}$	$46.8^{4.7-16.8}$	$49.6^{5.0-17.6}$	$52.3^{5.2-18.4}$
	Female	8	$10.2^{1.0-3.1}$	$21.2^{2.1-10.1}$	$26.2^{2.6-11.6}$	$30.9^{3.1-13.0}$	$55.4^{5.5-20.4}$	$64.4^{6.4-23.1}$	$68.2^{6.8-24.2}$	$71.9^{7.2-25.3}$
Alpha-tocopherol	Adults	15	5.5	20.0	27.0	33.1	6.7	20.5	27.5	32.7

[a] Data in superscripts relate to the micronutrient intakes corrected after considering their bioavailability.

dietary ligands were present (i.e. when the minerals were given as a part of a composite meal), the inhibitory effect of zinc on iron absorption was abolished. When calculated, the daily portion of white or wholegrain supplemented breads would provide lower molar doses of zinc-to-iron, ranging from 0.20:1 to 0.50:1 for white breads and from 0.20:1 to 0.47:1 for wholegrain breads, as well as doses of zinc much lower than 11.71 mg. Taking into consideration the above mentioned facts, it is not likely that zinc would negatively affect the iron absorption when breads supplemented with sunflower seeds are consumed.

The physicochemical similarities between iron and manganese, as well as common absorption pathways, imply that there is an interaction between iron and manganese absorption, too. High amounts of absorption of either of the two elements may potentially interfere with the absorption of the other element (Davidsson, Cederblad, Lönnerdal, & Sandström, 1991). Large amounts of dietary manganese were reported to cause anemia in rats (Hartman, Matrone, & Wise, 1955). In the study of Rossander-Hultén et al. (1991), it was shown that, at a manganese-to-iron ratio of 2.5:1, the iron absorption was decreased by 22% whereas, at a ratio of 5:1, the iron absorption was decreased by 34%. In the presence of a meal, the inhibition of iron absorption maintained at the same level, suggesting that dietary ligands do not affect the manganese–iron interaction. In the study of Davidsson et al. (1991) it was confirmed that the manganese absorption was not significantly affected by most dietary factors, except calcium. Consumption of a portion of supplemented breads would provide lower molar manganese-to-iron ratios, ranging from 0.59:1 to 056:1 for white breads (control and maximum supplementation level, respectively) and from 0.61:1 to 0.59:1 for wholegrain breads (control and maximum supplementation level, respectively) indicating that, after all, the elevated manganese levels found in the bread samples may not affect the iron bioavailability. On the other hand, high dietary iron has been shown to depress manganese absorption (Davis, Wolf, & Greger, 1992). Finley (1999) reported that subjects with high ferritin status showed the lowest level of manganese absorption.

3.2.5.3. Intake of macronutrients. The contribution of average daily portion of breads to the intakes of macronutrients is displayed in Table 6.

Average daily portions of white and wholegrain bread contribute similarly to the intakes of proteins covering 54.8%, i.e. 53.04% of DRIs for males, and 66.7%, i.e. 64.6% of DRIs for females, in the case of white and wholegrain bread, respectively. Since wholegrain bread contains less digestible carbohydrates (starch + reducing sugars), a portion of wholegrain bread contributes to 72.1% of DRIs for carbohydrates, unlike white bread that contributes to 96.3% of DRIs for carbohydrates. The addition of sunflower seed to both bread types slightly

Table 6
Contribution of macronutrient intakes to the relevant DRIs for consumption of an average bread portion (300 g)

Macronutrient (g/day)	Gender	DRIs (g/day)	Contribution to DRIs (%)							
			White bread				Wholegrain bread			
			Control	Bread + 8% sunflower seed	Bread + 12% sunflower seed	Bread + 16% sunflower seed	Control	Bread + 8% sunflower seed	Bread + 12% sunflower seed	Bread + 16% sunflower seed
Proteins	Male	56	54.8	55.3	55.8	58.5	53.0	54.4	54.3	54.8
	Female	46	66.7	66.7	68.0	71.2	64.6	66.2	66.1	66.8
Carbohydrate	Male	130	96.3	94.3	90.1	90.9	72.1	71.7	68.9	66.7
	Female									
Linoleic acid (n-6)	Male	17,000	6.3	16.6	21.3	25.7	8.6	18.3	22.6	26.8
	Female	12,000	9.0	23.5	30.1	36.4	12.2	25.9	32.1	37.9
α-linolenic acid (n-3)	Male	1600	6.3	6.7	6.8	6.9	7.1	7.4	7.5	7.6
	Female	1100	9.2	9.7	9.9	10.1	10.3	10.7	10.9	11.0

increases the contribution of breads to DRIs for proteins and decreases their contribution to DRIs for carbohydrates. A portion of control white bread meets around 6% (9%) of DRIs for males (females) for both linoleic (omega-6) and α-linolenic (omega-3) acids. Wholegrain bread contributes to slightly higher intakes for these fatty acids: 7–8.5% (10–12%) of DRIs for males (females) for linolenic (omega-3) and linoleic (omega-6) fatty acids, respectively. The addition of sunflower seed significantly increases the intakes of linoleic (omega-6) fatty acid for both bread types to around 25(30)–27(38)% of DRIs for males (females) for the 16% supplementation level. There is a non-significant increase in the intake of α-linolenic (omega-3) acid with the maximum supplementation level covering not more than around 7% (11%) of DRIs for males (females) consuming either white or wholegrain bread types.

4. Conclusions

From the present paper it may be inferred that sunflower seed could be added to bread up to levels of 16% (flour basis) without significant adverse effects regarding the crust colour, crumb grain structure and uniformity. Adding sunflower seed significantly decreases crumb elasticity compared to the control samples but not to the level that would disqualify the product. Adverse effects of supplementation on the crumb elasticity were not confirmed by penetrometer number. The breads containing sunflower seed at all levels were scored higher for flavour in comparison to the control breads. Besides sensory properties, sunflower seed supplemented breads were more acceptable in many nutritional aspects as they contained significantly more tocopherols, essential fatty acids, copper, zinc, fat, and crude fibre. Such breads may contribute to enhancement of zinc status of certain population groups since dietary surveys suggest that many individuals (particularly aged people or those taking medications such as hormone replacements or diuretics) fail to achieve the dietary reference value for zinc. According to data from this investigation, bread supplementation with sunflower seed would contribute to meeting up to 10% of DRIs for zinc with white breads, i.e. 20% of DRIs for zinc with wholegrain breads, whereas the control bread samples would contribute to only <1%, i.e. 4% of DRIs for zinc, respectively. Also, it was concluded that the zinc levels would not influence the iron bioavailability. It is also important that a portion of white (wholegrain) supplemented breads would contribute to meeting up to 26%, i.e. 40% of DRIs, for copper, respectively; copper deficiency is rare in Western populations but there are concerns that suboptimal copper status may be involved in developing many inflammatory and degenerative conditions such as osteoporosis and heart disease. In any case, consumption of breads supplemented with sunflower seeds could be beneficial for improving the nutritional status of the general population since Serbia is a poor developing country with high bread consumption.

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