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# Improving the nutritive characteristics of corn flakes enriched with functional components

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## Abstract

This paper investigates the effects of simultaneous addition of sunflower (3, 6 or 9 g/100 g of sample) and dry residue of wild oregano (0.5 or 1 g/100 g of sample) on the essential amino acids pattern and antioxidant potential of flake products. The accepted experimental design plan was 3×4. Data point that Score and PDCAAS values in flake products increase with increasing share of sunflower. Maximum value of phenolic compounds and antioxidant activity were experienced of TPC 2.84 mg/g, DPPH 0.75 mg/ml, FRAP 1.57 mg/g with the product having maximum shares of sunflower and dry residue of wild oregano. Tukey's HSD test showed statistically significant differences between most of the mean values of amino acids content and antioxidant activity in the observed corn flakes. The response surface method has been applied for evaluation of amino acid content and antioxidative potential of corn flakes. Sunflower in flake products positively contributed to the protein nutritive value and dry residues of wild oregano elevated antioxidant potential of flake products and also contributed to the food waste valorisation in the food industry. Corn flakes are new products with improved essential amino acid pattern, antioxidant activity and functional properties due to added dry residue of wild oregano and sunflower.

**Keywords:** amino acid, antioxidant potential, wild oregano, sunflower, corn flakes.

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Food wastes are today considered as a cheap source of valuable components since the existent technologies allow the recovery of target compounds and their recycling inside food chain as functional additives in different products [1]. Waste valorisation practices have attracted a significant amount of attention in recent years with the aim of managing waste in the most sustainable way [2,3]. Large quantities of waste material are generated annually from agricultural activities and processing of agricultural products. Waste can contain many reusable substances of high value [4]. Depending on the technology this residual matter can be converted into commercial products either as raw material for secondary processes, as operating supplies or as ingredients of new products [5] like residue after distillation of oil from wild oregano which is the natural source of antioxidants [6].

Food consumption is primarily determined by energy expenditure, a function of basal metabolic rate and physical activity level. However, basal metabolic rate, and consequently protein, varies with age, sex and body weight. Physical activity varies with a lifestyle

and patterns of behavior. Thus, when enough food is eaten to satisfy energy needs, the needs for protein or any other nutrient will also be satisfied if the ratio of protein or other nutrients to energy is appropriate [7]. Ingredients that could be included in flakes formulation are sunflower and wild oregano, which may significantly improve nutritive and antioxidant properties [8–11].

The differences between fabricated samples were tested using Tukey's HSD test. The response surface methodology (RSM) has been proven as useful method for determining the influence of process variables on a group of dependent parameters that are significant for the process and effects studies [12]. RSM is an effective tool for optimizing a variety of processes, especially in design of mixture experiments [13]. The RSM equations describe the effects of the test variables on the observed responses, determine test variables interrelationships and represent the combine effect of all test variables in the observed responses, enabling the efficient investigation of the process [12,13].

In this study the effect of varying the proportion of sunflower (3, 6 and 9 g) and wild oregano dry residue (0.5 and 1 g/100 g of sample) on the amino acid content and antioxidant potential of corn flakes was studied with the aim to obtain new products with improved nutritional and functional properties.

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## EXPERIMENTAL

### Material

Corn flour used in this study was obtained from the mill Žitoprodukt d.o.o., Bačka Palanka, Serbia, produced in 2014 with the following characteristics: moisture content of 13.3%, sugar, protein, cellulose, starch and lipid content (% dry matter basis) of 0.87, 5.59, 0.98, 79.43, and 1.57, respectively (AOAC 1990) [15].

Sunflower variety „Cepko“ was produced in 2014 by „Vitastil“, Erdevik, Serbia, with following characteristics of samples: protein, starch, lipid and cellulose, content of 26.67, 6.73, 54.05 and 8.21 (% dry matter basis), respectively (AOAC 1990) [15]. Sunflower was dehulled and milled in a Hammer mill 2300 rev/min with 2.5 mm sieve. Wild oregano (*Origanum minutiflorum* O. Schwarz & P.H. Davis) harvest 2013, was produced by Inan tarim ECODAB – Antalia, Turkey. Wild oregano (*Origanum minutiflorum*), harvest 2013, was supplied by Inan tarim ECODAB – Antalia, Turkey.

### Methods

#### Preparation of dry residue of wild oregano

Dry residue from wild oregano was prepared as follows: the distillation of wild oregano was carried out in the production plant of the Institute of Medicinal Plant Research “Dr Josif Pančić” from Pančevo, Serbia. A mini distiller based on water vapor principle was used for distillation. The duration of the distillation time was 2.5 h. The waste in the process of distillation (trope) is naturally cooled and dried by air flow in place, protected from sun and prepared for further research: milled at a facility Repro Trade Ltd., on the hammer mill Sever, Subotica (2300 rpm, sieve hole diameter 1.5 mm, particle size < 12 mm).

### Preparation of corn flakes

*Extrusion of flakes.* The corn flakes was obtained by extrusion in a twin-screw extruder (Yunnan Daily Extrusion, Yunnan, China) in industrial conditions on the factory Repro Trade Ltd., Industrial Zone bb, Temerin, Serbia. Extrusion parameters were as follows: the length of the screw 140 cm, diameter 3 mm×6 mm, rotor speed of 180 rpm, temperature profile: 131/125/114 °C. The moisture of raw material mixture prior extrusion was adjusted to 22%. Corn flour, was replaced by milled sunflower in the quantity 3, 6 or 9 g/100 g of sample, and dry residue of wild oregano added in the quantity of 0.5 or 1 g/100 g of sample based on corn flour and milled sunflower. Table 1 describes corn flour formulation enriched with different quantities of milled sunflower and dry residue of wild oregano. Obtained extrudates were dried in the drying unit at temperature of 84 °C, cooled for 30 min at controlled temperature 25±1 °C and stored at 4 °C in sealed plastic bags until required for analysis.

### Amino acids

The samples were prepared for analyses using the 24 h hydrolysis with 6 M HCl. Samples were analyzed on a liquid chromatograph Agilent 7890A GC system with a flame ionization detector FID-equipped automatic sampler (autosampler) and silica capillary column (SP-2560, 100 m×0.25 mm, ID, 0.20 μm). Amino acid peaks were identified by comparing the retention time of the individual amino acids in the sample with retention times of standards Amino Acid Standard (Sigma–Aldrich), as well as the internal data of the library data. The results are expressed in % as a proportion of the individual amino acids in the total amino acids.

### Antioxidant activity

Antioxidant activity was determined in samples of wild oregano (WO), dry residue of wild oregano (DR),

Table 1. Corn flakes formulation with different quantities of milled sunflower and dry wild residue oregano

Sample	Corn flakes composition		
	Quantity of corn g/100 g sample	Quantity of sunflower, g replacing 100 g flour	Quantity of dry residue of wild oregano g/100 g of sample
CF 1	100	0	0
CF 2	97	3	0
CF 3	94	6	0
CF 4	91	9	0
CF 5	100	0	0.5
CF 6	97	3	0.5
CF 7	94	6	0.5
CF 8	91	9	0.5
CF 9	100	0	1
CF 10	97	3	1
CF 11	94	6	1
CF 12	91	9	1

sunflower (SF) and corn flakes (CF 1–12). The material was grinded and extracts were obtained by subjecting 1 g (WO, DR, SF) and 2 g (CF 1–12) of powdered material to maceration in 100 ml 80% ethanol (in water) for 24 h at ambient temperature in the dark. The extracts were filtered using filter paper (Whatman No. 1) followed by centrifugation (U-320 R, Boeco, Germany) at 4750g (4 °C) for 15 min and kept refrigerated. These extracts were used for determination of total phenolic content (TPC), DPPH antiradical power and ferric reducing antioxidant power (FRAP). In all of the assays absorbances were measured using Thermo Scientific Evolution 220 spectrophotometer. Total phenolic content (TPC) was determined using a modification of the Foline–Ciocalteu method and the results expressed as mg of gallic acid equivalents (GAE) per g of extract sample [16]. The assay mixture contained 1 ml of Folin–Ciocalteu-phenol reagent (previously diluted 1:10 with distilled water) and 200 µl of the sample solution. The mixture was neutralized with 7.5% sodium carbonate solution after 10 min. For each sample three replicates were carried out and also a control (blank), where 1 ml of distilled H<sub>2</sub>O was added instead of FC reagent. The absorbance was determined after 60 min at 760 nm.

DPPH free radical scavenging activity was evaluated according to the method of Sánchez [17] with minor modifications. The assay procedure was the following: 15.7 mg of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was weighed and dissolved in absolute ethanol. A working solution of 90 µM DPPH was prepared diluting 22 ml of the primary DPPH solution to a volume of 100 ml in methanol. The assay mixture contained 2 ml working solution and different volumes of the sample solution. In the control, the exact amount of the extract was substituted with solvent (80% ethanol), and in the blank, only methanol and extract were mixed. The mixture was shaken vigorously on a Vortex mixer, then incubated 60 min at 25 °C in a water bath in the dark, after which the absorbance of the remaining DPPH was determined at 515 nm. For each sample three replicates were carried out. Radical percentage of inhibition of DPPH radical was calculated by the following equation:

$$I = 100(A_0 - A_1)/A_0 \quad (1)$$

Where  $A_0$  is the control and  $A_1$  is the sample solution absorbance. The concentration of extract that inhibits the DPPH radical formation by 50% is defined as  $IC_{50}$  (mg/ml). Finally, results were expressed as DPPH antiradical power (DPPH ARP) defined as:

$$\text{DPPH ARP} = 1/IC_{50} \quad (2)$$

Total antioxidant capacity was estimated according to the FRAP assay [18]. The reduction potential of extracts was calculated using the calibration curve of the standard solution of ascorbic acid ( $R^2 = 0.99$ ). The work-

ing FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), 2,4,6-tripyridyltriazine reagent (10 mM in 40 mM HCl) and  $FeCl_3 \cdot 6H_2O$  (20 mM) in the ratio of 10:1:1. The assay mixture contained sample and 2 ml of working FRAP reagent and absorbance (593 nm) was measured after 6 min incubation in the dark at room temperature. Due to the coloration of the extracts, it was necessary to prepare a control (sample and dH<sub>2</sub>O instead of the FRAP reagent) and blank (FRAP reagent and 80 % ethanol instead of the sample). All the samples were made in triplicate and mean values of reducing power were expressed as mg of ascorbic acid equivalents (AAE) per g of sample extract, calculated according to the standard calibration curve.

### Protein quality evaluation

Nutritional value of proteins expressed by the following indicators: the amino acid score determines the effectiveness with which absorbed dietary nitrogen can meet the indispensable amino acid requirement at the safe level of protein intake. This is achieved by a comparison of the content of the limiting amino acid (lysine) in the protein or diet with its content in the requirement pattern: amino acid score = mg of amino acid in 1 g test protein / mg of amino acid in requirement pattern.

Following data were used for digestibility (FAO/WHO/UNU, 2002) [7]:

- corn, 85%
- sunflower, 90%
- corn flakes with 3% of sunflower →  $85 \times 0.97 + 90 \times 0.03 = 85.15$
- corn flakes with 6% sunflower →  $85 \times 0.94 + 90 \times 0.06 = 85.30$
- corn flakes with 9% sunflower →  $85 \times 0.91 + 90 \times 0.09 = 85.45$

### Statistical analyses

Descriptive statistical analyses for all the obtained technological parameters were expressed as the mean ± standard deviation (*SD*). The evaluation of analysis of variance (ANOVA) and principal component analysis (PCA) analyses of the obtained results were performed using StatSoft Statistica 10.0® software. Collected data were subjected to ANOVA for the comparison of means, and significant differences are calculated according to post-hoc Tukey's HSD (honestly significant differences) test at  $p < 0.05$  significance level, 95% confidence limit.

The experimental data used for the study of experimental results were obtained using a 3×4 experimental design, with 12 runs (1 block).

The following second order polynomial (SOP) model was fitted into the data. The eleven models of the following form were developed to relate the five responses (*Y*) and two process variables (*X*):



$$Y_k = \beta_{k0} + \sum_{i=1}^2 \beta_{ki} X_i + \sum_{i=1}^2 \beta_{kij} X_i^2 + \beta_{k12} X_1 X_2 \quad k_1 = -11 \quad (3)$$

where:  $\beta_{k0}$ ,  $\beta_{ki}$ ,  $\beta_{kij}$ ,  $\beta_{k12}$ , are constant regression coefficients;  $Y_k$  is either essential amino acids (Lys, Thr, Val, Ile, Leu, Met, Phe and His), phenolic compounds or antioxidant activity values (TPC, DPPH<sup>\*</sup> and FRAP);  $X_1$  – oregano content and  $X_2$  – sunflower content.

## RESULTS AND DISCUSSION

Correct evaluation of protein in food products requires prediction of overall efficiency of protein utilization. According to FAO/WHO/UNU [7] measure of overall efficiency of protein quality should determine the capacity of food protein to meet metabolic demand for essential amino acids. As metabolic needs for amino acids and proteins depend on their part in biochemical pathways. The essential amino acids of corn flakes (CF1–12) with sunflower and wild oregano are presented in table 2. Addition of sunflower 6 and 9 g in corn flakes (CF3 and CF4; CF7 and CF8; CF11 and CF12) contributed to statistical significant increase in lysine. The addition of sunflower (3, 6 or 9 g) contributes to a statistically significant higher values of threonine, methionine and histidine content in comparison with samples without sunflower addition (CF1, CF5 and CF9). Amino acids of sunflower contributed to statistically insignificant decrease in leucine content and statistically significant decrease in isoleucine content of corn flakes with added sunflower.

Table 3 shows the ANOVA calculations of the prediction model for amino acid products with corn flakes containing sunflower and wild oregano. It can be seen that the SOP model used to calculate Lys content is mostly influenced by the linear term of sunflower con-

tent, statistically significant at  $p < 0.01$  level and the linear term of oregano content,  $p < 0.05$ . The greatest impact in the SOP models for Val, Ile, Met and Phe calculation is observed by the linear member of sunflower content ( $p < 0.05$ ).

The average error between the predicted values and experimental values (calculated by Eq. (1)) was below 10%. To verify the significance of the models, ANOVA was conducted and the results indicate that all models were significant with minor lack of fit, suggesting that they adequately represented the relationship between responses and factors.

Nutritional value was determined, based on the amount and composition of protein and essential amino acids. Score amino acid indicates the efficiency of protein utilization in food and presents the ratio of essential amino acid content in the tested protein and the specific categories of consumers needs. The lowest value score defined limiting amino acid.

The nutritive value of flake protein, expressed as amino acid score, is presented in Table 4. Score determines the effectiveness by which the absorbed dietary nitrogen can meet the essential amino acid requirement at a safe level of protein intake. WHO/FAO/UNU [7] marked lysine, sulfur amino acids, threonine and tryptophan as the most deficient aminoacids in food proteins. The results of the investigation show that lysine is the limiting amino acids in all corn flakes (CF1–12). All the values are less than 1, and addition of sunflower increased the score. This results point that sunflower addition contributed to better utilization of protein in corn flakes. As the score increases, these data also show that the applied temperatures of extrusion and drying (85 °C) do not lead to the loss of lysine. Which is also the most reactive amino acid in the Maillard reactions [9,19].

Table 2. Essential amino acids of corn flakes; the results are presented as mean±SD, different letter within the same column indicates the significant difference in mean values ( $p < 0.05$ ), according to Tukey's HSD test. Experimental cases are explained in detail in Table 1. Lys-lysine, Thr-threonine, Val- valine, Ile- Leu-Met- methionine, Phe-, His – histidine

Sample	Essential amino acids, mg/g protein							
	Lys	Thr	Val	Ile	Leu	Met	Phe	His
CF 1	24.99±0.20 <sup>a</sup>	46.80±0.13 <sup>e</sup>	31.03±0.43 <sup>d</sup>	27.81±0.43 <sup>de</sup>	97.14±0.83 <sup>abcd</sup>	19.02±0.14 <sup>b</sup>	33.31±0.25 <sup>de</sup>	20.01±0.18 <sup>f</sup>
CF 2	25.15±0.19 <sup>a</sup>	50.29±0.30 <sup>d</sup>	29.73±0.31 <sup>ab</sup>	25.94±0.12 <sup>b</sup>	98.01±1.28 <sup>abcd</sup>	19.17±0.20 <sup>b</sup>	31.17±0.09 <sup>a</sup>	21.18±0.15 <sup>a</sup>
CF 3	26.26±0.24 <sup>b</sup>	50.15±0.34 <sup>a</sup>	31.12±0.09 <sup>c</sup>	22.88±0.19 <sup>c</sup>	96.26±0.67 <sup>abcd</sup>	20.02±0.28 <sup>a</sup>	31.17±0.23 <sup>a</sup>	20.95±0.05 <sup>a</sup>
CF 4	27.23±0.20 <sup>c</sup>	52.11±0.40 <sup>a</sup>	28.87±0.18 <sup>ab</sup>	22.81±0.15 <sup>c</sup>	96.44±0.75 <sup>abcd</sup>	19.83±0.11 <sup>a</sup>	29.22±0.24 <sup>f</sup>	22.08±0.07 <sup>b</sup>
CF 5	25.18±0.07 <sup>a</sup>	54.14±0.18 <sup>bc</sup>	29.26±0.22 <sup>ab</sup>	27.08±0.22 <sup>d</sup>	98.05±0.78 <sup>cd</sup>	19.84±0.12 <sup>a</sup>	32.77±0.26 <sup>cd</sup>	19.12±0.20 <sup>e</sup>
CF 6	25.04±0.34 <sup>a</sup>	50.39±0.25 <sup>bc</sup>	33.19±0.25 <sup>d</sup>	24.87±0.20 <sup>f</sup>	95.37±1.09 <sup>a</sup>	19.00±0.21 <sup>b</sup>	31.00±0.30 <sup>a</sup>	21.88±0.12 <sup>b</sup>
CF 7	27.20±0.06 <sup>c</sup>	52.01±0.30 <sup>a</sup>	29.77±0.22 <sup>c</sup>	26.00±0.41 <sup>b</sup>	95.53±0.82 <sup>ab</sup>	20.83±0.20 <sup>c</sup>	32.09±0.17 <sup>c</sup>	21.97±0.08 <sup>b</sup>
CF 8	28.14±0.09 <sup>d</sup>	53.93±0.32 <sup>d</sup>	30.19±0.12 <sup>ac</sup>	25.76±0.26 <sup>b</sup>	95.55±0.47 <sup>abc</sup>	20.06±0.07 <sup>a</sup>	33.98±0.29 <sup>e</sup>	22.80±0.07 <sup>c</sup>
CF 9	26.03±0.09 <sup>b</sup>	49.23±0.40 <sup>c</sup>	29.01±0.11 <sup>e</sup>	28.22±0.24 <sup>e</sup>	98.76±0.55 <sup>d</sup>	17.84±0.16 <sup>d</sup>	34.92±0.09 <sup>g</sup>	21.12±0.17 <sup>a</sup>
CF 10	26.03±0.10 <sup>b</sup>	53.77±0.42 <sup>a</sup>	29.82±0.40 <sup>ab</sup>	23.92±0.25 <sup>a</sup>	97.35±0.79 <sup>abcd</sup>	18.95±0.23 <sup>b</sup>	30.12±0.39 <sup>b</sup>	24.12±0.20 <sup>g</sup>
CF 11	26.90±0.12 <sup>c</sup>	52.23±0.53 <sup>d</sup>	28.81±0.14 <sup>b</sup>	24.03±0.19 <sup>a</sup>	97.35±0.75 <sup>abcd</sup>	20.15±0.29 <sup>a</sup>	30.87±0.16 <sup>ab</sup>	22.90±0.08 <sup>cd</sup>
CF 12	28.09±0.11 <sup>d</sup>	51.52±0.44 <sup>b</sup>	29.88±0.06 <sup>c</sup>	23.82±0.23 <sup>a</sup>	96.29±1.47 <sup>abcd</sup>	20.88±0.14 <sup>c</sup>	30.93±0.45 <sup>a</sup>	23.22±0.16 <sup>d</sup>

Table 3. ANOVA table for the prediction of amino acid corn flakes with sunflower and wild oregano

Parameter	df	Lys	Thr	Val	Ile	Leu	Met	Phe	His
Sunflower	1	10.42 <sup>a</sup>	9.60	7.35 <sup>b</sup>	17.07 <sup>b</sup>	2.02 <sup>c</sup>	4.27 <sup>b</sup>	2.82	6.67 <sup>b</sup>
Sunflower <sup>2</sup>	1	0.75 <sup>c</sup>	0.00	0.75	5.33 <sup>c</sup>	0.75	0.00	10.08	3.00
Oregano	1	2.00 <sup>b</sup>	8.00	0.50	0.00	0.13	0.00	3.12	4.50 <sup>c</sup>
Oregano <sup>2</sup>	1	0.00	8.17	0.67	2.67	1.04	0.67	2.04	0.17
Sun.×Oreg.	1	0.00	1.60	1.60	0.90	0.02	0.90	1.23	0.40
Error	6	1.08	25.30	4.05	8.70	2.96	2.83	13.63	5.93
r <sup>2</sup>		0.924	0.520	0.728	0.749	0.572	0.673	0.586	0.713

<sup>a</sup>Statistically significant at  $p < 0.01$  level; <sup>b</sup>statistically significant at  $p < 0.05$  level; <sup>c</sup>statistically significant at  $p < 0.10$  level

Table 4. Nutritive value protein of corn flakes; the results are presented as mean±SD, different letter within the same column indicates the significant difference in mean values ( $p < 0.05$ ), according to Tukey's HSD test. Experimental cases are explained in detail in Table 1

Sample	Lys	
	Score amino acids	PDCAAS
CF 1	0.56±0.01 <sup>a</sup>	47.6±0.13 <sup>a</sup>
CF 2	0.56±0.02 <sup>a</sup>	47.7±0.23 <sup>a</sup>
CF 3	0.58±0.03 <sup>b</sup>	49.5±0.30 <sup>b</sup>
CF 4	0.60±0.01 <sup>c</sup>	51.3±0.33 <sup>c</sup>
CF 5	0.56±0.04 <sup>a</sup>	47.6±0.43 <sup>a</sup>
CF 6	0.56±0.05 <sup>a</sup>	47.7±0.63 <sup>a</sup>
CF 7	0.60±0.05 <sup>c</sup>	51.2±0.51 <sup>c</sup>
CF 8	0.62±0.03 <sup>d</sup>	53.0±0.48 <sup>d</sup>
CF 9	0.56±0.01 <sup>a</sup>	47.6±0.25 <sup>a</sup>
CF 10	0.56±0.02 <sup>a</sup>	47.7±0.21 <sup>a</sup>
CF 11	0.60±0.02 <sup>c</sup>	51.2±0.13 <sup>c</sup>
CF 12	0.62±0.03 <sup>d</sup>	53.0±0.20 <sup>d</sup>

The purpose of PDCAAS evaluation of protein nutritive value is the prediction of the overall efficiency of protein utilization in terms of its two components, digestibility and its amino acid score, *i.e.*, score of limiting amino acid [7]. PDCAAS values (Table 4) in corn flakes increases, statistically significant, with the augment of sunflower content (samples with added 6 and 9 g) in the corn flakes due to higher digestibility of protein sunflower (90%) compared to the digestibility of corn (85%) and also to sunflower compatible amino acid pattern [7].

Most of the antioxidant potential in plant foods is due to the properties of phenolic compounds, which can act as reducing agents, free radical scavengers and hydrogen donors [1,20,21]. It is known that oregano possesses high antioxidant capacity [22,23] but our study shows that dry residue of wild oregano (DR) is also very powerful phenolic compounds and antioxidant

source (TPC 23,55 mg/g, DPPH 4,98 mg/ml, FRAP 8,45µg, Table 5), which completely changes the aspect of its usage and well fits in the concept of better valorization of food wastes. As the residue after distillation of wild oregano oil was considered as waste material, applied technology of corn flakes allows the recovery of useful compounds and their recycling inside food chain as functional additives. The phenolic compounds and antioxidant activity of corn flakes (CF 1) were relatively low (TPC 0.98 mg/g, DPPH 0.13 mg/ml, FRAP 0.45 mg/g). Sunflower and dry residue wild oregano (DR) addition strongly influenced the content of phenolic compounds and antioxidant activity of corn flakes. The highest content of phenolic compounds and antioxidant activity (TPC 2.84 mg/g, DPPH 0.75 mg/ml, FRAP 1,57 mg/g) was experienced with CF 12, corn flakes with the highest amount of sunflower and dry residue. Statistically significant differences in TPC, DPPH and FRAP were observed between samples with increased oregano and sunflower content: CF 1–12, which confirms that the addition of oregano and sunflower increased the antioxidant activity of corn flakes, at statistically significant level (Table 5, Figure 1).

Table 6 shows the ANOVA calculations of the predictive model of the antioxidant activity values for corn corn flakes with sunflower and wild oregano. It can be seen from table that the SOP model for total phenols content and DPPH calculation is mostly affected by the linear terms of sunflower and oregano content, as well as the non-linear term of product Sun.×Oreg., statistically significant at  $p < 0.01$ . The calculation of FRAP in corn flakes is mostly affected by the linear terms of sunflower and oregano content and the quadratic term of sunflower content ( $p < 0.01$ ).

The results indicate that all models were significant with minor lack of fit, suggesting that they adequately represented the relationship between responses and factors.

Table 5. Antioxidant activity of corn flakes with sunflower and wild oregano; the results are presented as mean±SD; different letter within the same column indicates the significant difference in mean values ( $p < 0,05$ ), according to Tukey's test. Experimental cases (samples) are explained in detail in Table 1; number of repetitions:  $n = 10$ , WO- wild oregano, DR- dry residue wild oregano, SF-sunflower

Sample	TPC, mg GAE/g sample	DPPH * ARP (1/IC <sub>50</sub> )	FRAP, mg AAE/g sample
WO	42.87±0.85	8.25±0.32	8.69±0.08
DR	23.55±0.75	4.98±0.06	8.45±0.03
SF	11.68±0.36	5.78±0.15	11.01±0.05
CF1	0.98±0.03 <sup>a</sup>	0.13±0.00 <sup>a,h</sup>	0.45±0.01 <sup>a</sup>
CF2	1.36±0.20 <sup>b,e</sup>	0.20±0.00 <sup>b,e</sup>	0.65±0.01 <sup>b,f</sup>
CF3	1.62±0.10 <sup>c,f</sup>	0.29±0.01 <sup>c</sup>	0.77±0.01 <sup>c</sup>
CF4	1.88±0.05 <sup>d,g,j</sup>	0.37±0.00 <sup>d</sup>	1.38±0.02 <sup>d,h</sup>
CF5	1.18±0.01 <sup>e,i</sup>	0.14±0.00 <sup>a,h</sup>	0.46±0.01 <sup>e</sup>
CF6	1.57±0.02 <sup>f</sup>	0.21±0.01 <sup>e</sup>	0.68±0.01 <sup>f</sup>
CF7	1.80±0.06 <sup>g,j</sup>	0.34±0.00 <sup>f</sup>	0.95±0.00 <sup>g,j</sup>
CF8	2.13±0.03 <sup>h</sup>	0.45±0.02 <sup>g</sup>	1.35±0.03 <sup>h</sup>
CF9	1.30±0.01 <sup>i</sup>	0.16±0.02 <sup>h</sup>	0.50±0.01 <sup>i</sup>
CF10	1.82±0.03 <sup>j</sup>	0.26±0.00 <sup>i</sup>	0.78±0.03 <sup>j</sup>
CF11	2.44±0.07 <sup>k</sup>	0.40±0.01 <sup>j</sup>	1.08±0.02 <sup>k</sup>
CF12	2.84±0.10 <sup>l</sup>	0.75±0.00 <sup>k</sup>	1.57±0.01 <sup>l</sup>

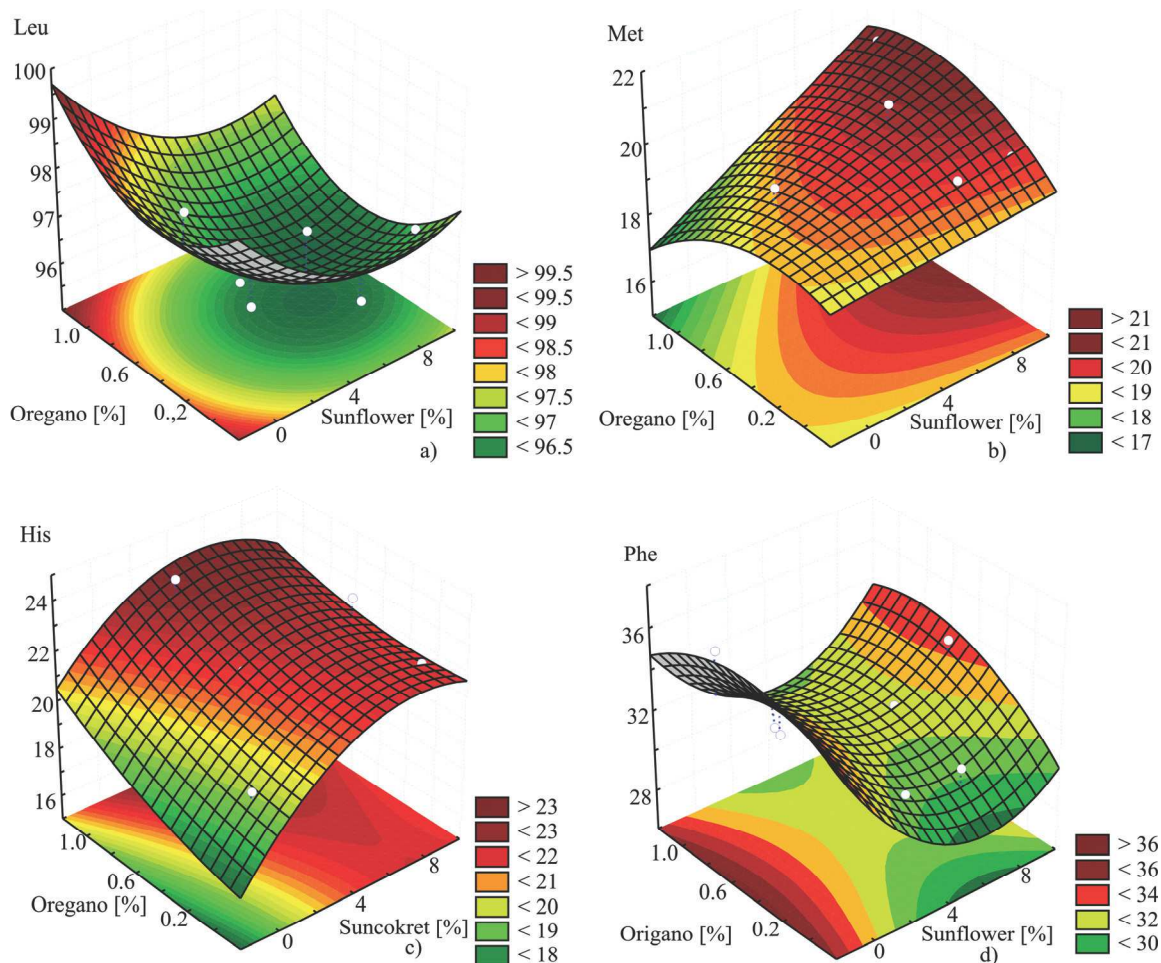


Figure 1. Observed responses of Leu, Met, His and Phe, based on the oregano and sunflower content.

Table 6. ANOVA table for prediction of antioxidant value corn flakes with flex sunflower and wild oregano

Parameter	df	Fenoli	DPPH	FRAP
Sunflower	1	2.377 <sup>a</sup>	0.270 <sup>a</sup>	1.629 <sup>a</sup>
Sunflower <sup>2</sup>	1	0.010	0.007	0.052 <sup>a</sup>
Oregano	1	0.913 <sup>a</sup>	0.049 <sup>a</sup>	0.067 <sup>a</sup>
Oregano <sup>2</sup>	1	0.030 <sup>b</sup>	0.003	0.003
Sun.×Oreg.	1	0.134 <sup>+</sup>	0.031 <sup>a</sup>	0.008
Error	7	0.048	0.014	0.030
r <sup>2</sup>	–	0.987	0.961	0.984

<sup>a</sup>Statistically significant at  $p < 0,01$  level; <sup>b</sup>statistically significant at  $p < 0,10$  level

## CONCLUSION

Based on data resulting from the investigations of quantity of sunflower and dry residue of wild oregano addition influenced on corn flakes amino acid and antioxidant potential it can be concluded:

- Statistically significant differences between most of the mean values of amino acids content and antioxidant activity in the observed corn flakes were observed.

- Sunflower and dry residue of wild oregano positively affect the phenolic compounds and antioxidant activity. Maximum value obtained was TPC 2.84 mg/g, DPPH 0.75 mg/ml, FRAP 1.57 mg/g with 9 g/100 g of sunflower and 1 g/100 g dry residue of wild oregano in CF 12.

- Flakes product with addition of dry residues wild oregano contributed to the food waste valorisation in the food industry to obtain a new product with antioxidant potential.

- Lysine is limiting amino acid for all corn flakes (CF1–12).

- The temperature extrusion (85 °C) does not lead to the loss of lysine as well as the most reactive amino acids.

- Score and PDCAAS value in corn flakes (CF1–12) increases with increasing of adding sunflower (3, 6, or 9 g).

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## IZVOD

### POBOLJŠANJE NUTRITIVNIH OSOBINA KORN FLEKSA OBOGAĆENOG FUNKCIONALNIM KOMPONENTAMA

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(Naučni rad)

Tokom procesa proizvodnje hrane stvara se velika količina sporednih proizvoda koji završe kao otpad. Sporedni proizvodi predstavljaju veliki problem prilikom odlaganja, potencijalno veliki izvor zagađenja i veliki gubitak biomase i hranljivih materija. Savremeno društvo, u kome postoji velika potražnja za prehrambenim proizvodima poboljšanog nutritivnog sastava, karakteriše rast troškova proizvodnje hrane i smanjena dostupnost sirovina. Trend rasta potražnje prehrambenih proizvoda poboljšane nutritivne vrednosti, sa dodatkom različitih bioaktivnih komponenti ili nenutrijenata sa pozitivnim efektima na zdravlje, uticalo je na povećanje iskorišćenja sporednih proizvoda. Podizanje svesti potrošača o značaju nutritivnog sastava prehrambenih proizvoda za očuvanjem zdravlja uticala je i na proizvođače. Danas je prehrambena industrija sve više usmerena prema poboljšanju kvaliteta i zdravstvene bezbednosti namirnica. U skladu sa savremenim stavovima nutricionista, proizvodi od žitarica, su najčešća hrana u svakodnevnoj ishrani kao što su „ready to eat“ žitarice za doručak fleks i snek proizvodi. Ekstruziona tehnologija omogućava primenu različitih tehnologija i različite izvore za obogaćivanje ekstrudiranih proizvoda na bazi žitarica. Ovaj rad istražuje efekte dodavanja suncokreta (3, 6 i 9 g/100 g uzorka) i suvog ostatka divljeg origana (0,5 g/100g uzorka i 1 g/100g uzorka) na aminokiselinski sastav i antioksidativni potencijal fleks proizvoda u cilju dobijanja novog proizvoda sa poboljšanim funkcionalnim svojstvima. Eksperimentalni plan je 3×4. Rezultati Score i PDCAAS vrednosti fleks proizvoda se povećavaju sa povećanjem udela suncokreta. Maksimalna vrednost fenolnih jedinjenja (TPC 2,84 mg/g) i antioksidativnog potencijala (DPPH 0,75 mg/ml, FRAP 1,57 mg/g) je zabeležena kod fleks proizvoda sa maksimalnim udelom suncokreta i suvog ostatka divljeg origana. Tukey's HSD test je pokazao statistički značajne razlike između aminokiselinskog sadržaja i antioksidativnog potencijala posmatranog korn fleks proizvoda. Metoda odzivne funkcije (površine) je primenjena za procenu fleks proizvoda. Dodatak suncokreta pozitivno utiče na nutritivnu vrednost, a dodatak suvog ostatka divljeg origana na antioksidativnu aktivnost fleks proizvoda i doprinosi boljoj valorizaciji sporednih proizvoda iz prehrambene industrije. Fleks proizvod sa dodatkom suncokreta i suvog ostatka divljeg origana je nov proizvod sa poboljšanim sastavom esencijalnih aminokiselina i antioksidativnim potencijalom pri čemu ima sve osobine funkcionalne hrane.

*Ključne reči:* Amino kiseline • Antioksidativnost • Divlji origano • Suncokret • Fleks proizvod