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PHENOLIC COMPOUNDS CONTENT AND RADICAL SCAVENGING CAPACITY OF WHEAT-LENTIL DOUGH

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Abstract. *The lentil (Lens culinarisL.) is a legume plant, one of the oldest known food crops and medicinal plants. The health benefits of lentil are well known: its consumption reduces the risks of cardiovascular diseases and some cancers. It has a low glycemic food index and is important in the dietary treatment of diabetes mellitus.Unfortunately, its consumption in many countries is low. Since bread is a daily consumed food this can be improved by adding the lentil in wheat flour. In this paper the content and DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging capacity of phenolic compounds from wheat dough and dough obtained by wheat flour supplemented with 40% of lentil flour were examined and compared. The dough with lentil flour had higher content of phenolic compounds than the dough with wheat flour only (2144.7 and 1592.5 μg of chlorogenic acid/g, respectively) and achieved higher DPPH scavenging capacity (SC_{50} value was 21.2 and 56.3 mg/mL, respectively). Results showed that, after baking, the dough retained the same value of DPPH scavenging capacity, while baked wheat-lentil dough had near three times higher antioxidant activity than baked wheat dough. These investigations indicate that the lentil flour is useful food ingredient for improving the antioxidative potential of wheat flour*.

Key words: *Lentil, dough, phenolic compound, scavenging capacity*

Introduction

Plant phenolic compounds are secondary plant metabolites synthesized by plants during their normal development or in response to stress conditions such as infection, wounding and UV radiation [1, 2]. They are a highly diversified group of compounds including the simple phenolics, phenolic acids, coumarins, flavonoids, hydrolysable and condensed tannins, lignans and lignins [3]. Phenolic compounds have free radical scavenging abilities, anti-mutagenic and anti-carcinogenic activities and the ability to reduce the risk of cardiovascular and carcinogenic diseases [4]. Their content in plants depends on many factors such as cultivar and stage of ripening [5, 6] and antioxidant activity depends on phenological stage [7].

The lentil (*Lens culinaris* L.) is a legume plant, one of the oldest known food crops as it has been cultivated for more than 8,500 years ago. Legumes are well known as "the poor man's meat", widely available and inexpensive, but they are not fully exploited [8]. Legumes are important crops due to their nutritional quality. It is an excellent and inexpensive source of protein, amino acids such as L-lysine and L-arginine, complex carbohydrates, fibre and minerals [9, 10]. The health benefits of lentils are also well known: its consumption reduces the risks of cardiovascular diseases and even some cancers. They have been identified as low glycemic index foods [11] and are important in dietary treatment of diabetes mellitus as they increase satiety and facilitate the control of food intake. In the *Caenorhabditis elegans* model system, legumes reduced intestinal fat [12]. Due to these lentil abilities, adding lentil flour to wheat flour could show potential to formulate functional foods.

Unfortunately, its consumption in many western countries is low. Since bread is daily consumed food in these regions, this can be improved by adding the legumes to bread. The legumes in food products in relation to currently used breads contribute to higher content of protein, minerals, fat and fiber, change cake volume [13] and lower the content of gluten and carbohydrate [14].

In available literature there is data about the content of the phenolic compounds from lentil and wheat flour and their antioxidant activity. However, they are not determined by the same procedure and equipment, and could not be used for comparison. The purpose of this paper is to determine and compare such data, and investigate the effects of the replacement of wheat flour by the lentil flour on phenolic compounds content and radical scavenging capacity. This is useful for an evaluation of the potential of lentil flour to improve the antioxidative potential of wheat flour, and in this way

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formulated functional foods. In this paper the phenolic content and the radical scavenging capacity of wheat (WF) and lentil flour (LF), wheat-lentil flour mixture in ratio of 60:40 (w/w) (WLM), wheat dough (WD) and dough obtained from WLM mixture (WLD), as well as baked wheat dough (WB) and baked wheat-lentil dough (WLB), were examined and compared.

Material and Methods

Flours and dough

Lentil flour was obtained by milling lentil seeds originating from Canada, grown in 2012 and sieving through a 0.30 mm sieve. The used flour was analysed for moisture, protein and ash content. The moisture content was determined by Scaltec SMO 01 (Scaltec instruments, Germany) instruments: 5 g of flour was put in the disk plate analyzer, dried at 110 ^{0}C to a constant weight, and the moisture content was read out on the display. Protein content was determined by the Kjeldahl method (Nx5.95) and the ash content by staking of sample at 800^{o}C during 5 h [15]. The wheat flour type 500 ("Padež", Bunibrod, Serbia) from the crop of 2012, was bought in a local store in Leskovac, Serbia, and the same analyses of the lentil flour were performed with wheat flour. The mixture wheat-lentil flour (WLM) was obtained by mixing the wheat and the lentil flour in ratio of 60:40 (w/w).

The dough from the wheat flour only and the wheat-lentil flour mixture, were obtained by mixing using farinograph (Brabender Model 8 10 101, Duisburg, Germany) according to ISO 5530-1 test procedure. In order to obtain a sample of dough, small slices of approximately of 1 × 0.5 cm, were cut out of dough, dried at 30^{o}C during 3 h and milled and sieved through a 0.30 mm sieve.

The separate sample of dough obtained by the same mixing procedure on the farinograph was shaped into round balls, of approximately 30 cm in diameter and 2.5 cm in height and baked at 180^{o}C, for 50 minutes in the oven (Candy, FPP403/1). The baked wheat dough (WB) and wheat-lentil dough (WLB) were cooled down to room temperature and sliced to a size approximately of 25 × 1.5 cm. The slices were dried for 3 h at 30 ^{o}C, milled and sieved through a 0.30 mm riddle.

Preparation of extracts

For measurements of the phenolic compounds content in LF, WF, WLM, WD and WLD, 5 g of the flour or sample was measured and 100 mL of 80% (v/v) ethanol was added. The mixture was stirred by MR1 magnetic stirrer (IKA-Werke, Staufen, Germany) for 10 minutes at 200 min^{-1} and vacuum filtered through No. 54 Wathman filter paper (GE Healthcare, Brondby, Denmark). The solids were re-extracted with 50 mL of 80% (v/v) ethanol, the filtrates combined and made to a final volume of 150 mL. For radical scavenging capacity (SC) measurements, 140 mL of each extract was evaporated in the vacuum at 45 ^{o}C until dry, and was dissolved in 30 mL of 96% (v/v) ethanol.

Phenolic compounds content

A standard curve for five chlorogenic acid (Sigma Chemical, St. Louis, Missouri, USA) concentrations covering the range from 10 to 300 μM (C=2319×Ab-10.2) was first made for phenolic content (PCC) determination. According to method of Glories (1978) [16], 4.50 mL of 2 g/mL HCl and 0.25 mL of chlorogenic acid standard solutions was added, mixed by vortex and allowed to stand for approximately 15 min, for PCC determination, in a test tube 0.25 mL of 0.1 g/mL HCl in 95% (v/v) ethanol. Then the absorbance (A) was read at 280 nm using UV 21000 Spectrophotometer (Cole Parmer Instruments Company, Vernon Hills, Illinois, USA).For measuring PCC in flours and dough, 0.25 mL of 0.1 g/mL HCl in 95% (v/v) ethanol, 4.50 mL of 2 g/mL HCl and 0.25 mL of filtered extracts was added into test tube and further treated as standard solutions of chlorogenic acid.

Radical scavenging capacity

The radical scavenging capacity (SC) of an extract diluted by ethanol to obtain concentrations ranging from 0.3 to 8 mg/mL, was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) test [17]. The ethanol solution of DPPH radicals concentration of 0.1 mM (1 mL) was added to 2.5 mL ethanol solution of the given concentration of the investigated extract and allowed to react at room temperature for 30 min. Then the A value was measured at 518 nm on UV 21000 Spectrophotometer (Cole Parmer Instruments Company) and converted to percentage of radical SC by using the equation defined by Mensor and Menezes (2001) [18]:

$$SC = 100 - \frac{(A_{sample} - A_{blank})}{A_{control}} \times 100$$

where A_{sample} is the absorbance at 518 nm of the ethanol solution of the extract treated by the DPPH radical solution; A_{blank} is absorbance at 518 nm of the ethanol solution of the extract (1 mL of ethanol added to 2.5 mL of extract), and $A_{control}$ is absorbance at 518 nm of ethanol solution of DPPHradical (1 mL of a 0.3 mM added to 2.5 mL of ethanol). The final results are presented as SC_{50} value, calculated by using Microsoft Excel ed50plus (v1.0) software by Mario H. Vargas, InstitutoNacionale de EnfermedadesRespiratories by inputtingthe data of SC and extract concentrations in appropriate columns and using the function "Interpolate" (www.sciencegateway.org/protocols/cellbio/drug/hcic50.htm). The value of SC_{50} represents the concentration of dry residue of studied extracts that causes a decrease in the initial DPPH concentration by 50%.

Statistical analysis

Statistical version 5.0 Software (StatSoft, Tulsa, Oklahoma, USA) was used to perform the statistical analysis: the mean, standard deviations and statistical dependence. The mean and standard deviations were obtained by using Descriptive Statistics, marking the Median & Quartiles and Confirm Limits for Means. Where appropriate, the

statistical dependence was tested by Excel 2003 and ANOVA Single factor test. Differences with $p<0.05$ were considered to be statistically significant.

Results

The moisture, protein and ash content in the tested samples is shown in Table 1. The investigated characteristics of obtained extracts from lentil and wheat flour and dough, extract yield (EY), phenolic compounds content and SC_{50} value, are also shown in Table 1. Values are the means and standard deviation (N=3) obtained by descriptive statistics and the same letters in superscript within the same column indicate significant differences ($p > 0.05$) obtained by ANOVA test.

The results of the dependence of the scavenging capacity on the concentrations of the polyphenols in the extract obtained from the investigated flours, wheat-lentil flour mixture (60:40 w/w), their dough obtained after mixing and corresponding baked dough are presented in Figure 1.

Discussion

The results presented in Table 1 show there are significant differences between flours in protein and ash content and the replacement of wheat by lentil flour increases the contents of these components, in dough as well as in final food products. In WLD, the protein content was 1.5 and ash content 3.3 times higher than in WD.

The extract yield (EY) of LF was higher than the EY of WF and the EY of the dough extract was higher than the EY of the flour extract from which they are made. The EY was 9.1 g/100 g for the extract obtained from WD and 6.6 g/100 g from WF. The EY of the extract of WLD was 12.1 and it was also two times higher than the EY of WLM, where it was 7.4 g/100g.

Han and Baik [19] published that the phenolic compounds content (PCC) in lentil (after extraction by 30% dimethylformamide and determination by using 4-aminoantipyrine and ferric cyanide and measuring absorbance at 505 nm) was ~12 mg/g expressed in galic acid equivalent. On the other hand, the PCC in lentil after extraction with acetone/water/acetic acid (70:29.5:0.5,

Table 1. The characteristics, phenolic compounds content and radical scavenging capacity of extracts obtained from lentil and wheat flour and dough

Sample /Parameter	Moisture (g/100 g)	Protein content (g/100 g)	Ash content (g/100 g)	EY (g/100 g)	PCC (μg/g)	SC_{50} (mg/mL)
LF	10.7 ± 0.6	21.9 ± 1.6^{a}	3.02 ± 0.6^{a}	8.1 ± 0.6	993.7 ± 32^{a}	2.2 ± 0.4^{a}
WF	13.1 ± 0.8	9.8 ± 0.8^{a}	0.48 ± 0.6^{a}	6.6 ± 0.8	789.6 ± 23^{a,d}	13.8 ± 0.4^{a,d}
WLM	12.8 ± 0.8	14.6 ± 1.1^{a}	1.49 ± 0.5^{a}	7.4 ± 0.9	878.9 ± 36^{a,e}	6.8 ± 0.3^{a,e}
WD	11.6 ± 0.9	9.2 ± 0.9^{b}	0.42 ± 0.6^{b}	9.1 ± 0.8	1592.5 ± 52^{b,d}	56.3 ± 0.8^{b,d}
WLD	12.3 ± 0.9	13.9 ± 1.8^{b}	1.41 ± 0.6^{b}	12.1 ± 0.9	2144.7 ± 71^{b,e}	21.2 ± 0.6^{b,e}
WB	11.8 ± 0.8	9.4 ± 0.8^{c}	0.43 ± 0.7^{c}	8.9 ± 1.1	1198 ± 64^{c}	61.4 ± 1.1^{c}
WLB	12.1 ± 1.1	14.1 ± 1.4^{c}	1.42 ± 0.4^{c}	10.9 ± 1.2	1897 ± 83^{c}	24.2 ± 0.4^{c}

Values are the means followed by standard deviation (N=3)
The same letters in superscript within the same column indicate significant differences ($p > 0.05$).

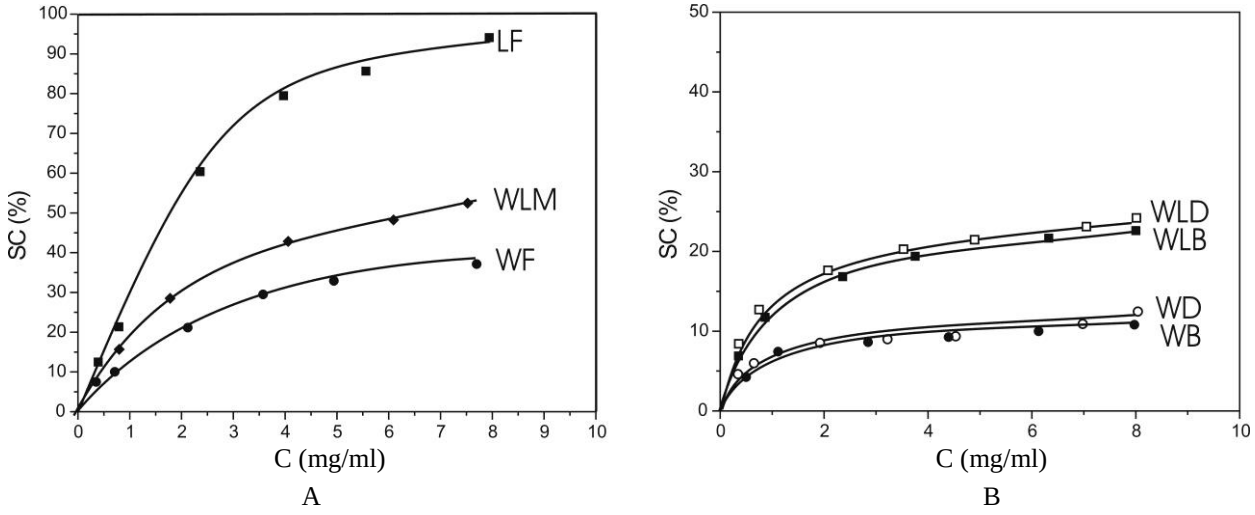

Fig. 1. The dependence of the scavenging capacity on the concentrations of polyphenols in extract obtained from the lentil flour (LF), wheat flour (WF), wheat-lentil flour mixture (60:40 w/w) (WLM) – A, and wheat dough (WD), wheat-lentil dough (WLD), baked wheat dough (WB), baked wheat-lentil dough (WLB) – B

v/v/v), determination with Follin-Ciocalteu assay, measuring absorbance at 765 nm, was 70.0 mg/g, expressed in gallic acid equivalent [20]. For wheat flour there is also an abundance of data where PCC was in the 254–499 μmol gallic acid equivalent/100g of wheat range, depending on the varieties [21] (obtained after extraction by 80% chilled acetone and by Follin-Ciocalteu reagent, measuring absorbance at 760 nm). Other studies have shown that the PCC was in the 119–201 μmol gallic acid equivalents/100g of wheat range, also depending on the varieties (obtained after extraction by 80% chilled ethanol and by Follin-Ciocalteu reagent, measuring absorbance at 760 nm) [22]. It is evident that making conclusions and comparisons based only on the presented literature data is not valid. The available literature does not provide data about the PCC and radical scavenging capacity in those doughs after processing, such as mixing and baking. These are the reasons why, in this paper, we presented and compared the results of PCC and radical scavenging capacity in lentil and wheat flour and their doughs.

The PCC in lentil flour that we have obtained was of 993.7 μg of chlorogenic acid/g and it was higher than in wheat flour (789.6). The PCC in dough was higher than in corresponding flour: WD contained 1592.5 μg/g while WLD had 2144.7 μg/g and it is 2.5 times higher than in wheat-lentil flour mixture, where it was 878.9 μg/g. Based only on these results, it is difficult to explain how the PCC appeared to be higher in a sample of dough than in corresponding samples of flour. These results could indicate that during the dough mixing process, when water was added, the reactions of hydration of phenolic compounds probably occurred. Also, phenolic compounds exist in their hydrate state and this probably increases the extractability of the phenolic compounds [23] and causes a higher value of EY and PCC in dough samples. Comparison of PCC in WLD and WD showed that value of PCC was considerably higher in the dough obtained from the mixture where wheat flour was replaced by lentil flour.

Furthermore, higher PCC in the lentil flour than in the wheat flour also caused a higher DPPH radical scavenging capacity of the extracts. The investigations showed the DPPH scavenging capacity depended on the extract concentration and it increased when the extract concentration increased. In extracts where the dry residue concentration was 8.0 mg/mL, the extract obtained from the lentil flour had a SC of 93.2%, while the extract from the wheat flour had a SC of only 34.9% (Figure 1A).

The extract obtained from WD and WLD had considerably lower SC than extracts from WF and WLM, respectively. The mixing of dough reduced DPPH scavenging capacity of WLD by approximately 25%, compared to the scavenging capacity of WLM. The reason for this might be the oxidation or hydration reactions of phenolic compounds which can occur during mixing. It is known that the processing of cereals and legumes, such as germination, may increase the level of phenolic compounds in foods when enzymatic reactions in seeds occur [24, 25].

Obtained SC_{50} values (Table 1) expressed as μg of chlorogenic acid per ml of extract were lower than the SC_{50} value obtained for ascorbic acid (9.8 μg/mL). Lower SC_{50} value indicates higher scavenging capacity which is in accordance with the results of SC. As WLD had higher SC and lower SC_{50} value than WD (Figure 1B), it was evident that the replacement of 40% of wheat flour by lentil flour improved antioxidant activity of dough, thus offering better health benefits.

Results obtained with baked wheat-lentil dough (WLB) were 21.8% for DPPH radical scavenging capacity and 24.2 mg/mL for SC_{50} value (Figure 1B). Based on these results, the baked dough retained the DPPH scavenging capacity which dough had had and bread from wheat-lentil flour mixture will have near three times higher antioxidant activity than bread made of wheat flour only: SC_{50} value for WB was 61.4 and for WLB, 24.2 mg/mL (Figure 1A). These results are in accordance with the results reported by Hye-Min and Bong-Kyung [26] when caffeic acid was added to dough and the recovery of caffeic acid after baking was 74–80%.

According to ANOVA test results, the lentil flour addition significantly affected the protein and ash content as well as phenolic compounds content and DPPH scavenging capacity ($p > 0.05$). Dough mixing also significantly affected the phenolic compounds content and DPPH scavenging capacity and baking had no significant effect on these parameters. The higher difference between F and F critical values (588 and 7.7, respectively) was observed for SC_{50} value of WF and WLM.

Conclusion

By replacing 40% of wheat flour with lentil flour, the obtained dough had a 1.3 times higher content of phenolic compounds and 2.7 times higher SC_{50} value than dough made of wheat flour only. By dough mixing, the DPPH scavenging capacity at the concentration of 8 mg/mL, for the extract obtained from wheat-lentil dough, was reduced by approximately 25%, compared to scavenging capacity of flour mixture from which it was made. Baked dough from wheat-lentil flour mixture had almost three times higher antioxidant activity than baked dough made from wheat flour only, so the addition of the lentil flour to the wheat flour showed potential to improve the antioxidant potential of wheat flour.

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