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Effect of Toasting and Microwaving on Gross Chemical Composition, Total Phenolics, Antioxidant Activity and Phenolic Acids Fractionation of White Beans Flour (*Phaseolus vulgaris* L.)

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Abstract: In this study, one variety of white beans was used to study the effect of toasting and microwaving on chemical composition and phenolic compounds of white beans flour (*Phaseolus vulgaris* L.). The antioxidant activity and phenolic acids of the aqueous extracts were studied. Maximal antioxidant activities was observed in the 45min toasted and 3min microwaved increased to 2.09, 1.86% total reduction activity, 4.49 and 3.95 H_2O_2 scavenging activity, respectively. Phenolic acids were identified by high performance liquid chromatography (HPLC). Chlorogenic acid was the most predominant amongst the ten phenolic acids identified in white beans samples. Syringic acid, p-coumaric acid, gallic acid and sinapic acid increased with an increase in toasting time. Our study has demonstrated that toasted white beans gave the most desirable quality of toasted white beans with respect to phenolic content and radical-scavenging activities.

Key words: Antioxidant activities · Toasting · Microwaving · Phenolic acids · White beans flour

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a traditional food in the human diet, as it is low in fat and rich in proteins, vitamins, complex carbohydrates and minerals. In addition to contributing nutritional requirements, consumption of dry beans had been linked to reduced risk of heart disease [1] obesity [2] and cancer [3-5]. However, widespread use of beans as a primary staple food had been limited by the presence of antinutritional factors, which might produce adverse effects for both human and animal nutrition. Some of these compounds include enzyme inhibitors, lectins, phytates, cyanoglycosides and phenolics [6]. Some publications on P. vulgaris had focused on antinutritional aspects of seed coat polyphenols [7]. However, polyphenols had contradicting positive effects on human health and it had been reported that they had anticarcinogenic and antioxidant properties [8]. It is generally believed that antioxidants scavenge free radicals and reactive oxygen species and can be extremely important in inhibiting oxidative mechanisms that lead to degenerative diseases [9]. Recently, antioxidant activity was reported in extracts, condensed tannins and pure flavonoids from colored genotypes of common bean seed coats [9-11].

Phenolics are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants. Plant polyphenols had drawn increasing attention due to their potent antioxidant properties as well as their marked effects in the prevention of various oxidative stress associated diseases such as cancer. In the last few years, the identification and development of phenolic compounds or extracts from different plants had become a major area of health- and medical-related research [12]. Phenolic acids can be divided into two classes: derivatives of benzoic acid such as gallic acid and derivatives of cinnamic acid such as coumaric, caffeic and ferulic acid. Caffeic acid is the most abundant phenolic acid in many fruits and vegetables, most often esterified with quinic acid as in chlorogenic acid, which is the major phenolic compound in coffee. Another common phenolic acid is ferulic acid, which is present in cereals and is esterified to hemicelluloses in the cell wall [13].

Antioxidants are defined as compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Oxidative stress is an imbalanced state where excessive quantities of reactive oxygen and/or nitrogen species (ROS/RNS, e.g., superoxide anion,

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hydrogen peroxide, hydroxyl radical and peroxynitrite) overcome endogenous antioxidant capacity, leading to oxidation of varieties of biomacromolecules, such as enzymes, proteins, DNA and lipids. Oxidative stress is important in the development of chronic degenerative diseases including coronary heart disease, cancer and aging [14]. Recently, phenolics had been considered powerful antioxidants *in vitro* and proved to be more potent antioxidants than Vitamin C and E and carotenoids [15, 16]. The inverse relationship between fruit and vegetable intake and the risk of oxidative stress associated diseases such as cardiovascular diseases, cancer or osteoporosis had been partially ascribed to phenolics [17, 18]. It had been proposed that the antioxidant properties of phenolic compounds could be mediated by the following mechanisms: (1) scavenging radical species such as ROS/RNS, (2) suppressing ROS/RNS formation by inhibiting some enzymes or chelating trace metals involved in free radical production, (3) upregulating or protecting antioxidant defense [19].

The purpose of the current study was to assess the effect of toasting, microwaving treatments, on some nutritional properties and chemical composition of white beans. Nutritionally, the content of total phenolics and its correlation with antioxidant activity and the phenolic acids fractions were investigated as well.

MATERIALS AND METHODS

Materials: 10 kg dried white beans ((*Phaseolus vulgaris* L.) Nebraska variety was purchased from the Agricultural Research Center – Giza – Egypt during the summer season 2012.

Sample Preparation

Raw Dry Beans (Control): Raw dry beans refers to beans as such (control), the samples were ground for 3 min in an electrical laboratory mill.

Toasted Beans (T): Toasted beans (T) refers to white beans flour toasted at 130°C for 15, 30 and 45 min using an electrical drying oven. (Model D-63450, Hanau, Germany).

Microwaved Beans (M): Microwaved beans (M) refers to white beans flour microwaved at 2450 MH for 1, 2 and 3 min using microwave oven.

Methods of Analysis

Gross Chemical Composition: Moisture, crude protein, crude oil, crude fiber and ash were determined as described in the AOAC Methods [20], while the carbohydrate content was calculated by difference according to Pellet and Sossy [21]. Triplicate determinations were carried out for each sample and the means were reported.

Phenolic Compounds: Phenolic compounds in the studied samples were determined spectrophotometrically using Folin–Denis reagent [22]. To each test tube and 30 mg of dry finely ground plant material. Add 10 ml of 50% aqueous methanol and extract for two hours shaking every 15 minutes. After samples have cooled and settled, pipette of 5 ml and save for analysis. The methanolic extracts (0.1 ml) of white been samples were diluted with distilled water (75 ml) in a volumetric flask. Folin-Denis reagent (5 ml) was added and the contents of the flask were mixed thoroughly. After 3 min, 10 ml of Na_2CO_3 solution (10 g /100 ml) was added and finally quantified to 100 ml with distilled water. The mixture was allowed to stand for 30 min with intermittent shaking. The blue color was measured by spectrophotometer at 750nm. The concentration of total phenolic compounds in samples was determined comparing with the absorbance of standard tannic acid at different concentration.

Determination of Phenolic Acid: The HPLC analysis of phenolic acids were carried out on a HPLC apparatus consisting of Merck-Hitachi L-7455 diode array detector (DAD) and pump L-7100 equipped with D-7000 HSM Multisolvent Delivery System. The separation was performed on a Li ChroCART® 125-3 Purospher® RP-18 (5 µm) Merck column. Column oven temperature was set to 30°C. 80% acetonitrile in 4.5% formic acid (reagent A) and 2.5% acetic acid (reagent B) were used as an eluent. The flow rate was 1 ml/min. The concentration of reagent A was stepwise increased to reach 15% after 7min, 20% after 15 min and 100% after 16 min. After 10 min of elution the concentration of reagent A was reduced to 0% to stabilize the column. During analysis the solvent were degassed in Merck degasser. Data logging were monitored at wavelength 280nm. Retention times and spectra were compared to those of pure standards [23].

Antioxidant Activities Assays:

Total Reduction Activity by Fe^{3+}- Fe^{2+} Transformation: The reducing activity of samples was determined by the method of Oyaizu [24]. The capacity of samples to reduce the ferric-ferricyanide complex to the ferrous-ferricyanide complex of Prussian blue was determined by recording the absorbance at 700 nm after incubation. Increased absorbance of the reaction mixture indicates greater reduction capability.

Hydrogen Peroxide Scavenging Activity: The hydrogen peroxide scavenging ability of samples was determined according to the method of Ruch *et al*. [25]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). Sample extract, at the 30 ìg ml/l concentration in 3.4 ml of phosphate buffer, was added to an H_2O_2 solution (0.6 ml, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm against blank solution containing the phosphate buffer without H_2O_2.

Statistical Analysis: All data collected were analyzed with analysis of variance (ANOVA) Procedures using the MSTAT-C Statistical Software Package [26]. Differences between means were compared by LSD at 5% level of significant [27].

RESULTS AND DISCUSSION

Effect of Toasting and Microwaving on the Gross Chemical Composition of White Beans Samples: The gross chemical composition of raw and treated white beans flour samples is given in Table 1. The obtained results indicated that the moisture content was 9.23% for raw white beans then decreased in the treated samples as compared with control one. It could be noticed that protein content was 20.47% for control and increased in all samples, while the crude fat content was 2.90% then increased in the treated samples as well. Crude fiber and total carbohydrates contents were 9.77, 53.83% for control then increased in the aforementioned studied samples. As shown in Table 1 the toasted and microwaved white beans contained relatively considerable amounts of ash ranging from 3.83 to 4.16% compared with 3.80% for the raw white beans. The result revealed that there were significant differences ($P<0.05$) in all chemical properties measured. T 45min had the highest value of ash (4.16%), crude protein (22.62%) and crude fat (4.26%). T 15min had the lowest value of ash (3.95%) while control had the lowest value of crude protein (20.47%) and crude fat (2.90%). Total carbohydrates, crude fiber ranged from 53.83- 56.71%, 9.77–11.89%, respectively, with control having the least value and M 3min having the highest value. The moisture also ranged between 0.65 and 9.23%. These results are very similar to that reported by Kereliuk & Kozub [28], Alonso *et al*. [29], Kahlon *et al*. [30] and Sai-Ut *et al*. [31] who found that moisture of white beans ranged between 8.52 to 11.07%, crude protein 16.36 to 25.30%, crude fat 1.5 to 2%, crude fiber 4 to 8%, ash 3 to 4% and total carbohydrates 54 to 59%. The different amount of any component in these beans possibly depends on the difference in spice of cultivar and growth conditions.

Total Phenolics and Antioxidant Activity of White Beans Samples: Phenolics content ranged from 0.32 to 0.76 in the heat-treated white beans as compared with 0.24 mg tannic acid/100 gm (dry weight) for raw white beans (Table 2). It is interesting to note that the toasted white beans flour for 45 min had a higher phenolic content than other products and control. The antioxidant activity of white beans samples is presented in Table 2. The raw white beans sample was found to have a total antioxidant activity 1.58% total reduction activity and 3.75% H_2O_2 scavenging activity. Due to the thermal treatment the total antioxidant activity in the toasted for 45min and microwaved for 3min increased to 2.09, 1.86% total reduction activity, 4.49 and 3.95 H_2O_2 scavenging activity, respectively. The heating treatments caused an increase in the availability of total phenolic that corresponded with an increased antioxidant activity in both toasted and microwaved beans. These changes appear to be related to the time the samples were exposed to high temperature. This finding may be explained on the grounds of previous suggestions that some phenolic compounds and their conjugated forms can be converted from one form to another during various technological processes [32, 33]. Such data indicated that thermal processing of beans under the specified conditions might liberate phenolic

Table 1: Mean value of the gross chemical composition of white bean samples (%)*.

Sample	Moisture	Ash	Crude protein	Crude fat	Crude fiber	Total carbohydrates
Control	9.23^{a}	3.80^{f}	20.47^{g}	2.90^{g}	9.77^{g}	53.83^{g}
T 15min	5.66^{b}	3.95^{g}	21.70^{e}	3.08^{e}	10.46^{e}	55.15^{f}
T 30min	3.60^{d}	4.05^{b}	22.25^{c}	3.56^{d}	11.14^{c}	55.40^{e}
T 45min	0.75^{f}	4.16^{a}	22.62^{a}	4.26^{a}	11.84^{b}	56.37^{b}
M 1min	5.14^{c}	3.83^{e}	21.58^{f}	3.06^{f}	10.30^{f}	56.09^{d}
M 2min	2.78^{e}	3.88^{d}	22.06^{d}	3.99^{c}	11.05^{d}	56.24^{c}
M 3min	0.65^{g}	3.96^{c}	22.60^{b}	4.19^{b}	11.89^{a}	56.71^{a}

*Means having different superscripts within the column are significantly different at $p < 0.05$

Table 2: Total phenolics and antioxidant activities content of raw and treated white bean samples.

		Antioxidant activities	
Sample	Total phenolics*	Total reduction activity**	H_2O_2 scavenging activity***
Control	0.24	1.58	3.75
T 15min	0.34	1.68	3.89
T 30min	0.59	1.82	4.15
T 45min	0.76	2.09	4.49
M 1min	0.32	1.60	3.78
M 2min	0.50	1.69	3.85
M 3min	0.64	1.86	3.95

*Calculated as mg tannic acid / 100 gm dry weight.

**Calculated as reducing ferric ions / 100 gm dry weight.

***Calculated as scavenging H_2O_2 molecules /100 gm dry weight.

Table 3: Phenolics acid fractionation of white beans samples (µg/ 100gm dry weight).

Phenolic acid	Control	T 15min	T 30min	T 45min	M 1min	M 2min	M 3min
Gallic	499.02	178.00	467.17	275.55	371.42	537.28	489.60
Protocatechuic	302.71	193.17	217.47	241.30	291.17	322.29	208.55
Catechin	474.98	241.30	524.25	204.76	585.03	529.01	527.59
Catechol	424.35	102.07	354.52	396.12	399.01	367.52	428.35
Chlorogenic	415.75	422.03	754.88	683.57	439.78	519.87	388.25
Caffeic	133.31	323.13	169.08	255.51	176.61	242.64	152.78
Vanillic	199.42	343.81	310.31	148.25	242.92	285.92	428.05
Caffeine	687.95	500.53	314.58	281.59	322.05	239.90	188.26
Benzoic	303.42	329.23	181.51	115.52	159.32	331.36	221.03
Cholchecien	431.08	210.34	187.93	315.63	381.33	407.88	694.84
p-hydroxybenzoic	607.59	797.55	140.46	590.20	401.13	218.48	1054.50
Chrysin	131.10	364.47	270.10	368.43	144.61	266.91	433.96

compounds and their derivatives from the wall cells. The liberated compounds may then contribute higher antioxidant potential if they are considered as a dietary antioxidant. Analyzing the level of bioactive compounds in cereal grains before and after hydrothermal processing, Zielin´ ski *et al*. [34] demonstrated an increase of up to 300% in the content of phenolic acids after extrusion cooking. The antioxidant activity of grape seed extracts had also been shown to be affected by heating conditions, showing a higher reducing power after various heat treatments [35]. However, some reports had shown a reduction of total phenolics in beans submitted to different heat treatments [36-38].

Phenolics Acid Fractionation of White Beans Samples: The composition of polyphenolic acids in white beans samples is presented in Table 3. From these data it is clear that beans samples contained many polyphenolic acids in variable levels. Protocatechuic, caffeine, chlorogenic, catechin and gallic acid were presented in reasonable amounts both in control and treated samples. The results of HPLC had identified also, catechol, vanillic, caffeic, *p*-hydroxybenzoic acid and chrysin acids as phenolic constituents of beans samples. Cholchecien and benzoic acids were also presented in white beans samples. The content of Cholchecien and benzoic acids were decreased in some samples, amounting to, 187.93, 181.51, 315.63, 115.52 and 381.33, 159.32, for T 30min, T 45min and M 1min, respectively, as compared with 431.08 and 303.42 µg acid/100 gm dry weight for the control. In general, the content of polyphenolic acids presented in beans samples was influenced by thermal treatment and time. The T 45min sample contained a higher content of vanillic, caffeic, catechin, cholchecien, *p*-hydroxybenzoic and chrysin than control. On the other hand, the contents of gallic, protocatechuic, caffeine acids were decreased in T 15min and M 1min samples as compared with control seeds. The increase in polyphenolic acids in M 3min seeds was due to the release of polyphenolics from the beans seeds during treatment. It is clear that the microwaving might accelerate more bound phenolic compounds releasing from the breakdown of cellular constituents.

CONCLUSION

The study showed that there are significant differences ($P<0.05$) in the gross chemical composition of the white beans samples. T 45min was the best in terms of most of the properties determined while control was

the least. The data obtained in the present investigation also pointed to that the changes in levels of all polyphenol classes and consequently the changes in the antioxidant activity of these compounds might be due to the effect and function of thermal treatment on white beans seeds.

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