Journal of Medicinal Plants Research Vol. 6(30), pp. 4562-4566, 8 August, 2012
Available online at http://www.academicjournals.org/JMPR
DOI:10.5897/JMPR12.248
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Antioxidant activity and phenolic content of germinated lentil (*Lens culinaris*)

Maryam Gharachorloo[1], Babak Ghiassi Tarzi[1]*, Marzieh Baharinia[1] and Amir Homan Hemaci[2]

[1]College of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[2]College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University, Tehran, Iran.

Accepted 6 June, 2012

In this study, the changes in phenolic compounds and antioxidant activity of lentil seeds before and after germination were investigated. Lentil seeds were germinated in dark chambers maintained near 100% relative humidity at 20°C. Three different solvents were employed to extract the phenolic compounds present in lentil seeds and sprouts. Total content of phenolic compounds were measured by Folin-Ciocalteau method and antioxidant activity determined by two different methods: assay of hydroxyl radical scavenging activity method and oven test method. For the later method, two different concentrations of extracts (0.02 and 0.1% w/w) were added to sheep tallow and the stabilities of the treatments were determined. Peroxide value and induction period measurements were used to evaluate the *in vivo* antioxidant activities. The results indicated that germination modifies the quantity and quality of phenolic composition of lentil, and the solvent used for extraction influence the antioxidant activity. The evaluation of *in vivo* antioxidant activity of the extracts measured by delay in tallow oxidation indicated that the activity was dependent on the phenolics concentration of extract which increased when higher concentrations of the extracts were applied. These data suggested that lentil sprout flour or extract can be used as a source of natural antioxidants in functional foods.

Key words: Antioxidant activity, germination, lentil, phenolic compounds.

INTRODUCTION

Legumes, the staple food in many regions of the world recently have been studied for their antioxidant properties, because of increasing interest about the health benefits associated with antioxidants. Meanwhile, studies on changes in antioxidant activity during processes such as germination have also been of great interest (Fernandez et al., 2009). Dietary antioxidants may play an important role in protecting the cell against damage caused by free radicals. Consumption of foods containing antioxidants may prevent some diseases and therefore, it is very important to determine their antioxidant capacity in order to estimate the repercussion on oxidative stress in living beings (Doblado et al., 2007).

Legumes contain several antioxidant compounds such as vitamins C and E, phenolic compounds and reduced glutathione (Fernandez et al., 2009), which are considered to be natural antioxidants, representing an important group of bioactive compounds in foods, and may prevent the development of many diseases. Phenolic compounds do not only effectively prevent oxidation in foods; they also act as protective factors against oxidative damage in the human body. The antioxidant activity of phenolics is related to their chemical structure (Lopez et al., 2006). Dietary antioxidants protect against reactive oxygen species in the human body by several mechanisms. An increased intake of antioxidants may therefore have a number of health effects, such as reducing the incidence of cardiovascular diseases and cancer (Fernandez et al., 2006). There is also an increase in the acceptance of formulated foods containing compounds extracted from natural sources which are known by their active substances such as the phenolic

*Corresponding author. babakghiassi@hotmail.com or b.ghiassi@srbiau.ac.ir. Tel: +98 21 44 86 5500 or +98 21 44 86 5400.

compounds (Vasantha et al., 2006). With the aim of improving the nutritive value of legumes, preparation techniques including germination have been developed to significantly raise the bioavailability of their nutrients (Urbano et al., 2005). Germination starts when the dry seed begins to take up water and is completed when the embryonic axis elongates. At this point, reserves within the storage tissues of the seed are mobilized to support seedling growth. From the moment the seed breaks dormancy, protective responses emerge through the synthesis of phenolics and other compounds. Testing 13 edible seeds for the levels of phenolic compounds and the antioxidant activity at different germination stages indicated that at initial germination stages phenolics may serve as radical scanvengers or antioxidants, while later they could become part of the structural framework of the growing plant and lose some of their antioxidant efficiency. Also, it has been reported that the increases in phenolic content on a dry base from dormant seed to 7 days sprout for lentil was about 185% (Cevallos and Cisneros, 2010).

With the current upsurge of interest about the efficiency and function of natural antioxidants in food and biological systems, the testing of antioxidant activity has received much attention. Thus, because of the effect of germination on nutritional value of legumes, this study was conducted to evaluate such bioprocess on lentil's (*Lens culinaris*) phenolics composition and antioxidant activity in order to improve the content of antioxidant compounds, and obtain processed lentil flours with added value that could be used by the food industry as functional ingredients (Fernandez et al., 2009).

MATERIALS AND METHODS

Lentil (*L. culinaris*) seeds were obtained from local market in Tehran. Seeds were sorted, cleaned and stored in darkness in polyethylene containers at 4°C. Mutton tallow was obtained by dry rendering of sheep tail fat under vacuum employing a rotary evaporator at 60 rpm and 80°C for 2 h. The fatty acid composition of tallow was determined by gas chromatography according to the AOAC official method (Method 969/33) employing a Varian Star 3400 gas chromatograph equipped with a DEGS capillary column (30 m 9 0.25 mm i.d.) and a flame ionization detector. The injector, column oven, and detector temperatures were 240, 200 and 280°C, respectively (Firestone, 1990). All the chemicals used were of analytical grade, purchased from Merck Chemical Company of Germany.

Germination process

Lentil seeds (200 g) were soaked in 1000 ml of 0.07% sodium hypochlorite for 30 min. These seeds were washed with distilled water to neutral pH and then were soaked in 1000 ml of distilled water for 5 h, shaking every 30 min. The hydrated seeds were located in germination trays on wet laboratory paper and covered, where water circulation by capillarity was created. The trays were introduced into the germinator (Model IKH-RH). The seeds were germinated at 20°C, 99% relative humidity in darkness for 5 days. The maximum time of germination was chosen when 95% seeds sprouted. The sprouted seed were collected, ground in the mill [Triplex, France], passed through a sieve of 0.5 mm and the obtained flour was stored in plastic bags, in darkness at 4°C (Lopez et al., 2006). A blank consisted of flour of ungerminated grains was also prepared.

Preparation of phenolic extracts

Flour of lentil seeds and sprouts were subjected to phenolics extraction using methanol, acetone and hexane separately. The extraction process was continued for 24 h at room temperature by stirring the flours in three solvents. The extracts were centrifuged at 4000 rpm for 15 min and filtered through filter paper (Whatman No. 41). The solvents were removed from the extracts using a rotary evaporator at 40°C under vacuum. The extracts were further dried using vacuum oven at 40°C and were kept in dry clean black glass bottle at 4°C for further analysis.

Assay of total phenolic compounds

Total phenolic compounds content was determined according to Fernandez-Orozco et al. (2006). The method is based on the colour reaction of Folin-Ciocalteu reagent with hydroxyl groups. Reaction absorbance was measured at 765 nm using a spectrophotometer (Optizen 2120, South Korea). The results were expressed as mg gallic acid per kg of extract.

Assay of hydroxyl radical (OH^-) scavenging activity

The assay was based on the benzoic acid hydroxylation method, as described by Chung et al. (1997). In a screw-capped tube, 0.2 ml sodium benzoate, (10 mmol) and 0.2 ml of $FeSO_4.7H_2O$ (10 mmol) and ethylene diamine tetraacetic acid (EDTA) (10 mmol) were placed. Then, an aliquot of the sample and phosphate buffer (pH 7.4, 0.1 mol) were added to give a total volume of 1.8 ml. Finally, 0.2 ml of a H_2O_2 solution (10 mmol) was added. The mixture was then incubated at 37°C for 2 h. After that, the fluorescence was measured at 407 nm emission (Em), and at 305 nm excitation (Ex). OH-scavenging activity was expressed as follows:

$$[\%] = [1 - (FIs-FIo) / (FIc-FIo)] \times 100$$

Where FIo is fluorescence intensity at Ex 305 and Em 407 nm with no treatment, FIc is fluorescence intensity at Ex 305 and Em 407 nm of treated control, and FIs is fluorescence intensity at Ex 305 nm and Em 407 nm of treated sample.

Assay of antioxidant activity by the oven test method

Mutton tallow which might contain minor natural antioxidants was used as a basic substrate for evaluation of the antioxidant activity of lentil sprout extracts. The collected hexane, acetone and methanolic extracts were added to 100 g mutton tallow at the concentrations of 0.02 and 0.1% (w/w) to examine their antioxidant activity. Induction period measurements for each treatment were performed on Metrohm Rancimat model 743 at 110°C with an air flow of 20 L/h. Peroxide value determinations were carried out by placing the treatments in the oven at 90°C and measuring the peroxides every 24, 48, 72, 96 and 120 h according to AOCS method, Cd 8 – 53 (Firestone, 1994).

Table 1. Total phenolic content of lentil seed and lentil sprout extracts obtained from three different solvents (mg/kg).

Sample/solvent	Hexane	Methanol	Acetone
Lentil	29.20 ± 0.05 [a]	53.00 ± 0.10 [b]	96.70 ± 0.00 [c]
Lentil sprout	51.80 ± 0.01 [b]	78.00 ± 0.00 [d]	105.80 ± 0.07 [e]

*The values are expressed as means ± standard deviation. There is no significant difference between similar letters ($p < 0.05$).

Table 2. Antioxidant activity of lentil seed and lentil sprout extracts obtained from three different solvents (%).

Sample/solvent	Hexane	Methanol	Acetone
Lentil	15.00 ± 0.5 [a]	63.00 ± 1.0 [b]	89.00 ± 0.5 [c]
Germinated lentil	87.00 ± 1.5 [c]	87.00 ± 1.0 [c]	90.00 ± 0.5 [c]

*The values are expressed as means ± standard deviation. There is no significant difference between similar letters ($p < 0.05$).

Statistical analysis

All the experiments and measurements were carried out in duplicated order. The data were statistically analyzed using the Statistical Analysis System software package on replicated test data. Analyses of variance (ANOVA) were performed by application of ANOVA procedure. Significant differences between the means were determined using Duncan multiple range test.

RESULTS AND DISCUSSION

The total phenolic content of extracts obtained from different solvents for lentil seeds and lentil sprouts is shown in Table 1. The highest extraction rate of phenolic compounds for both lentil seeds and sprouts was obtained by acetone. Statistical analysis indicated significant differences ($p \leq 0.05$) between total phenolic content of lentil seed and lentil sprout extracts obtained from each solvent. Lopez-Amoros et al. (2006) indicated that lentils contain different concentrations of the hydroxybenzoic phenolic compounds, protocatechuic, vanillic acid, aldehyde p-hydroxybenzoic, trans-ferulic acid, and trans-p-coumaric acid. They have also identified a derivative of trans-p-coumaric acid in very low concentration, the catechin together with the procyanidin dimers B2 and B3, the trimer C1 and a tetramer procyanidin.

The results showed that the amount of phenolic compounds increased significantly ($p \leq 0.05$) after germination. Although the increase of phenolic com-pounds is independent of the applied solvents, there was a significant difference ($p \leq 0.05$) in the amounts of extracted phenolic compounds among different solvents. Our findings are in accordance with Lopez-Amoros et al. (2006) which indicated that germination modifies the quantity and quality of phenolic compounds of legumes.

As shown in Table 2, the antioxidant activity of lentil sprout obtained from different solvents is not significant ($p \leq 0.05$), but the antioxidant activity of lentil seed is significantly different for various solvents. This might be due to difference in solvent polarity and consequently type of the extracted compounds. The results showed a significant increase in the antioxidant activity of lentil seeds after germination except in acetone extract. Further, increase in antioxidant activity was found to be significantly dependent on the applied solvent.

Acetone extracts from both seed and sprouts contained the highest amount of total phenolic compounds but antioxidant activity of germinated lentil was not significantly different among solvents, thus, it seems that additional antioxidants other than polyphenols may be present in the extracts. Legumes contain, together with phenolics, other bioactive compounds such as vitamins and carotenoids in different concentrations (Atienza et al., 1999) that can also affect the antioxidant activity of the samples. These compounds may exert a synergetic effect with phenolic compounds, which could be the reason for the observed differences in the antioxidant activity. These increases in phenolics content and antioxidant activity show potentially important role of phenolics during seed germination, as well as the potential enhancement of the nutraceutical value of seeds by the germination process (Cevallos and Cisneros, 2010).

Mutton tallow which contains minimal amount of natural antioxidants was used as a basic medium for evaluation of antioxidant activity of lentil sprout extracts by the method of ‘delay in fat oxidation’. Analyzing the fatty acids profile of tallow with gas chromatography indicated that tallow contains 49.3% unsaturated and 46.4% saturated fatty acids.

Peroxide value of tallow without the addition of any

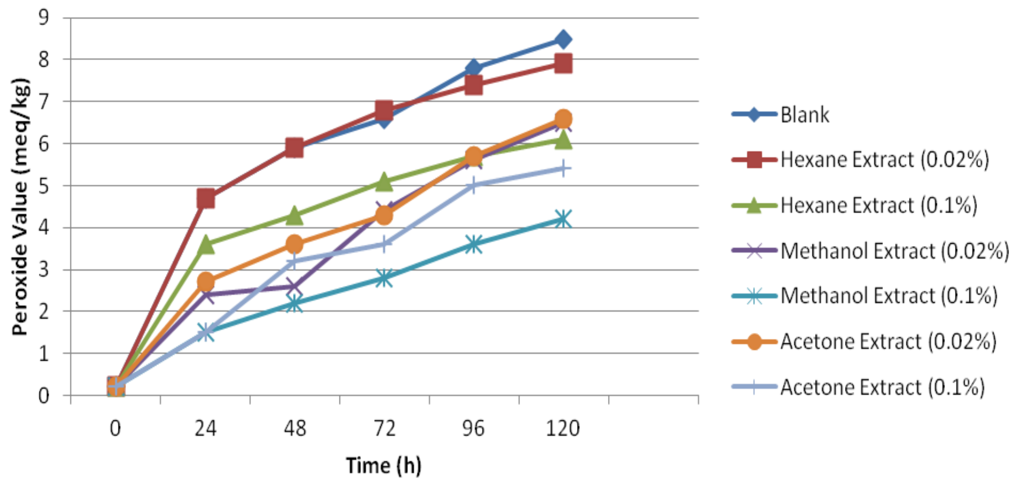

Figure 1. Peroxide values of tallow with different concentrations of lentil sprouts extracts at 90°C (meq/kg oil).

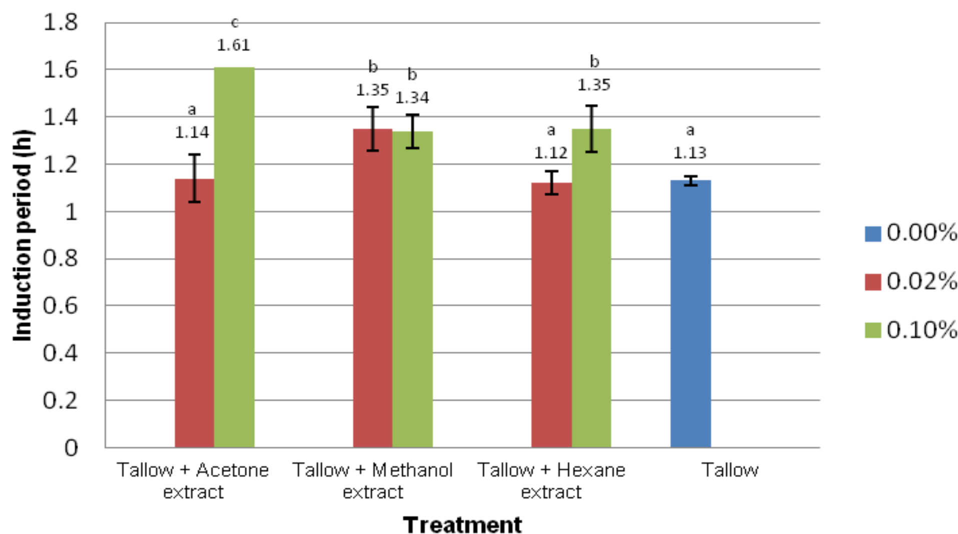

Figure 2. Induction period of tallow treated with 0.02 and 0.1% of lentil sprout extracts.

phenolic extract (blank) was 0.2 meq/kg, which may due to the prior oxidation. Figure 1 shows the peroxide values of tallow treated with different concentrations of extracts at 90°C for 120 h. The results indicated that all concentrations of lentil sprouts extract obtained from all solvents decreased the rate of tallow oxidation, and there were significant differences ($p < 0.05$) between all treatments, which emphasizes the positive effect of natural antioxidants in delaying the *in vivo* oxidation.

Methanolic extract (0.1%) of lentil sprouts showed lower rate of oxidation and consequently lower peroxide value as compared with hexane and acetone extracts. Although both hexane and acetone extracts of germinated lentil reduced the formation of peroxide, the effectiveness of phenolics present in the extract was depended on their concentration. After 120 h treatment, the lowest peroxide values were obtained for 0.1% methanol, acetone and hexane extracts which is significantly ($p < 0.05$) higher than the blank.

Figure 2 shows the induction period of tallow treated with the different concentrations (0.02% and 0.1%) of lentil sprout extracts. Acetone extracts increased the induction period of tallow more than others. Also, the results of the induction period which is related to the secondary oxidation products confirms the peroxide values, the primary oxidation products, that antioxidant activity increased when higher concentrations of the extracts were applied.

Conclusion

Germination process causes various changes in the phenolic compounds and modifies their antioxidant activity, therefore, lentil sprout flour or extract can be used as a source of natural antioxidants in functional foods.

REFERENCES

Atienza J, Sanz M, Herguedas A, Alejos JA, Jimenez JJ (1999). Beta-carotene alpha-tocoferol and gamma-tocoferol contents in dry legumes. Influence of cooking. Food Sci. Technol. Int. 4:437–441.

Chung SK, Osawa T, Kawakish S (1997). Hydroxy radical scavenging effects of spices and scavengers from brown mustard (*Brassica nigra*). Biosci. Biotech. Biochem. pp. 118-123.

Cevallos CBA, Cisneros-Zevallos L (2010). Impact of germination on phenolic content and antioxidant activity of 13 edible seed species. Food Chem. 119:1485–1490.

Doblado R, Frias J, Vidal-Valverde C (2007). Changes in vitamin C content and antioxidant capacity of raw and germinated cowpea *(Vigna sinensis var*. carilla) seeds induced by high pressure treatment. Food Chem. 101:918–923.

Fernandez-Orozc R, Frias J, Zielenski H, Munoz R, Piskula K, Kozlowska H, Vidal-Valverde C (2009). Evaluation of bioprocesses to improve the antioxidant properties of chickpeas. LWT - Food Sci. Technol. 42:885–892.

Fernandez-Orozco R, Piskula MK, Zielinski H, Kozowska H, Frias J, Vidal-Valverde C (2006). Germination as a process to improve the antioxidant capacity of *Lupinus angustifolius* L. var. Zapaton. Eur. Food Res Technol. 223:495–502.

Firestone D (1990). Official methods of analysis of the Association of Official Analytical Chemists, 15th edn. Association of Official Analytical Chemists, Arlington.

Firestone D (1994). Offical methods and recommended practices of the American Oil Chemists Society, 4th edn. AOCS Press, Champaign.

Lopez-Amoros ML, Hernrndez T, Estrella I (2006). Effect of germination on legume phenolic compounds and their antioxidant activity. J. Food Compost. Anal. 19:277–283.

Urbano G, Aranda P, Vilchez A, Aranda C, Cabrera L, Porres JM, Lopez-Jurado M (2005). Effects of germination on the composition and nutritive value of proteins in *Pisum sativum*, L. Food Chem. 93:671–679.

Vasantha RHP, Jayasankar S, Lay W (2006). Variation in total phenolics and antioxidant capacity among European plum genotypes. Sci. Hortic-Amsterdam. 108:243–246.