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Antioxidant Activity of Pulse Hydrocolloids: Classical Screening Methods Depending on Water Soluble Phenolic Antioxidants Need Revision to Measure True Antioxidant Potential of Pulses

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Abstract

The aim of this study is to show the presence of considerable amounts of antioxidants bound on pulse hydrocolloids. For this purpose, 6 lentil and 4 chickpea cultivars were tested for their free radical scavenging based antioxidant capacities in water soluble extracts and hydrocolloid extracts. The results clearly showed that the antioxidant potential of pulses based on hydrocolloid extracts accounts for 28 to 89% of those based on soluble phenolic antioxidants. Moreover, the antioxidant activity measurements in hydrocolloid extracts help much more than those in water extracts to determine differences among the antioxidant potentials of pulse cultivars. Extensive screening studies have been conducted by plant breeders to understand the importance of different phenotypic and genetic factors as well as the growth conditions on antioxidant status of the plants. This work clearly showed that not only water extracts, but also hydrocollid extracts should be considered to conduct realistic screening studies.

Keywords: Legumes; Chickpea; Lentil; Proteins; Antioxidant activity.

Introduction

An improvement of the antioxidant status of plants is not only important to increase their resistance or tolerance against stress factors such as diseases, drought and salinity, but it is also important to increase the health benefits of their edible parts on human. Thus, extensive screening studies have been conducted by plant breeders to understand the importance of different phenotypic and genetic factors as well as the growth conditions on plant antioxidants. The majority of these studies focus on water or solvent extraction of soluble phenolic antioxidants and measurement of their free radical scavenging based antioxidant potentials. However, recent studies have suggested that an important part of the phenolic antioxidants in some plant parts could be bound by the plant hydrocolloids including proteins and carbohydrates which need a particular extraction and testing procedure [1,2]. The phenolic compounds mostly bind hydrocolloids non-covalently since their phenolic hydroxyl groups are capable to form H-bonding with peptide carbonyl groups of proteins [3] and hydroxyl groups of carbohydrates [4,5]. Hydrophobic interactions also cause non-covalent binding of phenolic compounds on surfaces of carbohydrates and proteins [5,6]. Moreover, some oxidized phenolic compounds can bind proteins and carbohydrates covalently [1,7]. Thus, the contribution of hydrocolloids in antioxidant activity of phenolic, protein and carbohydrate rich sources like cereals and legumes should be considered very carefully. It was reported that in lentils, 82-85 % of total antioxidant activity was formed by bound phytochemicals, while this percentage changes between 25 and 39% in many other legumes including chickpeas, yellow and green beans, and soybeans [8]. Serpen, *et al.* [2] reported that the contribution of bound antioxidants in cereal based food is minimum 50%. There is a great interest in hydrocolloid bound phenolic antioxidants since it was thought that the digestible hydrocolloids like proteins and starch may act as carrier for the phenolic compounds along the digestive system. The release of bind phenolic compounds (or exposure of their bind antioxidant groups) following protein and starch digestion could be the major factor responsible for the health benefits of pulses and other legumes including soy beans. Recently, we showed the high bound phenolic content and antioxidant capacity of lentil proteins [9]. In this work, we compared the antioxidant potentials of water extracts and hydrocolloid extracts from different dry seeds of pulse cultivars. This work aimed to show significance of antioxidant activity present in pulse hydrocolloids and differences in ranking of antioxidant potentials of different pulse cultivars depending on type of extract used in screening. This work makes sense after the recent findings about the preventive/protective effects of legume phenolics on cardiovascular disease and cancer [3,10,11] and strong suggestions of the American Dietetic Association to increase consumption of pulses [12].

Materials and Methods

Materials: The cultivars Cevdetbey 98 and Sarı-98 were obtained from Aegean Agricultural Research Institute in Menemen, Turkey. The remaining 8 cultivars were obtained from General Directorate of Agricultural Research in Ankara, Turkey. The seeds were grown and harvested in the experimental fields for research purposes.

Preparation of water extracts from pulses: The pulses (10 g) were first rehydrated in 50 mL distilled water for 16-18h at room temperature and crushed in a ceramic mortar. Then, samples were further homogenized in a Waring blender in 90 mL distilled water for 3 minutes and filtered through 3-layers of cheesecloth to collect the filtrate. The 30 mL of the obtained filtrate from each sample was centrifuged for 30 min at 15000×g (+4° C) for clarification and assayed for its antioxidant capacity. The extracts were named as chickpea (CWE) and lentil (LWE) water extracts.

Preparation of hydrocolloid extracts from pulses: The pulses were first processed to acetone powder (AP) by repeated excessive washing and homogenization with cold acetone as described by Arcan

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and Yemenicioğlu [13]. The use of AP enables extraction of pulse hydrocolloids containing bound phenolic compounds, but lacking free phenolic compounds [9]. Then, 20 g of AP was suspended in 250 mL distilled water under continuous magnetic stirring. The pH of the suspension was adjusted to 9.5 by 1 mol/L NaOH solution to maximize solubilization of proteins. For preparation of chickpea hydrocolloid extract (CHE), the temperature of the obtained extract was brought to 85° C (to increase extraction yield). The extract was then stirred at this temperature for 30 min, and then it was cooled to room temperature by further stirring in ice water bath for 15 min. For preparation of lentil hydrocolloid extract (LHE), the extraction was conducted at room temperature for 45 min under continuous magnetic stirring. The hot extraction applied for CHE could not be employed for LHE since it showed extreme browning during heating. At the end of the extraction period, the pHs of both CHE and LHE were adjusted to 7.0 with 1 mol/L acetic acid solution. The extracts were clarified by centrifugation at 15000 x g (+4° C) for 30 min and then lyophilized for antioxidant activity measurements. During antioxidant activity tests 0.01 g/mL lyophilized CHE or LHE suspension was prepared in distilled water by stirring with a magnetic stirrer for 30 min at 30° C. This suspension was

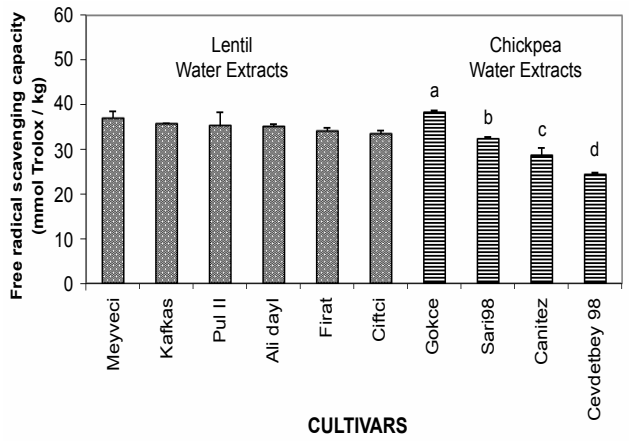

Figure 1: Antioxidant capacities in water extracts of pulses (values with different letters are significantly different at P<0.05 as determinedby Fisher's protected least significant difference).

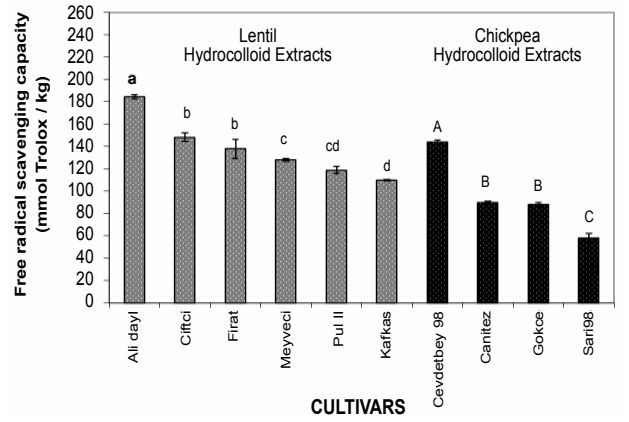

Figure 2: Antioxidant capacities in hydro colloid extracts of pulses (values with different letters are significantly different at P<0.05 as determined by Fisher's protected least significant difference).

then centrifuged at 15000×g (+4° C) for 30 min for clarification and tested for its antioxidant capacity. The average soluble protein contents of CHE and LHE obtained by these procedures were 23 % and 40 %, respectively (tested with the Bradford method using bovine serum albumin as standard).

Determination of antioxidant activity: The antioxidant activity tests were conducted spectrophotometrically by using the ABTS free radical by the Area Under the Curve (AUC) method given in Re et al (1999) [14]. All measurements were conducted three times and antioxidant activity was expressed as Trolox equivalents (mmol) per kg of dry legumes or per kg of lyophilized hydrocolloid extracts (for only LHE and CHE).

Determination of total phenolic content: Determined spectrophotometrically by the Folin-Ciocalteu reagent [15].

Results and Discussions

Antioxidant activities of water extracts: The antioxidant activities of pulse water extracts rich in free soluble phenolics were given in (Figure. 1) (The total phenolic content of CWEs and LWEs changed between 2869 and 4275 mg gallic acid/kg). The antioxidant capacities for LWEs of different cultivars did not show statistically significant differences (P >0.05) and changed between 30 and 40 mmol Trolox/kg of dry pulse. In contrast, significant differences exist in the antioxidant capacities of CWEs (P <0.05). The highest antioxidant capacity measured for CWE of Gökçe cultivar (38.20 mmol Trolox/kg of dry pulse) is almost 1.6 fold higher than the lowest antioxidant capacity measured for CWE of Cevdetbey 98 cultivar.

Antioxidant activities of hydrocolloid extracts: The antioxidant capacities of CHE and LHE extracted from AP to remove free phenolic antioxidants varied between 8.9 and 22.0, and 15.5 and 19.0 mmol Trolox/kg of dry pulses, respectively (Figure. 1). The highest antioxidant capacity measured for CHE of Cevdetbey-98 cultivar (22 mmol Trolox/kg of dry pulse) is 2.5 fold higher than the lowest antioxidant capacity measured for CHE of Sarı 98 cultivar. However, the highest antioxidant capacity measured for the LHE of Çiftçi cultivar is only 1.2 fold higher than the lowest antioxidant capacity measured for Pul-II cultivar. The total amount of soluble hydrocolloids obtained from different lentil and chickpea cultivars changed between 9 % and 15 %, and 14 % and 16 % of dry pulses (w/w), respectively. However, the antioxidant capacities of LHEs and CHEs equal to 44 % to 57 % and 28% to 89% of LWEs and CWEs, respectively. Thus, it is also important to consider the individual antioxidant capacities of CHE and LHE per kg of dry hydrocolloids. As seen in (Figure. 2), the antioxidant capacities of CHE and LHE varied between 60 and 180 mmol Trolox/kg of hydrocolloids and showed significant variations. The highest antioxidant capacity measured for LHE of Alidayı cultivar (185 mmol Trolox/kg hydrocolloids) is 1.7 fold higher than the lowest antioxidant capacity measured for LHE of Kafkas cultivar. The highest antioxidant capacity measured for CHE of Cevdetbey 98 cultivar (144 mmol Trolox/kg hydrocolloids) is also 2.5 fold higher than the lowest antioxidant capacity measured for CHE of Sarı 98 cultivar. Thus, it appears that the antioxidant activities calculated per kg of hydrocolloids is quite useful to determine the differences among the antioxidant potentials of lentil cultivars that could not be differentiated using water extracts. It is also worth to report that the Cevdetbey 98 cultivar that showed the lowest antioxidant capacity among CWEs, showed the highest antioxidant capacity among CHEs.

These results showed that the ranking of pulse cultivars based on their water soluble antioxidant potential could be insufficient to reflect

their real antioxidant potential. The hydrocolloids in pulses are among their major antioxidants and should be evaluated separately than water extracts that are rich mainly in soluble phenolic antioxidants. Future studies on antioxidant hydrocolloids might cause dramatic changes in classical extraction methods depending on soluble phenolic antioxidants.

Conclusion

In conclusion, this work clearly showed the presence of significant amount of antioxidant activity originating from pulse hydrocolloids. Digestible hydrocolloids like proteins and starch could bind excessive amounts of phenolic antioxidants and they may act as a carrier for these bioactive compounds along the digestive system. Further studies on bioavailability of hydrocolloid bound phenolic might cause dramatic changes in classical extraction and testing methods of phenolic antioxidants.

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