

## Effect of processing treatments on polyphenol removal from kernel of two Iranian acorns varieties

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### Abstract

In the present study, the effects of different process variables including boiling, autoclaving, roasting and soaking in examined solutions (water, acetic acid, NaOH and NaCl) on the removal of polyphenol compounds of two varieties of acorn, namely *Quercus branti* var. *persica* and *Quercus castaneifolia* var. *castaneifolia* were investigated. Polyphenol content in *Q. castaneifolia* and *Q. branti* were 9.11 and 4.33 (g/100g), respectively. All applied processes except roasting treatment, caused a significant ( $p < 0.05$ ) decrease in the polyphenol contents. Boiling reduced the concentrations of polyphenols of *Q. branti* by 14-52% and of *Q. castaneifolia* by 47-52%. In water soaking, with increasing of soaking time and temperature, the reduction percentage increased. Soaking in dilute NaCl solution (1%) eliminated around 39-56% and 42-47% of total phenol content from *Q. branti* and *Q. castaneifolia*, respectively. In conclusion soaking in alkali solutions can be mentioned as the most effective method in reducing levels of polyphenol content (*Q. branti* by 73-91% and *Q. castaneifolia* by 65-89%), especially in culture medium.

### Keywords

Acid treatment  
Acorn fruit  
Alkali treatment  
Heat treatment,  
Polyphenolic removal  
Soaking

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### Introduction

The acorn is an edible oval fruit of oak trees which has been extensively used as food and feed (Correia *et al.*, 2013). Oak acorn is rich source of carbohydrates, amino acids, proteins, lipids, various sterols and vitamins (Rakic *et al.*, 2007). The numbers of processed acorn products such as different types of breads and jelly, which are comprised principally of accord flour, have been increased recently.

Besides nutritious components, acorn contains considerable amounts of tannin and other phenolic substances (Rakic *et al.*, 2006). Polyphenols compounds as one of the most numerous and ubiquitous group of secondary metabolites of plant are an integral part of both human and animal diets with a great diversity ranging from simple phenolic molecules to highly polymerized compounds in their structures (Bravo, 1998). Although the pharmacological and therapeutic properties of some phenolic compounds are well demonstrated in the different literatures, there is no study related to their nutritional role in our diet. They are classified in category of antinutritional group along with

other components such as phytats, lectins, enzyme inhibitors, saponins, etc (Deshpande, 2002). Polyphenols have the potential to bind positively-charged proteins, amino acids and/or multivalent cations or minerals such as iron, zinc and calcium in foods (Gilani *et al.*, 2005). Thus, they could reduce the bioavailability of essential minerals in addition to reduce their content (Khandelwal *et al.*, 2009). The undesired astringent properties and bitter taste of polyphenols are thought to be caused by precipitation with salivary proteins (Lesschaeve and Noble, 2007).

Inactivation and/or removal of undesirable components are essential steps in improving the nutritional quality and organoleptic acceptability of plant food (Shimelis and Rakshit, 2007). Traditional processing techniques including dehulling, thermal treatment (ordinary cooking, pressure cooking and roasting), germination, soaking in different solutions (water, ash, alkali and acid) and fermentation could reduce antinutritional agents in many grains, seeds and vegetables. Cooking has often considered as means to improve the texture, palatability and nutritive value of cereal due to gelatinization of starch, denaturation of proteins, increased nutrient

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availability and inactivation of heat labile toxic compounds (Uppal and Bains, 2012). The profiles and quantities of polyphenols and tannins in foods are affected by processing due to their highly reactive nature, which may affect their antioxidant activity and the nutritional value of foods (Dlamini *et al.*, 2009).

In some region of Iran, acorn flour has been used as an additive for the bread preparation by the people. For this purpose, acorn kernels were dehulled and then soaked in water or ash solution overnight or boiling in water for a few minutes in order to remove tannin and reduce bitter taste. However, there are no scientific records on the polyphenol removal of acorn fruits, especially among the Iranian varieties. Therefore, current study was aimed to investigate the effects of different methods such as boiling, autoclaving, roasting and soaking in different solutions on polyphenol reduction. The utilization of acorns would be greatly enhanced if a more effective method would be discovered and acorn flour with desirable taste and light color prepared.

## Material and Methods

### Preparation of samples

Two different varieties of Iranian oak acorns, namely, *Quercus branti* var. *persica* (Qb) and *Q. castaneifolia* var. *castaneifolia* (Qc) were used in this study. Acorns of Qb and Qc varieties were collected in December 2008 from oak forest of Bazoft, Charmahal-Bakhtiari province and Ghorogh forest, Golestan province, Iran, respectively. The fruits were cleaned manually to remove all foreign materials, damaged and immature fruits. The acorns were dried at room temperature and then shelled out. Internal brown husk was removed with knife and then the kernels were crushed and ground to pass through 20-mesh screen. Samples were placed in plastic containers and stored at 4°C until the day of experiments. All chemicals and reagents used were of analytical grade.

### Boiling

Crushed kernels of two varieties (10 g) were boiled in distilled water (100°C) in sample: water ratio of 1/10 (w/v) for 20, 40 and 60 min.

### Autoclaving

Ten g of each type of two varieties was mixed with distilled water in sample: water ratio of 1:10 (w/v) and was autoclaved at 15 psi (121°C) for 10, 20 and 30 min.

### Roasting

Twenty g of each type of two varieties was placed in hot glassy plate as thin layer and roasted for 30 min at 120 and 150°C. Samples were cooled and stored in plastic containers at 4°C for further use.

### Soaking in water

Ten g of each type of two varieties was soaked in distilled water in samples: water ratio of 1/10 (w/v) for 6, 12, 18 and 24 h at two different temperatures of 25 and 50°C.

### Soaking in alkali, acidic and salt solution

Ten g of each type of two varieties was soaked in 100 mL of three different soaking solution including NaOH (0.1, 0.5 and 1M), acetic acid (0.1, 0.5 and 1M) and NaCl (1, 5 and 10%) for 6, 12, 18 and 24 h. The soaking solutions were discarded and samples were washed five times with distilled water.

### Preparation of samples for analysis

After each treatment, excess of water or other solutions were discarded; samples were rinsed with distilled water and dried at 50°C until to a constant weight. All the raw and processed materials were ground in household Mill (Sunny, SBG-450) to 60-mesh size and stored in plastic container at 4°C until day of experiment.

### Determination of total phenol content (TPC)

Total phenolic content (TPC) was determined by Folin-Ciocalteu assay and the standard curve ( $R^2=0.9945$ ) was prepared by using tannic acid (Esenwah and Ikenebomeh, 2008). Average values of triplicate estimations were expressed as g tannic acid equivalent /100 g of the samples on dry weight basis.

### Statistical analysis

Repeated measures analysis of variance (ANOVA) was applied using SAS software version 9.1 (Institute Inc, Cary, NC, USA).

## Results and Discussions

### Total phenolic content (TPC)

Results of analysis on unprocessed samples showed that the two varieties had high level of phenolic compounds,  $4.33 \pm 0.17$  and  $9.11 \pm 0.38$  for Qb and Qc, respectively. Significant differences ( $p < 0.05$ ) in TPC were observed between two varieties. Diversity in quantities of polyphenols and other phytochemical present in plant foods may be due to the variety of fruit, plant genetics, sunlight, soil composition, season, region of cultivation, stage of maturity and

Table 1. Effect of boiling and autoclaving on the polyphenol content (g TAE/100g) of two acorns varieties

Treatment	Time (min)	<i>Q. branti</i>		<i>Q. castaneifolia</i>	
		TPC	% Loss	TPC	% Loss
Raw	0	4.48 <sup>a</sup>	-	9.61 <sup>a</sup>	-
	20	3.88 <sup>b</sup>	13.39	5.13 <sup>b</sup>	46.61
	40	2.77 <sup>c</sup>	38.16	5.06 <sup>b</sup>	47.34
Boiling	60	2.14 <sup>d</sup>	52.23	4.60 <sup>d</sup>	52.13
	10	2.18 <sup>d</sup>	51.33	5.15 <sup>b</sup>	46.40
	20	2.03 <sup>d</sup>	54.68	5.02 <sup>bc</sup>	47.76
Autoclaving	30	1.66 <sup>e</sup>	62.94	4.81 <sup>cd</sup>	49.94

Mean values in same columns sharing different letters are statistically different ( $p < 0.05$ ).

All values are averages of three determinations.

Loss % was calculated using original unprocessed beans as starting materials.

post harvest maturity (Faller and Fialho, 2009). Total phenolic content determined for two varieties were lower than the value 12.33% which was reported for *Q. robur* (Rakic *et al.*, 2006). Shimada (2002) found that tannin content in *Q. serrata* and *Q. mongolica* was 7.3 and 11.17% (d.b), respectively. Values determined for two varieties were higher than those reported for cereals and legumes (Towo *et al.*, 2003).

### Boiling

The effect of boiling treatment on the TPC of two acorn varieties was shown in Table 1. Significant reduction ( $p < 0.05$ ) in the level of TPC was found when the boiling time was increased. Boiling Qb variety in water for 20, 40 and 60 min resulted in 13.31, 38.16 and 52.23% decrease in TPC, respectively. In the case of Qc variety, significant ( $p < 0.05$ ) decline in TPC was observed up to 20 min and thereafter, phenolic compounds were eliminated slowly. The TPC of chickpea, green pea and yellow pea were reduced by 37.5, 50.8 and 44.9% after boiling in tap water for 120 min (Xu and Chang, 2008). Reduction in the level of polyphenols during boiling was lower than 57-67% reduction, which was reported for *Bauhinia purpurea* (Vijayakumari *et al.*, 2007) but higher than 32%, which was reported for green gram (Grewal and Jood, 2006). Khattab and Arntfield (2009) reported that boiling resulted in significant reduction in tannin content and other antinutritional agents of legume seed. Since the phenolics are water-soluble and heat-labile substances, the reduction in TPC during boiling can be ascribed to the increase in their leaching out to boiling water under the

influence of concentration gradient or degradation by heat (Uzogara *et al.*, 1990). Boiling also causes rapid disruption of cellular compartments resulting in easier accessibility of protein and polyphenol interaction and promoting inactivation of the latter (Laurena *et al.*, 1987).

### Autoclaving

The phenolic content of two varieties was reduced significantly ( $p < 0.05$ ) during pressure-cooking (Table 1). The reported reduction of phenolic compounds for Qb and Qc varieties was 51-63 and 46-50%, respectively. Generally, extending autoclaving period increases the leaching out of phenolic substances. Autoclaving *Bauhinia purpurea* for longer periods resulted in additional tannin and phenolic losses (Vijayakumari *et al.*, 2007). The decrease in polyphenol content was high during the first 10 min and slowly declined up to 60 min. Some phenolic compounds such as tannic acid are not easily destroyed or eliminated in a short time period of a heating process; therefore a longer heating time is required in order to reduction in their content (Somsub *et al.*, 2008). Uzogara *et al.* (1990) suggested that the reduction in phenolic compounds may be attributed to leaching into cooking water, heat degradation and interaction with other components such as protein to form insoluble complexes. The decline in tannin content during pressure-cooking was also reported for K131 (carioca) dry beans (Nakitto *et al.*, 2015) and Indian pulses, and their cultivar (Khandelwal *et al.*, 2009) It is reported that the cooking and autoclaving remove most of the heat sensitive anti-

Table 2. Effect of roasting on the polyphenol content (g TAE/100g) of two acorns varieties

Temperature (°C)	<i>Q. branti</i>	<i>Q. castaneifolia</i>
	TPC	TPC
Raw	4.48 <sup>a</sup>	9.61 <sup>a</sup>
120	4.58 <sup>b</sup>	11.56 <sup>b</sup>
150	6.28 <sup>c</sup>	13.34 <sup>c</sup>

Mean values in same columns sharing different letters are statistically different ( $p < 0.05$ )

All values are averages of three determinations.

Loss % was calculated using original unprocessed beans as starting materials.

nutritional factors while the heat-stable compound can be removed at limited amount (Shimelis and Ralshit, 2007).

#### Roasting

Total phenolic content of raw and roasted kernel is presented in Table 2. In contrast to wet-heating methods (boiling and autoclaving), roasted samples showed significantly ( $p < 0.05$ ) higher TPC content than raw materials. TPC of raw Qb and Qc acorns increased to 40.5 and 39% after roasting at 150°C, respectively. Similar results were obtained with peanut skin that roasting resulted in 39.5% increase in its phenolic content (Yu *et al.*, 2005). Rakic *et al.* (2007) subjected *Q. robur* and *Q. cerris* kernel to be heated at 200°C for 10 min and found that the level of TPC was increased significantly during roasting. This can be explained by the effect of heat on polyphenol compounds, especially tannin. Hydrolysable tannin degrades at high temperature, causing an increase of non-tannin phenolics, gallic acid contents and consequently increases in TPC (Rakic *et al.*, 2007). Increase in the level of TPC during roasting was also reported for apricot kernel (Durmaz and Alpaslan, 2007) and *Canavalia cathartica* (Seena *et al.*, 2005). In contrast to the result of this research, the concentration of total phenolics was declined during roasting of pearl millet (Nithya *et al.*, 2007) and legume seeds (Khattab and Arntfield, 2009).

#### Soaking in water

The TPC content of two varieties after soaking in water at different times and temperatures were presented in Table 3. Soaking of acorns in water reduced phenolic content in two varieties, significantly ( $p < 0.05$ ). Reductions of TPC as a function of times and temperatures showed a similar trend for both acorns varieties. With increasing soaking time, the

removal of TPC was increased. For Qb variety, soaking for 6 h at 25°C resulted in 38.8% reduction in phenolics, which increased up to 42.6, 46.2 and 47.8% when the period of soaking increased to 12, 18 and 24 hours, respectively. The total phenolic content of Qb variety decreased from 4.5 to 1.6% with an increase in soaking time from 6 to 24 h at 50°C. However, there was no significant difference between the reduction of phenolic after soaking at 18 and 24 h. Soaking in water at 25°C and 50°C caused 42-58.2% and 62.3-72.5% reduction in TPC of Qc variety, respectively. Significant reductions ( $p < 0.05$ ) in the levels of phenolics were observed during the first 18 h of soaking in distilled water at two mentioned temperatures, afterward an increase of soaking time did not cause any significant ( $p < 0.05$ ). Absorption of phenolics by cotyledon in the water was observed by longer soaking times (Xu and Chang, 2008). Soaking temperature had a significant effect ( $p < 0.05$ ) on polyphenol elimination. In both varieties, at each soaking time, TPC reduced significantly ( $p < 0.05$ ) with an increase in temperature from 25 to 50°C. The maximum reduction of phenolic contents was observed when the samples were soaked in distilled water for 24 h at 50°C which resulted in loss of 65.6 and 71.8% of TPC in Qb and Qc varieties, respectively. The reduction in the phenolic content of acorn kernels during soaking in distilled water were higher than the values reported for Indian pulses (Khandelwal *et al.*, 2009). The reduction in the TPC and tannin content during water soaking has been reported for Green gram (Grewal and Jood, 2006). This reduction can be attributed to the solubility of these compounds and leaching them into the soaking water (Vijayakumari *et al.*, 1997). Ruenroengklin *et al.* (2008) reported that the extraction yield of phenolics from litchi fruit pericarp increased as the extraction temperature increased from 30 to 80°C. The increase in the temperature might soften the plant tissue and weaken the phenol-protein and phenol-polysaccharide interactions, thus more polyphenols would leach into the solvent (Shi *et al.*, 2003).

#### Soaking in alkali

The obtained results of soaking in sodium hydroxide solution on phenolic content of the acorn samples were shown in Table 4. All treatments caused significant effects ( $p < 0.05$ ) on the reduction of TPC in comparison to control samples. Soaking in different concentration of sodium hydroxide solution reduced the polyphenol content of the Qb and Qc by 73.4-90.9 and 65.3-89.1%, respectively. These results are comparable to reduction of phenolic contents of red sorgum (78%) during 24 h soaking in 5% NaHCO<sub>3</sub>

Table 3. Effect of soaking in water at various temperatures on the polyphenol content (g TAE/100g) of two acorns varieties

Treatment	Time (h)	<i>Q. branti</i>		<i>Q. castaneifolia</i>	
		TPC	% Loss	TPC	% Loss
Raw		4.48 <sup>a</sup>	-	9.61 <sup>a</sup>	-
	6	2.74 <sup>b</sup>	38.83	5.58 <sup>b</sup>	41.93
Soaking in water (25°C)	12	2.57 <sup>c</sup>	42.63	5.16 <sup>c</sup>	46.30
	18	2.41 <sup>d</sup>	46.20	4.28 <sup>cd</sup>	55.46
	24	2.34 <sup>d</sup>	47.76	4.02 <sup>d</sup>	58.16
Soaking in water (50°C)	6	1.90 <sup>e</sup>	57.58	3.62 <sup>e</sup>	62.33
	12	1.68 <sup>e</sup>	62.50	3.41 <sup>f</sup>	64.50
	18	1.61 <sup>f</sup>	64.06	2.81 <sup>f</sup>	70.75
	24	1.50 <sup>f</sup>	66.50	2.64 <sup>f</sup>	72.52

Mean values in same columns sharing different letters are statistically different ( $p < 0.05$ ).

All values are averages of three determinations.

Loss % was calculated using original unprocessed beans as starting materials

Table 4. Effect of soaking in NaOH at various concentrations on the polyphenol content (g TAE/100g) of two acorns varieties

Treatment	Time (h)	<i>Q. branti</i>		<i>Q. castaneifolia</i>	
		TPC	% Loss	TPC	% Loss
Raw		4.48 <sup>a</sup>	-	9.61 <sup>a</sup>	-
	6	1.19 <sup>b</sup>	73.43	3.33 <sup>b</sup>	65.34
Soaking in NaOH (0.1M)	12	0.94 <sup>c</sup>	79.01	1.98 <sup>cd</sup>	79.39
	18	0.64 <sup>de</sup>	85.71	1.15 <sup>e</sup>	88.03
	24	0.53 <sup>de</sup>	88.16	1.12 <sup>e</sup>	88.34
Soaking in NaOH (0.5M)	6	0.67 <sup>d</sup>	85.04	2.07 <sup>cd</sup>	78.45
	12	0.59 <sup>de</sup>	86.83	1.8 <sup>de</sup>	81.26
	18	0.48 <sup>ef</sup>	89.28	1.05 <sup>e</sup>	89.07
	24	0.44 <sup>f</sup>	90.87	1.05 <sup>e</sup>	89.07
Soaking in NaOH (1 M)	6	1.05 <sup>bc</sup>	76.56	3.56 <sup>b</sup>	62.95
	12	0.60 <sup>de</sup>	86.60	2.30 <sup>c</sup>	75.96
	18	0.51 <sup>de</sup>	88.61	1.57 <sup>ef</sup>	83.66
	24	0.49 <sup>ef</sup>	89.06	1.32 <sup>fe</sup>	86.26

Mean values in same columns sharing different letters are statistically different ( $p < 0.05$ ).

All values are averages of three determinations.

Loss % was calculated using original unprocessed beans as starting materials.

solution (Towo *et al.*, 2003). Considerable loss in the polyphenol and tannin content were reported for cowpeas (*Vigna unguiculata*) and *Prosopis chilensis* by Uzogara *et al.* (1990) and Vijayakumari *et al.* (1997), respectively. The decrease of TPC may be attributed to leaching to the soaking medium under the influence of concentration gradient (Saharan *et al.*, 2002), the increase in seed coat permeability in alkali environment and solubility of tannin and other phenolics in alkaline solutions (Ulloa *et al.*, 2002). Alkali treatment causes an opening and

rearrangement of the phenolic ring structures and promotes oxidative polymerization of condensed tannins (Cilliers and Singleton, 1990). During 24 h of alkali soaking of two varieties of kidney bean namely; Roba and Awash, tannin content decreased from 5.4 to 3.9 and 17.6 to 13.2 mg/g, respectively (Shimelis and Ralshit, 2007). Soaking in 0.5M NaOH solution was more effective in removal of polyphenol from both acorn species compared to 0.1 and 1M NaOH solutions. There was no significant difference in TPC of Qb samples treated with 0.5 and 1M NaOH after

Table 5. Effect of soaking in acetic acid at various concentrations on the polyphenol content (g TAE/100g) of two acorns varieties

Treatment	Time (h)	<i>Q. branti</i>		<i>Q. castaneifolia</i>	
		TPC	% Loss	TPC	% Loss
Raw	0	4.48 <sup>a</sup>	-	9.61 <sup>a</sup>	-
	6	2.34 <sup>b</sup>	47.76	3.83 <sup>b</sup>	60.14
Soaking in acetic acid (0.1M)	12	1.99 <sup>c</sup>	55.58	3.71 <sup>bc</sup>	61.39
	18	1.85 <sup>cd</sup>	58.7	3.39 <sup>cde</sup>	64.72
	24	1.76 <sup>de</sup>	60.71	3.11 <sup>efg</sup>	67.63
	6	2.01 <sup>c</sup>	55.13	3.56 <sup>bcd</sup>	62.95
Soaking in acetic acid (0.5M)	12	1.69 <sup>def</sup>	62.27	3.46 <sup>cde</sup>	63.99
	18	1.51 <sup>gh</sup>	66.29	3.13 <sup>efg</sup>	66.42
	24	1.43 <sup>h</sup>	68.08	2.95 <sup>fg</sup>	69.3
	6	2.31 <sup>b</sup>	48.43	3.28 <sup>def</sup>	65.86
Soaking in acetic acid (1 M)	12	1.71 <sup>def</sup>	61.83	3.02 <sup>fg</sup>	68.57
	18	1.68 <sup>efg</sup>	62.50	2.99 <sup>fg</sup>	68.88
	24	1.56 <sup>fgh</sup>	65.17	2.88 <sup>g</sup>	70.03
	0	4.48 <sup>a</sup>	-	9.61 <sup>a</sup>	-
Soaking in NaCl (1%)	6	2.72 <sup>c</sup>	39.28	5.10 <sup>c</sup>	46.93
	12	2.43 <sup>de</sup>	45.75	3.93 <sup>e</sup>	59.1
	18	2.23 <sup>f</sup>	50.22	3.36 <sup>f</sup>	65.03
	24	1.97 <sup>g</sup>	56.02	3.28 <sup>f</sup>	65.86
Soaking in NaCl (5%)	6	2.82 <sup>bc</sup>	37.05	5.51 <sup>b</sup>	42.66
	12	2.46 <sup>de</sup>	45.08	4.69 <sup>d</sup>	51.19
	18	2.32 <sup>ef</sup>	48.21	4.03 <sup>e</sup>	58.06
	24	2.05 <sup>g</sup>	54.24	3.95 <sup>e</sup>	58.89
Soaking in NaCl (10%)	6	2.96 <sup>b</sup>	33.92	5.84 <sup>a</sup>	39.22
	12	2.74 <sup>c</sup>	38.83	5.58 <sup>b</sup>	41.93
	18	2.48 <sup>d</sup>	44.64	4.76 <sup>cd</sup>	50.46
	24	2.35 <sup>def</sup>	47.54	4.56 <sup>d</sup>	52.54

Mean values in same columns (separately for NaCl or acetic acid) sharing different letters are statistically different ( $p < 0.05$ ).

All values are averages of three determinations.

Loss % was calculated using original unprocessed beans as starting materials.

18 and 24 h soaking. Polyphenol content of cowpea had notable reduction (50.6-84.9%) after 24 h of soaking in dilute NaOH solutions (0.005-0.5M). It was observed that at high alkaline concentration, samples lose their desirable texture and become soft and mushy with dark color that make high alkali concentration unsuitable for treatment (Laurena *et al.*, 1986).

#### Soaking in acetic acid

Various concentration of acid had significant effect ( $p < 0.05$ ) on the reduction of phenolic contents of Qb and Qc varieties (Table 5). In Qb variety significant reduction in phenolic compounds were observed during 6-18 h soaking in acid solution and afterward an increase in soaking time did not cause any significant reduction. However, in all investigated acid concentration the majority of reduction in polyphenol content was took place after 24 h soaking. Inherent differences in testa and membrane characteristics among species may also have contributed to the unequal results (Vijayakumari *et al.*, 2007). This reduction in phenolic contents was higher as compared with mung bean (37.5), kidney bean (8.7%) and cowpeas (38%) after 24 h soaking in 0.02% lactic acid solution (Towo *et al.*, 2003).

Soaking of Qc variety in 0.1, 0.5 and 1M acetic acid solutions reduced TPC by 60.1-67.6, 62.9-69.3 and 65.9-70.1%, respectively. These results indicated that the higher concentration of acid (1M) was more effective on phenolics removal from this variety. In contrast, maximum of phenolics reduction (55.13-68.08%) was observed by soaking of Qb samples in 0.5M acid acetic solution. Laurena *et al.* (1986) observed that phenolic contents of cowpea seed after 24 h soaking in 0.05M acetic acid, 0.5M hydrochloridric acid, 0.5 M sulfuric acid and 0.005 M local vinegar solutions decreased to 76.5, 56.7, 69.9 and 73.2%, respectively. The decrease in the TPC during soaking in acid solutions can be due to the interaction between protein and tannin, leaching out of polyphenol to aqueous medium and formation of higher oligomeric polymers which are insoluble in water and other solvent. Condensed tannin might undergo polymerization in diluted acid medium, resulting in formation of high polymers which may be insoluble because of their size and lose their ability to precipitate protein (Mahmood *et al.*, 2007).

#### Soaking in NaCl solution

After soaking in NaCl solutions, the TPC of processed samples was significantly ( $p < 0.05$ ) reduced

as compared to the raw acorns (Table 5). Soaking in dilute NaCl solution (1%) resulted in removal of about 39.3-56% and 46.9-42.7% of TPC from Qb and Qc, respectively. Similarly, the significant reduction (over 53%) in TPC of sunflower meal was observed after soaking in 1% NaCl solution for 24 h (Gandhi *et al.*, 2008). After 18 h of soaking, phenolics content of Qc variety remained unchanged, but maximum reduction of TPC for Qb varieties were observed after 24 h at all investigated concentrations. Effect of soaking in different salt solution in white and black velvet bean has been investigated by Vadivel and Pugalenti (2008). Soaking in 1% NaCl and CaCl<sub>2</sub> for 6 h resulted in significant reduction in total free phenolic content of both cultivars. The efficiency of salts to remove polyphenol may be due to their ability to interfere with ionic linkages that formed between this compounds and protein (Sripad *et al.*, 2008). The highest retentions of phenolics were observed at high concentration (10%) of NaCl for two varieties. The efficiency of 5% NaCl solutions for elimination of polyphenol from Qb was similar to water at room temperature, while in the case of Qc salt solution was more effective.

## Conclusion

All soaking treatments which applied in current study caused a significant ( $p < 0.05$ ) decrease in the polyphenol contents of two investigated varieties. The greater losses ( $p < 0.05$ ) were observed by soaking in alkali solutions, especially in medium concentration. Also, The highest content ( $p < 0.05$ ) of total phenolics in processed samples were found after roasting followed by soaking in water at room temperatures and 10% NaCl solution. The results of the present study showed that the wet thermal treatments of Qb (cooking and autoclaving) are more effective than soaking treatment in water, acid and sodium chloride solutions. The percentage of reduction in phenolic compounds during boiling of Qc was higher than autoclaving and soaking in NaCl and dilutes solutions of acid. These differences can be attributed to presence of various phenolic compounds with different heat stability in two varieties. No significant differences ( $p < 0.05$ ) was observed between TPC of boiled and autoclaved samples of Qc. However autoclaving was more effective for phenolics removal from Qb varieties than boiling. Phenolic complexity and structural configurations of these compounds among other factors, might have also contributed to the observed variation in phenolic content due to different treatments.

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