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Investigating the effect of various methods of soaking from *Vicia ervilia* in water and alkaline, acid and salt solutions on reduction of anti-nutritional compounds

Abstract: *Vicia* (*V.*) *ervilia*, commonly termed as bitter vetch, produces grains belonging to legumes family. One of the factors limiting the usage of *V. ervilia* is the existence of large amounts of anti-nutritional factors in the grains thereof. Considering the importance of the anti-nutritional factors, the present study investigated the effect of different methods of soaking from *V. ervilia* grains on the reduction of anti-nutritional factors rates. All of the soaking methods were found considerably reducing the anti-nutritional factors rates in all treatments as compared to *V. ervilia* grain flour. It was also found out in the present study that alkaline treatment, provides for the highest reduction of hydrolysable and dense tannin, phenolic ingredients in contrast to the other methods. Moreover, considerable increase in canavanine ooze-out from *V. ervilia* was documented for aqueous and saline treatments. Among different treatments, the highest reduction in total phenolic compounds and tannins was observed in alkaline maceration, followed by acidic, aqueous and salt saline. Also the lowest amount of canavanine was observed in aqueous treatment and the highest amount in alkaline treatment. The results show that treatment method can be selected depending on the type of need to remove anti-nutritional compounds of *V. ervilia* seeds. So soaking in water and use of saline solutions is preferred to soaking in basic and acidic solutions for the latter damages to some nutrients under basic and acidic conditions.

Key words: *Vicia ervilia*, water, alkaline, anti-nutritional, tannin, phenol, canavanine.

Introduction

Legumes are plants from *Fabaceae* (*Leguminosae*) family comprised of 16 to 19 thousand species and nearly 750 genera. Legumes are enumerated amongst the most important plant sources rich in proteins and valuable nutritional biogenic compounds can be prepared from them when combined with cereals for the fact that they contain a considerable amount of high-grade protein (17-38%). The protein rate in legumes crude grains is two to three times higher than that of the cereals (10-15%). Thus, legumes can be used as rich sources of protein in the production of plant protein byproducts in the form of flour (50-65% protein), concentrates (65-90% protein) and/or protein isolates (over 90%) [1; 3]. Efforts for finding alternative and cheap sources of protein for human nourishment have led to various researches regarding the use of some such less-known legumes in developing countries. The reason for such vast studies is the abundance and cheapness of the legumes as potential protein sources for the people of these countries who have less financial affordability in supplying protein from animal sources. *V. ervilia*, commonly termed bitter vetch, belongs to legumes family and the grains of this plant look red lentil when broken [2-5]. According to the producibility of this plant in Iran and its high percentage of protein content, its flours and protein byproducts can be evaluated in terms of performance characteristics and usability in food industry. Approximate analysis of *V. ervilia* is indicative of the idea that it has a chemical composition almost similar to legumes. Investigations performed on *V. ervilia* made it clear that carbohydrates account for a large quotient of *V. ervilia* grains volume. Protein, lipid, fiber, organics

(potassium, phosphorus, copper, iron and calcium) and vitamins are other ingredients constituting *V. ervilia* grains [3-6].

The majority of the legumes grains is relatively toxic and contains proteins or lipids controlling and inhibiting digestive enzymes and the proper processing methods or varieties breeding methods should be applied to overcome such a problem [6-9]. Like the majority of legumes grains, *V. ervilia* grains contain useful nutritional ingredients plus considerable amounts of anti-nutritional biologically active ingredients amongst which phenolic compounds, tannin, canavanine, gallic acid, ellagic acid and galloyl derivatives or hexahydroxydiphenol can be pointed out [9-11]. Furthermore, *V. ervilia* grains contain such toxic amino acids as canavanine (0.035% to 0.11%), cyanogenic glycoside and trypsin inhibitor (2.14 mg/g dry matter) [12]. Annually, a large amount of *V. ervilia* grains is produced in western and northwestern regions of Iran and, unfortunately, there is made no other useful application of this natural nutritional source in our country other than its limited use for feeding livestock and poultry. One factor restricting the use of *V. ervilia* is the existence of a large amount of anti-nutritional factors in the grains thereof. Besides having adverse health effects, anti-nutritional ingredients cause a bitter and astringent taste negatively influencing its agreeability by the consumer [5-12]. The present studys objective in the first stage is the determination of the amount of anti-nutritional factors existent in *V. ervilia* grains flour and various treatments; in a second stage, four methods of soaking from *V. ervilia* grains are compared so as to make it clear which soaking method is capable of exuding a higher rate of phenolic compounds, tannin and canavanine from the *V. ervilia*. The determination of an easy and less costly method with a high efficiency for reducing anti-nutritional factors is deemed as significant a step as possible in making use of *V. ervilia* grains (Table 1).

Table 1 – Some physical processes common for the reduction or elimination of the anti-nutritional factors existent in legumes (Bravo, 1998)

Physical process	Explanations
Autoclaving, cooking under pressure, vaporing	Heating using high temperatures (over 100ºC): the efficiency of the method depends on temperature, moisture and medium pressure
Blanching	Mild boiling (75ºC-95ºC) for deactivating the internal enzymes and prevention of complete cooking
Normal cooking	Before using this method, such pretreatments as skinning, soaking, germination, fermentation and other normal home-based methods are applied.
Extrusion	In this process, high temperature is exerted within a short time (HTST). The process is a combination of high temperature and use of pressure and shear process.
Parching	Dry heating (120ºC-250ºC)
Using chemical processes along with chemical modification (addition of chemical materials)	Using such chemicals as thiols, sulfites, copper salts, ascorbic acid-chemical modification via acylation and succinylation

Materials and methods

In the present study *V. ervilia* grains were procured from Agricultural Jihad branch of Lorestan Province. The grains were grounded in a laboratory mill. To make uniform flour particles, the flour was passed through a 1mm sieve and kept in a refrigerator until the application time. Then, different solutions were used to evaluate the effect of the process of soaking from *V. ervilia* on the reduction of anti-nutritional compounds. In all treatments, 100 ml of various solutions of sodium hydroxide and acetic acid 0.5 M, sodium chloride 5% and water solvent were added to 10 g of the sample separately. The samples were soaked for 24 hours at ambient temperature in these conditions. After 24 hours, the water used, as well as acidic, alkaline, and salt solutions were discarded and the samples were washed several times with distilled water to remove any remaining soluble residue. The specimens were dried for 12 hours to constant weight at 50 ºC [13; 14].

Measuring phenolic ingredients total rates. To retrieve the crude extract, samples were firstly vacuum-dried in an oven in 50 ºC. Then, the dried samples were grounded following which 20 ml 80% methanol was poured on 0.2 g of the dried and milled

sample and the mixture was shaken thoroughly and placed for 2 hours in bain marie in 50 ºC following which the extracts were passed through filter paper and increased in volume using 80% methanol solvent in a 20-ml volumetric flask. The total phenolic ingredients rates were measured in mg/g gallic acid based on Folin-Ciocalteau reagent using spectrophotometer at 765 nm [15; 16].

Measuring the hydrolysable tannin rate. To extract the hydrolysable tannins, 5 g of the milled and dried specimen was weighed. Extraction was carried out using 80% methanol. After remaining in room temperature for 24 hours and following the complete evaporation of methanol, 50ml boiled water was poured on each of the dried specimens and the sediments were separated from the containers and each were separately poured in 100-milliliter Erlenmeyer flasks. Then, ether was added and the solution was completely stirred. Next, the etheric phase was separated using decanter and discarded and the rest of the process continued with the aqueous phase in such a manner that the aqueous solution was poured inside two 50 ml Erlenmeyer flasks to which normal soda and normal hydrochloric acid had been respectively added. The contents of the flasks were correspondingly made completely acidic and basic. Then, both of the flasks were sealed using foil and placed inside hot water bath (bain marie) in 100ºC for a period of time between 20 min to 40 min. In the end of the process, following the cooling of both solutions, normal 6 M hydrochloric acid was added to the one that had become basic and the contents of both of the flasks were eventually mixed. Twenty milliliter ether was added to the obtained mixture for two to three times, each time separating the biphasic solution using decanter. The etheric phase containing hydrolysable tannin was poured inside a previously weighed container and allowed to be vaporized. Tannin measurement was carried out using Folin-Ciocalteau reagent in a spectrophotometer at 743 nm [13-16].

Measuring dense tannin rates. To extract dense tannins, 5 g of the dried and milled specimen was poured in 100 milliliters of 70% acetone solution and the mixture was placed in a 37 ºC hot water bath (bain marie) for 24 hours. After having the solution passed through filter paper, the sediments were washed two or three times using acetone. Then, ether was poured on the obtained solution twice or thrice and the solution was each time separated using decanter. The separated etheric solution contains dense tannin. To measure the amounts of the dense tannin, the etheric solution was dried in open air and dissolved in 10 ml ether. Out of the obtained etheric extract, 0.5 ml was poured inside a vial and 3ml butanol hydrochloric acid solution (95 parts butanol + 5 parts 37% hydrochloric acid) was poured thereon along with 0.1 ml ferric reagent (2% ferric ammonium sulfate in normal 2 M hydrochloric acid). Then, the mixture was stirred and the vial was sealed following which it was boiled in bain marie for 60 minutes. Afterwards, the solution was allowed to cool down. Then, the absorption rates of the obtained solutions were measured at 550 nm [13-16].

Measuring canavanine rates: preparing sodium pentacyanoaminoferrate reagent. 10g of sodium nitroprusside was dissolved in 55ml of condensed 32% ammonia solution. The obtained solution was kept in darkness and 0ºC for 24 hours. The yellow-greenish sediment containing sodium pentacyanoaminoferrate II and III was filtered and treated using absolute ethanol so that complete sedimentation could happen. The sediment was combined with the preliminary sediment and washed in absolute ethanol till ammonia was entirely removed. After partial omission of ethanol by filtering, the sediment was dried using sulfuric acid and kept along with calcium chloride in darkness in desiccator. The mixture has to be used within 48 hours after being prepared because pentacyanoaminoferrate begins breakdown after that time hence it loses its characteristics and becomes brownish green in color.

Preparing V. ervilia specimens. 2 g of sample was subjected to extraction using 0.1 M hydrochloric acid in a sample-acid ratio of 1:25 weight-volume. The mixture was stirred on a magnetic stirrer for 6 hours and kept at room temperature for one night. The solution was subsequently centrifuged in 1000 rpm for 20 min, the unsettled part (overlying solution) was stored, and the remaining (deposited) part was exposed to a secondary extraction stage for 6 hours under conditions similar to the first stage. The mixed extracts were set at pH values and final volumes of 7 and 100 ml, respectively, using 0.1 M soda solutions.

Measuring canavanine rates. 1 ml standard canavanine solution (1 mg/ml) was diluted by 0.1 M hydrochloric acid so that concentrations in a range from 0.005 to 0.08mg/ml of canavanine could be obtained. In a 10 ml volumetric flask, 6.5 ml 0.2 M potassium phosphate buffer (pH 7), 1 ml 1% potassium persulfate and 0.5 ml 1% pentacyanoaminoferrate solution (kept in dark) were added to 1 ml diluted canavanine solution and the obtained mixture was diluted to 10 ml using distilled water. The mixing process lasted 15 min and the absorption rates were measured at 520

nm. Similarly, a good volume of the sample solution was used instead of standard canavanine solution for quantitative determinations. Using standard curves, canavanine concentration rates in samples were determined based on dry matter weight [17].

Statistical data analysis. All methods of soaking from *V. ervilia* grain and the entire experiments were replicated thrice and the results offered herein are the mean values of the three repetitions. The present study investigated the effects of various treatments of soaking from *V. ervilia* grains on the anti-nutritional factors existent in *V. ervilia* within a completely randomized statistical design in three replications. The results were analyzed using SPSS 16 software.

Results and discussion

One of the factors limiting the usage of *V. ervilia* is the existence of large amounts of anti-nutritional factors in its grains. Percent chemical composition of *V. ervilia* grain flour based on dry matter is presented in Table 2.

Table 2 – Percent chemical composition of *V. ervilia* grain flour based on dry matter in the present study

Protein	Fat	Carbohydrate	Moisture	Ash	Crude fiber
25.36±0.25	4.50±0.2	52.56±0.3	6.30±0.2	3.64±0.19	6.83±0.12

In this regards, anti-nutritional factors existent in *V. ervilia* grain flour were as shown below.

Total phenolics content. The total phenolic ingredients existent in various plant food materials are different even in various varieties belonging to a single genus and they cover a vast range (Table 3) [19]. The total phenolic compound rates of *V. ervilia* grain flour have been obtained in equivalent gallic acid, 408.46±0.33mg/100g.

Table 3 – The amount of polyphenolic ingredients in various foodstuff based on dry matter (mg/100g)

Foodstuff	Total phenolic ingredients
Legumes	34-1710
Cereals	22-102.6
Nuts (kernels)	0.04-38
Vegetables	6-2025
Fruits	2-1200
Tea	150-210

The obtained values are higher than the rates reported by Golchin-Gelehdooni et al. (2014) of *V. ervilia* grain flour (202.1 mg/100g) has found amounts equal to 34-1710 and 22-102.6mg/100g for polyphenolic compounds in legumes and cereals based on dry weight [18]. Results presented in the current research paper are consistent with the other studies [19-21] and the amounts of phenolic ingredients found herein are in a range reported for polyphenolic compounds in legumes. In the study undertaken on oak, it was made clear that the fruits contain 12.33% phenolic ingredients out of which tannin and gallic acid account, respectively, for 9.06% and 0.142% [22]. It has been reported the total phenolic ingredients of crude oak fruit of *Quercus persica* to be 2.01% [23]. In addition, it was found total phenolic ingredient equal to 1.49% in such legumes as *Canavalia cathartica* and reported the total phenolic ingredients of such cereals as sorghum and wheat in a range between 1 and 2.3% and 0.07 and 1.4%, respectively [24; 25].

Total tannin (hydrolysable and dense tannins). Content of hydrolysable and dense tannins in the studied *V. ervilia* grain flour equaled to 232.54±0.29 mg/100g and 297.51±0.2 mg/100g, respectively. Amount of anti-nutritional factors in *V. ervilia* grain flour in the present study based on dry weight (mg/100g) is presented in Table 4.

Table 4 – Phenolics in *V. ervilia* grain flour based on dry weight (mg/100g)

Total phenolic content (gallic acid)	Hydrolysable tannin	Dense/ compact tannin	Canavanine
408.46±0.33	232.54±0.29	297.51±0.2	98.72±0.22

Golchin-Gelehdooni et al. [18] acquired 188.3 mg/100g and 230.2 mg/100g values for hydrolysable and dense tannins. As can be seen from the Table 4, corresponding values were lower than that.

Furthermore, in the other studies, the amounts of dense tannin in *V. ervilia* grain have been reported as 670mg/100g; 402mg/100g and in a range from 325mg/100g to 591mg/100g [26; 27]. In all of these studies, the amounts reported for dense tannins are higher than what was obtained herein. It can be related to weather conditions of the place that plants growth.

Canavanine. In the present study, the canavanine content of *V. ervilia* grain flour was equal to 98.72±0.22mg/100g. The other studies found canavanine rates of *V. ervilia* grain in ranges between 40 and 110 mg/100g, 10 and 170mg/100g, 5 and 110mg/100g and 10 and 260mg/100g [28-30].

As it can be observed in table 5, the amount of canavanine obtained in the present research paper is in the range reported by the other researchers hence consistent therewith (Table 5).

In other studies [25; 26] values equal to 76mg/100g and 78.5mg/100g correspondingly for canavanine rates of *V. ervilia* grain were reported. Their results are lower than what has been calculated herein. Generally, the difference in the amounts of anti-nutritional factors in *V. ervilia* grains, in contrast to what has been highlighted in the other studies, can be attributed to the effects of some climatic, environmental, soil and genetic conditions. Furthermore, the amount of anti-nutritional factors removal in soaking method is subject to factors like solution type, soaking duration, type of food material, solid to liquid phase ratio, temperature and solubility of the ingredients in the applied solutions [31; 32].

Table 5 – Amount of anti-nutritional factors in *V. ervilia* grain flour in various studies based on dry weight (mg/100g)

Total phenolic content	Hydrolysable tannin	Dense tannin	Canavanine	References
202.1	188.3	230.2	78.5	[25]
-	-	670	78.5	[26]
-	-	402	76	[27]
-	-	325-591	40-110	[28]
-	-	-	70-110	[28]
-	-	-	5-110	[29]
-	-	-	10-260	[30]

The content of anti-nutritional compounds in different soaking treatments.

Total phenolics content. The analysis of the results of the present study indicated that there is a significant relationship between the method of soaking from *V. ervilia* grain and the total phenolics content extracted in a 5% level (P<0.05). Results, presented in Table 6, also demonstrate significant difference between all four soaking methods in terms of reductions in the amounts of total phenolic contents of different treatments as compared to what was scored for the evidence sample.

Table 6 – The effect of various methods of soaking from *V. ervilia* grains in water and alkaline, acid and salt solutions on the anti-nutritional factors contents (mg/100g)

Treatment	Total phenolic content/gallic acid	Hydrolysable tannins	Dense/compact tannins	Canavanine
Unprocessed *V.ervilia* grains (control)	408.46±0.33[a]	232.54±0.29[a]	297.51±0.2[a]	98.72±0.22[a]
Soaking in sodium hydroxide o.5 M	[c]48.35±0.28	[c]28.29±0.06	[c]34.58±0.24	[b]32.49±0.3
Soaking in acetic acid 0.5 M	[d]63.49±0.34	[c]35.59±0.32	[d]47.72±0.22	[c]27.55±0.26
Soaking in water	[c]97.58±0.29	[c]58.41±0.25	[c]67.41±0.2	[c]11.43±0.18
Soaking in sodium chloride 5%	[b]131.45±0.27	[b]77.51±0.16	[b]87.73±0.24	[d]19.51±0.16
Note: Numbers with at least one similar letter are not statistically significant (P<0.05), where: a, b, c, and d are statistically significant (P<0.05), so that there is a difference significant between them.				

As can be seen from the Table 6, the highest reduction in phenolic content of different soaking treatments has been obtained in basic-acidic-aqueous-salty soaking methods, respectively. Basic and acidic solutions break down the plant tissues cell walls and cause the maximal discharge of anti-nutritional factors in comparison to saline and aqueous solutions.

Alkaline soaking method. Alkaline soaking method from *V. ervilia* based on offered the lowest total phenolic content (48.35±0.28 mg/100g) in contrast to those on other methods. According to the results obtained in various investigations, the extractability of the majority of phenolic ingredients, including phenolic acids and tannins, is increased in basic pH values attribute the reduction in the amount of the phenolic ingredients in basic environments to the loss of the cell walls integrity in plant tissues under basic conditions hence increase in the solubility and dispersion speed of these ingredients into the peripheral environment. Another reason for the reduction of the amounts of the phenolic ingredients and instability of them in basic environments is the essential changes created in the phenolic compounds structures as a result of absorbing electron. These changes in the phenolic compounds structure can be related to the phenolic ingredients tendency for interacting with proteins and forming complexes with them. Under basic conditions, the electrostatic interactions between polyphenols with positive charges and proteins with negative charges is increased linearly with the increase in pH as a result of which amounts of these compounds exude in the form of soluble protein-phenol complexes from plant tissues [13; 14]. Xu and Diosady [33] showed that concentration and condensation of simple phenolic ingredients is accelerated under basic conditions. The results obtained by the other researchers in using basic solutions for removing phenols and other anti-nutritional factors confirm what has been found herein. In an investigation of the effects of soaking in water and in normal basic 0.5 and 1 M sodium hydroxide on the reduction of the phenolic ingredients in two oak varieties [34] showed that normal 1 M sodium hydroxide solvent has the highest effect on the reduction of the amounts of phenolic ingredients in both Iranian oak species. To put it differently, in this study, the reductions in total phenol content were found respectively higher in soaking with various solvents, including normal 1M sodium hydroxide, water and normal 0.5 M sodium hydroxide. In another study, Ghaderi et al. [35] investigated the effect of soaking two Iranian oak varieties in water, 1M acetic acid, 1 M soda and 5% sodium chloride for the purpose of eliminating phenolic compounds from oak fruits. The results indicated that all of the processes considerably reduce the phenolic ingredients in both of the oak varieties in respect to evidence group. The highest amounts of phenolic ingredient removal from both of the oak varieties were observed respectively for soda, acetic acid, sodium chloride and water treatments and this is in compliance with what has been documented herein. Applied various concentrations (5% and 10%) of soda for reducing anti-nutritional factors from coffee indicated that the increase in soda concentration from 5% to 10% is followed by the omission of higher amounts of phenolic and tannin ingredients [36]. In other studies, the reductions equal to 21% and 64% in polyphenolic compounds were obtained for two types of plants, namely *Vigna aconitifolia* and *Vigna sinensis*, after six hours of soaking in sodium bicarbonate solution. The reductions in the polyphenolic compounds were attributed to the instability of these ingredients in higher (basic) pH values [13; 14]. Other researchers, as well, used basic solutions for the removal of anti-nutritional factors from plant food materials and delivered similar results. To eliminate polyphenolic ingredients from black-eyed peas and increase the digestibility of protein, these grains were soaked in various basic solutions like sodium hydroxide, potassium hydroxide, sodium carbonate and sodium bicarbonate for 48 hours. The highest amounts of reductions in the soaked specimens were respectively observed for sodium hydroxide, potassium hydroxide, sodium carbonate and sodium bicarbonate. In diluted basic solutions, parts of phenolic compounds are extracted in the form of soluble sodium phenate complexes. In line with the increase in the base concentration, hydroxyl groups of phenolic compounds are ionized hence other phenolic compounds become incapable of forming complexes with proteins and sodium hydroxide [37].

Acid soaking method. In this study, the obtained amount of total phenolic ingredients of the acid soaking method from *V. ervilia* grain was 63.49±0.34mg/100g. With a significant difference in 5% level, the acid treatment contains higher amount of phenolic ingredients as compared to that alkaline treatment. It attributed the reduction in the amount of phenolic ingredients in basic and acidic solutions to the loss of plant tissues cell wall integrity under basic and acidic conditions hence increase in their solubility and dispersion speed into the peripheral environment [13; 14]. Basic and acidic solutions can break the cell walls of the plant tissues thereby to release phenolic compounds. According to the obtained results, it seems that the basic treatment, in comparison to acid-

ic treatment, acts more intensively in breaking and destroying the plant tissues cell walls and resultantly maximal releasing of the phenolic ingredients. Possibly, this is one reason for the low amount of phenolic compounds in basic treatment in comparison to that in acidic treatment. The results obtained by the other researchers in using acidic solutions for removing phenols and other anti-nutritional compounds affirm the findings of the present research paper. Towo et al. [38] indicated that 70, 28, 38, 37 and 8% following 24 hours of soaking in lactic acid (0.02%) reduce the amount of phenolic ingredients extractable from red sorghum, millet, peas, mung bean and red bean, respectively. These researchers attributed the observed reductions to anthocyanidine breakdown into simpler phenols like flavan-3-ols. The results obtained by Laurena et al. [39], as well, is suggestive of the idea that various concentrations of acidic solutions like acetic acid, hydrochloric acid, sulfuric acid and home vinegar exert a large deal of effect on the reduction of phenolic ingredients in black-eyed peas. In between, the highest reduction (61%) was obtained after 24 hours of soaking in home vinegar. Acetic acid solutions, 0.05 moles, hydrochloric acid and sulfuric acid, 0.5 moles, and vinegar, 0.005 moles, have the highest effects on the exudation of these compounds and the increase in their concentrations brings augments the percentages of these grains polyphenol residues. The researchers also asserted that disregarding the percolation of the phenolic compounds from plant tissues into the acidic solution subject to concentration gradient and acceleration of soluble tannin-protein complexes formation in lower PH values, the formation of phenolic oligomers is another factor contributing to the reduction in the amounts of phenolic compounds in acid-soaked specimens because these compounds are insoluble hence immeasurable using ordinary methods. These findings are in accordance to the results obtained in the present paper. In a few number of the studies, acidic and basic and aqueous solutions were simultaneously used for the removal of phenolic or tannin compounds. A brief summary of these studies is provided as follows: It applied various solvents (sodium hydroxide, hydrochloric acid, sodium chloride and sodium sulfite) for the removal of phenolic compounds from sunflower seed cake [40]. The results indicated that sodium hydroxides, hydrochloric acid, sodium sulfite and sodium chloride respectively reduce phenolic ingredients from 4270mg/100g to 50 mg/100g, 220 mg/100g, 1995 mg/100g and 2905mg/100g. As it can be seen in table 6, the highest reduction in phenolic ingredients has been obtained in basic-acidic-saline media, respectively. In the another study, it used sodium hydroxide, hydrochloric acid and water to reduce the amounts of anti-nutritional factors in acacia leaves [41]. The results indicated that the highest reductions in polyphenols were found caused by sodium hydroxide, acid and water solutions and that these treatments reduced polyphenols for amounts equal to 74%, 69.6% and 40.9%, respectively. In another study, Shimelis et al. [42] soaked *Phaseolus vulgaris* grains in an acidic solution (citric acid), a basic solution (sodium bicarbonate) and water. These researchers stated that the highest reductions of tannin and total polyphenolic compounds in the aforesaid grains belong to basic, acidic and aqueous environments, respectively. The results of all the explored research sources approve the findings of the present study. As it is clear from the investigation of the results of the present and the other studies, the reductions observed in the amounts of phenolic compounds and tannins are higher in basic and acidic solutions than in aqueous solution. One of the most important reasons for these results pertains to the loss of cell wall integrity in acidic and basic environments hence increase in their solubility and diffusivity from the plant tissues into the peripheral environment [13; 14]. Under acidic conditions, enzymatic oxidation of phenolic compounds occurs in a lower speed. In addition, hydrolysis of polymeric phenols as well as the discharge of the ingredients bonded to the compounds existent in the cell membrane, like hydrocinnamic acid derivatives, are accelerated under such conditions. Soaking plant tissues under acidic and even aqueous conditions is accompanied by the formation of insoluble phenol oligomers. These compounds remain inside the plant tissue and their values are not taken into account in the measurements, Table 6 [43].

Methods of soaking in water and salt solution: In the present study, the total phenolic contents in the soaking procedures in water and salt solution, were respectively found equal to 97.58±0.29 mg/100g and 131.45±0.27mg/100g. As it can be seen, salt treatment contains the highest rate of total phenolic compounds, 131.45±0.27mg/100g, in contrast to the other treatments.

In the soaking procedures in water and salt solution, the observed reduction in phenolic compound rates can be attributed to the softening of the cell walls by aqueous solvent that is usually followed by an elevation in solubility of the bonded phenolic compounds [44]. Another reason for the reduction of phenolic compounds during soaking in water is the high solubility of the phenolic compounds in water and their diffusion into the aqueous environment (sa-

line solution) subject to the effect of concentration gradient hence enhancement of the cell membranes permeability [45]. Moreover, polymerization of phenolic compounds with low molecular weights and formation of insoluble compounds hence the obstruction of their exudation from the plant cells are other reasons for the observed reductions in the amounts of phenolic compounds in saline solutions. It ascribed the reductions in phenolic compounds following soaking in water to the interaction between polyphenol ingredients and proteins, carbohydrates and the subsequent formation of insoluble complexes and their conversion into an immeasurable form [13; 14]. Furthermore, these researchers pointed to the polyphenol oxidase activation and decomposition of phenolic compounds by this enzyme as another reason for the reduction of these ingredients during soaking. The results obtained by the other researchers in using saline solutions or aqueous solvents for the removal of phenols and other anti-nutritional factors confirm the findings of the present study. In another study that was conducted by Towo et al. [46], it was pointed out that red sorghum and millet grains respectively lose 23% and 19% of their phenolic compounds after 24 hours of soaking in water. These researchers considered high solubility of phenolic compounds in water and their diffusivity in aqueous medium as the most important reasons for the reductions in phenolic compounds of the specimens during soaking. The salts efficiency in extraction of phenolic compounds depends on their abilities in interfering with the ionic bonds formed between phenolic compounds and proteins and, in between them, bivalent salts exhibited larger effects.

Tannins (hydrolysable and dense tannins). The analysis of the results of the present study indicated that there is a significant relationship in a 5% level between the type of the method used for soaking from *V. ervilia* grains and the amount of tannins ($P<0.05$). The obtained results showed with a significant difference that all four methods of soaking reduce the amounts of tannins in different treatments as compared to the evidence specimen (*V. ervilia* grain flour). As it can be observed in table 6, the highest tannin reductions in different soaking treatments, has been obtained in basic-acidic-aqueous -salty soaking methods, respectively (Table 6).

It expressed that the total tannin rates of *V. aconitifolia* and *V. sinensis* are reduced by 57 and 74%, respectively, following six hours of soaking in sodium bicarbonate basic solutions. These researchers ascribed the reduction in tannin rates to their instability in higher PH values [13; 14]. In a research paper reported that such basic solutions as sodium carbonate and sodium bicarbonate are capable of reducing tannins by 40% to 50% in sorghum. It stated that oxidative polymerization of condensed tannins is accelerated under basic conditions [33].

Acid soaking method: In this study, hydrolysable and dense tannin rates of acid treatment, were 35.59±0.32mg/100g and 47.72±0.22mg/100g, respectively. It ascribed the reductions in total tannin of the basic and acidic solutions to the loss of plant tissues cell wall integrity under basic and acidic conditions hence increase in solubility and diffusivity of them into the peripheral environment. Basic and acidic solutions can break down the plant tissues cell walls thereby to discharge tannins [13; 14]. According to the results, it seems that the basic treatment, in respect to acidic treatment, acts more intensively in breaking and destroying the plant tissues cell walls and the consequent maximal discharge of tannins. This is possibly one reason for the low amount of tannins in alkaline treatment as compared to those in acidic treatment. On the contrary, the acidic treatment contains lower amounts of tannins in comparison to the aqueous and salty treatments with a significant difference in a 5% level. As it was explained previously, basic and acidic solutions break down the plant tissues cell walls and maximally release tannins in contrast to the saline and aqueous solutions hence they are more powerful in tannin extraction while the destruction of the plant tissues cell walls, playing a major role in releasing of the phenolic compounds and tannins, has been less frequently seen in aqueous and saline solutions.

Methods of soaking in water and salt solution: In this study, the amounts of hydrolysable tannin in aqueous and saline solutions were 58.41±0.25mg/100g and 77.51±0.16mg/100g, respectively. Furthermore, the amounts of dense tannins in aqueous and saline solutions were 67.41±0.20mg/100g and 87.73±0.24mg/100g, respectively. As it is observed in table 6, saline treatment contains the highest amount of total tannins (total hydrolysable and dense tannins), 165.24mg/100g, as compared to the amounts evidenced for the other treatments. One reason for the reductions in tannins rates during soaking in water is their solubility in water and diffusivity into the aqueous environment (saline solution) subject to concentration gradient hence elevation of the plants membrane permeability [47]. It applied such solutions as sodium hydroxide, hydrochloric acid and water to reduce anti-nutritional factors in acacia leaves [48]. Their results showed that the highest tannin reductions were respectively obtained for sodium

hydroxide, acid and water and that these treatments brought about reductions by 74.9%, 70.9% and 40.9%, respectively (Table 6).

Canavanine: The analysis of the present study results indicated that there is a significant difference in a 5% level between the type of the method used for soaking from *V. ervilia* grain and the amount of canavanine ($P<0.05$). The results also showed with a significant difference that all four methods of soaking reduce canavanine rates in different treatments in contrast to the evidence specimen (*V. ervilia* grain flour). As it can be seen in table 6, the highest rates of canavanine reduction in various treatments correspondingly belong to aqueous-saline-acidic-alkaline treatments. With a significant difference in 5% level, the lowest amount of canavanine reduction, 11.43±0.18mg/100g, was observed in aqueous treatment and the highest amount of canavanine reduction, 32.49±0.3mg/100g, was evidenced for alkaline treatment. The reduction in canavanine amounts in aqueous treatment, 11.43±0.18mg/100g, and in saline treatment, 19.51±0.16mg/100g, can be ascribed to the very high solubility of canavanine in water. The higher reduction in canavanine rates in aqueous treatment as compared to saline treatment is due to the very high solubility of canavanine in water (Table 6).

It soaked (drenched) *V. ervilia* grains in 1% acetic acid solutions in 40 ºC for 24 hours. These researchers reported that the actual digestion of lysine and arginine in acetic acid-treated *V. ervilia* grains is increased due to the canavanine extraction in 1% acetic acid [49]. The results obtained in these studies affirm what has been found herein. Although one of the effective and applied methods for reducing or eliminating anti-nutritional compounds in legumes is the use of thermal processes, these methods do not enable omission or reduction of canavanine from and in legumes due to its being inherently resistant to heat. On the contrary, canavanine readily dissolves in acidic and basic, especially aqueous, solutions. Therefore, canavanine rates can be reduced or eliminated in plants (legumes) by soaking and drenching them in water and acidic and basic solvents. Due to very good solubility of canavanine in water, drenching of plants in water has been put forth as one of the most effective methods of canavanine extraction and elimination from the plants [50]. Of course, it should be noted that overheating in processes and use of concentrated basic and acidic solutions for the maximal removal of anti-nutritional factors is not recommended for the degradation of the quality of such nutrients as proteins. Thus, the proper duration and temperature for the maximal elimination of these compounds should be selected so as to reduce the damage or wastage of the nutrients to the least possible extent [51].

Conclusion

Among different treatments, the highest reduction in total phenolic compounds and tannins was observed in alkaline maceration, followed by acidic, aqueous and salt saline. The alkaline soaking yielded the lowest amount of total phenolic compounds (48.35±0.28 mg/100g) and the saline soaking did the highest total phenolic compounds (131.45±0.27 mg/100g). In our study, the lowest amount of hydrolysable (28.29±0.06 mg/100g) and compact tannins (34.58±0.24 mg/100g) was observed in alkaline treatment, and saline treatment yielded the highest amount of total (hydrolysable and compact; 165.24 mg/100g) tannins compared to other treatments. According to the results of various studies, the reduction in the amount of tannins in alkaline environments is attributed to the loss of cell wall integrity of plant tissues in alkaline conditions and, consequently, to increased solubility and release rate of these compounds into the surrounding environment. In this study, the lowest amount of canavanine was observed in aqueous treatment (11.43±0.18 mg/100g) and the highest amount in alkaline treatment (32.49±0.3 mg/100g). Reducing the amount of canavanine in aqueous treatment can be attributed to the very good solubility of canavanine in water.

Generally, common processes for the reduction or removal of anti-nutritional factors from plants are heating and drenching or soaking in diluted basic and acidic solutions, saline solutions and water. In the current research paper, all of the soaking methods were found considerably reducing anti-nutritional factors in different treatments in contrast to *V. ervilia* grain flour. It was figured out herein that alkaline treatment significantly reduces such anti-nutritional factors as phenolic compounds and dense and hydrolysable tannins in a rate higher than the other methods. Furthermore, aqueous treatment was found with a significant increase in the amount of canavanine exudation from *V. ervilia* compared to other methods. Basic and acidic solutions break down the plant tissues cell walls and cause the maximal discharge of anti-nutritional factors in comparison to saline and aqueous solutions hence they enjoy a higher extractability of anti-nutritional factors whereas the plant tissues cell wall destruction that plays a special role in releasing of the phenolic compounds and tannins was less frequently seen in aqueous and saline solu-

tions. From nutritional perspectives, soaking in water and use of saline solutions is preferred to soaking in basic and acidic solutions for the latter damages to some nutrients under basic and acidic conditions.

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