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EFFECTS OF WATER SOAK AND LIME SOAK ON THE CHEMICAL COMPOSITIONS AND DRY MATTER LOSSES OF *HIBISCUS SABDARIFFA* SEEDS

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ABSTRACT

This study was carried out to assess the nutritional potential and the dry matter losses of *Hibiscus sabdariffa L.* seeds from two processing methods (water soak and lime soak) with each method having three different patterns in a completely randomized design (CRD). The water soak method involved three different duration of changing water (0hrs, 24hrs and 48hrs) which lasted for four days while the lime soak method involved three different inclusion levels (3%, 6% and 9%) of lime ($Ca(OH)_2$) in clean water for 24 hrs. Results indicated that there was a significant ($P<0.05$) difference observed in all the parameters. The results showed that the dry matter losses in the seeds increased significantly ($P<0.05$) in water soak with increased frequency of water change but decreased ($P<0.05$) in lime soak method with higher inclusion levels of lime, water soak had more dry matter loss than lime soak due to differences in soaking duration. There was a significant ($P<0.05$) increase in crude proteins, nitrogen free extract and crude minerals for the two processing methods but a decrease ($P<0.05$) was observed for ether extract and fiber fractions. Also, while a decrease ($P<0.05$) was observed in phytates and tannins, no effective trend was noted for oxalates contents of the seeds for both water and lime soak methods in comparison with the raw seeds. There was an increase in Ca, P and Fe but a statistical ($P<0.05$) decrease was observed in Mg and K as compared to the raw seeds. It was also noted that potassium is the major mineral in *H. sabdariffa* seeds though, significantly reduce when processed. It was therefore concluded that both water soak and lime soak methods of processing improved the chemical compositions and reduce the tannins and phytates of *H. sabdariffa* seeds with some amount of losses in dry matter.

Key words: *H. sabdariffa* seeds, Soaking, Chemical compositions, Anti-nutrients, Dry matter losses

INTRODUCTION

Hibiscus sabdariffa is a vegetable predominantly cultivated in Indonesia, India, West Africa and Many tropical regions (Tindal, 1986). It is widely grown for its pleasant red color calyx, commonly known for local drink (sobo) and wine production (Al-wandawi *et al*., 1984). *H. sabdariffa* seeds have been discovered to contain high content of oil (21.00%), crude protein (25.20%) and potentially rich in lysine and some amino acid like valine, isoleusine and tryptophan (Al-wandawi *et al*., 1984). Abu-Tarboush and Basher, 1995 reported that the seeds have reasonable amount of some inorganic elements like potassium, sodium, magnesium, calcium, iron, and zinc. Kwari *et al.* (2011) also found that *H. sabdariffa* seeds contain 38.75% crude protein, 13.50% ether extract, 16.50% crude fiber, 5.18% arginine, 2.58% lysine and 1.33% methionine. Crude protein values of 25.92% and 23.46% CP were reported by Isidahomen *et al.* (2006) and Abdu *et al.* (2008).

Anti-nutrients such as tannin, phytic acid, gossypol, hydrocyanic acid and trypsin inhibitor activity are one of the major factors affecting the nutritive value of *H. sabdariffa* seeds thereby militating against its utilization as an alternative feed source for livestock (Abu-tarboush and Basher, 1996; Ojokoh *et al.*, 2003; Abdu *et al.*, 2008). However, various processing methods have been adopted to alleviate its anti-nutrients. Soaking methods have been found to be effective in anti-nutrients destruction in plant materials (Joshi *et al.* 1989; Yagoub *et al.* 2008) while sprouting and cooking methods had been reported effective in improving the nutritive values of *H. sabdariffa* seeds by Ibrahim *et al.* (2019). Water soaking method had been reported by Joshi *et al*. (1989) to mitigate some adverse effects of anti-nutrients in seeds and other plant materials through repeated washing. Use of limewater ($Ca(OH)_2$) in seed soaking helps improve digestibility and absorption of amino acids by the body (Hambidge *et al*., 2005).

However, there are losses in feed dry matter which differ in amount with the type of feed ingredients (leaves, stems, seeds, nature of seeds coats) and processing methods adopted (Ibrahim *et al.,* 2018). The change in chemical compositions and dry matter losses in feedstuffs resulting from water soak and lime soak methods of processing have created a paucity of information. It is on this basis that this research is designed to assess the impact of water soak and lime soak methods on chemical compositions and dry matter losses of *H. sabdariffa* seeds.

MATERIALS AND METHODS

Collection, sorting and processing of *H. sabdariffa* seeds

The seeds of Red variety of *H. sabdariffa* were purchased from an open market in Yobe state during the harvest period. The seeds were sorted out to remove impurities before processing. Two processing methods on *H. sabdariffa* seeds with each having three different patterns and in triplicate were carried out using a Complete Randomized Design as follows:

Lime soak

H. sabdariffa seeds were treated with lime at 3, 6 and 9 percent. Three portions (200g each) of *H. sabdariffa* seeds were soaked in three plastic containers containing the lime solution at 3%, 6% and 9% for 24hrs and each replicated twice. The seeds were then removed, washed and oven dried at 60^oC for 24hrs and dry matter losses were determined. The dried treated seeds were then milled using laboratory hammer mill fitted with 1.5mm mesh size sieve and then stored in an airtight plastic container, until required for laboratory analysis.

Water soak

Three portions of *H. sabdariffa* seeds (200g each) were soaked in clean water for four days in a ratio of one part of the seeds to three parts of water (1:3w/v) into clean plastic containers so as to completely submerged the seeds, and a water changing pattern was followed; daily water changing (24hrs), changing water every two days (48hrs) and no changing of water (0hrs). The seeds were then removed, washed and oven dried at 60^oC for 24hrs and dry matter losses were determined. The soaked dry seeds were then milled using laboratory hammer mill fitted with 1.5mm mesh sieve and then stored in an airtight plastic container, until required for laboratory analysis.

$$\%DM\ loss = \frac{W1-W2}{W1} \times 100$$

Where; W1= Weight of seeds before processing

W2= Weight of seeds after processing

DM= Dry matter

Sample analysis

The proximate analysis of samples of both the raw, water soak and lime soak *H. sabdariffa* seeds was conducted according to standard methods (AOAC, 2005). The residual dry matter of the samples was determined by oven-drying at 60^{o}C for 48h. Nitrogen was determined by the micro Kjeldahl method with Tecator Product apparatus (Kjeltec^{TM} 2100), while crude protein was calculated by multiplying N×6.25. The Soxhlet extraction procedure was used for determination of crude fat (ether extract) using electromantle ME. The ash was measured by combustion of the dried material in a muffle furnace at 600^{o}C for 8 hrs. The crude fibre, sequential neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using Tecator Line (FT 122 Fibertec^{TM}) according to the method described by Van Soest (1991). The concentration of phytic acid was determined according to Wheeler and Ferrel (1971). A standard curve of ferric nitrate was plotted. Phytate phosphorus was calculated from the standard curve assuming a 4:6 Fe to P molar ratio. The concentration of total tannins present in raw, water soak and lime soak roselle seeds was assessed colorimetrically as described in AOAC (2005), whereby tannic acid was used as a reference standard. The total oxalates concentration was determined by calcium oxalate precipitation (titrimetric method) of Oke (1966); the method involved titration of acidic aqueous extracts of the sample with a standard solution of potassium permanganate. The analysis of minerals (calcium, phosphorus, potassium, iron and magnesium) was carried out using atomic absorption spectrophotometry in wet feed samples digested by concentrated nitric and perchloric acids, using PG instrument AA500 model.

All data collected during the experiment were subjected to statistical analysis using the general linear models (GLM) procedure of SAS version 9.13 (SAS 2002) according to a completely randomized design. Significance was declared at P<0.05 while significantly different means were compared using Duncan multiple range test (Duncan, 1955).

RESULTS

Effects of water and lime soak methods on the chemical composition of *H. sabdariffa* seeds

Table 1 shows the results of the chemical compositions of raw; water soak and lime soak *H. sabdariffa* seeds. There were significant (P<0.05) differences observed in all parameters measured. The dry matter losses obtained in this study ranged from 6.97% in 9% lime soak to 18.28% at 24hrs water changing pattern in water soak. The results showed that water soak *H. sabdariffa* seeds had higher (P<0.05) dry matter loss than lime soak. The percent crude protein (CP) ranges from 25.18% for raw *H. sabdariffa* seeds to 33.43% for 9% lime soak. The percent CP for 9% lime soak (33.43%) and water soak at 48hrs (32.75%) water changing pattern were statistically similar and higher (P<0.05) than all other treatments. The crude fiber contents for water soak *H. sabdariffa* seeds with 48hrs water changing pattern (23.87%) and lime soak at 6% (23.97%) were significantly lower (P<0.05) than all other treatments. The result for percentage ether extract (EE) showed that the unprocessed *H. sabdariffa* seeds (15.18%) was significantly higher (P<0.05) than all other treatments, while water soak at 48hrs water changing pattern (10.80%) was statistically higher (P<0.05) than 24hrs water changing pattern (9.22%) and 6% lime soak, while the 9% lime soak (8.12%) recorded the least numerical value.

The ash content of the untreated *H. sabdariffa* seeds (9.18%) was lower (P<0.05) than all other treatments while the ash content for water soak at 24hrs (12.00%) and 48hrs (11.91%) water changing pattern were statistically similar but higher (P<0.05) than all other treatments. The percentage nitrogen free extract (NFE) obtained from this study ranged from 20.40% in water soak at 48hrs water changing pattern to 29.12% at 24hrs water changing pattern which was significantly higher (P<0.05) than all other treatments. The acid detergent fiber (ADF), neutral detergent fiber (NDF) and hemicelluloses fraction recorded as 35.98%, 62.89% and 11.59%, respectively for the raw *H. sabdariffa* seeds were significantly higher (P<0.05) than the water soak and lime soak methods as observed in this study.

Effects of water and lime soak methods on some anti-nutrients and their percent reduction/increase in *H. sabdariffa* seeds.

Table 2 shows the effects of water soak and lime soak as processing methods on anti-nutritional factors of *H. sabdariffa* seeds compared with the unprocessed seeds. For phytate, only soaking at 0hrs water changing pattern (0.13%) was significantly (P<0.05) lower than raw (0.17%) *H. sabdariffa* seeds, though soaking at 24hrs and 48hrs water changing pattern and lime soak at different concentration also reduced phytate levels. The tannin value for raw *H. sabdariffa* seeds (2.40%) was significantly higher (P<0.05) than the processed seeds, with water soak methods at 24hrs (1.17%) water changing pattern having the least significant value for tannin. The values of oxalate for 3% lime soak (1.95%) was higher (P<0.05) than all other treatments.

Effects of water and lime soak methods on some mineral compositions in *H. sabdariffa* seeds

The results of mineral composition are presented in table 3. The calcium (Ca) level for 9% lime soak (7.74g/l) *H. sabdariffa* seeds was significantly higher (P<0.05) than all other treatments, while 6% lime soak (2.57g/l) which was statistically similar to 3% lime soak and water soak at 24hrs water changing pattern (1.73g/l) but was higher (P<0.05) than raw (1.10g/l), water soak at 0hrs and 48hrs water changing pattern. The 6% (7.71g/l) and 9% (7.87g/l) lime soak had statistically similar phosphorus which were significantly higher (P<0.05) than 3% lime soak (5.11g/l), water soak and raw (4.30g/l) *H. sabdariffa* seeds. The magnesium content of the raw *H. sabdariffa* seeds obtained in this study was significantly higher (P<0.05) than 9% (0.45g/l) and 6% (0.44g/l) lime soak. The magnesium content of 3% lime soak (0.41g/l) was statistically similar to the water soak methods. The untreated Hibiscus seeds recorded the least value for Iron (0.36g/l) which was statistically similar to 9% lime soak (0.76g/l) but lower (P<0.05) than other treatments. The potassium level however decreased numerically across the treatments with the raw *H. sabdariffa* seeds (11.98g/l) significantly higher (P<0.05) than both water soak and lime soak methods, while lime soak at 3% (3.85g/l), 6% (3.83g/l) and 9% (3.80g/l) were statistically similar but lower (P<0.05) than water soak methods.

DISCUSSION

The dry matter losses increased numerically with increase in the frequency of changing water for water soak *H. sabdariffa* seeds but decreased numerically with increase in percent lime treatments for lime soak. The results showed that water soak *H. sabdariffa* seeds, as a processing method had higher (P<0.05) dry matter loss than lime soak. This may be attributed to the differences in duration of soaking and quantity of water used as well as the possible metabolic activities resulting in loss of metabolites in soaking methods.

The crude protein value (25.18%) obtained in this study was slightly higher than the CP of 23.79% reported by Abdu *et al*. (2008) but within the range of the 25.20% CP reported by Al-Wandawi *et al*. (1984). These variations may be due to varietal difference, environmental factors and agronomic practices. The increase in CP of both water soak and lime soak as obtained in this study agrees with the work of Adanlawo and Ajibade (2006) who reported increase in the CP of red *H. sabdariffa calyx* soaked in wood ash. Also, Yagoub, *et al.* (2008) observed an increase in CP content of soaked *H. sabdariffa* seeds in sodium hydroxide solution (0.005M) for 12 hours. Also, the crude fiber (27.26%) obtained in this study was less than that of 36.36% reported by

Abdu *et al.* (2008). These differences may be due to the similar reasons as stated for CP. The decrease in the EE obtained in this study also agrees with the work of Adanlawo and Ajibade (2006) who observed similar trends in Hibiscus calyx soaked in wood ash and also Abdu *et al.* (2008) who made similar report for Hibiscus seeds cooked at different duration, though the EE in this study (15.18%) was higher than the 6.73% reported by Abdu *et al*. (2008). The percent ash content (9.18%) obtained in this study was far below the 23.80% observed by Abdu *et al*. (2008). Water soak and lime soak in this study significantly increased the percent ash content of *H. sabdariffa* seeds and the apparent increase for water soak may be attributed to the reduction in oil and fiber contents due to leaching out effect and series of microbial metabolic activities in soaked water while for lime soak *H. sabdariffa* seeds it may be due to the addition of the lime; though, Adanlawo and Ajibade (2006) reported a decrease in the percent ash content of soaked *H.* calyces in wood ash. The NFE (21.40%) obtained in this study was higher than the 14.75% recorded by Abdu *et al*., (2008) but far below the NFE content of Hibiscus calyces soaked in wood ash (ranging from 68.75% to 71.56%) as reported by Adanlawo and Ajibade (2006). This further supports the assertion of Babalola (2000) and Ojokoh (2003) that the Hibiscus calyces contain high soluble carbohydrate than the seeds.

The reduction in phytates suggests that there could be increase in bioavailability of minerals like phosphorus, calcium and magnesium as earlier reported by Yagoub *et al.* (2008) for metabolism. However, soaking of *H. sabdariffa* seeds in sodium hydroxide solution (0.005M) for 12 hours did not affect the level phytic acids according to Yagoub, *et al.* (2008). The results of the current study agree with the work of Adanlawo and Ajibade (2006) who reported reduction in both phytic acids and tannins of Hibiscus calyces soaked in wood ash. Abdu *et al*. (2008) also reported reduction in both phytic acids and tannins in *H. sabdariffa* seeds at different duration of cooking. From earlier report, fermentative heat degradation, leaching out effects and changes in chemical activity could be the factors responsible for tannin reduction in *H. sabdariffa* seeds (Saikia *et al*., 1999; Alonso *et al*., 2000; Yagoub *et al*., 2008). Also, the reductions in tannins and phytates confirmed the earlier report of Joshi *et al.* (1989) that the adverse effect of some anti-nutrients in feeds could be overcome by repeated washing with water.

The calcium levels increased numerically with increase in the frequency of changing water for water soak *H. sabdariffa* seeds but increased with increase in percent lime treatments for lime soak. The increase in Calcium content in lime soak *H. sabdariffa* seeds may be due to treatment with lime (Calcium hydroxide) as earlier observed by Hambidge *et al*. (2005) and Ibrahim and Yashim (2014). However, the apparent increase in calcium in water soak *H. sabdariffa* seeds may be due to losses in metabolites resulting from increase in the frequency of water change and possibly microbial degradation of some organic matter in the seeds. The increase in percent lime treatments was found to increase the levels of Phosphorus and Iron but on a contrary decreased the Magnesium and Potassium levels. The frequency of water change had no effects on the Phosphorus, Magnesium and Iron, except Potassium for water soak *H. sabdariffa* seeds, though soaking in water and lime had profound effects on these minerals as compared to the raw *H. sabdariffa* seeds. The reduction in Magnesium levels may be attributed to leaching of the mineral into the soaking water. This trend was observed by Abdu *et al*. (2008) who reported reduction in certain minerals like Mg, K, and Zn in *H. sabdariffa* seeds at different cooking duration. From this study, Potassium was found to be the most abundant mineral present in *H. sabdariffa* seeds. Also, Adanlawo and Ajibade (2006) reported similar trends for potassium content in

H. sabdariffa calyces. Potassium is not only for the chief electrolytes but also essential for the nervous systems, maintenance of fluid volume in the body, muscle contraction, maintenance of correct rhythm of heart beat as well as clothing of blood (Georgievskii *et al*., 1979; Soetan *et al*., 2010). This may strongly qualify Hibiscus seeds and calyces as potential sources of potassium in livestock diets.

Table 1: Effects of water and lime soaked methods on the chemical composition of *H. sabdariffa* seeds

	Raw	Water soak			Lime soak			
Parameters (%)		**0hrs**	**24hrs**	**48hrs**	**3%**	**6%**	**9%**	**SEM**
Dry matter	94.67^{a}	92.82^{b}	94.09^{a}	93.86^{a}	94.00^{a}	92.90^{b}	93.03^{b}	0.19
%DM loss	-	15.99^{b}	18.28^{a}	17.80^{a}	9.55^{c}	7.71^{cd}	6.97^{d}	0.63
Crude protein	25.18^{e}	27.00^{d}	27.88^{cd}	32.75^{a}	28.97^{c}	30.98^{b}	33.43^{a}	0.71
Crude fibre	27.26^{a}	28.20^{a}	26.22^{b}	23.87^{c}	25.26^{b}	23.97^{c}	25.30^{b}	0.50
Ether extract	15.18^{a}	10.00^{c}	9.22^{d}	10.80^{b}	10.00^{c}	8.97^{d}	8.12^{e}	0.23
Ash	9.18^{d}	10.83^{c}	12.00^{a}	11.91^{a}	10.88^{c}	10.66^{c}	11.26^{b}	0.12
NFE	21.40^{c}	24.08^{b}	29.12^{a}	20.40^{c}	25.03^{b}	23.42^{b}	21.53^{c}	0.85
ADF	35.98^{a}	19.95^{e}	21.32^{d}	22.23^{c}	24.01^{b}	20.33^{de}	20.08^{e}	0.36
NDF	62.89^{a}	47.95^{c}	48.81^{b}	44.71^{f}	45.91^{e}	45.40^{e}	47.19^{d}	0.28
Hemicellulose	11.59^{a}	7.10^{d}	9.01^{b}	9.08^{b}	8.14^{c}	8.41^{c}	8.28^{c}	0.19
Processing cost ₦/kg	0.00	2.00	7.00	4.00	7.50	15.00	22.50	-

[abcdef]=Means with different superscript show significant difference (P<0.05), SEM=Standard Error of Mean, NFE=Nitrogen Free Extract, ADF=Acid Detergent Fiber, NDF=Neutral Detergent Fiber, 0hrs=no water change, 24hrs=daily pattern of water change, 48hrs=two days pattern of water change.

Table 2: Effects of water and lime soaked methods on some anti-nutrients and their percent reduction/increase in *H. sabdariffa* seeds.

	Raw	WSS (4days)			LSS			
`Parameters (%)		0hrs	24hrs	48hs	3%	6%	9%	SEM
Phytate	0.17^{a}	0.13^{b}	0.15^{ab}	0.15^{ab}	0.16^{a}	0.16^{a}	0.15^{ab}	0.01
%	-	23.53	11.76	11.76	5.88	5.88	11.76	
Tannin	2.40^{a}	1.47^{b}	1.17^{c}	1.52^{b}	1.77^{b}	1.58^{b}	1.55^{b}	0.06
%	-	38.75	51.25	36.67	26.25	34.17	35.42	
Oxalate	1.46^{bc}	1.34^{cd}	0.87^{e}	1.29^{cd}	1.95^{a}	1.04^{d}	1.22^{cd}	0.17
%	-	8.22	40.41	11.64	+25.13	28.77	16.44	

^{abcde}=Means with different superscript along rows show significant difference (P<0.05), SEM=Standard Error of Mean, LLS= Lime Soaked Seeds, WSS=Water Soaked Seeds, 0hrs=no water change, 24hrs=daily pattern of water change, 48hrs=two days pattern of water change, += percent increase.

Table 3: Effects of water and lime soaked methods on some mineral compositions in *H. sabdariffa* seeds

	Raw	Water soak			Lime soak			
Parameters (g/l)		0hrs	24hrs	48hrs	3%	6%	9%	SEM
Calcium	1.10^{c}	1.19^{c}	1.73^{bc}	1.30^{c}	1.86^{bc}	2.57^{b}	7.74^{a}	0.21
Phosphorus	4.30^{b}	6.01^{ab}	5.56^{b}	5.11^{b}	5.11^{b}	7.71^{a}	7.87^{a}	0.42
Magnesium	0.56^{a}	0.40^{c}	0.41^{c}	0.40^{c}	0.41^{c}	0.44^{b}	0.45^{b}	0.01
Iron	0.36^{c}	2.38^{ab}	1.80^{b}	1.62^{b}	2.63^{a}	2.16^{ab}	0.76^{c}	0.16
Potassium	11.98^{a}	8.69^{b}	8.30^{b}	7.30^{c}	3.85^{d}	3.83^{d}	3.80^{d}	0.12

^{abcd}=Means with different superscript show significant difference (P<0.05), SEM=Standard Error of Mean, 0hrs=no water change, 24hrs=daily pattern of water change, 48hrs=two days pattern of water change.

CONCLUSIONS

The soaking of *H. sabdariffa* seeds in clean water and lime water as methods of processing was noted to increase the crude protein and ash of the seeds but decreased the ether extract and fiber fractions.

While water soak method increased the dry matter losses of *H. sabdariffa* seeds with increase in water change frequency, the dry matter losses was reduced in lime soak with increased in concentration of lime.

Also, while both water soak and lime soak methods of processing numerically decreased phytates and significantly reduced the tannin contents of *H. sabdariffa* seeds, there was no effective trends observed for oxalates.

The water soak and lime soak methods increased the Phosphorus and Iron contents of *H. sabdariffa* seeds while the Magnesium and Potassium contents on the contrary were decreased. However, while no effect was observed on Calcium level for water soak method, an effective increase in calcium content was noted for lime soak.

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