THE EFFECT OF SOAKING TIME AND SOAKING SOLUTION ON THE NUTRITIONAL QUALITY OF GRASS PEA SEEDS

Kelbessa Urga MSc. and Mengislu Gebretsadik:

ABSTRACT: The effect of soaking time and soaking solution on the nutritional quality of grass pea seeds were studied. The soaking solutions used were:plain water (pH 7.3); ImM HCl (pH 3.0); ImM NaOH solution (pH 11.0) and 0.1% (w/v) wood ash solution (pH 12.0). Mature whole grass pea seeds soaked for 72 hr in the four soak media that were used resulted in total solids loss of 1.3, 1.2, 5.2 and 6.2 percent, respectively. Total protein content increased by 11 and 13 percent, respectively for seeds soaked for 72 hr in plain water and ImM HCl. In contrast, a total protein loss of 12 and 16 percent, respectively was noted in seeds soaked for 72 hr in ImM NaOH solution and 0.1% wood ash solution. The soaking process resulted in the loss of non protein nitrogen, total soluble sugars and reducing sugars. Soaking also influenced the ash and crude fibre contents of the seeds. The pH of the soaking solutions were also altered in accordance to the soaking process. However 'kikn' showed significantly lower nutrient retention values compared to the whole seeds mainly due to the higher loss of solids during soaking. [Ethiop. J. Health Dev. 1993;7(2):79-83]

INTRODUCTION

The pulse grass pea also known as Lathyrus sativus is an important crop in the rain-fed regions of Ethiopia because of its resistance to drought and flood. It also grows well in depleted soils after harvest of the main crops.

Based on reports from some parts of Ethiopia (1,2) there is a strong epidemiological association between excessive consumption of grass pea and the development of an irreversible crippling disorder of the legs, known as neurolathyrism in humans and animals (3). Rao et aI. (4) have isolated and characterized the neurotoxin Beta-N-Oxalylamino-L- alanine {BOAA), the only neurotoxic amino acid implicated in human
lathyrism from grass pea- Grass pea seeds in Ethiopia are processed in various forms. The most common method of preparation is to soak them in water and then cook until they are soft.

The seed is usually soaked for the purpose of removing the toxic factors, reducing cooking time and to improve texture. Other traditional methods include sprouting, cooking of sprouted seeds; roasting of soaked seeds and fermerltation.

Grass pea flour is used alone where alternative crops are scarce or mixed with other cereal flours for making leavened and unleavened bread. In times when harvest is plentiful grass pea is eaten as 'shiro wot', which is an aqueous slurry cooked with spices, or mixed with other cereals to make bread, but in times of famine, the diet of the population of grass pea cultivating regions is forced to consist only grass pea.

The neurotoxin BOAA is water soluble, i.e., it can be removed by washing the seeds several times or soaking the grain for some time in water (5). There are reports that various processing and cooking procedures remove the toxic factors in Lathyrus sativus seeds (6, 7). However, a recent study involving these same processing methods for chick bioassays did not indicate that the toxic substances in Lathyrus sativus had been completely removed or destroyed (8). Jahan and Ahmed (9) also reported that autoclaving or cooking the seeds with water would not completely destroy BOAA in the seed. Just boiling in lime water for 30 min would remove the toxin from the powdered seeds but the taste of the meal would be slimy. This indicates that the Ethiopian traditional processing methods may not be effective in removing the neurotoxin BOAA. Soaking of legumes prior to processing in water, salt, alkaline, rock-salt and wood ash solutions is a common practice in many cultures (10, 11,12).

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Reports are available on biochemical changes in various legumes during soaking of the whole seeds in different salt solutions for several hours. However, data on biochemical changes in grass pea after soaking in various soaking solutions for different times are limited (13, 14). The objective of this study was to investigate the biochemical changes in grass pea seeds brought about by the nature of the soaking medium.

METHODS

Grass pea seeds preparation Grass pea seeds were purchased from a local market in Addis Ababa and stored at 4°C. The see were hand-selected to eliminate the broken ones arid those with cracked or otherwise damaged seed coats. Dehulled cotyledons split into halves are locally known as 'kikh'. 'Kikh ' was prepared from the selected seeds by using the traditional grinding stone. It was then hand-se1ected from the seed coats and powders and used for the study. Soaking treatments: The intact seeds were thoroughly mixed with soak solutions and incubated for the periods of 1, 3,6, 18, 24,48 and 72 hr at room temperature (23.:1:.2°C). 'Kikh' was soaked in the soaking solutions for only two days. The following soaking solutions were used:- (a) ImM HCI (PH 3.0); (b) tap water only (pH 7.3); (c) ImM NaOH solution (pH 11.0); (d) 0.1% (w/v) wood ash solution (pH 12.0). Throughout, the ratio of seeds or 'kikh ' to soak solution was I :5 (w/v). Samples were removed at regular intervals during soaking, rinsed with deionized water and the surface water removed by placing them on absorhent paper. The samples were then dried in cabinet tray driers to a final moisture content of 10-12 percent. The dried samples were ground in a Cyclotec Mill (Cyclotec, Tecator-AB, Sweden) to pass through 60 mesh sieve and further used for analysis. The pH of the soaking solutions were determined using an Expandomatic SS2 pH meter (Beckman, USA). The soaking procedures were replicated four times.

Leached solids: The seeds and 'kikh' samples were carefully removed at regular intervals from the soak solutions and the solutions were evaporated to dryness in a hot air oven at 800C overnight. The weight of the residue was taken and expressed in gram percent of the initial weight of seeds ' or 'kikh , before soaking.

Analytical methods: The proximate composition of the seeds and 'kikh' was determined by the standard methods of analysis (15). Non protein nitrogen was estimated by the method of Naczk et al. (16). Total soluble sugars were determined by the phenol-sulfuric acid method of Dubois et al. (17) with glucose as a reference standard. Producing sugars were extracted with distilled water and the col:nposition was colorimetrically estimated according to Nelsen (18). The neurotoxin BOAA in soaked seeds and 'kikh' was estimated colorimetrically according to Rao's method (19). All results were expressed on dry weight basis.

RESULTS AND DISCUSSION

Soaking is commonly used before the processing of legumes. During this process, there is a loss of solids manly low-molecular- weight substances (10). The amount of total solids recovered after soaking grass pea seeds for various lengths of time in different soak media are shown in Figure I. Under the conditions studied, soaking could leach out as much as 6.2 percent solids of grass pea seeds. Seeds soaked in ImM HCI and tap water for 24hr lost 1.2 and 1.3 percent of their solids, respectively. The rate of loss of solids for seeds soaked in ImM HCI was approximately linear for the first 24 hr it then decreased with increased soaking time; whereas for seeds soaked in tap water, it was linear for the first 24 hr and remained constant for the rest of the soaking period. When seeds were soaked in ImM NaOH and 0.1% wood ash solution the loss of total solids increased progressively with soaking time. Seeds soaked in 0.1% wood ash solution resulted in a greater loss of solids (6.2 percent).

The loss of solids during soaking may be influenced by the concentration gradient (differences in chemical potential) by regulating and limiting the rate of diffusion of water or soaking medium by means of the physical barrier in cotyiedon cell walls and need coat. The greater loss in solids of the seeds soaked in ImM NaOH and 0.1% wood ash solution in this investigation may be due to the effect these chemicals have in creating an ionic environment.

Bongirwar and Sreenivasan (20) reported that soak treatment softens the pellicle or bran of seeds by tenderizing them and boosting the solublization of protein in starch components. The effect of soaking treatment of grass pea

seeds on protein and non protein nitrogen (NPN) is shown in Table-l. Soaking of seeds in ImM HCI and tap water resulted in increasing the crude protein. The amount of total protein was increased by about 11 and 13 percent, respectively,in both soaking solutions after 72 hr. The apparent small increases in total protein in hydrated grass pea seeds were probably due to the loss of soluble solids during soaking. In contrast, soaking of seeds for 27 hr in ImM NaOH and 0.1% wood ash solution resulted in 12 and 16 percent loss of protein, respectively.

Soaking also resulted in the loss of non protein nitrogen content of the grass pea seeds. Seeds soaked for 72 hr in bOth ImM HCI and tap water lost 15 percent of their NPN content. The losses were much greater when the seeds were soaked for the same period in ImM NaOH and 0.1% wood ash solution which constituted 53 and 62 percent, respectively (Table-I). Nelson et al. (21) also reported that of the 5 percent loss of crude protein investigated, only less than 1 percent of the protein was leached out of the 8oybean while the rest leached was NPN constituents.

The protein loss of grass pea seeds Soaked in ImM NaOH and 0.1% wood ash solution may be due to the increase in protein solubility caused by alkalinity and an increase in permeability of the seed coat which is partially significant in terms of larger molecules.

True protein also increased by about 15 and 14 percent, respectively, when the seeds were soaked in ImM HCI and tap water for 72 hr. In contrast, the true protein content decreased by about 6 and 11 percent, respe.:tively, when the soaking media were ImM NaOH and 0.1% wood ash solution(Table-l).

Total carbohydrate by difference decreased by about 4-9 percent in ImM HCI, tap water and 0.1% wood ash solution whereas it remained unaffected in ImM NaOH soaking media (Table-2).

Total soluble sugars decreased by about 46 and 30 percent, respe\:tively in seeds soaked in ImM HCI and tap water for 72 hr . The losses were 40 and 5fpercent respectively in ImM NaOH and 0.1% wood ash solution soaking media (fable-2).

Reducing sugars were also lost to the soak solutions during the soaking process. Losses of reducing sugars to the four soaking media were 56, 67, and 78 percent, respectively, during 72 hr soaking process (table-2). Decreases in non-reducing sugars were also. noted in the four soaking media. Wang et al. (22) and Shun et al. (23) also observed partial removal of sugars during the hydration of whole soybeans.

The grass pea seeds may gain or lose minerals in the form of ash during the soaking process depending on the composition of the soaking solutions. Similarly, the ash content of the seeds soaked for 72 hr in the four soak media varied significantly (fable-3). Seeds soaked in 1mM HCI achieved a 50 percent reduction in ash content whereas the decreases were not significant in seeds soaked in water and ImM NaOH solution. The loss of ash content in the soaked seeds may be due to leaching of soluble mineral elements into the soaking media. In contrast, ash content of seeds soaked for 72 hr in 0.1% wood ash solution increased by about 68 percent which may be due to the absorption of minerals from filtrate.

The crude fibre content of the seeds increased throughout the incubation period whereas the total lipid content remained unaffected by the soaking process (fable-3). The soaking process also affected the pH of the soaking solutions. The pH of the tap water decreased from about neutral to acidic (5.1) during 72 hr soaking process.

The pH of the alkaline soaking solutions decreased by 2-3 log units. In contrast, the pH of the 1mM HCI soaking medium increased by about 2 log units (fable-1) .Such changes in pH may be attributed to the nature of the leachate.

During the soaking process, the neurotoxin BOAA leached out partially in all the soaking media (Fig. 2). The loss of BOAA in 1mM HCI and tap water was 70 and 54 percent, respectively whereas 72 and 80 percent losses were

recorded in seeds soaked for 72 hr in 1mM NaOH and 0.1% wood solution. Bhat (14) also reported that by soaking the grass pea seeds overnight in water at an initial temperature of 75OC and then drying, the toxin could be reduced by 94 percent.

Dehulling of pulses removes the seed coats and this results in increased protein and fat contents but decreases ash and fibre contents. 'Kikh' soaked for 48 hr in the four soak media resulted in higher losses of nutrients (Table-4). Higher losses in total protein, NPN, soluble and reducing sugars and leached soli1s were observed during the soaking process of seeds and 'kikh ' . The increase in pH of the soak media resulted in a significant decrease in BOAA content of 'kikh ' .After soaking for 48 hr in 1mM HCl and tap water, 1mM NaOH and 0.1% wood ash solution; decreases of 56, 65, 83 and 85 percents, respectively, were recorded. Consequently, 'kikh' showed significantly lower nutrient retention values compared to whole seeds mainly due to high loss of solids during soaking.

The soaking process resulted in light-coloured products when seeds were soaked in 1mM HCl and tap water. However, 1mM NaOH solution and 0.1% wood ash solution treatment produced seeds of less desirable colour after 48 hr soaking but the seeds had softer texture in comparison with those that underwent other soaking treatments. An increase in alkalinity was shown to increase discoloration caused by pigment leaching from the hilum of black eyed peas (24).

Soaking in 1mM HCI appeared to have significant edge over ordinary water in lowering the level of BOAA in grass pea seeds. However, 0.1% wood ash solution soaking proved to be effective as 1mM NaOH solution in removing BOAA. This study also demonstrated that during soaking in 1mM NaOH and 0.1% wood ash solution, significantly greater quantities of nutrients were leached at each interval from the seeds and 'kikh' than in the other two treatments. With judicious processing the loss of nutritive value of grass pea can be minimized and the neurotoxin BOAA content effectively reduced. Furthermore, processing of grass pea seeds and 'kikh ' may reduce their extended Cooking times and thus enhance their consumption by saving fuel cost and increasing nutritive values. Further studies on the sensory quality , characteristics and functional properties of presoaked grass pea foods are however, required before processing into various traditional food formulations.

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Erratum - Please insert pp 83a - 83f after page 83, Ethiop .J. Health Dev. Vol.7,No.2, 1993

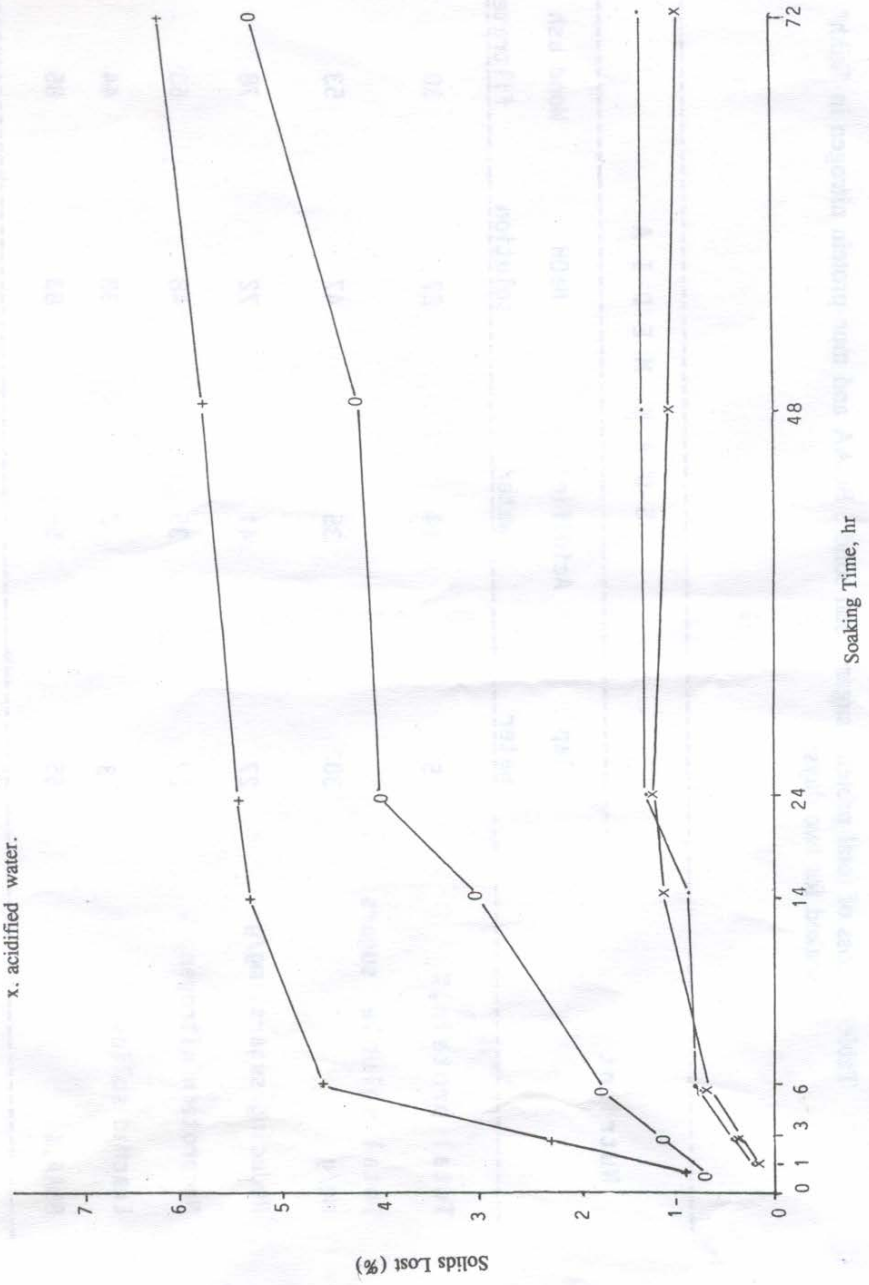

FIG. 1. Solids lost during Soaking. +, wood ash filtrate; O, NaOH solution; ., Plain Water; x, acidified water.

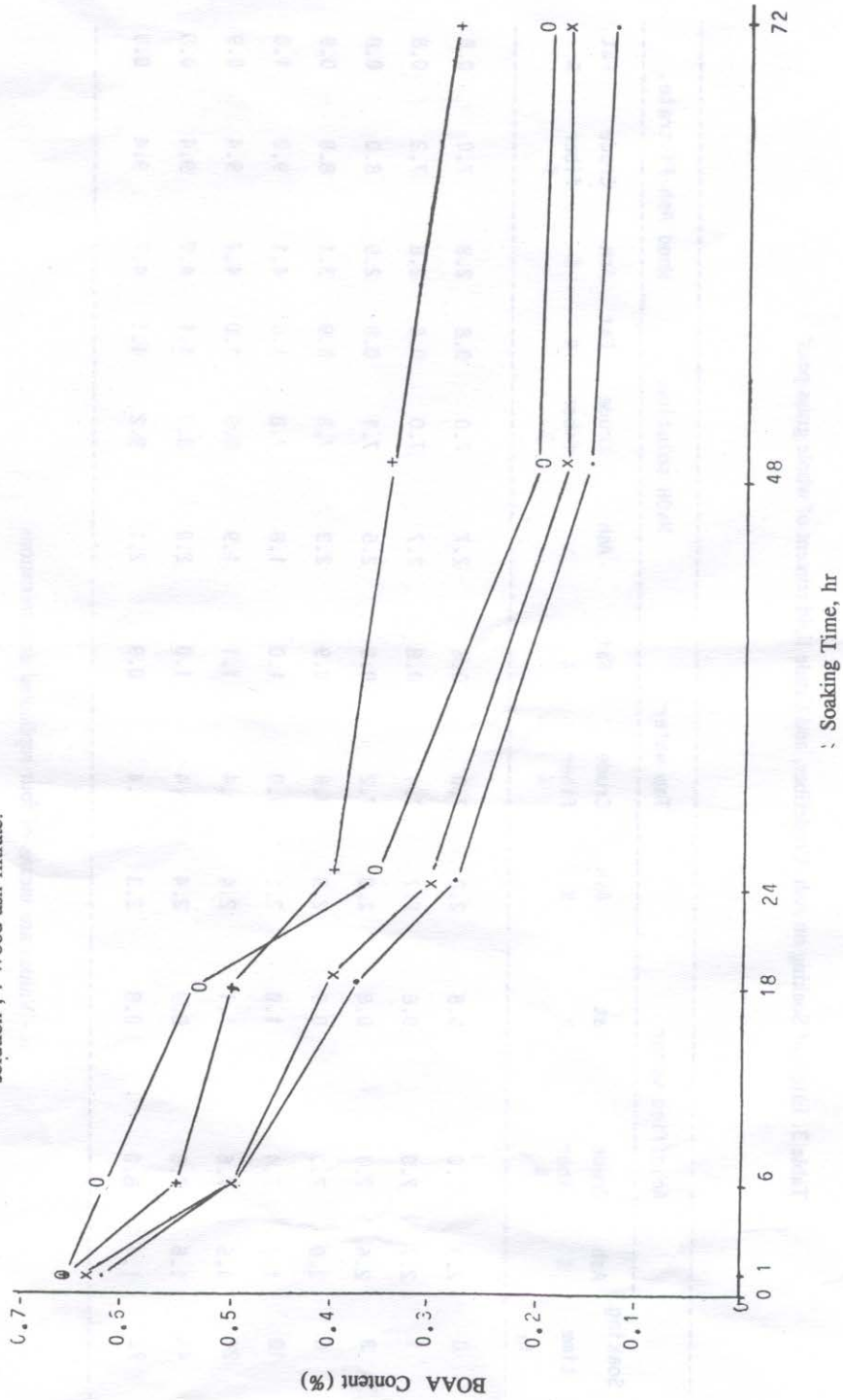

Fig. 2. Effect of Soaking on BOAA content . + , Plain Water ; O, acidified Water ; x , NaOH solution ; . Wood ash filtrate.

Table 1. Effect of Soaking on non protein, total protein and true protein contents of whole grass peas[a]

Soaking time hr	Acidified water				Tap water				NaOH solution				Wood ash filtrate			
	NPH	Total Protein	True protein	Final pH	NPH	Total Protein	True protein	Final pH	NPH	Total Protein	True protein	Final pH	NPH	Total Protein	True protein	Final pH
	%	%	%		%	%	%		%	%	%		%	%	%	
0	0.47	25.6	22.7	3.0	0.47	25.6	227	7.3	0.47	25.6	22.7	11.0	0.47	25.6	22.7	12.0
1	0.47	26.9	24.0	3.0	0.47	25.8	22.9	7.3	0.46	25.4	22.5	11.0	0.46	25.6	22.7	12.0
3	0.47	27.4	24.5	3.0	0.47	26.0	23.1	7.3	0.44	25.1	22.4	10.9	0.44	25.5	22.8	11.8
6	0.45	27.7	24.9	3.4	0.46	27.2	24.3	7.0	0.41	24.6	22.0	10.7	0.37	25.1	22.8	11.5
18	0.44	28.0	25.3	4.1	0.44	27.5	24.8	6.5	0.37	24.1	21.8	10.3	0.31	24.8	22.8	10.4
24	0.43	28.5	25.8	4.5	0.43	27.7	25.0	6.1	0.31	23.6	21.7	9.9	0.28	24.3	22.6	9.8
48	0.42	28.6	26.0	4.8	0.41	28.0	25.4	5.4	0.27	23.1	24.4	9.2	0.25	23.7	22.1	9.3
72	0.40	28.6	26.1	5.0	0.40	28.3	25.8	5.1	0.22	22.6	21.0	9.0	0.18	21.5	20.3	8.9

a- Values are means of four replicated determinations.

Table 2. Effect of Soaking on carbohydrates of whole grass peas [a]

Soaking time hr	Acidified water				Tap water				NoOH solution				Wood ash filtrate			
	Total Carbohydrate	Total sugar	Reducing sugar	Non-reducing sugar	Total Carbohydrate	Total sugar	Reducing sugar	Non-reducing sugar	Total Carbohydrate	Total sugar	Reducing sugar	Non-reducing sugar	Total carbohydrate	Total sugar	Reducing sugar	Non-reducing sugar
	%	Mg/g	Mg/g	Mg/g	%	Mg/g	Mg/g	Mg/g	%	Mg/g	Mg/g	Mg/g	%	Mg/g	Mg/g	Mg/g
0	53.6	87.3	1.8	85.5	53.9	87.3	1.8	85.5	53.9	87.3	1.8	85.5	53.9	87.3	1.8	85.5
1	53.6	79.4	1.7	77.7	53.6	87.3	1.8	85.5	54.1	79.5	1.7	77.8	53.6	87.0	1.7	86.3
3	52.1	76.0	1.6	74.4	53.4	87.2	1.7	85.5	52.5	77.7	1.5	76.2	52.8	81.7	1.7	80.0
6	52.1	64.6	1.5	63.1	50.8	86.9	1.6	85.3	53.4	77.2	1.3	75.9	52.1	77.1	1.6	75.5
18	51.6	59.0	1.4	57.6	50.2	83.3	1.4	81.9	53.3	72.5	1.1	71.4	51.1	64.5	1.4	63.1
24	51.3	53.1	1.3	51.8	49.4	79.7	1.1	78.6	52.9	61.1	0.9	60.2	50.2	52.3	1.3	51.0
48	51.2	51.2	1.2	50.8	49.2	68.3	0.8	67.5	52.9	55.9	0.7	55.2	51.4	48.2	0.7	47.5
72	51.1	47.3	0.8	46.5	49.1	61.2	0.6	60.6	53.0	52.4	0.6	51.8	51.8	431.	0.4	42.7

a-Value are means of four replicate determinations.

Table 3. Effect of Soaking Ash, Drudefiber, and lipd content of whole grass peas [a]

Soaking time hr	Acidfied water			Tap water			NaOH solution			Wood Ash Filtrate		
	Ash %	Crude fiber %	Fat %	Ash %	Crude fiber %	Fat %	Ash %	Crude fiber %	Fat %	Ash %	Crude fiber %	Fat %
0	2.8	7.0	0.8	2.8	7.0	0.8	2.7	7.0	0.8	2.8	7.0	0.8
1	2.7	7.0	0.8	2.7	7.1	0.8	2.7	7.0	0.8	2.8	7.2	0.8
3	2.6	7.1	0.8	2.6	7.2	0.8	2.5	7.1	0.8	2.9	8.0	0.8
6	2.0	7.3	0.9	2.3	8.8	0.9	2.3	7.3	0.9	3.1	8.8	0.9
18	1.7	7.4	1.0	2.3	9.0	1.0	1.8	7.8	1.0	4.1	9.0	1.0
24	1.5	7.6	1.1	2.4	9.4	1.1	1.9	8.6	1.0	4.7	9.4	0.9
48	1.5	7.8	0.9	2.4	9.4	1.0	2.0	8.9	1.1	4.7	9.4	0.8
72	1.4	8.0	0.8	2.3	9.4	0.9	2.1	9.2	1.1	4.7	9.4	0.8

Table 4. Loss of total protein, sugars total solids, BOAA and non-protein nitrogen in "kikh" soaked for two days:

Nutrient	SOAK MEDIA			
	Tap water	Acidified water	NaOH solution	Wood ash filtrate
Total protein	5	14	27	30
Total soluble mg/g	30	35	47	53
Reducing sugars, mg/g	27	41	72	78
Nonprotein nitrogen, %	27	35	48	63
Leached solids, %	3	7	33	44
BOAA, %	65	56	83	85