Original article

Effect of processing methods on the trypsin inhibitor, tannins, phytic acid and ODAP contents of grass pea seeds

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Abstract: Grass pea seeds were given different treatments including cooking boiling, autoclaving, dry heating and fermentation into tempeh. Changes in the levels of the antinutritional factors due to the treatments were estimated. Dry heat treatment completely eliminated phytic acid and greatly reduced tannins, trypsin inhibitor activity and ODAP (100%,64%,87.4% and 75%, respectively). Cooking reduced tannins (74%), trypsin inhibitory activity (81 %) and ODAP (77%) while phytic acid was less affected (59.4%). Autoclaving had the most pronounced lowering effect on trypsin inhibitor activity (91 %), whereas other anti-nutritional factors were less affected. Boiling also decreased the trypsin inhibitor activity by 89.3%. Preprocessing of grass pea for tempeh fermentation and fermentation into tempeh significantly removed large portions of the antinutritional factors in grass pea. [Ethiop. J. Health Dev. 1995;9(1):97-103]

Introduction

Like other legumes, grass pea (Lathyrus sativus) can synthesize a variety of undesirable chemical substances termed antinutritients that are known to exert a deleterious effect when ingested by man or animals. These substances include phytic acid, trypsin -inhibitors and tannins which can cause adverse physiological responses or diminish the availability of certain nutrients.

Phytate, widely distributed in food grains (1) lowers the bioavailability of minerals (2) and inhibits several proteolytic enzymes and amylases (3). All legumes studied to date have been found to contain trypsin inhibitors in different amounts. Trypsin inhibitors, when ingested by man in significant amounts, disrupt the digestive process and may lead to undesirable physiological reactions (4).

.- Tannins are also known to be present in food legumes and to inhibit the activities of trypsin, chymotrypsin, amylase and lipase, decrease the protein quality of foods and interfere with dietary iron absorption (5). Grass pea is known to contain ODAP (6-N- oxalyl-L-a,6 -diaminopropionic acid), a neurotoxic amino acid implicated as a cause to lathyrism (6). The disease is prevalent in Ethiopia, India and Bangladesh (7,8,9). Grain legumes in Ethiopia are processed and consumed in a variety of forms. The most common methods of preparation are usually cooking, boiling into a stew, gennination and fermentation. Teutenico and Knorr (10)
reported that traditional methods of processing such as soaking, cooking and fermentation improved the nutritional value of legumes.

Tempeli fermentation improved the nutritional and sensorial value of a wide variety of legumes allover the world (11). Effect of heat treatment and tempeh fermentation on trypsin inhibitors, tannins, and phytic acid in

other legumes nave oeen reponeo (12, 13, 14, 15) .Although the presence of antinutritional factors such as phytate (16) and trypsin inhibitors (17) in grass pea are reported, no study has documented the effect of heat
treatment on reducing them. 1 This paper is an account of an investigation on the effects of autoclaving, dry heat treatment, boiling, cooking and tempeh, fermentation on the trypsin inhibitors, tannins, phytic acid and ODAP content of grass pea grown in Ethiopia.

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Methods

Sample Preparation: Grass pea samples of local variety were obtained from a market in Ambo, Shoa. All the samples were cleaned manually to remove foreign matters and aliquots were ground in a cyclone mill for estimation of antinutritional factors. The remaining whole grain samples were used for decortication, boiling and/or cooking processes as described below.

Preparation of heat treated grass pea meals Dry heat treated grass pea meals: 10g of finely ground grass pea flour was heated in a beaker at a thickness not exceeding 1 cm in an oven maintained at 200°C for 30 minutes.

Autoclaved grass pea meals: the autoclaved grass pea meals were prepared by autoclaving 10g of finely ground grass pea flour in a beaker at a thickness not exceeding 1 cm at 12ooc (15 Ib/in2) for 10 minutes as described by Tan et al (18). The autoclave was preheated to minimize the time required to reach the desired temperature (approximately 7 minutes).

Boiled grass pea meals: about 10g of whole grass pea seeds were boiled in 100 ml of distilled water for 20 minutes. The cooking water was drained off, and the seeds were dried in an oven at 80OC overnight and were ground to yield the boiled grass pea meal.

Cooked grass pea meals: whole grass pea seeds (10g) were soaked in 30mI of distilled water at room temperature for 24 hours. After the soaking water was drained off, the seed were cooked in sufficient amount of boiling water 4 hours. The Cooked seeds mar were boiled to 'eating soft' were then dried in an oven at 80°C overnight before grinding fur fine flour.

Preparation of grass pea tempeh: grass pea tempeh was prepared using the procedure of Ko and Hesseltine (19). The pretreated grass peas were inoculated with a powder-form (10% of the substrate) of inoculum of Rhizopus oligosporus at 10s colony forming units/gram (cfu/g) prepared substrate. The inoculum of R. oligosporus was obtained from the Nutrition Research and Development Centre at Bogor , Indonesia.

Determination of .p-N-oxalyl-L-a,.p- diaminopropionic acid: the ODAP content of heat treated grass pea meals were determined using Rao's method (20). A hundred milligram of grass pea meals was extracted with 10ml of 60% ethanol with mechanical sh3;king, for 6 hours at room temperature. After centrifugation, 100p.l of the extracts were taken in duplicate in test tubes. To

one set of tubes, 0.2 mI of 3N KOH was added and the tubes were kept in a boiling water bath for 30 minutes. After cooling the tubes to room temperature, the samples were brought to 1 ml with water and 2 ml OPT (o-Phthalaldehyde dissolved in 95% ethanol and mercaptoethanol) reagent was added. The absorbency of the yellow solution was measured after 30 minutes at 420 nm against a reagent blank using Beckman DU-60 spectrophotometer (Beckman, USA).

Determination of the tannin content: the method of Bums (21) as modified by Max son and Rooney (22) was used for tannin determination. One gram of grass pea meal was extracted with 10 ml of 1% concentration
HCl in methanol for 24 hours at room : temperature. After centrifugation at l0000g e (Sorval, USA) for 5 min, lmI of,supematant was mixed with 5 ml of Vanillin-HCl reagent, a and the absorbency was read at 500 nm after :d 20 minutes. Values of tannins were expressed :r in mg of Dcatechin/gm of sample.

Determination of the phytic acid content: 19 Phytic acid content was determined using the method of Haug and Lantzsch (23). An aliquot (200mg) of grass pea meal was extracted with 10 mi of 0.2N HCI containing 10% Na2SO4 using a mechanical shaker for 3hours at room temperature. The extract (0.5 mi) was pipet ted into a test tube fitted with a ground glass topper. One mi of ferric solution (ammonium iron (III) sulphate dissolved in 2N HCI) was added. The tubes were heated in a boiling water bath for 30 minutes, and after cooling in ice water for 15 minutes, they were allowed to adjust to room temperature. After centrifugation for 10 minutes at 5000 g, 1 mi of the supernatant was transferred to another test tube and 1.5 mi of bipyridine solution was added. The absorbency was read at 519 Dm (Beckman, USA) against distilled water . Determination of trypsin inhibitor activity (FIA): Trypsin inhibitor activity of heat treated grass pea meals was determined using the method of Kakade et al (24). N-benzoyl-DL- arginine-P-nitroanilide (BAPNA) was used as the trypsin substrate. One trypsin unit (TU) was arbitrarily defined as an increase of 0.01 absorbency units at 410 nm per 10 mi of the reaction mixture. Trypsin inhibitory activity was expressed in terms of Trypsin Units Inhibited (TUI). Total TIA was expressed as TIU/g (dry weight).

Analysis of variance was used to all the data. Duncan's multiple range test was used to locate differences between means.

Results

Phytates, tannins TIA and ODAP contents of heat treated grass peas are presented in Table 1. The amount of phytic acid in raw whole seeds was 476.3 p.g/g. Phytic acid was greatly diminished by cooking (59.4%) and
substantially removed by autoclaving (21.2%) but decreased only slightly upon boiling (11%). Dry heat treatment, however, effectively eliminated all phytic acid in the grass pea seeds (Table 1). A reduction of
about 98.4% in the phytic acid content of grass peas, however, was shown to occur when grass peas were fermented into tempeh (Table 2).

The TIA of raw whole grass peas was found to be 23231 IU/g (Table 2). This value was significantly ($P<0.05$) reduced by heat treatments. Dry heat treatment of whole grass pea flour at 200OC for 30 minutes destroyed 87.4% of the trypsin inhibitor activity. Autoclaving of the whole

grass pea flour at 120°C (pressure of 15 Ib/in) for 10 minutes inactivated 91% of the trypsin inhibitor activity. On the other hand, cooking for 4
hours in boiling water to 'eating soft' reduced the trypsin inhibitor activity by 81% whereas boiling the whole seed for 20 minutes inactivated 89% of the TIA. Prefermentation treatments of grass pea for tempeh inactivated

Table 1: **Effect of different heat treatments on antinutritional factors in grass pea***

Heat treatment	Phytate mg/100g	Loss %	Tannin mg/100g	Loss %	TIA IU/g	Loss %	ODAP mg/100g	Loss %
Raw seeds	467.3[a]	-	670[a]	-	23231[d]	-	476.3[d]	-
Autoclaving	368.2[b]	21.2	533[b]	20.5	2091[b]	91.0	431.1[b]	9.5
dry heating	NIL[c]	100	244[c]	63.6	2927[c]	87.4	120.0[c]	74.8
Boiling	462.3[a]	10.5	438[d]	34.6	2486[d]	89.3	422.0[d]	11.4
Cooking	190.0[d]	59.4	178[e]	73.5	4460[e]	80.8	111.0[e]	76.7

* Data are mean values of three measurements
Means of the same column followed by different letters differ significantly ($P<0.05$)

Table 2: **Effect of tempeh preparation process on antinutritional factors in grass pea***

Processing methods	Phytate mg/100g	Loss %	Tannin mg/100g	Loss %	TIA IU/g	Loss %	ODAP mg/100g	Loss %
Dehulled seeds	467.0[a]	-	2.01[a]	-	23231[a]	-	476.3[d]	-
Boild 15m	408.9[b]	12.5	0.99[b]	51.2	2695[b]	88.4	125.7[b]	73.6
Soaked 18h	350.0[c]	25.1	0.62[c]	69.4	1673[c]	92.8	90.0[c]	81.1
Boiled 30m	256.0[a]	45.2	0.31[d]	84.7	1533[d]	93.4	54.3[d]	88.8
Tempeh fermentation	7.5[d]	98.4	13.27[e]	-	1185[e]	94.9	35.3[e]	92.6

* Data are mean values of three measurements
Means of the same column followed by different letters differ significantly (P<0.05)

from 88.4 to 93.4 % of the TIA. Fermentation of grass pea into tempeh further reduced the TIA by 94.9% (Table I). Dehulled grass pea seeds contained 23231 IU/g of TIA which was decreased upon fermentation to 88.4%. Tannin content of whole grass pea appeared to be high (6.7 mg/g) (Table 2). Pretreatment of dehulled grass pea prior to tempeh fermentation (soaking and boiling) significantly decreased the contents of tannin (51.2-84.7%). However, fermentation of pretreated dehulled grass pea into tempeh significantly increased the content of tannins in grass peas (Table 2). The content of tannin in final tempeh product increased 6.6-fold compared with dehulled seeds. The neurotoxin, ODAP, content of raw grass pea was 4763 p.g/g (Table 1). Cooking to 'eating soft' and heat treatment process effectively reduced the ODAP content by about 78 and 77% , respectively. The least effective processing of grass pea for fermentation into tempeh significantly (p<O.O5) reduced (74- 87%) the content of ODAP (Table 1). Similarly, tempeh fermentation of preprocessed grass pea further reduced the ODAP content by 93% (Table 2).

Discussion

fhe amount of phytic acid in raw whole grass pea is 476.3 p.g/g (dry weight). This value is significantly lower than the phytic acid content of moth bean and less than the phytic acid of the cowpea which is not likely to be of any nutritional importance (15). Upon comparing the absolute loss of phytic acid during different processing methods, it was observed that dry heat treatment completely eliminated the phytic acid content, while boiling and autoclaving processes were less effective in lowering phytic acid of grass pea grains. Ologhobo and Fetuga (14) showed that autoclaving slightly reduced the phytic acid content of cowpeas. The cooking process also significantly decreased the phytic acid content of grass peas. This was expected as the grass peas were incubated or steeped before cooking. Similarly, Chang et al. (25) reported that steeping of beans and incubation in water followed by cooking in boiling water hydrolysed 50% of bean's phytate. In the present study, the grass pea was also subjected to steeping process in water for 24 hours prior to cooking, and the reduction in phytic acid 1 content was mainly due to both the effect of It steeping and heat.

Prefermentation process also reduced phytic acid upto 45% and a further 98.4% reduction was achieved by mould fermentation. The loss s of phytic acid. during processing of grass pea seeds for tempeh preparation may be due to leaching while the decrease of phytic acid during fermentation could have resulted from phytic acid hydrolysis as a result of phytase activity. A reduction of about 33% in the phytic acid content of soybeans has been shown to occur when soybean was fermented into tempeh (26). Wang et al. (27) and Sutardi and Buckle (26) reported that enzymatic hydrolysis of phytic acid during fermentation was accomplished by fungal phytase.

Tannins in the raw grass pea seeds were much higher than those in chick peas, comparable with those in green gram and much lower than those in black gram, pigeon pea, horse gram, moth bean and winged bean (28) .The heat treatment processes decreased tannin content in grass pea grains. Cooking and discarding of the cook-water resulted in 73.5% decrease in tannin content of grass pea. Reddy et al (28) also observed 38- 77% reduction in tannin content due to cooking of legumes. It was suggested that the apparent decrease in tannins during cooking was most likely not due to an actual decrease in tannins but due to a change in their solubility or chemical reactivity (28).

The observed decrease in tannin content of grass pea as during cooking may be due to binding of tannins with other organic substances and proteins, or from alteration in the chemical structure of tannins that cannot be determined by chemical methods used in this study.

In a separate study, Tan et al. (18) reported that autoclaving and dry heat treatment reduced the assayable tannin content in winged beans by 56-75%, whereas cooking process reduced it by 89100%. For cowpeas, Ologhobo and Fetuga (14) found that cooking and autoclaving reduced tannin content only by 43 and 21% , respectively.

Since tannins are located mainly in the testa or seed coats of dry legumes, the physical removal of seed coats by dehulling may decrease the tannin content in legumes and improve their nutritional quality. In the present study, dehulled grass pea contained about three times less tannins compared with raw whole grass pea. Significant reduction in tannin content of dry beans (68-99% of tannins) by removal of seed coat by dehulling have been reported recently (28).

Prefermentation processing further reduced tannin content of grass pea by 87.4 % while fermentation of grass pea into tempeh by the tempeh inoculum increased the tannin content by 6.6fold. Fermentation by R. oligosporus similarly increased the tannin content of the sorghumcomrnonbean tempeh, probably from moulds and release from the tannin-protein complexes by enzymes during fermentation (29).

Trypsin inhibitors are known to be heat liable and inactivating protease inhibitors in several food legumes results in increased protein quality. TIA in the raw whole grass pea compares favourably with that of the cowpea varieties (14) but are lower than those of winged beans (18) and lima bean varieties (12).

Among the processing methods studied, autoclaving, boiling and dry heat treatment were equally effective in decreasing TIA compared with cooking although none of the methods completely eliminated the TIA in grass pea grains. In the present study, 9-19 % of TIA remained unaffected

after the heat treatment processes. It may be assumed from these results that trypsin inhibitors found in grass pea grains are heat-stable. Complete
loss of TIA of cowpeas was, however, reported by Ologhobo and Fetuga (14) after autoclaving and soaking. Such heat treatments have also been reported to be effective in inactivating the trypsin inhibitors in several food legumes (12,15).

Prefermentation processing greatly reduced the TIA of dehulled grass peas (93.4%). Fermentation by the tempeh inoculum for 48 hours further significantly decreased ($p < 0.05$) this antinutritional factor by 94.9% .Similar loss of TIA was observed by Roozen (30) during the fermentation of soybean tempeh .

Wang et al (31) also reported that R. oLigosporus is capable of hydrolysing the trypsin inhibitor of soy beans.
The results also indicate that the ODAP content of the grass peas was greatly diminished by cooking and dry heat treatment but reduced only slightly upon autoclaving and boiling. However, a recent study using chick
bioassay with these same processing methods did not indicate the toxic substances in Lathylls sativus to be completely removed or destroyed (32). In a more recent study, the loss of ODAP, however, was 52-82% when grass pea seeds were soaked for three days in different soaking media (33).

The present investigation indicated that compared to other common legume grains, grass pea contained considerably less antinutritional factors exclusive of ODAP . This could reflect an overall better nutritional value of the seeds of this legume.

Of the processing methods, dry heat treatment and fermentation into tempeh appeared to be very advantageous in removing some of the antinutritional factors, especially phytic acid which was removed in substantial amounts.

Both autoclaving and boiling removed TJA and tannins to some extent. Cooking to 'eating soft' after soaking for 24 hours had the most pronounced effect on reducing tannins, phytic acid, TIA and ODAP. TIA was also lowered considerably due to boiling treatment.

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