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Effect of processing on the content of β-N-oxalyl-α, β-diaminopropionic acid (β-ODAP) in grass pea (*Lathyrus sativus*) seeds and flour as determined by flow injection analysis

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The effect of cooking, roasting, autoclaving and fermentation on the content of β-ODAP in the whole seeds and flour of grass pea (*Lathyrus sativus*) were studied at different levels of temperature, time, pH, degree of soaking and moisture content. The method of determination used was flow injection analysis, with immobilised glutamate oxidase and horseradish peroxidase. The whole seeds flour was found to contain about 922 mg $100g^{-1}$ β-ODAP in dry weight basis. The reduction of β-ODAP content, in samples which were cooked for 60min and roasted (150°C for 60 min) was 57% and 82%, respectively. The content of β-ODAP in dry seeds autoclaved for 30 min also showed a significant ($p=0.05$) reduction by 39%, as compared to that of raw whole seeds. Similarly, by cooking of presoaked seeds the content of β-ODAP was reduced by up to 67%. Neither the back-slopped fermentation process nor the spontaneous fermentation were effective in reducing the content of β-ODAP. Whereas roasting and autoclaving of the milled samples caused significant ($p=0.05$) reduction in the content of β-ODAP up to 30% and 50%, respectively, compared to that of raw whole seeds.

INTRODUCTION

Grass pea is a leguminous crop cultivated in different parts of the world. It has a number of advantageous biological and agronomic qualities such as extensive tolerance to draught and water logging, high grain yielding capacity, and resistance to insects and pests. Moreover, these seeds are comparatively cheaper than many other legumes (Rao *et al.*, 1969; Lambein and Haque, 1993). In a nutritional survey report on the preparation and consumption of grass pea in 224 families of two villages in north western Ethiopia, this pulse is reported to have an excellent taste, and its flour possessed unique water absorbing and swelling properties which are advantageous for preparation of sauce (Teklehaimanot *et al.*, 1993).

However, a major draw back to its full utilisation is the occurrence of a toxic syndrome associated with the consumption of these seeds, called neurolathyrism or lathyrism. The causative agent was reported to be the non-protein amino acid β-N-Oxalyl-α, β-diaminopropionic acid (β-ODAP). In human, the disease generally appears whenever a diet consisting one-third to one-half of grass pea seed is consumed for a period of 3–6 months (Rao *et al.*, 1969).

Ramanujam *et al.* (1980) who analysed the ODAP content of different parts of grass pea plant found highest concentration of ODAP in the whole seeds ranging from 0.1% to 2.5% on dry weight basis. ODAP is found to occur in nature in two isomeric forms and the amount of α-isomer is about 5% of the total quantity of ODAP. The α-isomer is reported to be less toxic than the β-isomer (Harrison *et al.*, 1977; Chase *et al.*, 1985).

Most of the previous research dealing with ODAP in grass pea were done using a colorimetric method of analysis. In this case, both isomers of ODAP are converted quantitatively to DAP by alkali hydrolysis. Thus,

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the less toxic α-ODAP is determined along with the β-form (Rao, 1978).

In a recent study, Padmajaprasad *et al.* (1997), by using electrophoresis for separation of the isomers and colorimetric method for determination of ODAP, showed that the isomerisation of ODAP present in grass pea seeds is affected, to a significant degree, by household cooking procedures. In the present work, the effect of cooking, roasting, autoclaving and fermentation at different levels of temperature, time, soaking, pH and moisture on the content of β-ODAP in whole grass pea seeds and its flour was determined. An enzymatic flow injection analysis (FIA) using immobilised glutamic oxidase specific to the β-isomer, according to Moges (1994) was used for the determination of ODAP in all the samples.

MATERIALS AND METHODS

Sample

Seeds of grass pea (*Lathyrus sativus*) which were harvested in Enewary woreda, Tegulet and Bulga Agricultural Office, Ethiopia, were used for this study.

Chemicals

L-Glutamate oxidase (EC 1.4.11, 6.9 units mg^{-1} of solid at pH 7.4 and 30°C, Yamasa corp., Japan), Catalase (EC 1.11.1.6, 19900 units mg^{-1} of solid, sigma C-40), Horseradish peroxidase (HRP, EC 1.11.1.7, 268 purpurogallin units mg^{-1} of solid, Sigma p-8375), Glutaraldehyde (25%) and standard ODAP were from Sigma Chemical Co., St Louis, Missouri, USA. All other chemicals used were of analytical grade.

Sample preparation

The seeds were cleaned manually to remove foreign materials, washed with tap water, rinsed with distilled water and immediately dried in an oven (Nino lab. E. Nilsson instrument. AB, Sweden) at 50°C for 8 h under air circulation before it was divided into two lots. One lot was milled to a particle size of 40–60 mesh using a standard mill (Cyclotec 1093 sample mill, Tecator, Sweden). The flour and the second lot of whole seeds were stored in a cold room (4°C) until required for analysis or processing.

Processing

Cooking

The whole seeds were placed in boiling tap water (1: 2.5 w/v seed to water) at 100°C and cooked for 30 or 60 min at pH 5 or 8. Whole seeds were also cooked in this way after soaking (1:2 w/v seed to water) for 3 h or 12 h.

Autoclaving

Whole seeds were autoclaved (Getingeverken, Sweden) at 1.3 kg cm^{-2} for 15 or 30 min. During wet autoclaving, the seeds were kept in the autoclave submerged in water and for dry autoclaving the seeds were kept without water.

Roasting

The seeds were roasted uniformly in an oven (AB Termoglas, Göteborg, Sweden) placed in a rotating container at 110°C or 150°C for 30 or 60 min.

Fermentation

Fermentation of the whole grass pea flour was carried out in two ways. For spontaneous fermentation, the flour was mixed with water at 40°C (1:2 w/v flour to water) and kept at 25°C in a water bath without agitation until its pH became 4.0. For the back slopped fermentation, a portion of the earlier fermented slurry (by spontaneous fermentation) was used as starter culture, to a level of 5% by weight.

All the processed samples (except roasted and dry autoclaved) were dried in an oven at 50°C for 8 h and milled to fine flour with laboratory mill (Cyclotec, Tecator AB Höganäs, Sweden) to a particle size of 40–60 mesh and stored at 4°C until required for analysis.

Determination of β-ODAP by flow injection analysis

Immobilisation of the enzymes

Pre-treatment (Silanization and activation) of the controlled porous glass (CPG-10 with 0.12–0.20 mm particle size and 50 nm pore size, Serva) on which the enzymes were immobilised, was done according to Johansson and Ögren (1976). Further procedures for imobilization of the enzymes were carried out according to Moges (1994).

Glutamate oxidase (4 mg) from *Streptomyces* species was immobilised on 150 mg activated CPG in 0.1 M phosphate buffer (pH 7.0). The estimated coupling yield (immobilisation efficiency) was 85%, expressed as the ratio between the absorbance (280 nm) of the clear enzyme solution after and before immobilisation. Catalase (0.5 mg) was immobilised on 50 mg of activated CPG, giving an estimated coupling yield of 66%. Horseradish peroxidase was immobilised by the diazotization method, giving an estimated coupling yield of 85%.

FIA equipment

The set-up of the equipment for the determination of β-ODAP was according to Moges (1994). It consisted of two pumps which delivered the carrier buffer and the reagent, respectively. The extracted sample was injected from a 40 μl loop and taken by the carrier (0.1 M phosphate buffer), which passes through the reactors containing immobilised glutamate oxidase catalase and hydrogen peroxidase. This sample stream was mixed

with the reagent stream (phosphate buffer consisting of 2.5 mM DCPS, 0.5 mM 4-AP and 0.25 mM DCP), in a 0.5 m knitted Teflon tube before it entered the reactor with immobilised horseradish peroxidase. Possible interference from the presence of any glutamic acid in the sample was prevented by using two prereactors containing glutamate oxidase and catalase. The flow rate of the carrier and reagent was 0.3 ml min^{-1} and 0.12 ml min^{-1}, respectively. Standard Teflon tubing of 0.5 mm internal diameter was used in all connections of the flow system. Quantitative detection of the red quinone imine dye was performed at 512 nm using a spectrophotometer. A linear standard curve for the determination of β-ODAP was obtained in the range of 0 to 250 μmol ml^{-1}.

Extraction

For the determination of β-ODAP using FIA, 50 mg of the milled sample was extracted with 10 ml of 0.1 M degassed phosphate buffer (pH 7.0). The extraction was done at room temperature, using a vertical rotator (6 rpm) in a tightly closed centrifuge tubes for 90 min. Particulate matter was separated by centrifugation at 4000 rpm (3000×*g*) for 10 min and filtration through a membrane filter with pore diameter of 0.45 mm. In addition to this, protein and other macro molecules were removed by using ultra filtration through a membrane (Amicon, cut-off at 10 000 molecular weight) and centrifugation at 4000 rpm for 1 h (Moges, 1994).

Statistical analysis

The results were analysed at 95% confidence level using Microsoft excel version 5.0 applying F-test for comparing variances and multiple comparison t-test procedure. Simple regression analysis was also done using the same software.

RESULTS AND DISCUSSION

Several methods for the determination of ODAP in grass pea were carried out in different laboratories mainly in connection with development of low ODAP varieties, for large scale cultivation. The most widely used method, which was described by Rao (1978), utilises the reaction of O-phthaldehyde with diaminopropionic acid formed from hydrolysis of both the α and β-isomer of ODAP. In the present study, however, a flow injection method of determination, selective for the β-isomer, described by Moges (1994) was used.

The β-ODAP content of whole grass pea seeds was 920 mg 100 g^{-1} on dry matter basis, which is comparable with the earlier results of Moges (1994), who reported the content to be 870 mg 100 g^{-1} whole seed.

Traditionally, different processing methods including roasting, boiling, preparation of sauce and unleavened bread are used for the consumption of grass pea in the daily diet (Teklehaimanot *et al.*, 1993). Therefore, data on the effect of these processes on the content of β-ODAP in the finished product could be very useful.

Cooking

Figure 1 shows the content of β-ODAP in the seeds cooked at different pH and duration. The content of β-ODAP in the whole seeds was significantly ($p=0.05$) reduced by 26% and 57% after cooking at pH 5 for 30 min and at pH 8 for 60 min, respectively. Gupta (1980) showed the neurotoxic compounds of grass pea (mainly ODAP) to be water soluble and a reason for this reduction could be leaching of ODAP with the water used for cooking, which seems to have increased with time and alkalinity of the media. However, there was no significant difference between the content of β-ODAP of cooked seeds and that of cooked flour. The maximum reduction in the β-ODAP content of the cooked flour as compared to that of seeds was 9%. When the seeds were cooked after soaking them (Fig. 2), their β-ODAP level reduced by 56 ± 5% as compared to that of the raw whole seeds. Nevertheless, change in the cooking time from 30 to 60 min, pH from 5 to 8 and soaking time from 3 to 12 h, did not show remarkable change in the content of β-ODAP. As in the cooking, this reduction seems to be mainly due to the leaching of ODAP in to the water used for soaking and cooking. Soaking of the seeds prior to cooking, within the stated experimental ranges, resulted in a significant ($P=0.05$) reduction in the β-ODAP content by up to 37% in comparison with the seeds cooked without soaking. The reduction could be due to softening of the seeds after soaking (for 3 or 12 h) causing changes in the matrix, which facilitate the diffusion and transport of soluble components. However, there was no significant difference in β-ODAP content of the seeds soaked for 3 and 12 h. However, Geda *et al.* (1995), reported a reduction (25–28%) of ODAP in grass pea after soaking in cold

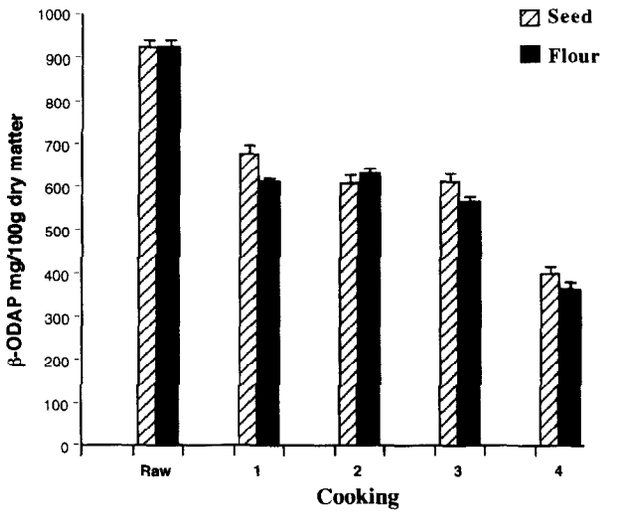

Fig. 1. β-ODAP content of cooked grass pea seeds and flour. **1** = 30 min, pH 5. **2** = 60 min, pH 5. **3** = 30 min, pH 8. **4** = 60 min, pH 8.

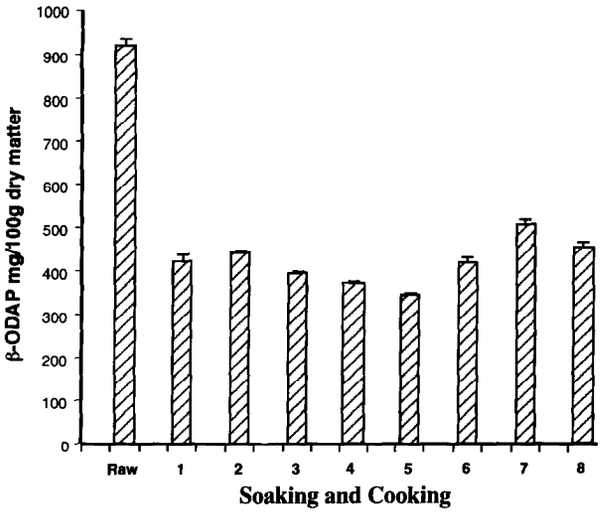

Fig. 2. β-ODAP content of soaked and cooked grass pea seeds. **1** = pH 5, soak 3 h, cook 30 min. **2** = pH 5, soak 3 h, cook 60 min. **3** = pH 8, soak 3 h, cook 30 min. **4** = pH 8, soak 3 h, cook 60 min. **5** = pH 5, soak 12 h, cook 30 min. **6** = pH 5, soak 12 h, cook 60 min. **7** = pH 8, soak 12 h, cook 30 min. **8** = pH 8, soak 12 h, cook 60 min.

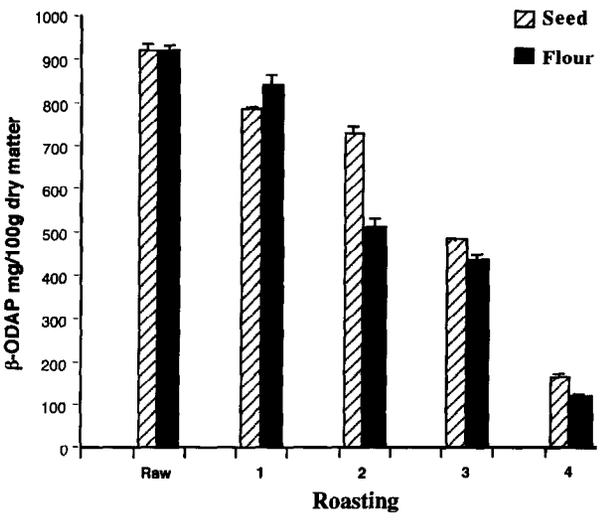

Fig. 3. β-ODAP content of roasted grass pea seeds and flour. **1** = 110°C, 30 min. **2** = 110°C, 60 min. **3** = 150°C, 30 min. **4** = 150°C, 60 min.

water and this reduction was comparatively higher (37–39%) in hot water soaking (at 50°C, for 3 h), requiring a shorter cooking time of 55 min. In addition, Urga and Gebrestadik (1993) also reported a loss 70% and 54% of ODAP when soaked in 1 mM HCl and tap water, respectively. Whereas, they reported loss of 72% and 80% ODAP, using the colorimetric method of determination, in seeds soaked for 72 h in 1 mM NaOH and 0.1% wood ash solution, respectively. Steeping of the seeds in excess water has also been reported by Teklehaimanot *et al.* (1993) to reduce the ODAP content by 50%.

Roasting

Figure 3 shows the content of β-ODAP in the seeds and flour roasted at two different temperature and duration. The content of β-ODAP in the seeds was lower only by 14% after roasting at 110°C for 30 min. However, the 922 mg 100 g^{-1} β-ODAP in raw whole seeds was reduced to 164 mg 100 g^{-1} after roasting at 150°C for 60 min, which accounts for a significant ($p = 0.05$) reduction by 82%. Although both roasting time and temperature showed significant effect on the content of β-ODAP, it appears that the roasting temperature was more effective than the duration, as could be expected. The kinetics of the reaction which caused the reduction in the β-ODAP content is yet to be elucidated. Thus, it seems that it is possible to lower the β-ODAP content of grass pea seeds to a significant degree by raising the roasting temperature a little, to about 200°C for a shorter period of about 35 min. In contrast, after a nutritional survey in the north west of Ethiopia, Teklehaimanot *et al.* (1993) reported elevated levels of ODAP in a roasted grass pea seeds of a local product called 'Kolo', when compared to that of raw seeds. The reason for such an elevation is not clear, however, in this case, the analysis of ODAP was done using the colorimetric method of determination.

The content of β-ODAP in all the roasted flour samples followed the same pattern as that of roasted seeds and there was a significant change in the β-ODAP content of the roasted flour when compared with that of roasted seeds. The highest reduction was obtained after roasting the flour at 150°C for 60 min. The content of β-ODAP of this sample was 115 mg 100 g^{-1}, which means a reduction of up to 88%. This significant reduction achieved after roasting a milled sample must be due to the disruption of the seed structure (during milling) and effective heating of the contents due to the increased surface area.

Autoclaving

Figure 4 shows the β-ODAP content of the autoclaved seeds as dry or submerged under water for 15 or 30 min. Autoclaving of the dry seeds for 15 min reduced the content of β-ODAP by 13%. Whereas a significant ($P = 0.05$) reduction of 39% was found in seeds after dry autoclaving for 30 min. Autoclaving of wet seeds resulted in an average reduction of 32 ± 1% when compared to that of raw whole seeds. This shows that autoclaving of wet seeds was less effective than the dry seeds in reducing the content of β-ODAP. However, autoclaving of the flour showed significant reduction in the content of β-ODAP (an average of 27 ± 14%), when compared to that of whole seed. The reason for this reduction could also be the same as described in roasting.

Fermentation

During spontaneous fermentation, the pH reached 4.0 after 48 h while during back-slopped fermentation,

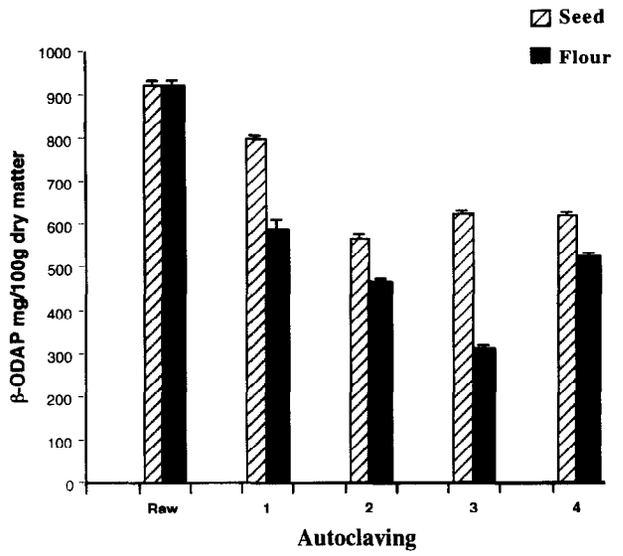

Fig. 4. β-ODAP content of autoclaved grass pea seeds and flour. **1** = 15 min, dry. **2** = 30 min, dry. **3** = 15 min, wet. **4** = 30 min, wet.

using the earlier dough as starter culture (5%), pH reached 4.0 within 24 h. Neither the spontaneous nor the back slopped fermentation seems to have affected the ODAP content of the flour to a significant extent. In contrast, Kuo *et al.* (1995) using the colorimetric method of analysis, reported a reduction of ODAP in the seeds of *Lathyrus sativus* var. Jamalpur by 90% after a solid state fermentation of the seeds using starter culture of *Asperigillus oryzae* and *Rhizopus oligosporous*. It seems that certain micro-organisms are able to use β-ODAP as a substrate. Sachdev *et al.* (1995) isolated and identified soil borne micro-organisms (*Pseudomonas stutzeri*) encoding genetic information for ODAP metabolism. They reported the strain to be capable of utilising ODAP/DAP as the sole source of nitrogen and carbon.

Earlier work showed the β-isomer to isomerize at room temperature to the α-isomer until an equilibrium ratio of 40:60 (α:β), which could be enhanced upon heating (Kahn *et al.*, 1993). This finding was also supported by Padmajaprasad *et al.* (1997) who reported a maximum of 40% conversion of β-ODAP to its α-form after 90 min cooking of dal and standard β-ODAP.

CONCLUSION

Food processing methods like roasting, cooking autoclaving and soaking have significant effect in reducing the content of β-ODAP in the grass pea seeds and flour. Milling of the seeds prior to roasting as well as soaking prior to cooking facilitates the reduction of β-ODAP content. Highest reduction was attained after roasting (150°C for 60 min), whereas, there was no significant change in the content of β-ODAP after spontaneous fermentation (24 and 48 h). The process optimisation can therefore be based on the processing methods which reveal significant reduction of the β-ODAP content and study on the nutritional consequences of the methods.

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