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EFFECT OF DOMESTIC PROCESSING ON AMINO ACID COMPOSITION OF RICEBEAN (Vigna umbellata).

KEYWORDS Germination, Fermentation, Amino acids, Amino acid scores.

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ABSTRACT *The effect of various processing methods viz., soaking, pressure cooking, open pan cooking, germination followed by pressure cooking and roasting of whole rice bean and pressure cooking, open pan cooking and fermentation and frying of fermented batter of dehulled rice bean was assessed for its crude protein content, sulphur amino acids, tryptophan and lysine contents. Dehulling caused a significant reduction in the protein content. The maximum amino acid content in whole rice bean was found in 24 and 48h germinated legume. The highest losses were observed for cystine followed by methionine after processing. The highest content of amino acids in dehulled rice bean was found in 12 and 18h fermented batters. Based on the amino acid scores it can be concluded that germinated and fermented legumes are nutritionally superior to the pressure cooked and open pan cooked rice bean. Tryptophan and lysine were present in adequate amounts while methionine and cystine were marginally adequate.*

INTRODUCTION

Legumes are widely grown throughout the world and their dietary and economic importance is globally appreciated and recognised. They occupy a predominant place in the diets of the people living in the developing countries like India (Sood, et. al, 2002) the majority of whom are vegetarians and fulfil their requirements for protein from vegetarian diets mainly legumes. The nutritive value of dietary proteins is governed by the pattern and quantity of essential amino acids present in it. The presence of one or more of the essential amino acids in inadequate amounts would decrease the nutritive value of proteins.

Pulses are rich in proteins (20 – 40 %) but they are of relatively low biological value because of the deficiency of sulphur amino acids, which are methionine and cystine (Iqbal et. al, 2006). However, they are rich in lysine and are of good supplementary value to cereal diets. In some parts of the world, the per capita consumption of legumes has recently decreased. Keeping in view the declining per capita availability of pulses, efforts are being made to increase the production of legumes through breeding strategies. Therefore, greater attention is being paid to the exploration of non – conventional pulses such as jack bean, ricebean etc.

Ricebean as a grain legume is attracting attention throughout the world as a potential source of high quality protein for the future for bridging the 'protein gap'. Scanty data are available on the amino acid profile of ricebean and the effect of traditional methods of processing on their composition. This study was undertaken to study the effects of various processing methods on the amino acid profile of ricebean.

MATERIALS AND METHODS

Ricebean variety 'RBL – 6' was procured from the Department of Seed Science and Technology, Punjab Agricultural University, Ludhiana. The samples for analysis were withdrawn from the same stock. The beans were cleaned, freed of extraneous substances and whole and dehulled beans were subjected to various processing methods described below and analysed for their crude protein and amino acid contents.

Whole Ricebean:

Soaking: Ricebean was washed and soaked (12h) at room temperature. The seed to water ratio was 1:5 (w/v). The unimbibed water was discarded and the soaked seeds were rinsed thoroughly.

Pressure Cooking: The soaked (12h) and unsoaked seeds, were pressure cooked at 15 lbs pressure. The ratio of seed to water in case of soaked and unsoaked seeds was 1:4 and 1:6 (w/v), respectively. The cooking time for soaked and unsoaked seeds was 5 and 10 minutes, respectively.

Open Pan Cooking: The soaked (12h) and unsoaked seeds, were cooked in an open pan. The ratio of seed to water in case of soaked and unsoaked seeds was 1:6 and 1:10 (w/v), respectively. The cooking time for soaked and unsoaked seeds was 20 and 30 minutes, respectively.

Germination and Pressure Cooking: The soaked seeds (12h) were taken in sterile petri plates lined with wet filter paper and kept in an incubator at 37 ± 1° c for 24h and 48h. A part of the sprouted seeds were analysed as raw sample and the rest were pressure cooked (seed: water, 1:1) for a period of 4 minutes.

Roasting: Ricebean was roasted in sand at 250° c for 2 minutes.

Dehulled Ricebean (Dhal): Whole ricebean was split in a grain pearler and soaked (12h). The beans were rubbed between the palms to remove the outer covering and dried in the sun. A part of the raw sample was stored for further analysis.

Pressure Cooking of Dhal: The soaked (12h) and unsoaked ricebean *dhal* was pressure cooked at 15 lbs pressure. The ratio of *dhal* to water in case of soaked and unsoaked seeds was 1:2 and 1:4 (w/v), respectively. The cooking time for soaked and unsoaked seeds was 4 and 8 minutes, respectively.

Open Pan Cooking of Dhal: The soaked (12h) and unsoaked seeds, were cooked in an open pan. The ratio of *dhal* to water in case of soaked and unsoaked seeds

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was 1:7 and 1:8 (w/v), respectively. The cooking time for soaked and unsoaked seeds was 20 and 25 minutes, respectively.

Fermentation and Frying: The soaked *dhal* (12h) was ground to a coarse paste (*peethi*) which was naturally fermented for 12 and 18h at 37 ± 1° c in an incubator. A part of the fermented batter was analysed as raw sample and the rest was shaped into small balls and fried in medium hot (190° c) refined groundnut oil for 2 minutes.

The samples were dried in a hot air oven at 60+2 c, ground to a fine powder and stored in airtight containers till further analysis.

Analysis: The crude protein content (N x 6.25) of both the raw and processed samples was estimated by the macrokjedhal method (AOAC, 1985). Cystine was analysed from the acid hydrolysate by using the method of Lidell and Saville (1956). Methionine was analysed by the method of Horn et al (1946). The available lysine was estimated by the method of Carpenter (1960) modified by the method of Booth (1971). Tryptophan was analysed by the method of Concon (1975). All the analyses were carried out in triplicates. The amino acid scores of lysine, sulphur amino acids and tryptophan were calculated using the suggested pattern of amino acid requirements for preschool children (FAO, 1991) where values of 58, 25 and 11 mg/g of protein for respective amino acids have been used. The results were statistically analysed for analysis of variance using Sigma Plot 10, computer software.

RESULTS AND DISCUSSION

The crude protein content (Table 1) of 26.03% was observed in raw whole ricebean. Katoch, R (2013) reported a crude protein content ranging from 23.17 to 25.57% in 16 ricebean genotypes. Crude protein content varied from 20.34 to 22.97% in different Vigna species (Srivastava et. al, 2001). A higher protein content observed in this study could be due to varietal differences. On subjecting it to various processing methods, the crude protein content ranged from 22.29 – 26.69%; being highest in sprouted raw (24h) legume and being lowest in unsoaked open pan cooked ricebean. An increase in the protein content on germination was observed. A significant increase in protein content was seen in field beans germinated for 40h and 60h (D'Souza, M. R, 2013). During germination, degradation of storage proteins takes place for the seed embryo to develop but at the same time there is synthesis of new proteins and the synthesis of other nutrients also takes place (King & Puwastein, 1987; Pawar & Ingle, 1988).

Table 1: Protein and Amino Acid Content of Raw and Processed Whole Ricebean.

Sample	Crude Protein (g)	Methionine (g/16g N)	Cystine (g/16g N)	Tryptophan (g/16g N)	Available Lysine (g/16g N)
Raw	26.03	0.98	1.39	1.22	5.85
Soaked	25.24	0.96 (2.04)	1.28 (7.91)	1.17 (4.09)	5.83 (0.34)
Soaked + Pressure Cooked	23.85	0.75 (23.46)	1.06 (23.74)	1.06 (13.11)	5.70 (2.56)
Unsoaked + Pressure cooked	23.93	0.83 (15.30)	1.13 (18.70)	1.10 (9.83)	5.68 (2.90)
Soaked + Open Pan Cooked	22.72	0.72 (26.53)	1.02 (26.61)	0.97 (20.49)	5.75 (1.70)
Unsoaked + Open Pan Cooked	22.29	0.79 (19.38)	1.10 (20.80)	1.02 (16.39)	5.73 (2.05)
Sprouted (24h) Raw	26.49	0.98 (0.00)	1.42 (+2.15)	1.15 (95.73)	5.80 (0.85)
Sprouted + Pressure Cooked	25.61	0.89 (9.18)	1.28 (7.91)	1.14 (6.55)	5.80 (0.85)
Sprouted (48h) Raw	26.69	1.00 (+ 2.04)	1.42(+2.15)	1.15 (5.73)	5.82 (0.51)
Sprouted + Pressure Cooked	25.72	0.98 (0.00)	1.36 (2.15)	1.14 (6.55)	5.82 (0.51)
Roasted	26.09	0.80 (18.36)	1.15 (17.26)	0.95 (22.13)	5.44 (7.00)
Reference amino acid pattern[1]	--	------------------------2.5----------------------		1.10	5.80

FAO (1991)
The figures in parenthesis indicate the percent loss or gain of the amino acid.

The methionine contents of all the processed samples were significantly (P<0.01) lower than the raw contents except in the case of sprouted (48h) raw legume where the methionine content increased by 2%. Igbedioh et. al. (1995) reported the methionine content to increase by 5% in pigeon pea germinated for 3 days. No differences in comparison to raw were found for sprouted (24h) and sprouted (48h) and pressure cooked legume. The losses were highest for methionine in soaked and open pan cooked legume followed by pressure cooked legumes. A maximum loss of 13.07% in the methionine content has been reported on pressure cooking of mash beans (Rani & Hira, 1998). Both, open pan cooking and pressure cooking involve the application of high temperatures which resulted in the destruction of methionine.

In the similar manner, a significant (P<0.01) increase of 2.1% in the cystine content was observed for both 24 and 48h sprouted raw legume which decreased by 9.8 and 4.2% in the respective pressure cooked samples. However, the maximum loss of cystine was observed in soaked and pressure cooked legume. The tryptophan content of sprouted (24 and 48h) and pressure cooked legumes was significantly (P<0.01) lower than the raw sprouted (P<0.01) legumes which had the maximum tryptophan contents. The maximum available lysine contents were observed for sprouted (48h) raw and pressure cooked legumes. Higher contents were observed for open pan cooked legume than for pressure cooked legume. Datta and Datta (1978) also observed that losses of available lysine for pressure cooked legumes are higher than for open pan cooked legumes. Though less time is required in a pressure cooker but the excessive application of heat and pressure makes the pulse more deficient in available lysine content than that cooked without a pressure cooker. The maximum loss of 24% in the available lysine content was found in the roasted ricebean mainly due to the reaction of free epsilon- amino group of lysine and carbonyl group of carbohydrate. The maximum losses for tryptophan and available lysine were observed in case of roasted ricebean. Ihsanullah et. al. (2008) reported significant losses in the lysine, methionine and cystine contents on roasting and pressure cooking of chickpeas.

Overall, soaking and open pan cooking caused the maximum loss of methionine and cystine. The longer cooking time and leaching effects might have caused the loss of these amino acids. Maximum losses of tryptophan and lysine were observed on roasting followed by open pan cooking, pressure cooking and then sprouting. Rani and Hira (1998) have also reported maximum losses of available lysine on roasting, pressure cooking and sprouting of mash beans. Losses were found to be highest for cystine (7.91 – 26.61%), followed by methionine (2.04 – 26.53%), tryptophan (4.09 – 22.13%) and lowest for available lysine (0.34 – 7.00%).

Table 2: Amino Acid Scores of Raw and Processed Whole Ricebean

Sample	Methionine+ Cystine	Tryptophan	Available Lysine
Raw	94.8	110.9	100.8
Soaked	89.6	106.4	100.5
Soaked + Pressure Cooked	72.4	96.4	98.2
Unsoaked + Pressure cooked	78.4	100.0	97.9
Soaked + Open Pan Cooked	69.6	88.2	99.1
Unsoaked + Open Pan Cooked	75.6	92.7	98.8
Sprouted (24h) Raw	96.0	104.5	100.0
Sprouted + Pressure Cooked	86.8	103.6	100.0
Sprouted (48h) Raw	96.8	104.5	100.3
Sprouted + Pressure Cooked	93.6	103.6	100.3
Roasted	78.0	86.3	93.8

Amino acid scores were calculated using values of 25, 58 and 11 mg/g of protein, respectively for sulphur amino acids, tryptophan and available lysine from FAO (1991).

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The amino acid scores (Table 2) of processed ricebean samples ranged from 69.6 to 96.8, 86.3 to 104.5 and 93.8 to 100.3 g/16g N, for sulphur amino acids, tryptophan and available lysine, respectively. The maximum scores were observed for sprouted raw (48h) legume while minimum scores were observed in roasted ricebean for tryptophan and available lysine. The amino acid scores suggested that tryptophan and available lysine were present in adequate amounts but the sulphur amino acids were marginally adequate when compared to the suggested values.

Table 3: Protein and Amino Acid Content of Raw and Processed Dehulled Ricebean.

Sample	Crude Protein (g)	Methionine (g/16g N)	Cystine (g/16g N)	Tryptophan (g/16g N)	Available Lysine (g/16g N)
Raw	23.70	0.83	1.37	1.05	5.44
Soaked	23.00	0.77 (7.22)	1.28 (6.56)	0.98 (6.66)	5.41 (0.55)
Soaked + Pressure Cooked	22.89	0.65 (21.68)	1.06 (22.62)	0.94 (10.4)	5.15 (5.33)
Unsoaked + Pressure cooked	23.08	0.74 (10.84)	1.13 (17.51)	0.96 (8.57)	5.02 (7.72)
Soaked + Open Pan Cooked	22.78	0.62 (25.30)	1.04 (24.08)	0.91 (13.3)	5.11 (6.06)
Unsoaked + Open Pan Cooked	23.04	0.70 (15.66)	1.10 (19.70)	0.92 (12.38)	5.07 (6.80)
Fermented (12h) Raw	24.55	0.81 (2.40)	1.38 (0.72)	1.00 (4.76)	5.42 (0.36)
Fermented & fried	23.96	0.79 (4.81)	1.30 (5.10)	0.98 (6.66)	5.35 (1.65)
Fermented (18h) Raw	25.52	0.84 (+1.20)	1.41 (2.91)	1.00 (4.76)	5.44 (0.00)
Fermented & fried	24.86	0.82 (1.20)	1.38 (0.72)	0.98 (6.66)	5.40 (0.73)
Reference amino acid pattern[1]	--	------------------------2.5----------------------		1.10	5.80

[1] FAO (1991)

The figures in parenthesis indicate the percent loss or gain of the amino acid.

The crude protein content (Table 3) of dehulled raw ricebean was observed to be 23.70%. There was a reduction of 8.9% in the protein content on dehulling of raw whole ricebean. A decrease in the protein contents of chickpea and pigeon pea have been reported with an increase in dehulling time (Singh et. al. 1989) which indicates that the outer layers of cotyledons are rich sources of protein. The protein contents increased significantly (P<0.01) in fermented and fried legumes when compared to the raw. Similar findings have been reported by Padmashree and Putturaj (1987) upon fermentation of cowpea flour batter. The amino acid contents (Table 3) decreased significantly (P<0.01) on dehulling of whole ricebean. The maximum reduction of 15.3% was observed in the methionine content on dehulling. The methionine contents increased by 1.2% in 18h fermented batter but there was a loss of 2.45% on frying of this batter. Similarly, cystine contents increased by 0.8% and 6.5% on fermentation for 12 and 18h, respectively. However, there were losses of 5.79 and 7.53% in *vadas* prepared from 12 and 18h fermented batters. Bujang and Taib (2014) observed a significant increase in the amounts of all amino acids in garbanzo beans and soybeans on fermentation for 18 h. Further fermentation at 24 h showed a greater increase in amino acid contents of these legumes.

Kaur and Mehta (1993) reported losses of 7.1 and 10.0% in the methionine contents of *vadas* made from black gram and ricebean flour with husk, respectively. The corresponding losses for cystine were 15.3 and 9.09%, respectively. Amongst the various processing methods the maximum losses of methionine + cystine were observed in soaked and open pan cooked dehulled ricebean.

The maximum tryptophan contents were observed in fermented (12 and 18h) batters. Similarly, the available lysine contents were highest for fermented 18h batter. Maximum loss of tryptophan occurred in soaked and open pan cooked legume while available lysine was lost marginally in pressure cooked dhal without soaking. The losses in dehulled ricebean samples ranged from 1.20 – 25.30, 5.10 – 24.08, 4.76 – 13.30 and 0.36 – 7.72% for methionine, cystine, tryptophan and available lysine, respectively.

Table 4: Amino Acid Scores of Raw and Processed Dehulled Ricebean

Sample	Methionine+ Cystine	Tryptophan	Available Lysine
Raw	88.0	95.5	93.8
Soaked	82.0	89.1	93.3
Soaked + Pressure Cooked	68.4	85.5	88.8
Unsoaked + Pressure cooked	74.8	87.3	86.6
Soaked + Open Pan Cooked	66.4	82.7	88.1
Unsoaked + Open Pan Cooked	72.0	83.6	87.4
Fermented (12h) Raw	87.6	90.9	93.4
Fermented & fried	83.6	89.1	92.2
Fermented (18h) Raw	90.0	90.9	93.8
Fermented & fried	88.0	89.1	93.1

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Amino acid scores were calculated using values of 25, 58 and 11 mg/g of protein, respectively for sulphur amino acids, tryptophan and available lysine from FAO (1991).

The amino acid scores (Table 4) ranged from 66.4 – 90.0, 82.7 – 90.9 and 8.6 – 93.8 for sulphur amino acids, tryptophan and available lysine, respectively. The scores were highest for fermented ricebean samples followed by pressure cooked and then open pan cooked legumes for sulphur amino acids, tryptophan and available lysine. The scores suggested that all the amino acids were marginally adequate in the dehulled ricebean samples.

Thus, it can be concluded that dehulling significantly lowers the protein content of ricebean. Germination in case of whole legume and fermentation in case of dehulled legumes are considered to be the best processing methods for the optimum utilization of methionine and cystine which are the limiting amino acids of legumes and also for the availability of tryptophan and lysine. Amino acids are the essential nutrients which are particularly important in the diets of infants, preschool children, pregnant and lactating women, especially in the developing nations who depend on vegetarian diets for their protein needs. So, it would be desirable that germination and fermentation of legumes are adopted at the household level and these processing methods can be used for formulation of legume based weaning foods for infants and supplementary foods for other vulnerable groups so as to attain optimum utilization of legumes.

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