EFFECT OF DIFFERENT PRE-TREATMENTS ON ANTINUTRIENTS AND ANTIOXIDANTS OF RICE BEAN (*VIGNA UMBELLATA*)

– Research paper –

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Abstract: Rice bean (*Vigna umbellata*) is a legume that belongs to *Vigna* genus. Native to Indo-Chinese region, it is considered to be an ‘under-utilized’ or ‘orphan’ crop. Rice bean is known to possess high nutritional potential and antioxidant activity. But the use of rice bean supplementation in routine diet is limited despite its high nutritional profile due to the presence of non-nutritional factors. Thus, various pre-treatments like soaking, germination, oven roasting, sand roasting, boiling and pressure cooking at different time and temperature were carried out to reduce the anti-nutritional content of rice bean and to study its effect on antioxidant activity and phytochemical content. All the pre-treatments were able to significantly reduce the anti-nutrient content in rice bean, but germination showed the maximum reduction. Also germinated rice bean showed the maximum antioxidant potential and maximum content of total phenols, total flavonoids, vitamin C and carotenoids. Rice bean has been underutilized so far, owing to its antinutrient content and low popularity. This experiment attempted to use low cost processing to reduce the content of antinutrients and track the antioxidant content in rice bean. The concluded processing could be adopted for commercial applications for dietary supplementation.

Keywords: *Vigna umbellata*, Pre-treatments, Anti-nutrients, Antioxidant activity, Phytochemicals

INTRODUCTION

Beans are known to be an important nutritional source especially in developing countries with low socioeconomic group. Beans are the best source of quality proteins, dietary fibre, minerals, vitamins, slow digestible starch and bioactive phytochemicals which aid in reducing the risk of several chronic and metabolic diseases (Bepary, 2016). The rising population has emphasized the need for the utilization of underutilized legume like rice bean (*Vigna umbellata*). It is a multipurpose legume crop belonging to genus *Vigna*. It is an ‘under-utilized’ or ‘orphan’ crop, cultivated in the Indo-china plain. Rice bean is easily established on exhausted soil and has a good

nitrogen fixing capacity. In some places, rice bean has a reputation of “poor man’s food” (Andersen, 2012). In India, Northern and Eastern regions are mostly site of cultivation for rice bean. In recent years, rice bean has received immense attention owing to its high nutritional profile. Rice bean has a low level of glycaemic index, oligosaccharide and high protein with balanced amino acid profile, high calcium and zinc bioavailability and other minerals like iron, zinc, potassium, manganese and copper (Andersen, 2012; Bepary et al., 2016). It has high methionine content in comparison with other members of same genus. Certain phenolic compounds like vitexin, isovitexin and catechin exhibit antioxidant activity in certain rice bean varieties (Sritongtae et al., 2017).

Despite its high nutritional profile, its consumption in routine diet is limited owing to anti-nutritional

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factors like phytic acid, tannins, enzyme inhibitors, saponins and phenolic compounds. These anti-nutritional factors interact with other nutrients like protein, carbohydrates and minerals and reduce their absorption in the body. Phytic acid reduces the bioavailability of minerals like zinc, calcium and iron, owing to its chelating properties and also binds with protein and starch (Bepary et al., 2016). Tannins in rice bean also affect the nutritional quality of food by forming complexes with

minerals, protein, vitamin B12 and digestive enzymes. It also causes browning in foods by polyphenol oxidase action and thus reduces the acceptability of foods (Bepary et al., 2016).

Various processing techniques like soaking, germination, cooking and roasting are effective in reducing these anti-nutritional content. This paper aims at evaluating the effect of different processing techniques on anti-nutrient content, phytochemical content and anti-oxidant activity of rice bean.

METHODOLOGY

Raw material and chemicals

Rice bean was procured from the local market of Manipur, India. All the chemicals used were procured from LOBA Chemic. Pvt. Ltd. Mumbai, India.

Pre-treatments

Soaking: Samples of 50g rice beans were soaked in potable water in seed to water ratio of 1:10 w/v for 6, 12 and 18 hour each at room temperature.

Germination: Samples of 50g rice beans were soaked in 1.5% sodium hypochlorite solution followed by washing with tap water for 3-4 times to prevent the fungal growth. The washed seeds were soaked for 18 hour in potable water at room temperature and germinated for 24, 48 and 72 hours each in dark. The seeds were moistened at a regular interval of 12 hour.

Roasting: Rice beans were subjected to sand and oven roasting treatment. Samples (50g) were roasted at 200 °C for 10, 15 and 20 min each in sand taken in open pan. Oven roasting was performed at 170 °C for 10, 15 and 20 min.

Cooking: Two types of cooking treatment viz., boiling and pressure cooking were performed. Boiling of rice beans was done for 5, 10 and 15 min. with seed to water ratio of 1:6 [w/v]. Pressure cooking was done with seed to water ratio of 1:2 [w/v] for 5, 10 and 15 min.

Preparation of samples after pre-treatments: All the samples were tray dried at 60 °C and then grinded to a uniform size. The grinded samples were packed in air tight pouches and stored for further analysis. All the analyses were carried out on powdered samples in triplicate experiments carried out to get triplicate samples.

Determination of anti-nutrients

The samples were evaluated for phytic acid and tannin content. Phytic acid and tannin content was determined by method described by (Sadasivam and Manickam, 2008).

Determination of phytochemicals

Total phenolic content was determined by Folin Ciocalteu's method and Total flavonoid content was determined using aluminium chloride method. Total phenolic content (TPC) results were expressed as gallic acid equivalent (GAE), whereas total flavonoid content (TFC) results were expressed as quercetin equivalent (QE) (Kamtekar et al., 2014). Total carotenoid content was estimated by method described by Sadasivam and Manickam (2008). Ascorbic acid content was determined using 2,6-dichlorophenol indophenol dye method as described in Ranganna (2016).

Determination of antioxidant activity

Different antioxidant properties viz., free radical scavenging activity (FRSA), ABTS radical scavenging activity and ferric reducing antioxidant power (FRAP) were evaluated using spectrophotometer at different Optical Density's (OD's). Free radical scavenging activity was determined using DPPH method. ABTS radical cation decolorization method was used for determining free radical scavenging activity of the sample (Rajurkar and Hande, 2011).

FTIR analysis

Different rice bean samples viz; raw, soaking (18hour), germination (72hour), oven roasting (20 min), sand roasting (15 min.), boiling (15 min.) and pressure cooking (15 min.) were subjected to FTIR analysis (Shimadzu 8400S FTIR spectrometer, equipped with KBr bean splitter) (Handa et al., 2017).

Statistical Analysis

All the experiments were carried out in triplicate experiments to derive triplicate samples. The data was represented using mean of all the values. The statistical analysis was carried out using SPSS 22.0 software (SPSS Italia, Bologna, Italy).

RESULT AND DISCUSSION

Effect of pre-treatments on phytic acid content

Table 1 illustrates the effect of pre-treatments on the phytic acid content of rice bean. A significant difference ($p < 0.05$) was observed in the phytic acid content for all the pre-treatments under study. The phytic acid content of raw rice bean was found to be $0.58 \pm 0.14\%$. Among soaking, a maximum phytic acid reduction of 43.10% was observed in rice bean soaked for 18 hours. A percentage reduction of 60.34% was observed in phytic acid content after germinating for 72 hours. Among roasting treatment, oven roasting showed a reduction of 13.79-29.31%, while sand roasting showed a reduction of 27.58-32.72% in phytic acid content. Pressure cooking was found to be effective in reducing the phytic acid content of rice bean samples. On the other hand, Boiling for 5 min did not show promising results in reducing the phytic acid content, whereas boiling for 10 and 15 min did significantly reduce the phytic acid content. However, this increases the harshness of the process.

Leaching of phytate ions during soaking could be a possible reason for the reduction of phytic acid content. In the soaked water, phytic acid exists as water-soluble salt (potassium phytate) in dried legumes. Similar reduction in phytic acid of rice bean from soaking was found by few significant researches (Kaur and Kapoor, 1990; Khattab and Arntfield, 2009). Soaking resulted in leaching of

phytate and hence phytic acid reduction in *Vigna unguiculata* (Kalpanadevi and Mohan, 2013). Germination also resulted in a significant in phytic acid reduction, this loss may be due to the hydrolytic activity of phytase enzyme, followed by diffusion. A similar reduction in phytic acid content was observed in *Vigna unguiculata* (Sinha and Kawatra, 2003). Reduction of 42.6% in phytic acid in germinated red kidney beans (Yasmin et al., 2008; Mugendi et al., 2010). This increased phytase synthesis, increased the phytic acid degradation and hence reduced the phytic acid content in soaked-germinated beans. The phytic acid reduction in cooked and roasted samples was due to the destruction of phytate ions due to high temperature. Khattab and Arntfield (2009) observed heat treatments like cooking and roasting resulted in phytic acid reduction in various beans, owing to their sensitive to heat and tendency to form insoluble complexes between minerals and phytates. Saikia et al. (1999) reported that pressure cooking for 15 min was more effective in reducing the phytate content as compared to boiling. A similar reduction in phytic acid content was found in *Vigna unguiculata* during soaking and cooking by Sinha and Kawatra (2003). Yang et al. (2014) reported a phytate reduction in soybean during thermal treatment. Conclusively, the reduction is due to insoluble compounds formed particularly magnesium and calcium phytate that are thermally unstable and hence results in phytic acid reduction.

Table 1. Effect of different pre-treatments on anti-nutrient content of rice bean

Treatments	Phytic acid (%)	Tannins (mgTAE/100g)
Raw	0.58 ± 0.14^a	933.33 ± 43.89^a
Soaking 6h	0.47 ± 0.07^a	808.33 ± 43.89^b
Soaking 12h	0.42 ± 0.05^a	658.33 ± 38.18^c
Soaking 18h	0.33 ± 0.03^b	579.16 ± 26.02^f
Germination 24h	0.33 ± 0.03^b	579.16 ± 26.02^f
Germination 48h	0.30 ± 0.03^b	575.00 ± 37.50^f
Germination 72h	0.23 ± 0.05^c	537.00 ± 37.50^g
Oven roasting 10min	0.50 ± 0.15^a	904.16 ± 31.45^a
Oven roasting 15min	0.43 ± 0.17^a	862.50 ± 37.50^b
Oven roasting 20min	0.41 ± 0.13^b	845.83 ± 26.02^b
Sand roasting 5min	0.42 ± 0.08^a	883.33 ± 31.45^b
Sand roasting 10min	0.40 ± 0.06^a	800.00 ± 25.00^c
Sand roasting 15min	0.39 ± 0.02^b	725.00 ± 25.00^d
Boiling 5min	0.40 ± 0.02^a	758.33 ± 52.04^d
Boiling 10min	0.37 ± 0.03^b	704.16 ± 50.51^d
Boiling 15min	0.31 ± 0.05^c	675.00 ± 37.50^e
Pressure cooking 5min	0.38 ± 0.05^b	737.50 ± 25.00^d
Pressure cooking 10min	0.33 ± 0.09^b	691.66 ± 43.89^e
Pressure cooking 15min	0.29 ± 0.13^c	658.33 ± 43.89^e

Values represented with different small superscripts differ significantly in a row ($p < 0.05$)

The values of the result represented in the form of mean $\pm$ standard deviation

The values are obtained from triplicate experiments ($n=3$)

Effect of pre-treatments on tannin content

Table 1 illustrates the effect of pre-treatments on the tannins of rice bean samples. When compared to raw rice bean all the pre-treated samples had a significant reduction ($p<0.05$) in tannin. The tannin content of raw sample was found to be 933.33 ± 43.89 mgTAE/100g. Soaking for 18 hours resulted in a reduction of 42.46%, while germination resulted in 37.94-42.46% reduction in tannin content. Oven roasting showed a reduction of 3.12-9.37%, while sand roasting showed a reduction of 5.35-22.32%. Pressure cooking was more effective in reducing tannin content as compared to boiling. Boiling showed a reduction of 18.75-27.67%, while pressure cooking showed a reduction of 20.98-29.46%.

As tannin is readily soluble in water the reduction is probably due to its leaching in the soaked water. During germination, enzymes get activated and this results in tannin reduction due to hydrolysis of various components like carbohydrates, fiber, lipids, protein and phenolic compounds. Similar reduction was seen in *Vigna unguiculata* during germination (Kalpanadevi and Mohan, 2013). Cooking treatments also resulted in a significant reduction which might be due to molecular alterations or binding of tannins with other organic compounds. Similar results were obtained by Kaur and Kapoor (1990). Saikia et al. (1999) reported a reduction in tannin content during boiling and pressure cooking was due to diffusion of tannin in cooking water. Yang et al. (2014) also reported a reduction in tannins during thermal treatment. This

is due to water soluble and heat sensitive nature of tannins and the thermal treatment like cooking and roasting degrades the tannin and hence resulted in their reduction. The present results are in harmony with those of Khattab and Arntfield (2009) who reported a reduction of the anti-nutritional factors in various beans upon physical treatments.

Effect of different pre-treatments on antioxidant activity of rice bean

Table 2 presents the effect of different pre-treatments on antioxidant activity tests. All the pre-treatments showed a significant difference ($p<0.05$) in antioxidant activity as compared to raw rice bean. The FRSA, ABTS test and FRAP of raw rice bean was found to be $24.23\pm0.28\%$, $22.75\pm0.11\%$ and 19.52 ± 0.11 mgTE/g respectively. As the soaking period increased, the antioxidant activity was found to decrease significantly ($p<0.05$). Moreover, the maximum antioxidant activity was found at 72 hours of germination in rice bean. Both the roasting treatments viz. oven roasting and sand roasting and cooking treatments viz. boiling and pressure cooking showed an increase in antioxidant activity. Handa et al. (2017) reported a decrease in antioxidant activity in horsegram upon soaking. This was due to the leaching of polyphenols in liquid medium which were responsible for reducing the antioxidant activity subsequently. Similarly, Xu and Chang (2008) also observed a reduction in the antioxidant activity in soaked cold season legumes.

Table 2. Effect of different pre-treatments on antioxidant activity of rice bean

Treatments	DPPH (%)	ABTS (%)	FRAP (mgTE/g)
Raw	24.23 ± 0.28^j	22.75 ± 0.11^g	19.52 ± 0.11^h
Soaking 6h	23.15 ± 0.20^k	21.01 ± 0.29^h	16.00 ± 0.09^i
Soaking 12h	21.03 ± 0.19^l	20.75 ± 0.16^h	14.36 ± 0.14^j
Soaking 18h	19.36 ± 0.11^m	17.17 ± 0.16^i	12.54 ± 0.07^k
Germination 24h	28.77 ± 0.15^f	23.17 ± 0.20^e	20.10 ± 0.13^f
Germination 48h	33.58 ± 0.21^b	25.01 ± 0.15^b	21.45 ± 0.18^c
Germination 72h	40.30 ± 0.10^a	27.11 ± 0.22^a	22.63 ± 0.12^a
Oven roasting 10min	26.63 ± 0.22^h	23.01 ± 0.18^f	19.74 ± 0.14^g
Oven roasting 15min	28.63 ± 0.20^f	24.15 ± 0.20^c	20.10 ± 0.09^f
Oven roasting 20min	30.01 ± 0.19^d	24.87 ± 0.22^b	21.44 ± 0.10^c
Sand roasting 5min	28.48 ± 0.14^f	24.10 ± 0.10^c	19.88 ± 0.13^g
Sand roasting 10min	30.15 ± 0.20^d	24.65 ± 0.10^b	20.32 ± 0.15^c
Sand roasting 15min	32.10 ± 0.11^c	25.01 ± 0.16^b	21.87 ± 0.11^b
Boiling 5min	26.48 ± 0.18^h	22.96 ± 0.17^f	19.76 ± 0.17^g
Boiling 10min	27.24 ± 0.24^g	23.10 ± 0.12^e	20.12 ± 0.12^f
Boiling 15min	29.43 ± 0.14^e	23.45 ± 0.20^d	20.57 ± 0.10^d
Pressure cooking 5min	25.10 ± 0.20^i	23.11 ± 0.11^e	19.66 ± 0.15^g
Pressure cooking 10min	27.32 ± 0.13^g	23.64 ± 0.19^d	20.00 ± 0.11^f
Pressure cooking 15min	30.14 ± 0.17^d	24.00 ± 0.13^c	20.74 ± 0.19^d

Values represented with different small superscripts differ significantly in a row ($p<0.05$)

The values of the result represented in the form of mean $\pm$ standard deviation

The values are obtained from triplicate experiments (n=3)

However, the present results contradicted the finding of Siah et al. (2014) and Boateng et al. (2008) who showed an increase in antioxidant activity in faba and dry bean upon soaking. Aguilera et al. (2013) reported an increase in the antioxidant activity of some germinated nonconventional legumes. It was reported that this increase in the antioxidant activity in germinated legumes is probably due to the release of some bioactive compounds which possess some antioxidant properties. Similar observations were made by Lopez-Amoros et al. (2006). The results suggested that the increment in antioxidant activity is owing to synergistic effect observed between the various bioactive compounds and phenolic compounds present in the legumes in different concentration. A similar increase in germinated legumes was observed in black beans, mung beans, peanuts, white chickpeas, adzuki beans and soybeans (Khang et al., 2016).

Gujral et al. (2013) observed an increase in the antioxidant capacity in sand roasted Bengal gram, due to formation of Maillard products and also owing to the release of intact and low molecular weight phenolics. It was also reported that the thermal treatments like roasting resulted in the formation of dark colored melanoidins pigment which possess potent antioxidant activity (Sharma et al., 2012). Acar et al. (2009) reported an increase in the antioxidant activity in the oven roasted pulses was probably due to the formation of new Maillard reaction products. The antioxidant

activity of rice beans is also influenced by the cooking treatment. Similar increase in the FRSA during cooking was observed in various pulses. Ranilla et al. (2009) reported an increase in the antioxidant activity in *Phaseolus vulgaris* L. species. Owing to the low molecular weight phenolic compounds like flavonols and phenolics, the antioxidant activity was profound even after thermal treatment. Most probably because cooking results in better solubility of non-phenolic compounds and thus increases the antioxidant activity. Also the Maillard reaction products formed during thermal treatment increases the free radical scavenging properties of pulses. Similar work was reported by Segev et al. (2011) in cooked chickpea.

Effect of different pre-treatments on phenolic content of rice bean

Table 3 illustrates the effect of different pre-treatments on rice bean TPC and TFC. All the pre-treatments significantly ($p < 0.05$) influenced the phenolic content of rice bean. The TPC and TFC of raw rice bean was found to be 28.71 ± 0.29 mgGAE/100g and 40.26 ± 0.25 mgQE/100g. This loss in TPC and TFC ranges from 22-42% and 15-46%, respectively upon soaking. Germination was found to significantly ($p < 0.05$) increase the samples phenolic content. Also roasting showed a similar significant ($p < 0.05$) increase in phenolic content. Sand roasting resulted greater change in the phenolic content compared with oven roasting.

Table 3. Effect of different pre-treatments on total phenols, flavonoid, carotenoid and vitamin C content of rice bean

Treatments	Total phenolic content (mgGAE/100g)	Total flavonoid content (mgQE/100g)	Carotenoids (g/L)	Vitamin C (mg/100g)
Raw	28.71 ± 0.29^j	40.26 ± 0.25^c	0.111 ± 0.10^a	1.60 ± 0.10^d
Soaking 6h	22.15 ± 0.26^k	34.07 ± 0.29^f	0.090 ± 0.07^a	1.58 ± 0.11^d
Soaking 12h	19.16 ± 0.11^m	33.80 ± 0.11^f	0.087 ± 0.06^a	1.50 ± 0.14^d
Soaking 18h	16.84 ± 0.20^n	21.52 ± 0.09^k	0.085 ± 0.08^a	1.42 ± 0.12^d
Germination 24h	32.30 ± 0.15^g	43.68 ± 0.24^c	0.115 ± 0.10^a	11.36 ± 0.20^c
Germination 48h	36.25 ± 0.29^d	46.06 ± 0.24^b	0.119 ± 0.11^a	15.74 ± 0.19^b
Germination 72h	41.28 ± 0.26^a	49.06 ± 0.26^a	0.122 ± 0.11^a	19.00 ± 0.16^a
Oven roasting 10min	30.30 ± 0.25^i	42.07 ± 0.28^d	0.097 ± 0.09^a	1.46 ± 0.11^d
Oven roasting 15min	34.12 ± 0.29^f	43.64 ± 0.29^c	0.091 ± 0.07^a	1.29 ± 0.09^d
Oven roasting 20min	37.49 ± 0.20^c	45.86 ± 0.26^b	0.088 ± 0.08^a	1.17 ± 0.10^c
Sand roasting 5min	31.23 ± 0.11^h	41.80 ± 0.11^d	0.091 ± 0.08^a	1.36 ± 0.12^d
Sand roasting 10min	35.03 ± 0.26^e	43.89 ± 0.26^c	0.086 ± 0.08^a	1.24 ± 0.11^d
Sand roasting 15min	39.04 ± 0.14^b	46.16 ± 0.24^b	0.081 ± 0.07^a	1.06 ± 0.07^c
Boiling 5min	19.48 ± 0.11^l	32.00 ± 0.28^g	0.109 ± 0.08^a	1.52 ± 0.09^d
Boiling 10min	17.03 ± 0.29^n	30.50 ± 0.16^h	0.101 ± 0.09^a	1.35 ± 0.10^d
Boiling 15min	16.65 ± 0.24^n	27.89 ± 0.17^j	0.098 ± 0.07^a	1.11 ± 0.11^c
Pressure cooking 5min	19.48 ± 0.21^l	30.73 ± 0.26^h	0.107 ± 0.09^a	1.42 ± 0.12^d
Pressure cooking 10min	15.64 ± 0.11^o	28.78 ± 0.10^i	0.099 ± 0.08^a	1.06 ± 0.14^c
Pressure cooking 15min	13.58 ± 0.20^p	26.73 ± 0.12^j	0.092 ± 0.09^a	0.94 ± 0.09^c

Values represented with different small superscripts differ significantly in a row ($p < 0.05$)

The values of the result represented in the form of mean $\pm$ standard deviation

The values are obtained from triplicate experiments ($n=3$)

The decrease in the phenolic content upon soaking was due to the fact that the water soluble polyphenols leaches out in the water upon soaking. Similar result was found by Tajoddin et al. (2013) in mung bean who reported a reduction of 18-35% in polyphenols upon soaking. Xu and Chang (2008) also observed a loss of 2-12% in phenolic content of peas and chickpea upon soaking. This loss was particularly due to leaching of phenolic content in soaked water. Khang et al. (2016) reported positive results of germination on the phenolic content of six selected legume varieties. This is significant as new phenolics is generated during germination. Lopez-Amoros et al. (2006) reported both quantitative and qualitative changes in the phenolic compounds in legumes that accounted for an substantial increase in the phenolic content of legumes post-germination. Similar increase in polyphenols after germination was observed by Tajoddin et al. (2013) in mung bean. It was stated that this increase is either due to the synthesis of new phenolic compounds or due to the polymerisation of already existing phenolic compounds. The breakdown of high molecular weight insoluble polymers to low molecular weight

Effect of different pre-treatments on vitamin C and carotenoid content of rice bean

Table 3 illustrates the different pre-treatments and its effect on ascorbic acid and carotenoid content of rice bean. All the pre-treatments does not show any significant difference in carotenoid content in comparison to raw rice bean. The carotenoid content of raw rice bean was found to be 0.111 ± 0.10 g/L. Soaking resulted in a loss of carotenoid content but this loss was insignificant. A loss of 18-23% was observed after 18 hours of soaking. Germination showed an increase in carotenoid content of up to 0.09%. Both roasting and cooking treatments resulted in an insignificant loss of carotenoids.

The carotenoid reduction during soaking could be attributed to its leaching in the liquid medium. The similar reduction in β -carotene content was observed by Sihag et al. (2015) in pearl millet. The present results were agreement with those of Afify et al. (2012) who also reported a similar reduction in carotene content upon soaking. Okudu and Ojinnaka (2017) also reported a loss of carotenoid in *Vigna subterranean* upon soaking. The present results are in agreement with those of Suryanti et al. (2016) who also reported an increase in carotene content upon germination. Tomer et al. (2018) also reported an increase in carotenoid content in germinated mung gram, black gram, pigeon pea and cowpea. An increase in carotenoid

soluble polymers can also accounts for this increase in polyphenols upon germination.

An increase in the TPC and TFC upon oven and sand roasting was observed by several studies. This increase upon roasting might be due to the formation of Maillard reaction products or the release of bound polyphenols. Segev et al. (2012) also reported an increase in the phenolics and flavonoids content in chickpea upon roasting. This was attributed to the Maillard reaction products formed. Kim et al. (2011) showed that roasting alters the cell wall and cell membrane of legumes and releases the soluble phytochemicals particularly phenolic acids from insoluble ester bonds and this accounts for increased total phenolic content. Gujral et al. (2013) also reported an increase in phenolic content in Bengal gram upon roasting. The reduction of phenolic content in both the cooking treatments viz. boiling and pressure cooking can be due to leaching of water soluble phenolic in cooking water. Similar results were obtained by Segev et al. (2012) in chickpea. Gujral et al. (2011) also reported a reduction of 26% in total phenolic content in pulses upon cooking. Ranilla et al. (2009) observed a decrease in phenolic content in cooked Brazilian bean content with increasing germination period was also reported by Khyade and Jagtap (2016) in cowpea, chickpea, black gram and yellow mustard seeds. The present results are in agreement with those of Suryanti et al. (2016). Nwafor et al. (2017) reported that the loss of carotenoid in *Adenanthera pavonina* L. seeds upon roasting can be due to its degradation upon oxidation. Oulai et al. (2016) also observed a loss in carotenoid content upon roasting. This decrease is attributed to the isomerization and oxidation of β -carotene upon roasting. Zhang et al. (2014) reported that a decrease in carotenoid content upon cooking is due to the fact that heating disrupts the cell wall and cell membrane and hence releases the lipophilic compounds like carotenoids and thus results in its degradation. A similar decrease in β -carotene content was observed by Sihag et al. (2015) in pearl millet upon pressure cooking. Nwafor et al. (2017) also found that the decrease in carotene content during cooking of *Adenanthera pavonina* L. seeds is due to its thermo labile nature and also due to its leaching in the cooking medium. Shin et al. (2016) also reported a decrease of carotenoid content in mung bean after boiling. This decrease is due to the fact that heating disrupts the hard tissues in pulses and results in denaturation of protein and their components. These loosened tissues facilitate the leaching of carotenoids in the

surrounding medium and hence accounts for its loss upon cooking.

All the pre-treatments except germination resulted in a reduction in vitamin C content. The vitamin C content of raw rice bean was found to be 1.60 ± 0.10 mg/100g. Soaking caused a decrease in vitamin C content in rice bean, but this decrease was insignificant ($p > 0.05$). Soaking for different time period accounted for 1-11% loss of vitamin C. A significant increase in vitamin C content was observed in rice bean with prolonged germination period. An increase of up to 10% was observed in 72 hours germinated rice bean. Both the thermal treatments viz. cooking and soaking resulted in vitamin C reduction. A loss of 8-26% was observed in oven roasted rice bean, while a loss of 15-33% was observed in sand roasted rice bean. Boiling resulted in 5-30% loss, whereas pressure cooking resulted in 11-41% loss in vitamin C content.

Loss of vitamin C during soaking period was also reported by Kakati et al. (2010) in *Vigna radiata* (L.) and *Vigna mungo* (L.). This loss is particularly due to water soluble nature of vitamin C. With prolonged soaking, more vitamin C is leached out in the soaked water and hence results in more reduction of vitamin C. The present results were in accordance with Luo et al. (2013) who also reported a loss of vitamin C content in faba beans, azuki bean and mung bean upon soaking. However, the results were contradictory to those found by Handa et al. (2017) who reported an increase in vitamin C content in horsegram upon soaking. The increase in vitamin C content during prolonged germination is due to the biosynthesis of vitamin C which results from the reactivation of L-galactono- γ -lactone dehydrogenase. This enzyme is responsible for oxidation of L-galactono 1,4 to vitamin C (Handa et al., 2017). An increase in vitamin C content was observed in germinated cowpeas by Doblado et al. (2007). Similar increment in vitamin C content was reported by Frias et al. (2005) in *Lupinus albus* seeds and by Sood and Malhotra (2002) in chickpea. The loss of vitamin C content during sand roasting and oven roasting is due to the heat sensitive nature of this vitamin which resulted in its degradation. This reduction in vitamin C content upon cooking was observed by Sood and Malhotra (2002) in chickpea. Loss of vitamin C during cooking is due to its thermo labile and water soluble nature which resulted in its leaching in the cooking water. Luo et al. (2016) reported a similar reduction in vitamin C content during cooking of faba beans, mung bean and azuki bean.

FTIR analysis

The presence or absence of particular functional groups in raw and treated samples was determined through FTIR spectra. The different peaks obtained for different rice bean samples in FTIR spectra are illustrated in Table 4.

The FT-IR spectra obtained for raw rice bean is shown in Figure 1a. The spectrum shows absorbance at 999, 1242, 1396, 1535, 1643, 1741, 1982, 2046, 2173, 2364, 2926, 3321 and 3755 cm^{-1} . The spectral peak obtained at 999 cm^{-1} is due to $=\text{C-H}$ out of plane bending of aliphatic hydrocarbons. The absorption band at 1242 cm^{-1} attributes to C-O stretching of aliphatic ester group. The peak obtained in the spectral region of 3755 cm^{-1} is attributed to O-H stretching and the peak obtained in the spectral region of $3200\text{-}3300\text{ cm}^{-1}$ (3321 cm^{-1}) is due to N-H stretching of secondary amides. The peaks obtained in the spectral region of $1430\text{-}1650\text{ cm}^{-1}$ is due to skeletal vibration representing C=C stretching. The peak ranging at $2000\text{-}1700\text{ cm}^{-1}$ indicated the presence of benzene ring. The absorption band at $2100\text{-}2700\text{ cm}^{-1}$ indicates phosphorous acid and ester O-H stretching of phytic acid (Stuart, 2004).

Similar bands were obtained for treated samples viz. 18 hour soaking shown below in Figure 1b. 18 hour soaking followed by 72 hour germination shown in Figure 1c, 20 min. oven roasting shown in Figure 1d, 15 min. sand roasting shown in Figure 1e. 15 min. boiling shown in Figure 1f. and 15 minutes pressure cooking is shown in Figure 1g.

The formation and absence of certain bands in various treated samples indicate the presence and absence of certain functional groups. In germinated samples, the absence of absorption band at $2100\text{-}2700\text{ cm}^{-1}$ indicates the absence of phytic acid. The high temperature during cooking treatment disrupts certain chemical bonds due to decreased vibrational energy and hence results in the absence of certain absorption bands as compared to raw samples (Mir et al., 2016). The spectral regions at $1400\text{-}1200\text{ cm}^{-1}$ in oven roasted, boiled and pressure cooked samples is associated with changes to N-O esters, pyridines, t-butyl and ether group. The valley obtained at $3000\text{-}3600\text{ cm}^{-1}$ in oven roasted, boiled and pressure cooked samples represents unsaturation due to oxidation reaction (Jogihalli et al., 2017).

Table 4 a. FT-IR spectra of different rice bean samples.

S. No.	Raw			Soaked (18 hours)			Germinated (72 hour)			Oven roasting (20 min.)		
	Peak	Area	Compound	Peak	Area	Compound	Peak	Area	Compound	Peak	Area	Compound
1	999.16	1.54	Oxime N-O stretching	605.67	0.264	=C-H bending (alkyne)	995.3	3.754	Oxime N-O stretching	997.23	5.267	Oxime N-O stretching
2	1242.2	0.741	C-O stretching (alcohols and phenols)	1001.09	0.414	=C-H out of plane bending (alkene)	1149.61	0.818	C-O stretching (alcohols and phenols)	1240.27	1.023	C-O stretching (alcohols and phenols)
3	1396.51	0.23	Azo compound N=N stretching (nitrogen containing compounds)	1460.16	0.042	Methylene scissoring	1396.51	0.662	Azo compound N=N stretching (nitrogen containing compounds)	1396.51	0.631	Azo compound N=N stretching (nitrogen containing compounds)
4	1535.39	0.107	C-N stretching	1527.67	0.079	C=O stretching	1541.18	1.282	C-N stretching	1518.03	0.637	C=C stretching
5	1643.41	0.366	C=C stretching	1653.05	0.088	C=C stretching	1643.41	1.324	C=C stretching	1639.55	1.188	C=C stretching
6	1741.78	0.155	Aliphatic C=O stretching	1745.64	0.036	Aliphatic C=O stretching (ester)	2926.11	0.938	O-H stretching (carboxylic acid)	2931.9	0.439	O-H stretching (carboxylic acid)
7	1982.89	0.022	Overtone and combination bands	1955.88	0.038	C=O stretching	3304.17	0.7	O-H stretching (water)	3254.02	0.548	=C-H stretching
8	2046.54	0.083	Benzene ring	2023.4	0.011	Benzene ring				3743.96	0.107	O-H stretching (water)
9	2173.85	0.084	Phosphorous acid and ester O-H stretching	2166.13	0.018	C=C stretching (alkyne)						
10	2364.81	0.352	Phosphorous acid and ester O-H stretching	2335.87	0.042	C=C- stretching (alkyne)						
11	2926.11	0.407	O-H stretching (carboxylic acid)	3616.65	0.004	O-H stretching (water)						
12	3321.53	0.051	O-H stretching (water)	3742.03	0.025	O-H stretching (water)						
13	3755.53	0.029	O-H stretching (water)	3853.9	0.004	O-H stretching (water)						

The values represented obtained are for the best of the triplicate experiment, doesnot represent the mean value (n=1)

Table 4 b. FT-IR spectra of different rice bean samples.

S. No.	Sand roasting (15 min.)			Boiling (15 min.)			Pressure cooking (15 min.)		
	Peak	Area	Compound	Peak	Area	Compound	Peak	Area	Compound
1	657.75	0.06	=C-H bending	999.16	6.218	Oxime N-O stretching	1010.73	5.322	C-O stretching (alcohols and phenols)
2	1003.02	0.321	C-O stretching (alcohols and phenols)	1147.68	1.588	C-O stretching (alcohols and phenols)	1238.34	1.528	C-O stretching (alcohols and phenols)
3	1516.1	0.194	C=C stretching	1240.27	2.905	C-O stretching (alcohols and phenols)	1315.5	0.616	Aromatic C-N stretching
5	1539.25	0.124	C-N stretching	1315.5	1.506	Aromatic C-N stretching	1396.51	0.447	Azo compound N=N stretching (nitrogen containing compounds)
6	1645.33	0.144	Oxime C=N-OH stretching	1396.51	2.055	Azo compound N=N stretching (nitrogen containing compounds)	1456.3	0.412	C=C stretching
7	1741.78	0.053	Aliphatic C=O stretching	1539.25	2.285	C-N stretching	1523.82	0.338	C=C stretching
8	1942.38	0.026	Overtone and combination bands	1631.83	5.028	C=C stretching	1639.55	0.194	C=C stretching
9	2027.25	0.028	Benzene ring	2926.11	2.026	O-H stretching (carboxylic acid)	2926.11	0.888	O-H stretching (carboxylic acid)
10	2169.99	0.026	Phosphorous acid and ester O-H stretching	3273.31	6.022	=C-H stretching	3254.02	0.196	=C-H stretching
11	2320.44	0.057	Phosphorous acid and ester O-H stretching	3751.67	0.132	O-H stretching (water)	3751.67	0.057	O-H stretching (water)
12	2376.38	0.001	Phosphorous acid and ester O-H stretching						
13	3618.58	0.013	O-H stretching (water)						

The values represented obtained are for the best of the triplicate experiment, doesnot represent the mean value (n=1)

Figure 1. FT-IR spectra

Figure 1. FT-IR spectra (continuation)

CONCLUSION

The processing methods studied in the present work had significant effect on the various attributes including the antioxidant, anti-nutritional and phytochemical properties of rice bean. The study revealed that all the processing treatments were able to reduce the anti-nutrient content. Germination showed maximum reduction in anti-nutrient content in rice bean. Also, the germinated

rice bean possessed maximum antioxidant activity along with increased amount of phytochemicals (phenolics, flavonoids, vitamin C and carotenoids) as compared to other processing methods and raw samples. Though rice bean is underutilized, but its nutritional value cannot be neglected. Also with the proper processing technique, its nutritional value can be enhanced and hence rice bean's nutritional potential can be fully exploited.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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