Effect of domestic processing methods on anti-nutritional factors and its impact on the bio-availability proteins and starch in commonly consumed whole legumes

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ABSTRACT

The biological utilization of protein is influenced by the presence of anti-nutritional compounds. This study aims to find the best domestic processing methods (soaking, sprouting, boiling, pressure cooking and roasting) in reducing the anti-nutritional factors thereby improving the rate and extent of starch and protein digestion by in-vitro condition. Results revealed that all the treatments were effective in significantly reducing the anti-nutritional content and application of single domestic processing method is insufficient for complete removal of anti-nutritional factors (tannin, phytic acid, Trypsin Inhibitor activity). The most effective combination method for reducing tannin, phytic acid and trypsin inhibitor activity content is soaking, roasting and pressure cooking. Hence, the best processing treatments was soaking/roasting followed by pressure cooking. For better digestibility of in-vitro protein and starch soaked pulses followed by pressure cooking was found effective.

Key words: Anti nutritional factors, Domestic processing, Legume, In vitro protein, Starch digestibility.

INTRODUCTION

Legumes are the most important food crop due to their dietary and economic importance that is globally appreciated and recognized. It is the second important constituent of Indian diet after cereals. Legumes in India have long been considered as the poor man’s meat as they are rich in protein. Although legumes constitute one of the richest and least expensive sources of protein in human/animal diet, the nutritional value of the protein in legumes is often reduced due to the presence of anti-nutritional compounds such as tannin, phenols, phytic acid, trypsin, chymotrypsin inhibitors etc.

Tannin (Polyphenol) are high molecular weight structures (>500) known to form complexes with proteins under certain pH conditions and render them unavailable for absorption by the human body and is responsible for decreased amino acid availability, cellulose for enzyme digestion. Phytic acid is the principal storage form of phosphorus in many plant tissues, especially bran and seeds. In-vitro studies had revealed that polyphenols present in field beans inhibit the activity of salvary α amylase and diminish the overall digestibility of carbohydrates in rat intestinal tract. It also reduces the digestibility of proteins, and fats. Phytic acid can bind minerals in the gut before they are absorbed and influence the digestive enzymes. Trypsin inhibitor is a type of serine protease inhibitor that reduces the biological activity of trypsin. The quality and quantity of these factors vary greatly due to factors such as plant genotypes, soil composition, growing condition and state of maturity (Khandelwal et al., 2010).

Raw pulses are subjected to a variety of processing technique prior to consumption including milling, dehulling, cooking etc., to remove or to reduce the anti-nutritional factors. However, polyphenols and tannins retain the inhibitory properties even after heat treatment of the food. Limited information is available regarding the effect of variety on the anti-nutritional factors. Further there were less studies on the effect of commonly applied domestic processes on concentration of polyphenols and tannin and correlating with the protein and starch digestion (Hefnawy 2011) Joshi Jyoti and Rahal Anshu (2018)

The present study, therefore estimated the concentration of total tannin, phytic acid, trypsin inhibitors in popular pulse varieties of commonly consumed whole legume grown in different areas of Tamil nadu viz. cow pea (Vigna catjang), blackgram (Vigna mungo) and green gram (Vigna radiata) and to validate the best domestic processing methods (soaking, sprouting, boiling, pressure cooking and roasting) in reducing the anti -nutritional factors such as tannin, phytic acid and Trypsin Inhibitor Activity (TIA) with reference to the rate and extent of bio availability of starch and protein digestion by in vivo studies.

MATERIALS AND METHODS

Legumes and processing: Sample collection and preparation: The high yielding and commonly grown seed samples of four varieties of cowpea (Vigna catjang) Paiyur...
1. Co 4, Co 6, VBN 2 four varieties of blackgram (Phaseolus mungo) VBN (Bg) 4, VBN (Bg) 6, VBN (Bg) 7, MDU (Bg) 1 and two varieties of green gram, (Phaseolus aureus) VBN (Gg) 3, Co (Gg) 8 were obtained from Research stations of Tamilnadu Agricultural University. Seeds were cleaned to remove dirt, grit, shriveled seed and broken grains and then packed in airtight containers. The samples were well mixed and four lots were drawn by the quartering method. One lot was used for determination of total polyphenols, tannins and TIA, in-vitro protein digestibility (IVPD) starch digestibility (IVSD), in the raw sample and the other lot was used to study the effect of domestic processing techniques soaking, sprouting, boiling, pressure cooking and roasting. From each lot samples were taken in triplicates for analysis. The main focus of the present study was to investigate the maximum reduction or elimination of anti-nutritional factors in the whole legumes using the domestic processing technique which were representative of those practiced in Indian households.

**Processing of treatments**

**Soaking:** All pulses were soaked separately in 1.5 part of tap water at 30 °C for 3 hours. The soaking solution was drained off and seeds were rinsed twice with distilled water and dried in hot air oven at 55 °C for 24 hours

**Sprouting:** All pulse varieties were soaked overnight for 12 hours in the water (1: 5 w/v) at room temperature. Excess water was drained and the samples were wrapped in a moist cloth for 12 hours at room temperature and the seeds were dried in a hot air oven at 55 °C for 24 hours.

**Boiling:** All pulses were put in water and open cooking was done until they become soft as felt between two fingers. After cooking, excess water cooked on a hot plate was drained and the seeds were dried in a hot air oven at 55 °C for 24 hrs.

**Pressure cooking:** All pulses were cooked in one liter vessel containing water in ratio of 1: 4. Tops of the vessel were covered with aluminum foil. After cooking in a pressure cooker at 15lbs/ at 105± 5 °C for 15 minutes excess water was drained and then seeds were dried in a hot air oven at 55 °C for 24 hours.

**Roasting:** All pulses were dry roasted in pre heated vessel for three minutes at 120 °C and seeds were packed air tight.

**Chemical analysis:** All legumes were estimated for their moisture, ash, fat, starch and protein (Nx 6.25) content by employing the standard methods of analysis (AOAC 2000). Anti-nutritional factor tannin content of the sample were estimated using a spectrophotometer at 760nm Sadasivam and Manickam (1992). Phytic acid content was extracted and separated by ion-exchange chromatography determined as per Sadasivam and Manickam (1992). Trypsin Inhibitor Activity (TIA) was determined as per the method described by Thimmiah (1999) In-vitro protein digestibility of selected pulses were determined after digestion with a pepsin HCL solution at 37.5 °C for 24 hours as per Sreerama et al., (2008) In-vitro starch digestibility of selected pulses were determined as per procedure suggested by Singh et al., (2015). All the results of triplicate samples were statistically analyzed by separate two-way analysis of variance ANOVA between varieties and processed samples. A probability value of P<0.01, was considered significant

**RESULTS AND DISCUSSION**

The amount of starch and protein was almost the same between the varieties but differed between the pulses. The starch content of cowpea ranged from 49-52 g/100g. In black gram it ranged from 48.10-50.1 g/100g and in green gram starch was in range of 56.87 and 57.23 g/100g. The protein content of cowpea ranged from 24.60 to 25.75 g/100g in blackgram it ranged from 26.2 to 28.20 g/100g and in greengram from 22.96-23.96 g/100g. The protein and carbohydrate component are important in determining nutritive quality and processing quality of cowpea seeds Henshaw (2008).

**Anti-nutritional factors in raw pulses:** The unprocessed raw seeds recorded the highest total tannin, phytic acid and TIA concentration. The total tannin concentration in pulses ranged from 509 to 965 mg/100g. It was lowest in green gram and highest in blackgram. In blackgram dark colored seed variety VBN(Bg)8 and MDU(Bg) 1 contained higher concentration of tannin, Beninger and Hosfield (2003) reported that dark colored seeds of Phaseolus vulgaris contained higher concentration of tannin than did those of light colored cultivars. The total phytic acid concentration was lowest in cowpea and it ranged from 145.67 to 153.8 mg/100g when compared to blackgram and green gram where it ranged from 633 to 944 mg/100g highest observed in VBN (Bg) 7. The total TIA was in blackgram (2000.56 to 2463.25 TIA/100 g) when compared to cowpea (2450.23 to 2866.85 TIA /100 g) and green gram (2509.98 and 2566.65). The concentration significantly varied among the legumes/variety. Unprocessed raw seeds of green gram recorded the highest level of TIA (2509-2566) than and blackgram it was (2442-2463 ). However values determined for green gram and black gram were in agreement with those reported by Kakati et al. (2010). Differences between varieties of pulses may be attributed to differences in the color of seed coat, storage time, seed size and stage of development (Dobhal, and Raghuvarshi, 2018).

**Effect of processing:** Effect of different processing treatments to remove anti-nutritional factors viz., Tannin mg/100g, Phytic acid mg/100g and Trypsin inhibitor activity TIA/A100g in the selected pulses are presented in Table 1. Tannin content present in the raw seeds of all the pulses showed sequential decline with processing treatments as well as with the varieties. The losses of tannin in processing was greatest in roasted legumes averaging from 48.7 to 57.1 %
in cowpea and in blackgram 37.2 to 41.3%. Next to roasting, pressure cooking had the highest reduction in tannin and ranged from 36.6 to 44.7% in cowpea and 34.3 to 41.3% in blackgram. Among the blackgram MDU (Bg)1 variety had very poor reduction of tannin in all the processing methods. The loss of tannin in heat treatment methods *viz.* roasting, boiling and pressure cooking were found higher for cowpea and blackgram as compared to sprouting and soaking methods. Losses of tannin were similar in magnitude for the legume cowpea and blackgram. Khattab *et al.* (2009) Kaketi *et al.* (2010) also reported that highest reduction of tannin content with boiling followed by autoclaving and microwave cooking in some legumes such as cowpea and kidney beans. Sprouted green gram seeds had greater reduction in tannin content (39.7 and 46.8%) followed by soaking. Several possible reason have been suggested for reduction in tannin due to leaching in water. In roasting the reduction was 21.4 to 28.5% and in pressure cooking it was only 17.9 to 28.9%.

From the present study it was observed that single technique is not sufficient to completely remove the tannin. Hence combination of domestic processing is essential. Thus for complete removal of tannin the best suited processing treatments are soaking, roasting followed by pressure cooking. However the reduction was only 74.6% in the blackgram variety MDU (Bg) 1. In Green gram sprouting, roasting and pressure cooking has to be employed for 100% removal. Baddi *et al.* (2016)

Experimental finding on phytic acid content revealed that cow peahad highest reduction per cent when compared to black gram and green gram. The reduction was similar in soaking and sprouting treatments ranging from 20.5 to 26.9 % and in boiling and pressure cooking it ranged from 21.2 to 28.3 % irrespective of variety. In blackgram there was wide difference in reduction percent between soaking, sprouting and pressure cooking and the difference was two fold for the variety VBN (Bg 4) *viz.* 11.3% in

| Processing | Anti nutritional factors | Raw pulse | Soaking (3 hr) | Sprouting (24 hr) | Boiling (30 min) | Pressure cooking (15 min) | Roasting (3 mts) |
|------------|--------------------------|----------|----------------|------------------|-----------------|-------------------------|-----------------|
| **Pulse**  |                          |          |                |                  |                 |                         |                 |
| Paiyur 1   | Tannin                   | 760      | 510            | 490              | 420             | 420                     | 390             |
|            | Phytic acid              | 153.80   | 122.22         | 119.22           | 110.21          | 112.22                  | 120.31          |
|            | TIA                      | 2866.85  | 1865.52        | 1725.20          | 890.15          | 870.55                  | 1140.56         |
| Co 4       | Tannin                   | 770      | 500            | 450              | 420             | 440                     | 370             |
|            | Phytic acid              | 150.71   | 110.21         | 115.10           | 118.71          | 119.81                  | 120.31          |
|            | TIA                      | 2822.10  | 1845.24        | 1462.24          | 750.13          | 780.58                  | 1111.23         |
| Co 6       | Tannin                   | 710      | 540            | 470              | 440             | 450                     | 340             |
|            | Phytic acid              | 149.62   | 112.34         | 109.96           | 109.91          | 105.61                  | 106.71          |
|            | TIA                      | 2546.35  | 1657.28        | 1548.28          | 780.96          | 780.00                  | 990.86          |
| VBN 2      | Tannin                   | 700      | 480            | 400              | 410             | 420                     | 300             |
|            | Phytic acid              | 145.67   | 109.71         | 106.59           | 105.61          | 104.71                  | 100.60          |
|            | TIA                      | 2450.23  | 1558.35        | 1308.35          | 710.50          | 712.50                  | 910.58          |
| VBN(Bg)4   | Tannin                   | 859.3    | 664.3          | 526.4            | 506.6           | 504.6                   | 540             |
|            | Phytic acid              | 674      | 598            | 468              | 400             | 400                     | 553             |
|            | TIA                      | 2463.25  | 1077.01        | 1071.05          | 652.50          | 648.50                  | 1002.45         |
| VBN(Bg)6   | Tannin                   | 868.2    | 676.7          | 514.5            | 571.3           | 570                     | 570             |
|            | Phytic acid              | 633      | 432            | 316              | 337             | 337                     | 412             |
|            | TIA                      | 2442.38  | 1281.41        | 1490.35          | 570.89          | 560.85                  | 1000.10         |
| VBN(Bg)7   | Tannin                   | 980      | 720            | 690              | 600             | 600                     | 575             |
|            | Phytic acid              | 944      | 744.60         | 718              | 546.78          | 546                     | 520             |
|            | TIA                      | 2008.39  | 1111.56        | 0989.52          | 686.50          | 499.59                  | 1148.92         |
| MDU(Bg)1   | Tannin                   | 965      | 765            | 710              | 700             | 700                     | 710             |
|            | Phytic acid              | 854      | 734            | 734              | 616             | 615                     | 600             |
|            | TIA                      | 2000.56  | 0985.32        | 0975.32          | 490.39          | 480.15                  | 1054.67         |
| VBN(Gg)3   | Tannin                   | 509.3    | 329.3          | 307              | 420.3           | 418.3                   | 400.3           |
|            | Phytic acid              | 664.76   | 550.80         | 452.80           | 430.62          | 430.62                  | 410             |
|            | TIA                      | 2566.63  | 1145.36        | 1341.84          | 596.63          | 496.58                  | 1150.26         |
| Co (Gg) 8  | Tannin                   | 534.3    | 382.5          | 284.3            | 383.8           | 380                     | 380             |
|            | Phytic acid              | 692.40   | 545.47         | 460.99           | 489.35          | 480                     | 480.0           |
|            | TIA                      | 2509.98  | 1251.61        | 1271.38          | 591.95          | 448.32                  | 1156.83         |

P value for testing overall difference between variety of same pulse for various processing treatment using ANOVA F test was <0.01except the TIA content in TIA of green gram between variety.
soaking and 30.6% in sprouting. In blackgram, the variety MDU (Bg)1 had very poor reduction of phytic acid in all processing methods lowest of 14.1% in soaking and highest of 29.7% in roasting. In green gram the reduction was highest in all heat treatment methods 35.2 to 38.3 % for VBN (Gg)3. In sprouting the reduction was up to 31.9 to 33.4%. Sidduraja and Becker (2001) remarked that loss of phytic acid during germination may be caused by hydrolytic activity of the enzyme phytase. The observed reduction during heat treatment may be attributed to heat labile nature of phytic acid due to the formation of insoluble complex between phytase and other compounds. For reducing phytic acid content up to 100% in cow pea and blackgram soaking along with roasting and pressure cooking, has to be employed for maximum removal except MDU (Bg) 1 all pulses showed complete removal of phytic acid and MDU (Bg) 1 had only reduction of 71.8%. In green gram along with sprouting, roasting and pressure cooking, has to be employed for complete removal.

Effect of processing on TIA content clearly showed all the treatments had considerable reduction and pressure cooking was more effective. This might be due to heat labile nature of trypsin inhibitors. Among the treatments the reduction of TIA level was highest by pressure cooking (69 to 82) as cooking inactivates TIA. In blackgram VBN (Bg) 6 and MDU (Bg) 1 77 and 76 per cent respectively. In green gram there was not wide difference between varieties and ranged 81 and 82%. Similar report was stated by Grewal and Jood (2006). Therefore pressure cooking was found to be the best treatment in reducing TIA and for reducing TIA content completely any two processing treatments is enough viz., soaking/roasting and pressure cooking. There exist significant difference in tannin phytic acid, TIA concentration among pulses, varieties and different processing methods at P <0.05 in all cases.

**In vitro starch digestibility (IVSD) of selected pulses:**

The IVSD of raw and processed seeds are presented in Table 2. The IVSD of raw seeds of all the selected pulses ranged from 28.5 to 38.7 %. The IVSD of all the pulses increased significantly during processing with maximum increase observed with pressure cooking for cow pea (35.3 to 40.7%) blackgram 49.3 to 53.3% and in green gram it was. The reduction was statistically significant 59.7 and 62%. Legumes in general are markedly resistant to pancreatic amylase attack but cooking led to dramatic increase in susceptibility to this enzyme. Moreover, cooking improves digestibility of starch through gelatinization and destruction of anti-nutrients. An increase in the total starch content in cooked samples compared to the respective raw seed samples was also reported by Kasote et al., 2014 Kaur et al (2015). Giang et al (2015). On statistical analysis the best performed treatment was pressure cooking.

**In vitro protein digestibility (IVPD) of selected pulses:**

The IVPD of raw seeds of all the selected pulses are given in Table 3. The IVPD of raw seeds of all the selected pulses ranged from 48.6 to 61.8 %. A similar improvement of IVPD by hydrothermal treatment, dry heating and germination has also been reported in several legumes by

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Table 2: Per cent In vitro Starch availability of raw and processed pulses.

| Processing (T) | Whole pulse | Soaking (3 hr) | Sprouting (24 hr) | Pressure cooking (15 min) | Roasting (3 mts) |
|---------------|-------------|----------------|-------------------|--------------------------|-----------------|
| Paiyur 1      | 32.8        | 33.4           |                   |                          | 35.2            |
| Co 4          | 31.4        | 31.1           |                   |                          | 33.5            |
| Co 6          | 31.2        | 32.7           |                   |                          | 36.9            |
| VBN 2         | 30.2        | 31.1           |                   |                          | 32.6            |
| T             | SED= 0.16   | CD =0.53**     |                   |                          | 38.2            |
| V             | SED =0.32   | CD = 0.87**    |                   |                          | 33.6            |
| VBN(Bg)4      | 29.9        | 30.5           |                   |                          | 40.6            |
| VBN(Bg)6      | 28.5        | 30.3           |                   |                          | 40.1            |
| VBN(Bg)7      | 29.9        | 31.7           |                   |                          | 41.6            |
| MDU(Bg)1      | 31.0        | 32.0           |                   |                          | 40.1            |
| T             | SED= 0.28   | CD =0.94**     |                   |                          | 49.3            |
| V             | SED =0.26   | CD = 0.72**    |                   |                          | 45.6            |
| VBN (Gg)3     | 36.7        | 43.0           |                   |                          | 45.1            |
| Co (Gg) 8     | 38.7        | 43.7           |                   |                          | 45.0            |
| T             | SED= 0.628  | CD = 2.29**    |                   |                          | 45.0            |
| V             | SED =0.26   | CD = 1.08**    |                   |                          | 47.1            |

P value for testing overall difference between variety of same pulse for various processing treatment using ANOVA F test was <0.01 except the green gram between variety.
Table 3: Per cent *in vitro* protein availability of raw and processed pulses.

| Pulse       | Processing          | Whole pulse | Soaking (3 hr) | Sprouting (24 hr) | Pressure cooking (15 min) | Roasting (3 mts) |
|-------------|---------------------|-------------|----------------|-------------------|----------------------------|------------------|
| Paiyur 1    |                     | 52.8        | 53.2           | 54.4              | 65.0                       | 63.0             |
| Co 4        |                     | 48.6        | 50.0           | 54.0              | 68.8                       | 61.5             |
| Co 6        |                     | 50.6        | 53.0           | 59.9              | 67.9                       | 60.5             |
| VBN 2       |                     | 55.6        | 56.6           | 61.6              | 67.9                       | 66.6             |
| VBN(Bg)4    |                     | 50.8        | 51.2           | 54.0              | 70.0                       | 64.3             |
| VBN(Bg)6    |                     | 50.0        | 53.9           | 56.0              | 76.8                       | 66.9             |
| VBN(Bg)7    |                     | 53.7        | 54.4           | 58.7              | 72.8                       | 65.0             |
| MDU(Bg)1    |                     | 55          | 57.8           | 59.9              | 74.9                       | 68.2             |
| VBN (Gg)3   |                     | 58.9        | 61.9           | 72.9              | 79.5                       | 73.5             |
| Co(Gg) 8    |                     | 61.8        | 64.8           | 77.1              | 82.8                       | 77.3             |

T x V SED 1.39 CD at (0.01) 3.89**

**Black gram**

| VBN(Bg)4    |                     | 50.8        | 51.2           | 54.0              | 70.0                       | 64.3             |
| VBN(Bg)6    |                     | 50.0        | 53.9           | 56.0              | 76.8                       | 66.9             |
| VBN(Bg)7    |                     | 53.7        | 54.4           | 58.7              | 72.8                       | 65.0             |
| MDU(Bg)1    |                     | 55          | 57.8           | 59.9              | 74.9                       | 68.2             |

T x V SED 1.25 CD at (0.01) 3.57**

**Green gram**

| VBN (Gg)3   |                     | 58.9        | 61.9           | 72.9              | 79.5                       | 73.5             |
| Co(Gg) 8    |                     | 61.8        | 64.8           | 77.1              | 82.8                       | 77.3             |

T x V SED 1.72 CD at (0.01) 5.61**

P value for testing overall difference between variety of same pulse for various processing treatment using ANOVA F test was <0.01 except the green gram between variety.

Sreerama et al. (2008) protein digestibility of various raw legumes in the range of 33.8 -37.8% such as lentil, greengram, bengal gram, red kidney bean, and soya bean. The IVPD of all the pulses increased significantly during processing with maximum increase observed on pressure cooking. On pressure cooking green gram had the highest increase of IVPD for both the variety tested (79.5% and 82.8%). In roasting treatment the increase in IVPD ranged from 9.9 to 16.9% in all legumes. Siddhuraju and Becker (2001) Chakrabarty and Bhattacharyya (2014) reported significant increase of IVPD value by processing methods soaking, autoclaving, dryheating and germination in two varieties of macuna beans cooking or autoclaving treatments result in more improvement (13-18.6% and 10.3 to 18% in two varieties of macuna bean. In their study, it was also stated that antioxidant capacity of some of the pulses could be improved by thermal processing methods that resemble cooking.

Correlation of IVPD values with Trypsin inhibitor activity for the processing treatments revealed that there exists highly significant relationship between the two parameters with negative correlation as the IVPD increases there is a decrease in TIA value for all the pulses.

CONCLUSION

All the selected pulses showed sequential decline of anti-nutritional factors *viz.*, tannin, phytic acid and trypsin inhibitor activity in all processing methods. It is revealed that application of single domestic processing technique is frequently insufficient to completely remove all the anti-nutritional factors and combination of domestic cooking processing has to be employed. The most effective method to eliminate tannin and phytic acid content can be achieved by applying at least three processing methods *viz.*, roasting, soaking and pressure cooking. For complete elimination of TIA any two processing treatments *viz.*, soaking/roasting followed by pressure cooking is effective. The IVSD and IVPD of all the pulses increased significantly during the processing with maximum increase in pressure cooking. There exists highly significant relationship between the two parameters with negative correlation as the IVPD increases there is a decrease in TIA value for all the pulses.

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