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ESTIMATION OFANTI-NUTRITIONAL PROPERTIES IN PULSESAND THEIR MITIGATION USING VARIOUSPROCESSINGMETHODS

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ABSTRACT

An experimental study was conducted to study the possible processing methods that can be used for the reduction of anti-nutritional factors from six commonly used pulses in India. The six selected pulses were Biri(Black Gram),Rajma (Red Kidney Beans),Toor(Yellow Lentils),Kolatha (Horse Gram), Mung(Mung Beans), and Masoor (Orange Lentils). Chemical as well as spectrometric methods were used to estimate the possible reduction in anti-nutritional components. According to our study boiling and roasting were observed to be the best methods for the removal of saponins and lectins from the pulse samples. We thus concluded, that boiling and roasting can be effectively used as pre-cooking processing methods before consumption of pulses.

KEYWORDS: Phyto-chemicals, Anti-nutritional properties, Pulse processing

Pulses are otherwise called grain legumes. They are leguminous products yielding one to two seeds inside a case (R.N. Tharanathan, 2003). As indicated by the Food and Agriculture Organization (FAO) the term is saved for crops reaped for dry seeds. In this way, it rejects green pods andoilseeds like soybeans and peanuts. Pulses have been the most easily accessible protein source for the world's population and nutritionally fulfilling, this is due to the fact that pulses comprise a good concoction of almost all essential ingredients, such as, protein (20-30%), carbohydrates (50 – 60%) and fat (1%) (Adriana N. Mudryj, 2012).

However, Pulses do suffer from some disadvantages. Pulses during their growth in the fields synthesize certain compounds which help them to fight through the harsh environment around them. These compounds protect them from being ingested by pests as well as other insects that can lead to the destruction of the pods.These protection mechanisms are unfortunately carried by the pulses throughout the processing and packaging process.

Such compounds are called as anti-nutritional factors. Some of the anti-nutritional factors are: Trypsin inhibitors, Hemagglitinins or Phytoagglutinins, Cyanogen glycosides, Saponins etc.

The present study is focused on the estimation of the amount of anti-nutritional factors in the above mentioned verities of pulses and to test different processing methods for mitigating of these anti-nutritional factors to increase the overall health benefit.

MATERIALS AND METHODS

Pulses and Chemicals

Pulses used in this study were obtained from the company- 24 Mantra organic (India). All the reagents used in the experiments were analytical grade and obtained from HiMedia (France) and Sigma-Aldrich (USA).

Sample Preparation for Phytochemical Analysis

The air-dried pulse samples were grinded in a clean grinder individually and stored at 4^0C. An aqueous extract was prepared for the estimation of different phytochemicals.

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Extract Preparation

5gm of each powdered sample was dissolved in 50ml of double distilled water and kept for 24hrs at 4^0C. The samples were then filtered using Whatman filter paper and stored at 4^0C for further analysis.

Phytochemical Analysis

2.4.1-Detection of Alkaloids

Mayer's Test:-Filtrates were treated with few drops of Mayer's reagent (Potassium mercuric iodide).1.36gm of Mercuric chloride in 60ml of distilled water.3gm of Potassium iodide in 20ml distilled water.Potassium iodide solution was made and added to Mercuric chloride solution and diluted by raising volume to 100ml.Formation of a cream colored precipitate indicates the presence of alkaloids.

Wagner's Test:-Filtrates were treated with few drops of Wagner's reagent (Iodine in Potassium Iodide). 1.27gm of Iodine and 2gm of Potassium iodide were dissolved in 5ml of water and the solution was diluted to 100ml with distilled water.Formation of brown reddish precipitate indicates the presence of alkaloids.

2.4.2 Detection of Phenols

Ferric Chloride Test:-Extracts were treated with 3-4 drops of Ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

2.4.3 Detection of Tannins

Lead Acetate test:-To 5ml of extract, few drops of 1% Lead acetate was added, appearance of yellow precipitate indicates presence of tannins.

2.4.4 Detection of Flavonoids

Alkaline Reagent Test:-Extracts were treated with few drops of Sodium hydroxide solution (1N). Formation of intense yellow color,which becomes colorless on addition of dilute acid (10% Sulfuric acid), indicates the presence of flavonoids.

Ferric Chloride Test:-Test sample was added with a few drops of Ferric chloride solution. Formation of blackish red color indicates the presence of flavonoids.

Anti-Nutritional Analysis

Sample Preparation

The Experiment was divided into four different processing techniques viz., Germination, Fermentation, Boiling, and Roasting, to study the effectiveness of different processes for the reduction of anti-nutritional factors.

Germination

Whole pulses were sandwiched between two water soaked filter papers and wrapped in a muslin cloth and kept inside a tray with water sprinkled over them for 48hrs., After the germination the pulses were freeze-dried using liquid nitrogen and stored at -80^0C.

Fermentation

Whole pulses were added in the ratio of 1:2 (pulse: water) soaked for 24hrs, decanted and grinded using motar pestle. Packaged curd was confirmed for active *Lactobacillu*s bacteria on MRS media and kept for 24hrs incubation. After confirmation of active lactobacillus colonies, 15mg curd was added to grinded pulse samples withminimal amount of waterto make a thick slurry. The slurry was incubated at 37^0C for 24hrs fermentation. Fermented samples were freeze-dried and stored at -80^0C.

Boiling

Pulses were added in the same pulse: water ratio and boiled at a temperature of 100^0C for 20 min. in a waterbath. The water was decanted and samples were freeze-dried after bringing them to room temperature and stored at -80^0C.

Roasting

Pulses were roasted one sample at a time, in a microwave at 900 watt for 4 minutes. The roasted pulses were sealed with cotton plugs to avoid moisture, and were freeze-dried and stored at -80^0C.

2.5.2Test for Cyanogen glycosides

Cyanogen glycosides were estimated by Ferri-Ferro Cyanide test as described in (Singh, 1999). Rectangular Whatman paper strips were dipped in 10% $FeSO_4$ solution and dried, then the strips were put in 20% NaOH solution and dried, 2 – 3 drops of Toluene were added to

the crushed pulses samples and incubated at 60^0C for 2 hours with the strips attached at the top of the test tube, strips were then immersed in 30% H_2SO_4, a prussian blue color is observed by the formation of Sodium ferric ferrocyanide.

2.5.3 Test for Saponins

Saponins were colorimetrically determined as method described by (S Hiai, 1976).Vanillin (analytical grade) was made to a concentration of 8% by dissolving in 99.5% (v/v) ethanol, prepared freshly. Equal volume of the clarified extract and vanillin solution were added to a test tube and 5ml of Sulphuric Acid was added to it and subjected to 60^0C in water bath, absorbance was taken at 460 nm.

2.5.4 Test for Lectins

Lectins were tested by erythrocyte agglutination test as described by (Saleem Khan, 2011). A 2% erythrocyte suspension was made and clarified extract of pulses was added with the erythrocyte suspension in equal volumes. The result of agglutination was observed after 30 minutes. The results were calculated as Hemagglutination titre.

2.6 Statistical Analysis

Results were analysed using one way ANOVA to find the significance of the values and were expressed using bar graphs with standard deviation and mean.GraphPad PRISM was used for all the graphical and statistical analysis.

RESULTS

3.1Phytochemical Analysis

The presence of different phytochemicals (2.4) were estimated using various chemical methods (2.4.1, 2.4.2, 2.4.3, 2.4.4)which detected the presence or absence of the phytochemicals under study, the data is presented in Table 1.

Table 1.Phytochemical analysis of legumes from extracted samples

			Samples					
Sl. No.	Phytochemical	Tests	Black Gram	Yellow Lentils	Orange Lentils	Red Kidney Bean	Mung Bean	Horse Gram
1	Alkaloids	Mayer's Reagent	-	-	-	-	-	-
		Wagner's Reagent	-	-	-	-	-	-
2	Phenols	Ferric chloride	+	-	+	-	-	-
3	Tannins	Lead Acetate	-	+	+	-	+	+
4	Flavonoids	Alkaline Reagent	+	-	+	-	+	+
		Ferric Chloride	+	-	+	-	+	+

Present = Positive (+); Absent = Negative (-)

According to the experiment no presence of alkaloids were found in any of the samples. Presence of phenolic compounds were detected in Masoor (Orange lentils) and Biri (Black Gram). Tannins were present in Toor (yellow Lentils), Masoor (Red Lentils), Mung Bean, and Kolatha (Horse Gram). Biri (Black Gram), Masoor (Orange Lentils), Mung Bean, and Kolatha (Horse Gram) were rich in flavonoids.

3.2 Anti-nutritional Analysis

Very significant reduction in the anti-nutritional concentrations (2.5.2, 2.5.3, 2.5.4) present in minimally processed pulses were seen after the use of methods like fermentation, boiling, roasting and germination.

3.2.1 Test for cyanogen glycosides

There was no presence of cyanogen glycosides in any of the pulses under consideration, a similar result was found in the processed pulses as well.

3.2.2 Spectrophotometric analysis for Saponins

A very sharp decrease was found in the concentration of saponins in the minimally processed pulses to the processed pulses. The samples of Rajma (Red Kidney Bean), Toor (Yellow lentils) and Kolatha (Horse Gram) did not germinate as Rajma might require more time than 48hrs., the latter two were in split form, so the data for these three samples is not available. Boiling and roasting of pulses gave the best results. The individual changes in pulses due to each processing method are depicted in Figure 1 and the mean reduction of anti-nutritional factors by each process is depicted with the help of one way ANOVA in figure 2.

Similar results were found in other researchers which provided confirmation that such processing methods doesreduce the saponin concentration. Autoclaving was the method suggested to give best results but our study provides boiling as a good processing method as that is done in general household (Panozzo, 2007). Beans were suggested to have high concentration of saponins which was also evident in our study (John Shi, 2004).

Figure 1: graphical representation for reduction in saponin concentration using various processing techniques. The unprocessed samples had very high absorbance which has been significant reduced after the processes of Boiling, Roasting, Fermentation and Germination. The absorbance was determined at the maxima of the control of vanillin without adding the sample

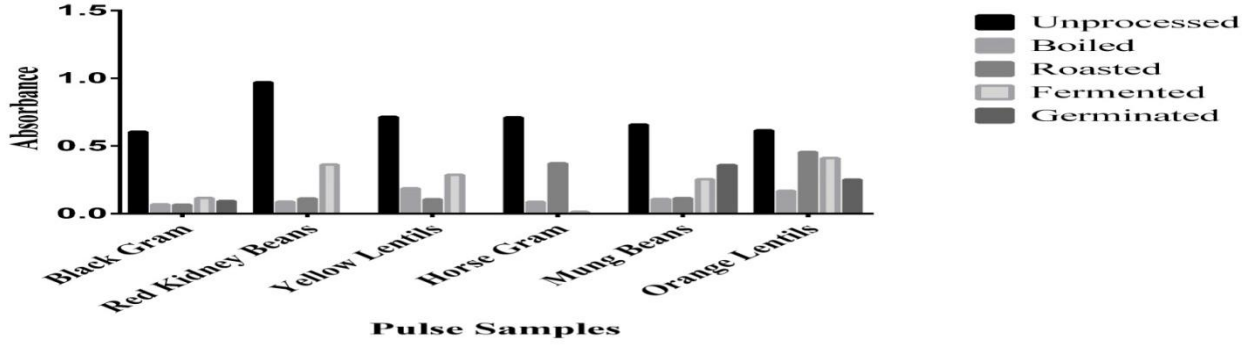
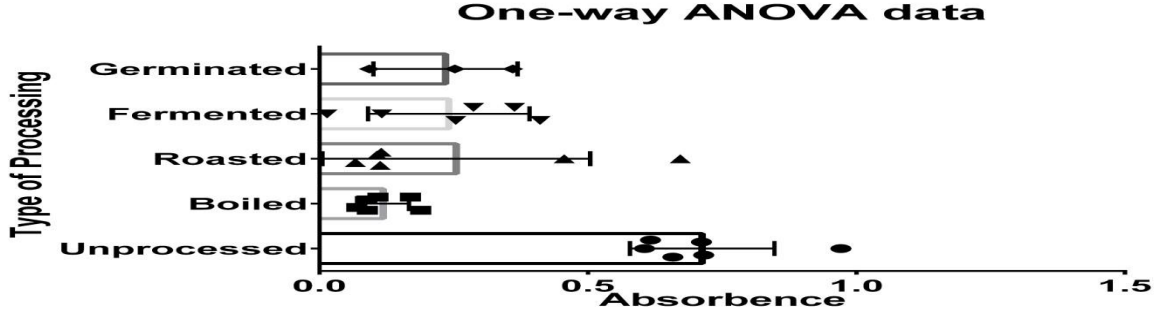


Figure 2: Graphical representation of grouped data with one way ANOVA depicting the various processes that were followed for removal of anti-nutritional factors. As the data suggests, boiling has reduced the absorbance obtained from the test to a significantly lower limit, while other processes have also reduced the saponin concentration. The mean was significant (P< 0.05).



3.2.3 Lectins and erythrocyte hemagglutination

The presence of lectins which is a striking feature of Rajma (Red Kidney Beans) as it was observed in the unprocessed sample, the lectin content was significantly reduced when the tests were conducted on the processed samples. Boiling tends to reduce the lectin concentration drastically but at the same time it was observed that the germinated samples tend to enhance the effect of agglutination in many samples as compared to the control sample. Germination may play an important role in the release of lectins or due to the release of other glycoprotein attachment compounds, released during the germination process. Fermentation also showed a similar agglutination activity which was previously not found in the unprocessed samples. The agglutination results were predicted on the basis of visual grading method. The results for the grading are presented in Table 2.

Table 2.The effect of various processing methods on the reduction of lectins in different samples.

Processing Methods	Biri (Black Gram)	Rajma (Red Kidney Beans)	Toor (Yellow Lentils)	Kolatha (Horse Gram)	Mung (Mung Beans)	Masoor (Orange Lentils)
Unprocessed	++	+++++	++	++	++	++
Boiling	-	++	+	+	-	-
Roasting	+	++++	++	++	+	+
Fermentation	+	+++	+++	+++	+++	+++
Germination	+++	x	x	x	++	++

Nil = (-); Satisfactory = (+); Fair = (++); Good = (+++); Very good = (++++); Excellent = (+++++); x = Not Applicable

DISCUSSIONS

The study presently conducted was based on the fact that pulses consist a large amount of anti-nutritional compounds. These anti-nutritional compounds are known to have deleterious effects on human body (WG, 1980) (Liener I. , 1979).We considered six most commonly consumed pulses in India for our study, viz, Biri(Black Gram),Rajma (Red Kidney Beans),Toor(Yellow Lentils),Kolatha (Horse Gram),Mung(Mung Beans), andMasoor (Orange Lentils).

The harmful effects of anti-nutritional factors is still a topic of research as the long-term effects and amount of consumption can be critical parameter for diseases. Although, some of these anti-nutritional factors are known to cause more immediate effects, like high levels of saponins reduced the bioavailability of micronutrients (Liener I. , 1982). Potatoes also contain saponins and when eaten in large quantity cause abdominal pain, vomiting and diarrhea. Saponins do have beneficial effects like the reduction in plasma cholesterol levels in animals and reducing the risk of heart disease (Amit Kumar Jain, 2009) (Olaboro G, 1981). Phytic acid and its interaction with protein is considered for limiting the nutritive value of legumes (Tabekhia M M, 1980).

Lectins are extensively present among pulses. Studies based on microbial and immunochemistry of these compounds have suggested that a higher intake of lectins can lead to toxic effects, inhibiting growth and death (WG, 1980). Studies suggest that binding of lectins occurs in the intestinal mucosa cells, leading to malfunction, disruption and lesion in small intestine blocking absorption of nutrients from gut (WG, 1980) (IE, 1974) (Pusztai A, 1982).

In our study we found that lectins were present in highest amount in raw Rajma (Red Kidney Beans) as compared to other samples. The use of processing methods like boiling and roasting had a very significant impact in the reduction of lectins thus reducing the amount of hemagglutination. Thus boiling or roasting of legumes before consumption plays a very crucial role as far as the reduction in anti-nutritional factors of the consumed pulses in our body is concerned. Study on the amount of lectins in Red kidney

beans and their beneficial effects as a retroviral drug therapy have been suggested by (George Grant, 1995).

The significant decrease in the concentration of saponins and lectins using boiling and roasting had provided us concrete evidence on the use of such processing methods, although, these anti-nutritional factors in the ongoing research do suggest evidence ofbeing healthy and helpful in curing ailments. Further, study on their exact consumption and if proven to be healthy their safe isolation can be a better alternative for people suffering with such diseases. Pulses are a diverse group and needs further understanding so that they can prove more nutritious for the vegetarian and vegan population.

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