IJFANS
International Journal of Food And Nutritional Sciences

Volume 4, Issue 4, Jul-Sep 2015, www.ijfans.com e-ISSN: 2320-7876

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

IMPACT FACTOR ~ 1.021

Official Journal of IIFANS

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

e-ISSN 2320 –7876 *www.ijfans.com*
Vol.4, Iss.4, Jul-sep, 2015

Research Paper *Open Access*

PROXIMATE COMPOSITION, ANTINUTRITIONAL FACTORS AND PROTEIN FRACTIONS OF *TAMARINDUS INDICA L* SEEDS AS INFLUENCED BY PROCESSING TREATMENTS

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Received on: 21th May, 2015 **Accepted on: 14th September, 2015**

ABSTRACT

Tamarind (*Tamarindus indica* L) is an economically important tree of India, belongs to the phyla Leguminosae. Legume seeds are an important component of the human diet. Legumes have to be processed prior to consumption due to their content of antinutritional compounds, such as trypsin inhibitors, phytic acid, tannins. Processing techniques such as soaking, dehulling, cooking, autoclaving and also germination and fermentation have been found to reduce significantly the levels of phytates and tannins by exogenous and endogenous enzymes formed during processing. Increasing attention is also paid to the use of tannins as antimicrobial agents, for example in wood preservation or prevention of dental caries. In the present study, the effect of different processing methods on chemical profile of Tamarindus indica seeds was investigated. Raw seeds were collected for the study. Five different processing methods such as soaking, dehulling, cooking, autoclaving and germination were used. All the processed seeds were dried and powdered before chemical analysis. The proximate composition, antinutritional factors and protein fractions of *Tamarindus indica* seeds are determined before and after soaking, followed by dehulling, cooking, autoclaving and germinating treatments. The chemical composition varied between the treatments. Soaking of seeds followed by dehulling and germinating have reduced tannin content and phenolics in comparison to the control seeds. While tannin content and phenolics significantly increased in autoclaved seeds. Albumin and glutelin were the two major proteins present. The results indicated that Globulin and Prolamin fractions of the seeds decreased during processing while albumin greatly increased.

Key words: Tamarind seed, antinutrients, tannins, albumin, globulin.

INTRODUCTION

Tamarind *(Tamarindus indica* L.) is a perennial herb belonging to the dicotyledonous family of Leguminosae. Its local names include Indian date (English), *asam jawa* (Malay), *siyambala* (Sinhala),*sampalog* (Philippines) and *puli* (Tamil). The tree averages 20-25 m in height and 1 m in diameter, has a wide spreading crown and a short, stout trunk. It is slow growing, but long lived, with an average life span of 80-200 years. Today, tamarind grows widely in most tropical and subtropical regions of the world. Tamarind is well adapted to semiarid tropical conditions and also grows well in many humid tropical areas with seasonally high rainfall. Tamarind is grown commercially in plantations and homestead gardens for its product, and along avenues as a ornamental plant in towns and cities. Tamarind has many uses and it is best known for its fruits.

The seed comprises the seed coat or testa (20-30%) and the kernel or endosperm (70-75%) (Coronel, 1991; Shankaracharya, 1998). Whole tamarind seed and kernels are rich in protein (13-20%), and the seed coat is rich in fibre (20%) and tannins (20%). Panigrahi *et al.* (1989) reported that whole tamarind seed contains 131.3 g/kg crude protein, 67.1g/kg crude fibre, 48.2 g/kg crude fat, 56.2 g/kg tannins and trypsin inhibitor activity (TIA) of 10.8, with most of the carbohydrate in the form of sugars. According to Ishola *et al.* (1990), the seed also contains 47 mg/100g of phytic acid, which has minimal effect on its nutritive value. It also contains 14-18% albuminoid tannins located in the testa. According to Purseglove (1987), the seeds contain 63% starch and 4.5-6.5% of semi drying oil. Both pulp and the seeds are good sources of protein (269.3 g/kg), oil (109.1 g/kg) and calcium (Ishola *et al.,* 1990). Tamarind seeds are reported as a source of food or food ingredients due to the presence of proteins (Marangoni *et al.*, 1988). Alkali extraction of the seeds showed that about 70% of the proteins were extractable. The protein isolated was relatively high in lysine (406 mg/g N), phenylalanine, tyrosine (520 mg/g N) and leucine (496 mg/g N) (Marangoni *et al.,* 1988). The seeds are an important source of proteins and valuable amino acids (Shankaracharya, 1998). In India, legumes

constitute an important foodstuff and are an economic source of protein in the diets of economically weaker sections of population (Kumar *et al*., 1991). Some of the wild nuts and seeds used as food in several parts of the world have considerable promise as protein source (Amubode and Fetuga, 1983). The proteins are an essential component of the diet, needed for survival of animals and humans. Proteins basic function in nutrition is to supply adequate amounts of required amino acids (Friedman, 1996). Large segments of human population and animals in developing countries suffer from protein malnutrition (Conway and Toenniessen, 1999). Although grain legumes have been identified as cheap potential source of protein, the per capita availability is meager. The availability and consumption of protein foods in India will remain inadequate due to population explosion and urbanization and results in Protein Energy Malnutrition (PEM). The PEM problem can be alleviated by finding alternative cost effective sources of proteins (Prakash and Misra, 1988; Waterlow, 1994).With an increasing interest in new food sources, the seeds of wild plants including the tribal pulses receive more attention, because they are highly resistant to disease and pests and exhibit good nutritional qualities (Janardhanan and Vadivel, 1994). The underutilized legumes / wild tribal pulses have tremendous potential for commercial exploitation but remain ignored. They offer a good scope to meet the ever-increasing demands for vegetable protein. Although they have high protein content and possess good nutritional value, their utilization is limited by the presence of some antinutritional / antiphysiological / toxic substances.

Legumes such as lentil contain a high concentration of proteins, carbohydrates and dietary fiber and make an important contribution to human diet in many countries. Legumes have to be processed prior to consumption due to their content of antinutritional compounds, such as trypsin inhibitors, phytic acid, galactosides (Vidal-Valverde et al.,2002). Processing techniques such as soaking, dehulling, cooking or autoclaving, germination and fermentation have been found to reduce significantly the levels of phytates and tannins by exogenous and endogenous enzymes formed during processing (Mosha and Savanberg 1990; lorri and Savanberg 1995; WHO,1998). The objective of this study was to investigate the effect of different processing methods on proximate composition, antinutritional factors and protein fractions of *Tamarindus indica* seeds.

MATERIALS AND METHODS

CHEMICALS

All Chemicals used in this study were of analytical grade.

PLANT MATERIAL

The seeds of *Tamarindus indica* were collected using random sampling technique (RST) from local areas of Bangalore district, Karnataka State, India. After dehulling the fruits, equal samples of seeds were combined to give one bulk population sample from which sub samples were taken. The seed samples were dried in the sunlight for 24 hrs. After removing immature and damaged seeds, the matured seeds were washed under tap water, dried and stored in refrigerator until further use.

PROCESSING TREATMENTS:

The seeds were subjected to five different types of processing.

SOAKING:

The seeds were soaked in water for 5 days, dried at 60° C and ground to a fine powder using a blender.

DEHULLING:

The seeds were soaked in water for 5 days and then hand pounded to separate the hull. The dehulled seeds were then dried at 60° C and ground to a fine powder.

COOKING:

The seeds were cooked for 30 minutes, mucus was removed from seed coat and washed. The cooked seeds were then dried at 60° C and ground to a fine powder.

AUTOCLAVING:

The seeds were autoclaved, cooled and then dried at 60° C and ground to a fine powder.

GERMINATION:

The seeds were treated with 50% H_2SO_4 for 30 minutes. After 30 minutes, it was washed and sowed onto a medium containing coco pith and sand in the ratio 1:1. After 10 days, the seeds were cleaned, dried overnight at 60° C and ground to a fine powder.

CHEMICAL COMPOSITION

PROXIMATE COMPOSITION

The proximate composition – moisture, ash, ether extract, crude fiber and protein contents – were determined for all the five processed samples as described below.

DETERMINATION OF MOISTURE

The moisture content of the samples were determined by the methods described by AOAC (Official Methods of Analysis of the Association of Official Agricultural Chemists, 10th edn., Washington, D.C. 1965). The crucibles were washed and dried at 105° C overnight. The crucibles were then placed in desiccators, cooled and weighed. The sample (2.0 gm) was weighed into the crucible and dried overnight at 105° C, placed in desiccators, cooled and weighed.

DETERMINATION OF CRUDE LIPID

Ether is continuously volatized, condensed and then allowed to pass through the sample to extract ether soluble materials. When the process is completed, the ether is distilled, collected in another container, remaining crude fat is dried, weighed and percent oil is calculated. The crude lipid was determined by the continuous solvent extraction method in a Soxhlet extraction apparatus as described by AOAC (1965). The sample (2.0 gms) was taken in the thimble and extracted with petroleum ether for 16 hours. The miscella obtained was evaporated on hot water bath, dried at 105° C for 30 mins, cooled in desiccator and weighed.

DETERMINATION OF CRUDE FIBER

The crude fiber was determined by the method of Maynard, A.J. edn (1970). The sample (2.0 gm) was extracted with 200 ml of 0.255N H_2SO_4 for 30 mins, filtered through muslin cloth and washed with boiling water until acid free. The residue was further extracted with 200 ml of 0.313N NaOH for 30 minsss, filtered through muslin cloth and washed successively with 25 ml of hot 1.25% H_2SO_4, 50 ml of water (thrice) and 25 ml of alcohol. The residue obtained was dried for 2 hours at 130° C, cooled in dessicator and weighed.

DETERMINATION OF TOTAL PROTEIN

The protein content of the samples was determined by the micro-kjeldhal method as reported by Layne, E. (1957). This is generally the method of choice for estimating the protein content of food materials and agricultural and clinical samples.

100 mg of the sample is weighed and transferred to a 30 ml digestion flask. To this 0.5 ml of 14% of mercuric sulphate solution in 4 N sulphuric acid and a pinch of potassium sulphate is added along with 2.5 ml of concentrated sulphuric acid. The sample is digested till the solution becomes colorless. After cooling the digest, it is diluted with a small quantity of ammonia-free distilled water and transferred to the distillation apparatus and 10 ml of sodium hydroxide-sodium thiosulphate solution is added. The sample is steam distilled and the liberated ammonia is collected in boric acid solution. This is then titrated against standard H_2SO_4 (1 ml of 0.1 N H_2SO_4 acid is equivalent to 1.401 mg N). The total nitrogen content is multiplied with 6.25 to obtain total protein content.

DETERMINATION OF THE ASH

The ash content of the sample was determined by the method described by AOAC (1965). This includes an inorganic fraction – the total of the incombustible sample left after ignition (ash). The sample (2.0 gm) is ignited at 600° C for 6 hours to burn all organic material. The inorganic material which does not burn or volatilize at that temperature is called ash.

DETERMINATION OF THE CARBOHYDRATE

The nitrogen-free extract (NFE) of a feed is determined by difference after the analysis has been complete for ash, crude fiber, crude fat and crude protein by using the arithmetical difference method as described by Pearson (1976) and James (1995). The carbohydrate content was calculated and expressed as the nitrogen free extract. The nitrogen-free extract (NFE) represents the difference in dry weight of the fraction obtained by subtracting the total dry weight of the sample from the sum of the values of ash, crude fiber, crude fat and crude protein on dry weight basis. The chief components of NFE are the sugars and starches (soluble) whereas crude fiber is chiefly of cellulose-like (insoluble) components.

ANTINUTRITIONAL FACTORS

EXTRACTION OF TANNINS

100mg of sample is mixed with 5ml of 2.5N HCl on boiling water bath for 2.30 hours, cool to room temperature. This mixture is neutralized with solid sodium carbonate. The volume in each case is made up and centrifuged.

DETERMINATION OF TANNIN CONTENT

Quantitative estimation of tannin for each sample was carried out using the modified vanillin-Hcl in methanol method. A standard curve was prepared expressing the results as catechin equivalents, i.e. amount of catechin (mg per ml) which gives a colour intensity equivalent to that given by tannins after correcting the blank.

TOTAL PHENOLS DETERMINATION

Total phenols were determined according to Malick, CP and Singh, MB (1980). A standard curve was prepared expressing the result as tannic acid equivalents i.e. amount of tannic acid (mg per 100g) which gives a colour intensity equivalent to that given ny phenols after correction to blank.

PROTEIN FRACTIONATION

Protein fractions were extracted according to their solubilities in different solvents. The ground powder (1.0g) of each sample was extracted twice with 50ml distilled water for 30 mins at room temperature. The extract was centrifuged at 6000rpm for 30 mins and the supernatant was used for the determination of a water-soluble protein (albumin). The residue was then extracted successively in a similar manner with 1.0 M NaCl, 70% ethanol and 0.2% NaOH. The supernatant of each extact was collected separately and used to estimate the salt-(globulin), alcohol-(prolamin), and alkali-(glutelin) soluble fraction. The residue remaining after successive extractions represents the insoluble proteins.

RESULTS

Plant seeds are easily available and are the richest source of antioxidants, phenolics and proteins. Legumes have to be processed prior to consumption due to their high content of antinutritional compounds, such as lectins, polyphenols, tannins, trypsin inhibitors, phytic acid and galactosides.

Various processing treatments such as soaking, dehulling, cooking, autoclaving and germination have been carried out on the tamarind seed and proximate composition, antinutritional factors and proteins on fractionation were determined.

PROXIMATE COMPOSITION

Table 1 illustrates the proximate composition of control (unprocessed) and processed tamarind seeds. The moisture content of control (untreated seeds) is 6.0%. The ash, crude fiber and oil content were found to be 4.58%, 14.9% and 5% respectively. The protein content of untreated seeds was found to be 18.8% which was higher than that of green peas. The moisture content was low in case of autoclaved seeds (2.5%) whereas it was high with respect to soaked and germinated seeds (7.5%). The low levels of moisture in seeds enable them to be preserved for long hours. The moisture content of some of the edible oil seeds such as cotton seeds (6.46%), peanuts (4.58%), palm kernel (5.31%), sesame (4.60%) and sunflower seeds (6.58%), soybean (11.07%) and coconut seeds (14.3%)

(FAO, 1982). The ash content was reduced in all the processed cases by 1.0 – 1.5%. These values are similar to those of soybean (5.0%), cotton seed 9.4%), sesame (3.8%) and sunflower seed (4.1%) (FAO, 1982). The fibre content in autoclaved seeds remained same as that of the control but it reduced in all the other processed samples drastically by 3 – fold. The protein content in the control group (unprocessed seed) was 18.8% which got reduced to 3.22% in case of germinated seeds. But in all the other seeds there was a slight reduction of up to 2%.

Table 1.1 : Proximate composition (%) of processed tamarind seeds.

No.	Treatments	Moisture	Ash	Fibre	Oil/Fat	Proteins	Carbohydrates
1.	Control	6.0	4.58	14.9	5.0	18.8	50.72
2.	Soaked	7.5	3.36	4.6	2.5	16.11	65.93
3.	Dehulled	4.5	3.6	4.0	4.0	16.11	67.79
4.	Cooked	3.8	3.76	3.9	4.75	11.51	72.28
5.	Autoclaved	2.5	3.48	14.0	3.75	13.81	62.46
6.	Germinated	7.5	2.35	0.68	1.68	3.22	84.57

Samples in duplicates were taken and the average values are reported

ANTI-NUTRITIONAL FACTORS

Fig 1 shows the effect of processing methods on tannins. The results indicate that soaking followed by dehulling of seeds and germinated seeds have reduced tannin content in comparison to the control seeds. Since most tannin is located in the testa, its physical removal reduced tannin content. Tannin content increased with autoclaving. The result is confirmed by thin layer chromatography (Fig 2).

Fig 3 shows the effect of processing treatments on total phenolics of tamarind seeds. The results indicate that the autoclaved seeds showed an increase in phenolics while a reduction was noticed in dehulled and germinated seeds.

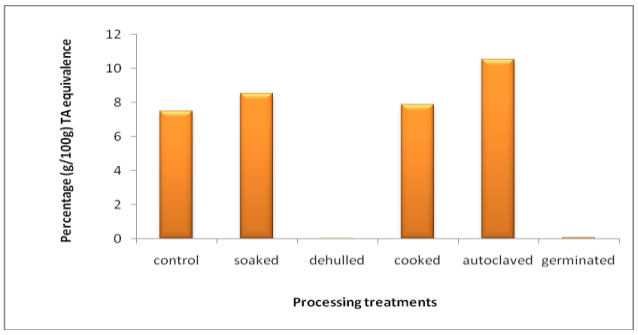

Fig 1 : Effect of various processing treatments on the levels of tannins in *Tamarindus indica* seeds.

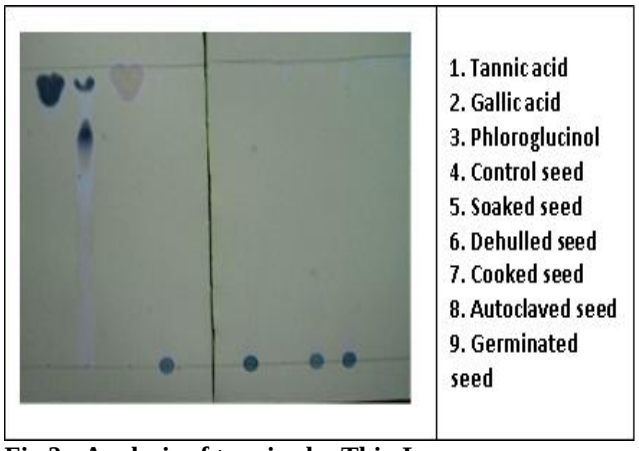

Fig 2 : Analysis of tannins by Thin Layer Chromatography

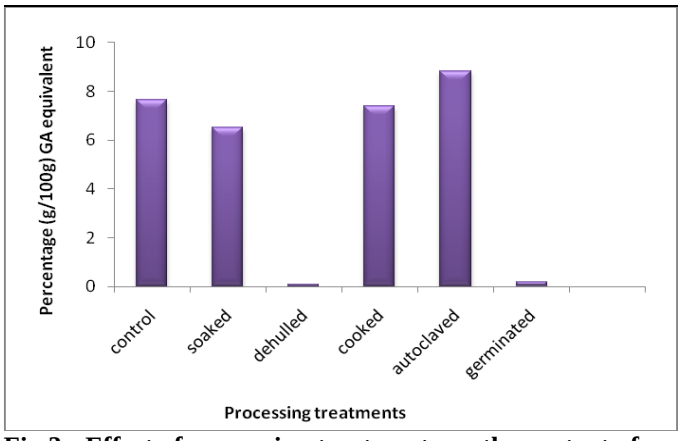

Fig 3 : Effect of processing treatments on the content of total phenolics in *Tamarindus indica* seeds

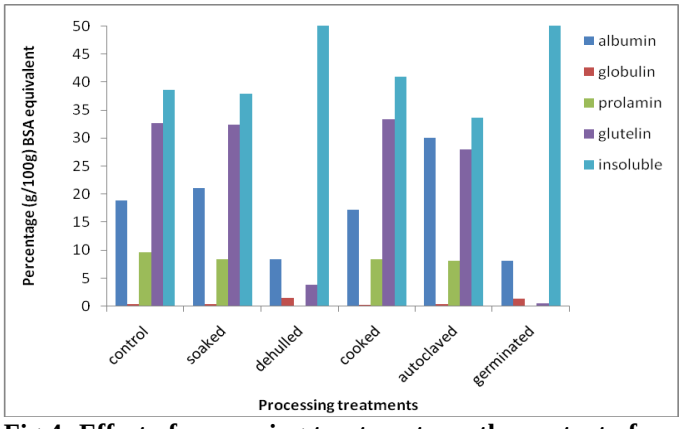

Fig 4: Effect of processing treatments on the content of total proteins in *Tamarindus indica* seeds

PROTEIN FRACTION

Fig 4 shows the protein fractions of treated and untreated seeds based on their solubilities. Albumin and glutelin were the two major proteins present. The results indicate that Globulin and Prolamin fractions of the seeds soluble in 1.0 M NaCl and 70% ethanol respectively decreased during processing while albumin which is water soluble greatly increased.

DISCUSSION

Bridging the gap between the teeming population and food production is one of the most important tasks of developing countries. Most developing tropical countries obtain proteins from conventional legumes and animal based sources for livestock feeding as well as for human nutrition. The major conventional legumes are soybean (*Glycine max*) and groundnut or peanut (*Arachis hypogea*). The demand for these items has given rise to a disproportionate increase in their prices and consequently on the cost of livestock and feeds. Protein - Energy Malnutrition (PEM) has therefore been recognized as the most common form of malnutrition in regions where people depend on starch based diets for survival (FAO, 1994; Pellelier, 1994; Michaelson and Henrik, 1998). There is a need for identification and exploitation of other novel legumes, which fortunately are in abundance in these regions to fulfill the growing need of plant based proteins and underutilized legumes as inexpensive and good sources of protein than the conventional sources of protein (Chel-Guerrero *et al*., 2002; Krause *et al*., 1996; Siddhuraju *et al*., 2000). The presence of some anti-nutritional factors in the raw seeds has limited its use in non-ruminant animals.

Tamarind seed is an underutilized byproduct of the tamarind pulp industry. Only a small portion of the seed, in the form of tamarind kernel powder (TKP), is used as a sizing material in the textile, paper and jute industries. Though many applications of this seed are possible, there have been hardly any other uses for it including using it as an additive in food formulations. Panigrahi *et al.* (1989) reported that whole tamarind seed contains 131.3 g/kg crude protein, 67.1g/kg crude fibre, 48.2 g/kg crude fat, 56.2 g/kg tannins and trypsin inhibitor activity (TIA) of 10.8, with most of the carbohydrate in the form of sugars. The trypsin inhibitor activity is higher in the pulp than in the seed, but both are heat labile. According to Ishola *et al.* (1990), the seed also contains 47 mg/100g of phytic acid, which has minimal effect on its nutritive value. It also contains 14-18% albuminoid tannins located in the testa. According to Purseglove (1987), the seeds contain 63% starch and 4.5-6.5% of semi drying oil. Both pulp and the seeds are good sources of protein (269.3 g/kg), oil (109.1 g/kg) and calcium (Ishola *et al.,* 1990).Tamarind kernel powder is used in developing food products such as jelly and marmalades.

ACKNOWLEDGEMENT

The study was supported by UGC under UGC – Major Research Project (2010 – 2012) New Delhi. The authors wish to acknowledge DOS in Biochemistry, Bangalore University for offering their facilities for the analysis.

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