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# PROTEIN SOLUBILITY AND HAEMAGGLUTINATING ACTIVITY OF TAMARIND SEED EXTRACTS

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**ABSTRACT:**

Tamarind (Tamarindus indica L.) is a plant that belongs to the Leguminosae. The seeds are used as feed for cattle and pigs, as a valuable remedy in diarrhea and dysentery in pharmaceutical industry. The objective of the study was to determine the influence of processing treatments (soaking, dehulling, cooking, autoclaving and germinating) the seeds on protein fractionations based on solubility and haemagglutinating activity. Proteins maximum extracted with NaOH. Germinated seeds showed least proteins. Albumin fractions except dehulled samples showed strong agglutinating activity with A blood group. It shows partial clumping with B and AB blood group with respect to three fractions (control, soaked, germinated). Globulin fractions exhibit strong agglutinating activity without any specificity to blood groups in case of dehulled and cooked fractions. In germinated fractions it is weak agglutination. Globulin fraction of the seeds decreased during processing while albumin greatly increased. Haemagglutinating activity decreases during germination as observed in other legumes.

**KEYWORDS:** Tamarind, Haemagglutinating activity, Blood groups, Albumin, Globulin

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## INTRODUCTION

In India, legumes constitute an important foodstuff and are an economic source of protein in the diets of economically weaker sections of population[1]. Some of the wild nuts and seeds used as food in several parts of the world have considerable promise as protein source[2]. 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[3]. Large segments of human population and animals in developing countries suffer from protein malnutrition[4]. 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[5, 6]. 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[7]. 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.

Tamarind is an arboreal fruit. The fruit pulp is most acidic and has a uncommon plant acid, tartaric acid. The pulp of the fruit is used in the preparation of beverage and to flavor confections, curries and sauces. Tamarind

kernel powder is used in developing food products such as jelly and marmalades. The seed kernels have been used as food either alone or mixed with cereal flours. Certain hill tribes eat kernels mixed with flowers of mahua.

Lectins are carbohydrate binding proteins that are widely distributed among plants, animals and microbes. Mature seeds are the main source of plant lectins, however, lectis are also present in small amounts in other tissues such as leaves and roots. The majority of plant lectins are secretory proteins that accumulate either in the vacuole or extracellular matrix. Most plant tissues contain one lectin, but in some cases they contain two (or more) lectins that differ in their biological properties.

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[8]. 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[9,10,11].

## 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 for test. Collected seed samples were dried in the sunlight for 24 hours. After removing immature and damaged seeds, the dried matured seeds were washed under tap water, dried and stored in plastic containers or refrigerator at room temperature (25$^{o}$C) until further use.

**Processing treatments :**

**Soaking :** The seeds were soaked in water for 5 days. Then the soaked seeds were dried at 60$^{0}$C and ground to a fine powder.

**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$^{0}$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$^{0}$C and ground to a fine powder.

**Autoclaving :** The seeds were autoclaved, cooled and then dried at 60$^{0}$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 cocopith and sand in the ratio 1:1. After 10 days, the seeds were collected, cleaned and dried overnight placing in petridish in hot air oven at 60$^{0}$C and then ground to a fine powder.

**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 minutes at room temperature. The extract was centrifuged at 6000rpm for 30 minutes 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 extract 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.

**Erythrocyte preparation :**

Human blood (A, B ,AB ,O) was procured from individuals of the lab on an informed consent prior to the study. Immediately after the blood draw, erythrocytes were separated from the plasma and buffy coat by centrifugation at 3000 rpm for 10 minutes. The crude erythrocytes were washed three times with 5 volumes of phosphate buffered saline pH 7.4. During the last wash, the erythrocytes were centrifuged at 3000 rpm for 20 minutes to obtain a packed cell preparation. The packed erythrocytes were then suspended in 4 volumes of phosphate buffered saline solution.

**Assay for haemagglutinating activity :**

Albumin and globulin protein fractions obtained under fractionation of different solubility classes of seed proteins were employed as protein samples for determining haemagglutinating activity[13]. 5 drops of protein fractions were mixed with different blood group and allowed to stand for 20 minutes and centrifuged at 1000rpm for 3 minutes. After centrifugation, the tubes were shaken and the presence or absence of agglutination activity was noticed.

## RESULTS AND DISCUSSION:

Legume seeds are valuable source of protein, oil, carbohydrates, minerals and vitamins. They are playing an important role in human nutrition mainly in developing countries[17, 24].

Solubility of proteins is considered as the most important factor and an excellent index for their functionality of dehydrated products. In addition, this is an important factor because of its relevance to other properties such as viscosity, gelation, foaming and emulsification[27]. Protein solubility refers to the amount of total protein that goes in to solution under specified conditions[31] and depends on protein structure, pH, concentration of salt, temperature, duration of extraction and many intrinsic factors. The conformation of proteins, which is related to the environment to which the protein is exposed, plays an important role in defining the functional properties of the proteins. The method of processing affects the solubility of proteins specially if they are exposed to heat[29]. Denaturation and aggregation of proteins are associated with the formation of disulfide bond formation[28].

Signs of denaturation of proteins are reflected in changes in solubility. Method of processing affects the solubility of proteins especially if they are exposed to heat[29]. Reduction in protein solubility due to heat processing has been reported[30, 26]. However, in our study the protein solubility has not changed when compared to control due to heat processing. The degree of protein solubility in an aqueous medium is the result of electrostatic and hydrophobic interactions between proteins is greater than hydrophobic interactions[31].

Fig 1 shows the protein fractions of treated and untreated seeds based on their solubilities. The results indicated 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.

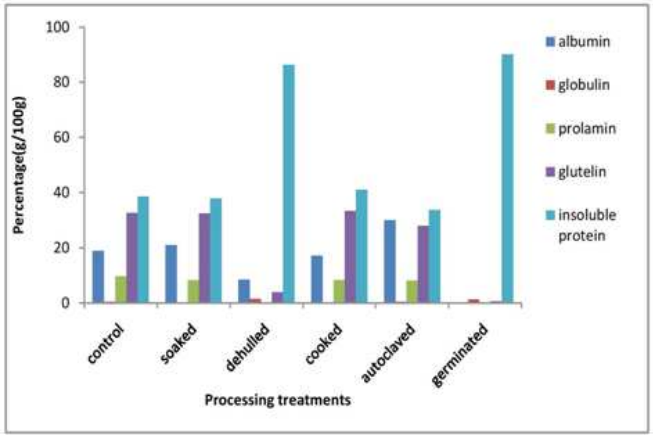


**Fig 1:** Effect of processing treatments on the content of total proteins in *Tamarindus indica* seeds

In general the globulin constitutes the major seed storage protein in legumes except in T.indica. This is in consonance with some earlier reports in Cassia obtusifolia and Entada scandens and Mucuna monosperma[16]. In T.indica, glutelin fraction forms the major seed protein followed by albumin.

Generally there seems to be a reduction in nutrient values when the seeds are processed, as shown by the difference in values for undehulled and dehulled samples.

In the present investigation, soaking of seeds followed by dehulling significantly decreased protein content and this may be due other water soluble constituents. The effect of processing methods on protein fractions indicate that albumin fraction increased in soaked and autoclaved seeds, globulin remained negligible, prolamin and glutelins remained almost similar to that of control ( Table 1).

The protein fraction of treated and untreated seeds on the basis of solubility is shown (Table 1). Untreated seeds were fractioned into albumin (18.92 %), globulin (0.35 %), prolamin (9.60 %) and glutelin (32.6 %). The results obtained indicated that about 61.44 % of the total could be extracted by solvents and the remaining percentage account for the non-protein nitrogen and insoluble proteins. Soaking of seeds followed by dehulling significantly decreases fraction of (albumin, prolamin, and glutelin ) to 40.11, 100 and 11.88 %s respectively. Autoclaving of seeds had caused further reduction in globulin, prolamin and glutelin fractions. The albumin fraction was significantly increased upon processing treatments.

**Table 1:** Amount of proteins present in the treated seeds

| samples | albumin g/100g | globulin g/100g | Prolamin g/100g | glutelin g/100g | Insoluble components g/100g |
|---|---|---|---|---|---|
| Control | 18.92 | 0.328 | 9.60 | 32.6 | 38.55 |
| Soaked | 21.04 | 0.312 | 8.32 | 32.44 | 37.88 |
| Dehulled | 8.44 | 1.496 | 0.00 | 3.84 | 86.224 |
| Cooked | 17.16 | 0.208 | 8.32 | 33.32 | 40.992 |
| Autoclaved | 30.08 | 0.336 | 8.04 | 27.92 | 33.624 |
| Germinated | 0.04 | 1.328 | 0.00 | 0.56 | 90.072 |

CM lectin stimulates significantly proliferation of human lymphocytes, shows hemagglutination activity towards human erythrocytes of group B[12].

Lectins are toxic glycoproteins that have the ability to bind with carbohydrate moieties on the surface of the human red blood cells (RBC) and cause them to agglutinate. Lectins can combine with intestinal mucosal cells and cause interference with the absorption of available nutrients[14].

The course of evolution of plant lectins and of proteins associated with them has not been so far elucidated. Similarly, little is also known about their biological function. It has been suggested that they play a role in plant defense against different pathogens and animals

as well as in carbohydrate interactions. Most of them might function as storage proteins.

The protein display two types of activities: hemagglutination activity and enzymatic activity has been found previously in mung bean and Vicia faba[25]. The mung bean protein can be reversibly converted by pH changes from tetrameric form, which possesses both enzymatic and haemagglutination activities to a monomeric form which possesses enzymatic activity only. Lectins are highly sensitive to heat treatment[19]. Cooking and autoclaving i.e., Albumin4 and Albumin5 shows weak agglutination with blood group B, AB and O which proves the previously said statement. Haemagglutinating activity decreases during germination in Glycine max, Phaseolus vulgaris, Vicia faba and Vigna radiate[20]. But in our study, germination of seeds (Albumin6) shows strong agglutination with blood group A and also agglutinates blood group B, AB and O (Table 2).

**Table 2:** Data on Heamagglutinating activity using albumin fractions

| RBC | Albu--min1 | Albu--min2 | Albu--min3 | Albu--min4 | Albu--min5 | Albu--min 6 |
|---|---|---|---|---|---|---|
| A | ++ | ++ | _ | ++ | ++ | ++ |
| B | + | + | _ | _ | _ | + |
| AB | + | + | _ | _ | _ | + |
| O | ++ | + | _ | _ | _ | + |

But Globulin 6 (germinated seed) show weak agglutination with B and AB (Table 3). A significant reduction in lectin activity has been noticed when the seeds of certain pulses were subjected to dry heat treatment and autoclaving[21] and cooking and autoclaving[21,22]. The globulin fraction of T.indica exhibits strong agglutinating activity without any specificity against A blood group except the control (untreated seeds). Nonetheless, albumin protein specifically agglutinates the human A, B, AB and O in control, soaked and germinated seeds.

**Table 3:** Data on Heamagglutinating activity using globulin fractions

| RBC | Glob--ulin 1 | Glob--ulin 2 | Glob--ulin 3 | Glob--ulin 4 | Glob--ulin 5 | Glob--ulin 6 |
|---|---|---|---|---|---|---|
| A | - | ++ | ++ | ++ | ++ | + |
| B | ++ | - | ++ | ++ | - | - |
| AB | ++ | ++ | ++ | ++ | ++ | - |
| O | ++ | ++ | ++ | ++ | - | + |

## CONCLUSION

In the present investigation, the albumin fraction was fluctuated while globulin, prolamin and glutelin remained unchanged during processing treatment. But in case of germinated seeds, all the four fractions decreased drastically. In contrast, the globulin fraction showed strong agglutinating activity without any specificity against A, AB and O human blood groups except the germinated fraction and albumin showed weak agglutinating activity with A, B, AB and O with respect to dehulling and heat treated seeds. Of the five processing methods employed, heat treatment is found to be more effective in reducing heagglutinating activity for albumin fractions and its vice versa for the globulin fractions.

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## REFERENCES

1. S. Kumar, S.Kumar, G. K. Singh, R. Kumar, N. K. Bhatia and C. P. Awasthi. Variation in quality traits of pigeon pea (Cajanas cajan L. Mill sp) varieties, Journal of Food Science and Technology 28: 173 – 174 (1991).
2. F. O. Amubode and B. L. Fetuga. Proximate composition and chemical assay of the methionine, lysine and tryptophan concentrations of some forest tree seeds, Food Chemistry 12: 67 – 72 (1983).
3. M. Friedman. Nutritional value of proteins from different food sources, A review, Journal of Agriculture and Food Chemistry 44: 6 – 29 (1996).
4. G. Conway and G. Toenniessen. Feeding the world in the twenty-first century, Nature 402 (suppl): 55 – 68 (1999).
5. D. Prakash and P. S. Misra. Protein content and amino acid profile of some wild leguminous seeds, Plant Foods for Human Nutrition 38: 61 – 65 (1988).
6. J. C. Waterlow. Childhood malnutrition in developing nations: Looking back and looking forward, Annual Review of Nutrition 14: 1 – 19 (1994).
7. K. Janardhanan and V. Vadivel. Biochemical composition of different germplasm seed materials of South Indian tribal pulse, *Canavalia gladiata.* In: Proceedings of the National Seminar on Biodiversity: Strategies for Conservation and Future challenges. (Eds.) K. Udaiyan, K. Janardhanan, S. Manian and V. R. K. Reddy, Bharathiar University, Coimbatore, India. October 16 & 17, 1993. pp.93–97.

8. C. Vidal-Valverde, J. Frias, I. Sierra, I. Blazquez, F. Lambien and Y. H. Kuo. New functional legume food by germination. Effect on the nutritive value of beans, lentils and peas, Eur. Food Res. Tec., 215: 472 - 476 (2002).
9. A. C. Mosha and Svanberg. The acceptance and food intake of bulk reduced weaning. The liganga village study, Food Nutr. Bulletin 12: 69 – 74 (1990).
10. W. Lorri and Svanberg. An overview of the use of fermented food for child feeding in Tanzania, Ecp. Food Nutr 34: 65 – 81 (1995).
11. WHO. Complementary feeding of young children in developing countries: A review of current scientific knowledge – Geneva (1998).
12. E. Fik, A. Gozdzicka-Jozefiak, I. Mirska and W. Kedzia. New plant glycoprotein against methicillin resistant Staphylococci and Enterococci, Acta Microb. Pol 46: 325 – 327 (1997).
13. I. E. Liener. Phytohaemagglutinins (phytolectins), Annual Review in Plants Physiology 27: 291 – 319 (1976).
14. I. E. Liener. Implications of antinutritional components in soybean foods, Critical Review of Food Science and Nutrition 34: 31 – 67 (1994).
15. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with the folin-phenol reagent, Journal of Biological Chemistry 193: 265 – 275 (1951).
16. M. Arulmozhi and K. Janardhanan. The biochemical composition and nutritional potential of the tribal pulse, *Mucuna monosperma* DC. ex Wight, Plant Foods for Human Nutrition 42: 45 – 53 (1992).
17. A. I. Mohamed and M. Rangappa. Screening soybean (grain and vegetable) genotypes for nutrients and antinutritional factors, Plant Foods for Human Nutrition 42: 87 – 96 (1992).
18. P. Siddhuraju, K. Vijayakumari and K. Janardhanan. Chemical composition and nutritional evaluation of an underexploited legume, *Acacia nilotica* (L.) Del, Food chemistry 57: 385 – 391 (1996b).
19. U. Singh. Antinutritional factors of chick pea and pigeon pea and their removal by Processing, Plant Foods for Human Nutrition 38: 251 – 261 (1988).
20. P. Valdebouze, E. Bergeron, T. Gaborit and J. Delort-Laval. Content and distribution of trypsin inhibitors and haemoagglutinins in some legume seeds, Journal of Plant Science 60: 695 – 701 (1980).
21. K. Vijayakumari, P. Siddhuraju and K. Janardhanan. Chemical composition, amino acidcontent and protein quality of the little known legume, *Bauhinia purpurea* L, Journal of Science Food Agriculture 73: 279 – 286 (1997).
22. K. Vijayakumari, P. Siddhuraju and K. Janardhanan. Effect of various water or hydrothermal treatments on certain antinutritional compounds in the seeds of the tribal pulse, *Dolichos lablab* var. *vulgaris* L, Plant Foods for Human Nutrition 48: 17 – 29 (1995).
23. K. Vijayakumari, P. Siddhuraju and K. Janardhanan. Effect of different post harvest treatments on antinutritional factors in seeds of the tribal pulse, *Mucuna pruriens* (L.) DC, International Journal of Food Science Nutrition 47: 263 – 272 (1996).
24. E. Yanez, I. Zacarias, M. Aguayo, M. Vasquez and E.Guzman. Nutritive value evaluated on rats of new cultivars of common beans (*Phaseolus vulgaris*) released on chile, Plant foods for Human Nutrition 47: 301 – 307 (1995).
25. E. J. M. Van Damme, A. Barre, K. Smeets, S. Tarrekens, F. Van Leuven, P. Ruuge and W. J. Peumans. The bark of Robinia pseudoacacia contains a complex mixture of lectins, Plant Physiolology 107: 833 – 843 (1995).
26. T. Bourtoom, M. S. Chinnan, P. Jantawat and R. Sanguandeekul. Recovery and characterization of proteins precipitated from surimi wash-water, Food Science and Technology 42: 599 – 605 (2009).
27. G. M. Hall. Fish Processing Technology. USA and Canada: VCH Publishers, Inc., New York (1992).
28. S. T. Jiang, F. J. Wang and C. S. Chen. Properties of actin and stability of the actomyosin reconstituted from milk.sh (Chanos chanos) actin and myosin, Journal of Agriculture and Food Chemistry 37: 1232 – 1235 (1989).
29. A. Kilara and V. R. Harwalkar. Denaturation. In Nakai, S., Modler, H. W. (eds). Food proteins properties and characterisationn. 1996. New York: VCH. 71 – 165.
30. A. Romero, F. Cordobes, M. C. Puppo, A. Villanueva, J. Pedroche and A. Guerrero. Linear viscoelasticity and microstructure of heat-induced crayfish protein isolate gels, Food Hydrocolloids 23: 964 – 972 (2009).
31. J. F. Zayas. Solubility of proteins. In: Functionality of proteins in food. Springer (1997).