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Shlini P
Department of Chemistry,
Mount Carmel College,
Autonomous. Bangalore,
Karnataka, India

KR Siddalinga Murthy
DOS in Biochemistry, Bangalore
University. Bangalore,
Karnataka, India

Correspondence
Shlini P
Department of Chemistry,
Mount Carmel College,
Autonomous. Bangalore,
Karnataka, India

Isolation of tamarind seed proteins, polysaccharides, and cellulase from the seeds of *Tamarindus indica*

Shlini P and KR Siddalinga Murthy

Abstract
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. Tamarind is an economically important species. India exports tamarind products to Pakistan, Arab countries, Europe and North America. Other Asian countries also produce and export tamarind, but on a much smaller scale. Tamarind trade has expanded over the last decade and is continuing to do so. Proteins of Tamarind seed kernel are of a higher biological value than those of wheat and corn, and lower only than those of millets. In the present investigation tamarind seed protein were isolated using different solvents. The studies indicate that maximum amount of protein can be extracted with NaOH and in a one – step process both proteins and polysaccharide can be separated very easily. Further, tamarind seed polysaccharide was extracted and its viscosity was determined to be 800cps. The germinated seeds were studied for their cellulase activity. Seeds of 16-17 days indicated maximum activity and can be used for the production of cellulases.

Keywords: tamarind seed, cellulase, viscosity, proteins, polysaccharide

1. Introduction
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 (Mohamed and Rangappa, 1992; Yanez *et al.,* 1995) [9, 14]. Pugalenthi *et al* (2004) [10] have shown the presene of high content of crude protein (31.08%) in *Sesbania bispinosa* than the other two species *Tamarindus indica and Erythrina indica. T. indica* contain high levels of crude protein than the levels reported earlier (Ishola *et al.,* 1990; Bhattacharya *et al*., 1994; Siddhuraju *et al.,* 1995a) [5, 2, 13]. Information on the levels of crude protein in *Tamarindus indica* seems to be meager. *T. indica* contained a high level of crude lipid content (7.84%).
The seeds are used as feed for cattle and pigs, as a valuable remedy in diarrhea and Dysentery, as a base in cosmetics, in pharmaceutical industry, as a curative against rheumatism and as a soil stabilizer (Anon, 1955) [1]. India is the main producer and consumer of tamarind in the world. (Shankaracharya, 1998) [12]. Tamarind kernel powder is used in developing food products such as jelly and marmalades (Bhattacharya *et al., 1*983) [3]. Rao and Subramanian (1984) [11] and Marangoni *et al* (1988) [8] have attempted to produce protein concentrates or meals from kernel proteins.
Cellulose is a linear polysaccharide of glucose residues connected by β – 1, 4 linkages. Like chitin it is not cross – linked. Cellulose is the most abundant organic source of food, fuel and chemicals. There is a growing demand for xyloglucan gels as vehicles for oral delivery of drugs. Since TSP is a galactoxyloglucan (cellulose backbone with side branches), it can be enzymatically modified to produce xyloglucan. Xyloglucan has the unique property of forming a gel on heating and reverting to a sol state on cooling. There is a growing demand for specific, efficient and cheap cellulase for use in formulation of washing powders, in textile production.
Processes have not been developed to produce tamarind kernel protein concentrate, which can be used to supplement other legume proteins. Hence, a lot of interest is shown by the chemists, technologists, nutritionists, and biochemists, on the chemical aspects of tamarind seed.
The country is constantly facing crop failure due to erratic rainfall, leading to shortage of food grains. India produces about 1,00,000 tons of seeds annually. Therefore, the production of protein, polysaccharide and oil, from the seed will improve the food security of the country. Therefore, the present investigation is very relevant to the Indian situation and specifically from the point of view of unpredictable weather. the present study deals with the isolation of proteins, polysaccharide and cellulase from the tamarind seeds.

2. Materials and methods

2.1 Chemicals: sodium chloride, ethanol, sodium hydroxide, TKP, sodium carbonate, sodium potassium tartarate, copper sulphate, Folin – Ciocalteu's reagent, glycine, hydrochloric acid, TEMED, H_3PO_4, acrylamide, bis-acrylamide, potassium persulphate, glycerol, bromophenol blue, SDS, acetic acid, chloroform, sodium thiosulphate, silver nitrate, sulphuric acid, acetone, KH_2PO_4, K_2HPO_4, TSP, dinitrosalicylic acid.

2.2 Protein fractionation: The proteins of commercial sample of defatted tamarind kernel powder (TKP) were extracted according to their solubilities in different solvents. Defatted TKP flour (1.0 g) was extracted twice with 10.0 ml distilled water for 30 min at room temperature. The extract was then centrifuged at 3000 rpm for 30 min 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 10 ml of 1.0 M NaCl, 70% ethanol and 0.2 % NaOH. The supernatant of each extract was collected separately and used to estimate salt – (globulin), alcohol – (prolamin) or alkali – (glutelin) soluble fraction. The residue remaining after successive extractions represents the insoluble proteins and polysaccharide.

2.3 Protein estimation: Protein was estimated according to the method of Lowry et. al (1951) [7].

2.4 Native Polyacrylamide gel electrophoresis: Qualitative analysis of the proteins were carried out by Native PAGE on a 10 % gel according to the method of Davis and Ornstein (1964) [4]. The separated proteins were stained by using silver staining method.

2.5 SDS – Page: Qualitative analysis of the proteins were carried out by SDS – Page on a 10 % gel according to the method of Laemmli (1970) [6]. The proteins were stained by using Silver Staining method.

2.6 Isolation of Protein and Polysaccharide: The proteins of defatted TKP (10 gm) were extracted twice using 100 ml of 0.1 M NaOH for 5 mins at 4° C. The extract was then centrifuged at 3000 rpm for 15 mins. The residue (Tamarind Seed Polysaccharide – TSP) remaining after extraction was washed with alcohol and dried at 37° C. The protein was estimated according to the method of Lowry *et al* (1951) [7].

2.7 Germination Studies: The black tamarind seeds were pretreated by immersing in 50 % sulfuric acid for 1 hr and then thoroughly washed with water before germination. The seeds were then germinated for 7 days on coco pith and sand mixture.

2.8 Preparation of Acetone Powder: Acetone powder of the germinated seeds were prepared by blending 10 gm of the seeds in 100 ml of chilled acetone using a blender for 5 mins. It was then filtered under vacuum and dried at 37°C. The acetone powder was used for the extraction of enzyme.

2.9 Extraction of Enzyme: A 10 % extract of the enzyme was prepared by stirring 1 gm of acetone powder in 0.1 M phosphate buffer, pH 7.0 over a magnetic stirrer for 1 hr at 4° C. The extract was then centrifuged at 10,000 rpm for 10 mins at 4° C. The supernatant was collected and used for assay of cellulase activity.

2.10 Assay of Cellulase activity: Cellulase activity was determined by DNS method (Miller, G.L., 1959) [15]. at pH 7.0 (0.05 M phosphate buffer) by incubating the enzyme with 1 % solution of TSP (w/v) in a reaction volume of 1.0 ml for 15 mins. The enzyme activity was stopped by the addition of 0.5 ml of DNS reagent. The tubes were then heated over a boiling water for 15 mins., cooled and the volume made up to 7.0 ml with water. The absorbancy was read at 540 nm against a suitable blank.

2.11 Viscosity Measurement: A 1.5 % solution of TSP was prepared by dissolving 15 gm of TSP in boiling water and the volume was made upto 1 ltr. The viscosity of the solution was measured at 25° C at 30 rpm using spindle No. 4 in a Brookefield Viscometer.

2.12 Viscosity Reduction Determination: Absorbance & percent transmittance of the reaction mixture (0.5 ml enzyme + 3.5 ml substrate) recorded continuously at 5 minute intervals for a time period of 70 minutes at 600 nm using a spectrophotometer.

3. Results and discussions

The protein fractionation was carried out using different solvents. The results (Table 1) showed that maximum amount of proteins in the glutelin fraction (11 %) followed by albumins, globulins and prolamins (1 %, 0.5 %, and 0.5 %). However, extraction with 0.1 N NaOH resulted in the extraction of about 15 % of the soluble proteins and the residue consisted of polysaccharide and insoluble proteins.

Table 1: Protein fractionation

Albumin fraction (%)	Globulin fraction (%)	Prolamin fraction (%)	Glutelin fraction (%)	Insoluble components (%)
1.0	0.5	0.5	11.0	87.0

The insoluble proteins had very low density and were easily separated by centrifugation to obtain pure tamarind seed polysaccharide. By employing this method, proteins and polysaccharide were separated in a single step.

Native PAGE was carried out for the four different fractions of proteins. The electrophoretic pattern (Fig. 1) showed five major albumin fractions, two major and minor globulin fractions. Prolamins were not observed in the gels. Unresolved broad bands of glutelins were obtained.

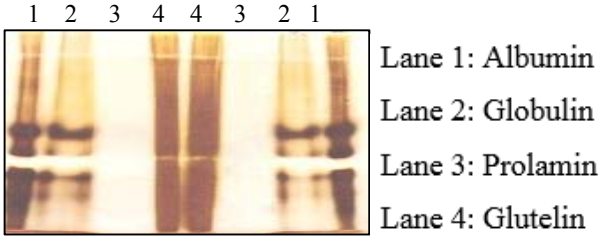

Fig 1: Native Page

SDS – Page analysis (Fig. 2) showed maximum amount of very low molecular weight proteins. Based on electrophoretic studies it can be inferred that the proteins present in the seeds are of very low molecular weight and are highly acidic in nature.

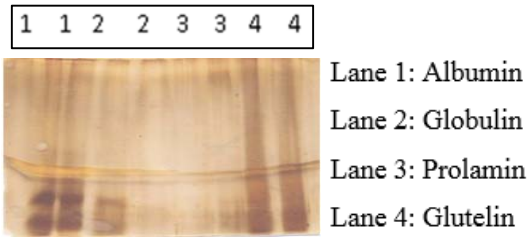

Fig 2: SDS - Page

The tamarind seeds contain polysaccharide which consists of glucose residues linked by β – 1, 4 linkage and xylose and galactose linked by 1, 6 – linkages.

The germinated seed extract was assayed for cellulase activity as illustrated in figure 3. The extract exhibited very high cellulolytic activity. The determination of viscosity of the purified tamarind seed polysaccharide showed that the enzyme is an endocellulase, since about 50 % of the viscosity was reduced in 30 mins.

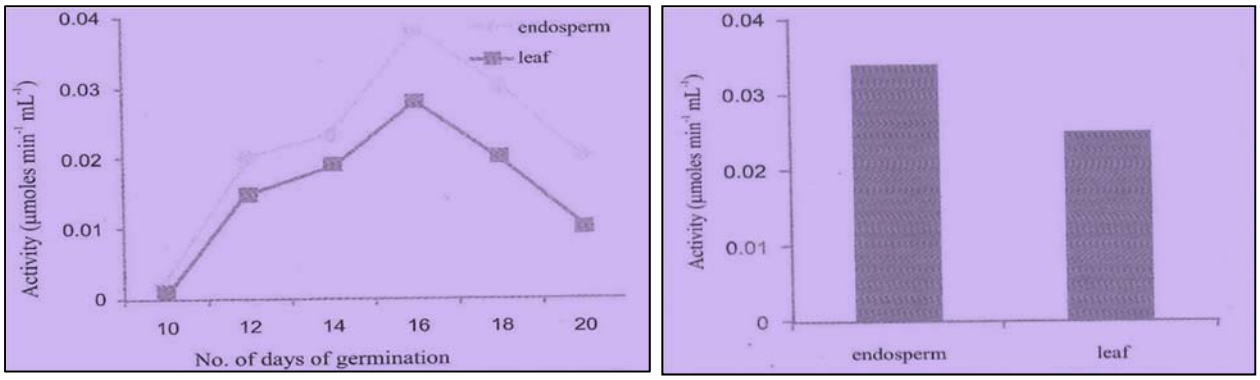

Fig 3: a) Germination profile of cellulase production; **b)** Cellulase activity of endosperm and leaf using FPA.

The viscosity measurement of purified tamarind seed polysaccharide was carried out using Brookefield Viscometer and a 1.5 % solution of the pure polysaccharide showed a viscosity of around 800 cps (Figure 4).

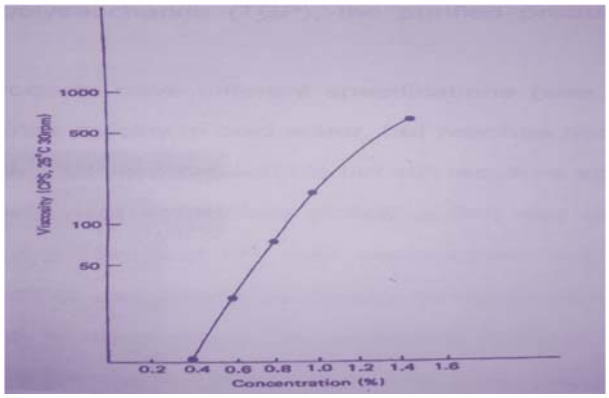

Fig 4: Viscosity measurement.

Tamarind products are highly developed and widely used in Asia and so far little used in Africa. In India and Thailand especially, cultivars are grown and the food industry is active. Tamarind gum (or hydro-colloid) is a polysaccharide polymer (d-galactose, d-xylose and d-glucose) obtained from the endosperm of the seeds. In India it is the chief acidifying agent in curries, chutneys, and sauces. The gum can also be used as a binder in pharmaceutical tablets, as a humectant and emulsifier.

Two main products are used by the food industry: (i) Tamarind kernel powder (TKP), which contains about 50% gum and (ii) Tamarind gum polysaccharide (TGP), the purified product that is virtually 100% pure. These two products have different specifications and uses. TKP hydrates quickly in cold water, but reaches maximum viscosity if heated for 20± 30 min. TGP is more soluble but still requires some heat. A typical 1.5% gum solution will yield a viscosity of 500 ± 800 cps at 25° C. TGP has excellent stability over a range of pH, with electrolytes (e.g. 20% salt) and at temperatures below 65° C and degrades rapidly at higher temperatures and low pH. The xyloglucan from tamarind seeds offers no chemical advantage over guar gum as a viscosifier, but tamarind flour is cheaper, indicating that a bioprocess to upgrade the tamarind polysaccharide might be commercially viable.

4. Conclusion

The major industrial use for the seeds is in the manufacture of TKP, which is an important sizing material for jute and textile industries and TSP, a galactoxyloglucan (50 – 58 %) that is used as gelling agent, thickner and stabilizer. TSPs contains glucose units linked by β – 1, 4 linkages. TSPs can be commonly added to flour as improvers during baking and amelioration of the wheat flour. The seeds are gaining importance as an alternative rich source of proteins and essential amino acids. Also the seeds have been used as food in times of scarcity either alone or mixed with cereal flours. The present study is undertaken to examine tamarind seed protein concentrate, tamarind seed polysaccharide, and cellulose. The studies shows that maximum amount of protein can be extracted with NaOH and in a one – step process both proteins and polysaccharide can be separated very easily. Hence, this process can be employed to obtain high protein concentrates and polysaccharide which can be used to supplement the diet. The polysaccharide can be used as thickening agent in food industry. The technology of bioprocessing of raw materials or their constituents into bioproducts is gaining momemtum. The cellulases can be used to modify the characteristics of Tamarind seed polysaccharide for use in various food formulations (like production of low viscosity polysaccharide and oligosaccharides to be used as bulk material since it has no caloric value) and textile industries for the manufacture of derivatives. It is also being researched for drug delivery and release. It is therefore of interest to gain more information about the cellulases produced by the Tamarind seeds. The

germinated seeds of tamarind exhibits high Cellulase activity and can be used for the production of cellulases, which has wide application in desizing of textile fibres and modification of the viscosity. However, further studies are needed to commercialise the process.

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