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# Physicochemical Properties of Tamarind (*Tamarindus indica*) Seed Polysaccharides

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## Abstract

The polysaccharides of two samples of *Tamarindus indica* (Tamarind) seed; namely Light brown (LB) and Dark brown (DB), were extracted and studied for their physicochemical and functional properties. The physiochemical properties were determined and compared to those of commercial pectin to investigate their potentials as probable substitute of pectin. The two polysaccharide extracts were similar in most of the physical properties such as solubility in hot water, pH, and refractive indices as compared to commercial pectin. The intrinsic viscosity, molecular weights and equivalent weights were similar in both extracts and higher than that of commercial pectin. The polysaccharides were the major components in the extract comprising 88.85%, 85.21% and 92.43% for LB, DB and commercial pectin, respectively. The tamarind seed polysaccharides form gels over a wide pH range in the presence of sucrose with or without acid and base, while commercial pectin forms gels over a narrow range (acidic) in the presence of sucrose. Although the protein levels in the polysaccharides were higher compared to commercial pectin, their gel formatting ability was found more applicable as compared to commercial pectin. This indicated that the protein level did not interfere with gel formation. The functional properties of both extracts, as a gelling agent, indicated that polysaccharides of both samples contained residual amount of uronic acid, no galacturonic acid, no acetyl group, small amount of methoxyl group, and high degree of estrification. The commercial pectin, however, contained a high level of uronic acid and relatively low degree of estrification. The high performance liquid chromatography showed that the two polysaccharide extracts contain pentose sugars (xylose and arabinose) and hexose sugars (glucose and galactose). The molar ratios of these sugars were 2:1:3:1 in both extracts. Commercial pectin contains similar sugars but with fructose instead of xylose.

**Keywords:** Tamarind seed polysaccharides; Pectins; Methoxyl contents; Galacturonic acid; Gel formation

## Introduction

*Tamarindus indica* L, (Tamarind) a member of the family Leguminosae (Fabaceae), is native to dry Savanna of tropical Africa [1]. It is an important woody perennial fruity species known for its adaptability to variable climatic and edaphic conditions and for fruit production [2]. In Sudan, the tamarind grows wild on sandy soils and near Khors (water cources) in short grasses Savanna in Kordofan, Darfur, Blue Nile, Bahr ElGhazal [3]. India is the main producer and consumer of tamarind in the world [4]. The edible pulp of ripe fruit is used as a flavouring agent in cooking, soups, jams chutneys, sauces, and juices and the preparation of beverage [5]. In Sudan, the tamarind pulp is used as a beverage [6] and the seeds are discarded.

Pectin, which is chemically similar to cellulose, may be thought of as the "glue" that holds the cellulose together in the cell walls of plants. Pectin and cellulose are both polysaccharides, but pectin is primarily a-linked polygalacturonic acid (partly esterified with methyl ester) in which rhamnose may be found, and cellulose is essentially a polymer chain made up of b-1,4 linked sucrose units with no esterification. Not all plant materials are rich in pectin. The oldest pectin source is apple pomace left over from juicing. The most common source of pectin nowadays is from citrus peel, primarily lemon—but lime, orange and grapefruit may also be used. Novel sources of pectin include sugar beets and sunflower heads, but these are not, at the moment, commercially significant. In order to qualify as pectin, the anhydro-galacturonic acid must make up at least 65% of the ash-free dry matter in pectin sold as a commercial product [7].

Jam is one of the important fruit products with considerable economic and dietary importance in Sudan [8]. The pectin used in jam making is imported as there is no pectin industry yet in Sudan [9,10] so more attention should be directed towards utilization of pectin obtained from local substitutes. From this point of view, the agricultural by-product like tamarind seed kernel can be used as a cheap source for functional food to increase the added value of tamarind seeds. Most researches, carried out in Sudan on tamarind, were on the pulp and for medicinal purposes [11] the seed was found to be rich in protein, sugar and potassium, thus can suffice the human needs and was consumed as famine food. Research concerning the chemical, technological and usage of tamarind kernel in Sudan are scarce. The aim of this study was to extract polysaccharides from tamarind kernel and study its physicochemical and functional properties compared to commercial pectin.

## Material and Methods

Two samples of tamarind seeds; light brown (LB) and dark brown (DB),one kilogram each were obtained from Ali Ibrahim shop for tamarind juice. Pectin was obtained from Saeed Food Industry Factory. All chemicals and reagents used were of analytical grade.

Samples were prepared according to the method described by Ghose and Krishna [12].

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Whole seeds of tamarind were sun dried, and the immature and damaged seeds were discarded. The mature whole seeds were then soaked in sodium hydroxide solution (10%) for 30 min then crushed and washed with water several times. The crushed seeds were dried, grounded into powder to pass through a 60 –mesh size. The powder was then divided into two portions and kept in plastic containers at room temperature. One part was used for proximate analysis where the other part was reserved for polysaccharides extraction.

## Proximate analysis

Determination of moisture, protein, fat, ash, and crude fiber were carried out in triplicate.

The moisture content was determined by weighing the kernels before and after drying in an oven at 105°C for 3 hours [13].

The nitrogen content was estimated by the micro- Kjeldahl method as modified by Cocon and Dian [14], and then crude protein was calculated by multiplying the N value with the factor 6.25 [15].

The fat content was determined by extracting the sample with ether in sohxlet apparatus for 16 hours [16].

The ash content was calculated by the method mentioned in AOAC [16]. The carbohydrate content was obtained by difference.

All proximate results were expressed as a percentage of the weight of samples analyzed.

## Extraction of tamarind seed polysaccharide

The tamarind gum was extracted from the powder kernel by Khullar et al. [17] method.), in three batches at laboratory scale. To 200 grams of tamarind kernel powder, 2000 ml of cold distilled water was added and slurry was prepared. The slurry was poured into 2400 ml of boiling distilled water and boiled for 20 min with stirring in a water bath. The resulting thin clear solution was kept overnight so most of the protein and fiber settle down. The solution was then centrifuged at 5000 rpm for 20 min. The supernatant was separated and poured into twice the volume of absolute ethanol with continuous stirring. The precipitate was pressed between cheese cloth and the product was washed with absolute ethanol, diethyl ether and petroleum ether. The material is then dried at 50-60°C under vacuum, then ground and sieved and stored for further purification.

## Physical properties

The solubility and refractive index of polysaccharides were determined according to the method of the AOAC [16] and viscosity by the method of Gidley et al. [18].

The methods described by Owen et al. [19] were used for the analysis of pectin. Methods of analysis used for polysaccharide were those mentioned by Marlino [20].

## Chemical properties

**pH-value:** The method described by mts was used for the determination of the pH. A 1% aqueous solution of LB and DB polysaccharides and pectin were prepared and measured using a Beckman Zeromatic pH meter at room temperature.

**Acetyl content:** The acetyl content of the polysaccharide samples and the pectin were determined by a hydroxamine acid colour reaction [21].

**Equivalent weight:** The equivalent weight was calculated as follows Owens et al. [19]:

$$\text{Equivalent Weight} = \frac{\text{Weight of sample (mg)}}{\text{meq. Of sodium hydroxide}}$$

where: meq. of sodium hydroxide = normality×titre value

This titre is known as initial titre (IR) or free acid titre.

**Uronic acid percentage:** It was calculated by multiplying by 100 and dividing the apparent equivalent weight of the sample as follows:

$$\text{Uronic acid} = \frac{194 \times 100}{\text{Eq. weight}}$$

**Methoxyl content:** The methoxyl was determined according to Owens et al. [19] as follows:

$$\text{Methoxyl content} = \frac{\text{meq. ofNaOH} \times 31 \times 10}{\text{Weight of sample}}$$

**The degree of estrification and anhydrouronic acid:** Content were calculated as follows:

$$\text{Degree of esterification} = \frac{ST \times 100}{ST + \text{corrected IT}}$$

The IT was corrected for ash alkalinity.

$$\textbf{Anhydrouronic acid content (AUA)} = \frac{176 \times 100}{Z}$$

Where

Z = Weight of sample meq. of alkali for free acid + meq. of alkali for methoxyl

**Sugar composition:** Acid hydrolysis of the three samples was done according to the method adopted by Balabon [22]. Separation of sugars was done by high performance liquid chromatography (HPLC). Using HPLC system (HPLC UFLC, Shimadzu, Japan), with refractive index detector (RID – 10A), coloum ShodexAsahipak (type $NH_2P$ – 504E), mobile phase (175 ml acetonitrile + 25 ml water), oven temperature 30°C, and column temperature 30°C. Analyses were performed at ambient room temperature (30°C), and the flow rate maintained at 1 ml/min. The retention times of the monosaccharide were monitored using differential refractometer (RID – 10A). The retention times were compared to those determined using D-glocose, D-galactose, D-xylose and L-arabinose (Sigma chemical Co. Ltd.) as standards. The area percentage of each peak was calculated by Shimazduprogramme which is connected with RI detector (RID – 10A). The sugar percentage was calculated as follows:

$$\text{Component sugar (i)} = \frac{\text{Area percentage of component (i)} \times 100}{\text{Total area percentage}}$$

## Gel formation, strength and setting rate

The gel formation was carried according to the method described by Bhattacharya et al. [1]. One gram of the sample in each case was added to 22 g of sucrose and stirred well with 30 ml distilled water. About 5 ml of 3% citric acid was then added in a beaker and boiled for 15 min. In a second scenario, 5 ml of 3% sodium citrate was added instead of citric acid. Without addition of acid and base in the third scenario only the samples with sugar and water. All these scenarios were done in triplicate (Figure 1).

Gel strength was measured by the knife raising method similar to that suggested byRao [23]. About 50 ml of gel suspension prior to setting was poured into jelly glass containing a knife and allowed to cool at 24°C, for 24 hours. The relative gel strength was compared with standard jelly of pectin. Gel strength was reported as the time required raising the knife from the bottom of the 50 ml jelly of 3 cm thickness by a weight of 50 g applied by Lever system.

The gel setting time was determined according to Rao [23]. About 50 ml of gel suspension prior to setting was poured into 100ml beaker at room temperature (28-30°C) and the time required for complete setting of the jelly was noted using stop watch. Complete setting was judged by inverting the beaker by pressing with the finger and by scooping with a spoon. Triplicate observations were made.

### Sensory evaluation of prepared jellies

The gels were prepared from dark and light brown seeds and pectin in three scenarios (A- acid, B- base and S- sugar only). Sensory evaluation was conducted using a panel of 20 persons. Trained panelists evaluated 7 samples of gels for appearance, texture, taste and flovour, using ranking scale of 1 to 10.

### Statistical analysis

Sensory evaluation was assessed by randomized block design and analysis of variance (ANOVA) as shown by Scendecor and Cochran [24] and the least significance difference (LSD) was used to separate the means.

## Results and Discussion

The moisture content, on fresh weight basis, of the two seed samples (Table 1) was higher than the value of 9.4% estimated by Marangoni et al. [25], and lower than the range 11.4%-22.7% reported by Ishola et al. [26], Bhattachanya et al. [27] and Morad et al. [28]. The protein contents of two samples was in agreement with the results of 21-25% reported by Leaky and Yusuf et al. [29] and higher than the values of (15.5%,17%) respectively. The oil percentage of the LB sample lied within range of

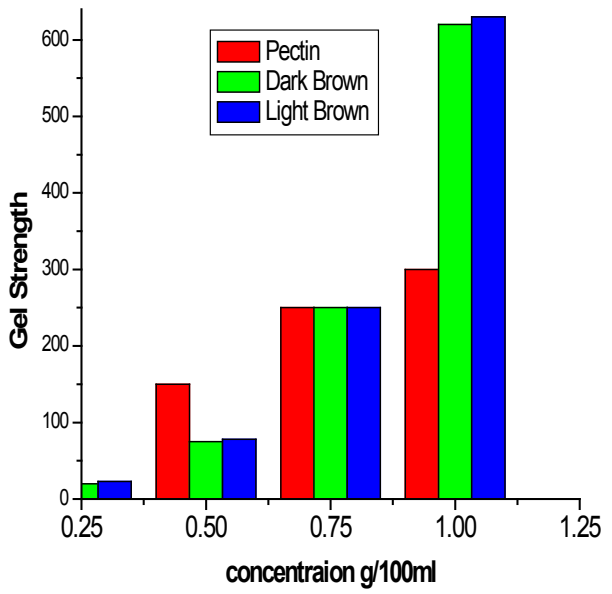


**Figure 1**: Effect of polysaccharides (LB and DB) and commercial pectin concentration on gel strength.

| Parameters (%) | Seed Sample | |
|---|---|---|
| | Light Brown (LB) | Dark Brown (DB) |
| Moisture | 10.99b ± 0.1002 | 11.21a ± 0.0058 |
| Protein | 20.23b ± 0.5658 | 23.75a ± 0.0839 |
| Oil | 3.90a ± 0.0500 | 3.17b ± 0.0462 |
| Ash | 2.50a ± 0.0000 | 2.17b ± 0.0577 |
| Fiber | 3.08a ± 0.0493 | 2.51b ± 0.0058 |
| Carbohydrate (by difference) | 59.303b ± 0.4041 | 57.33a ± 0.2452 |

Seed colour: LB = Light brown and DB = Dark brown respectively
Each value is a mean of three determinations.
Means followed by similar superscript letters in a column are not significantly different at ($P \geq 0.05$)

**Table 1:** Proximate chemical composition of the *Tamarindus indica* seed.

| Parameter | Polysaccharide | | |
|---|---|---|---|
| | Light Brown (LB) | Dark Brown (DB) | Pectin |
| Intrinsic viscosity (dL/g) | 4.62 | 4.42 | 3.8 |
| Molecular weight. | $7.3\times10^4$ | $7.1\times10^4$ | $6.3\times10^4$ |
| Equivalent wt. | 5623 | 5697 | 803 |
| Refractive index | 1.334 | 1.334 | 1.334 |

Seed colour: LB=Light brown and DB=Dark brown respectively.

**Table 2:** Physical properties of Tamarind seed polysaccharides as compared to commercial pectin.

3.9%-16.2% estimated by Morad et al., Ishola et al. and Bhattacharya et al. [27]. While the DB sample gave 3.17 ± 0.0462 oil content which was lower than LB sample.

The ash values were in agreement with the range 2.17%-4.2% reported by Moradet al., Isholaet al.and Battacharya et al. [27], but lower than 2.8%-4.58% reported by Marangoni et al. and Bhattacharya et al. [1,25]. The fiber content of the two samples was within the range of 0.7%-8.2% reported by Bhattacharya et al. and Leakey [1], but higher than the values of 2.33%, 8% mentioned by Marangoni et al. and Yusuf et al. [25]. The carbohydrate content in the two samples was similar to the values of 59.4%, 58% and 58.83% reported by Marangoni et al. and Yusuf et al. [25] and lower than the range of 65%-73.48% reported by Morad et al. [28], Ishola et al. [26], Bhattacharya et.al., [27] and Leakey [30]. The percentage of carbohydrates in the two samples was sufficient for the extraction, purification and subsequent characterization of the polysaccharide. Marathee [31] claimed that the carbohydrate in tamarind kernels is not less than 50%. The two samples showed significant difference in all components of the approximate chemical composition.

The extraction yielded 34% polysaccharides for LB sample and 29% for DB sample. The results obtained for both samples differ from the findings of Kooimanwho reported 32%, and findings of Morad et al. [28], Ishola et al. [26] and Bhattacharya et al. [1] who reported 59% and higher than range of 20%-25% estimated by Khannaet al. [32]. These variations may be due to the extraction conditions and the varieties of the tamarind itself. Graham and Uan [33] and Marlett et al. [34] indicated that the yield of polysaccharide depends on the extraction condition (temperature and time).

The viscosity and average molecular weight of the polysaccharides LB and DB were remarkably higher than that of commercial pectin (Table 2) and slightly higher than the findings of Chakravorti et al. [35] who reported $5.5\times10^4$ molecular weight and 4.17 dl/g viscosity for tamarind seed polysaccharide and lower than the value 8.9 dl/g reported by Reid [36]. These differences may be attributed to the differences in temperature. The average molecular weight for the two

samples was higher than that of the commercial pectin. The equivalent weights of LB and DB polysaccharides were higher than that of pectin, and this may be due to difference in their polymers. The refractive indices indicated the similarity of tamarind kernel polysaccharides and commercial pectin.

Polysaccharides of both LB and BD, have the same moisture content (3.81% and 3.80%) which was lower than 5.18% for commercial pectin (Table 3). A significant difference was obtained between the two samples (LB and DB) polysaccharide in protein content and ash, both were higher than the pectin used as control. There was no significant difference between the two samples in oil content and fiber content. There was significant difference obtained between the two samples of polysaccharides in carbohydrate content and the values were higher than the value of 74.94% obtained by Savur and Sreenivasan [37] and lower than 95.8% of Marlino [20].

The absence of acetyl group in the polysaccharides extracted from both samples was indicated by no red colour formation as shown in Table 4 while a value of 0.034% was obtained for citrus pectin. There was a difference in the methoxyl content in the polysaccharides of the two samples. While commercial pectin showed much higher

| Parameters (%) | Polysaccharide | | |
|---|---|---|---|
| | Light Brown (LB) | Dark Brown (DB) | Pectin |
| Moisture | 3.810 ± 0.070 | 3.800 ± 0.000 | 5.18 |
| Protein | 3.900 ± 0.000 | 6.500 ± 0.017 | 1.24 |
| Oil | 1.323 ± 0.070 | 1.360 ± 0.540 | Trace |
| Ash Content | 1.043 ± 0.060 | 2.933 ± 0.060 | 1.90 |
| Fiber | 1.007 ± 0.010 | 1.030 ± 0.660 | 0.13 |
| Carbohydrate (by difference) | 88.850 ± 0.600 | 85.210 ± 0.530 | 92.43 |

Seed colour: LB=Light brown and DB=Dark brown respectively.

**Table 3:** Chemical constituents of Tamarind seed polysaccharides compared to reference commercial pectin.

| Parameter | Polysaccharide | | |
|---|---|---|---|
| | Light Brown (LB) | Dark Brown (DB) | Pectin |
| Acetyl content Hydroxamic acid | No red colour formed | No red colour formed. | Red colour formed. |
| Titration | 0 | 0 | 0-034 |
| Methoxyl content | 0.124 | 0.062 | 8.82 |
| Degree of esterification | 100 | 100 | 66.82 |
| Uronic acid | 3.44 | 3.45 | 62.90 |

Seed colour: LB = Light brown and DB= Dark brown respectively.

**Table 4:** Chemical properties of Tamarind seed polysaccharides and Commercial Pectin.

| Sample / Time(min) | Pectin Jelly | Polysaccharide Jelly (Brown) | Polysaccharide Jelly (Black) | LSD |
|---|---|---|---|---|
| Time | 10.86c ± 0.079 | 13.37 ± 0.170 | 12.40 ± 0.022 | 0.97 |

**Table 5:** Gel setting rate of Tamarind kernel polysaccharides and commercial pectin.

| Test | Sample | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Pectin | LB | | | DB | | | |
| | A | S | A | B | S | A | B | LSD |
| Appearance | 1.959 ± 0.033 | 1.932 ± 0.068 | 1.931 ± 0.068 | 1.930 ± 0.056 | 1.911 ± 0.068 | 1.914 ± 0.067 | 1.880 ± 0.070 | 0.10 |
| Texture | 3.960 ± 0.030 | 3.946 ± 0.032 | 3.923 ± 0.031 | 3.933 ± 0.031 | 3.903 ± 0.031 | 3.926 ± 0.031 | 3.894 ± 0.030 | 0.05 |
| Taste | 1.966 ± 0.021 | 1.962 ± 0.019 | 1.956 ± 0.019 | 1.951 ± 0.020 | 1.936 ± 0.020 | 1.934 ± 0.023 | 1.915 ± 0.027 | 0.03 |
| Flavour | 1.965 ± 0.025 | 1.956 ± 0.027 | 1.951 ± 0.029 | 1.946 ± 0.027 | 1.932 ± 0.03 | 1.928 ± 0.029 | 1.908 ± 0.027 | 0.05 |
| Total score | 9.836 ± 0.086 | 9.536 ± 0.407 | 9.756 ± 0.095 | 9.536 ± 0.407 | 9.628 ± 0.232 | 9.701 ± 0.092 | 9.588 ± 0.104 | 0.31 |

A =Acid S = Sugar B = Base

**Table 6**: Sensory evaluation of pectin and polysaccharide jellies.

percentage of methoxyl content. Marlino [20] reported a value 0.02% and Damodaran and Rangachari [38] found 1.08%. A similar value of 100% of esterification was obtained for LB and DB samples which were higher than commercial pectin. A similar value of 3.44% of uronic acid was obtained for samples of tamarind polysaccharides which agree with the value of 3.44% reported by Savur and Sreenivasan [39] and lower than that of commercial pectin (62.9%).

## Sugar identification

The results indicated that the polysaccharides obtained from LB and DB samples contained two aldopentose sugar (xylose and arabinose) and two aldohexose sugars (glucose and galactose), while the commercial pectin contained ketohexose sugar (fructose) instead of the aldopentose sugar xylose. The presence of arabinose in the two polysaccharide agrees with the findings ofDamodran and Rangachari [38], Cremate and Rodrigves [40] and Marlino [20]. In addition to arabinose, galactose and xylose. McCready and Gee [41] and Mohamed [10] reported the presence of rhamnose in commercial pectin.

## Gel formation

The tamarind Polysaccharides were able to form gels over a wide pH range in the presence of sucrose (with or without acid and base), while commercial pectin form gels over a narrow pH range (acidic) in the presence of sucrose. The maximum gel strength was obtained using concentration of 0.75% tamarind polysaccharides and commercial pectin, 65-70% sucrose concentration and 0.80% citric acid at pH range from 2.50-2.75. A significant difference ($P \geq 0.05$) was obtained in the gel setting rate of gels made from the three gelling agents (Tables 5 and 6). The organoleptic evaluation of the gels showed that there were insignificant differences in appearance, texture, taste, and flavour under all conditions investigated for LB tamarind polysaccharide and commercial pectin. However, the appearance, taste, and flavour of the gels formed by DB tamarind polysaccharide under the base condition were significantly different. Although the gels made from commercial pectin obtained the higher panelists score (9.836) compared to that made from LB and DB polysaccharide (9.756, 9.701, respectively), the statistical analysis showed insignificant difference.

## Gel setting rate

The results indicated that the degree of esterification, rather than acetyl content and methoxyl content enhanced the gel formation. This result agrees with the findings of Savur and Sreenivasan [37], Ghose and Krishna [12] who attempted to relate gel-formation capacity to some factor like degree of esterification due to absences of methoxyl group in tamarind polysaccharides.

# Conclusion

The tamarind seed, which is considered as waste can be converted into a useful agricultural by-product by extracting the kernels' polysaccharide. The tamarind polysaccharides could be used as

substitute for commercial pectin, thus reducing the import bill and the foreign currency expenditure. The findings of this study may provide a base for the possible industrial utilization of tamarind seed polysaccharide.

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