Extraction and Characterization of Stabilizer from Tamarind Seeds and Its Utilization in Pomelo Juice

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Abstract

The present research work emphasized on the preparation of stabilizer from tamarind seeds and its utilization in pomelo juice. The tamarind seed testa and its kernel were separated by roasting and subsequent pounding, grinding and screening to obtain tamarind kernel powder. The tamarind seed polysaccharide was extracted from tamarind kernel powder by extraction with ethanol. The effects of amount of citric acid, amount of distilled water and soaking time on the yield percentage of tamarind seed polysaccharide were determined. The maximum yield percent 42.8 %w/w of tamarind seed polysaccharide was obtained using (50) g of tamarind seed kernel powder, (5) g of citric acid and 1200 ml of distilled water at 25-30˚C soaking temperature for (12) hours. The physico-chemical characteristics such as moisture content, ash content, crude fiber content, crude fat content, protein content, carbohydrate content, total solids content, acidity and pH of prepared tamarind kernel powder and tamarind seed polysaccharide were determined. The identification tests such as solubility test, gel formation test and precipitate formation test of prepared tamarind kernel powder and tamarind seed polysaccharide were also investigated. The elemental compositions of prepared tamarind seed polysaccharide were determined by Energy Dispersive X-Ray Fluorescence (EDXRF), which gave high concentration of potassium. The prepared tamarind seed polysaccharide was utilized as stabilizer in the preparation of pomelo juice.

Keywords: Tamarind kernel powder, tamarind seed polysaccharide, stabilizer, pomelo juice

Introduction

Tamarind (*Tamarindus indica* L.) is as an important economical plant being widely grown in Myanmar. According to the taste of tamarind fruit, it can be mainly classified into two: sour tamarind and sweet tamarind. Tamarind is a multipurpose tropical fruit tree used primarily for its fruits, which are eaten fresh or processed as a seasoning or spice or the fruits and seeds are processed for non-food uses. Tamarind fruits have outers-shell, pulp and the seeds. These seeds are flat and irregular, rectangular shapes. The seeds have about 25% testa and 75% kernel. Testa is of brown colour and kernel is of light cream. The kernel consists of protein, fibers, carbohydrates and oil (https://www.hort. purdue.edu/newcrop/moton/ tamarind.com).

Tamarind seed is very hard, strong and is covered with black brown husk. Tamarind seeds are roasted and soaked to remove the seed coats and ground to a flour or starch. The decorticated kernels contained 46 to 48% of a gel-forming substance, a purified product, called jellose, polyose or pectin like substance which has been found superior to fruit pectin in the manufacture of jellies, jams and marmalades. Currently purified and refined tamarind kernel powder (TKP) is produced and permitted in Japan as a thickening, stabilizing and gelling agent in the food industry (Morton, 1987).

The polysaccharide constituent of tamarind kernel powder is also known as gellose and polyose. It is a polysaccharide consisting mainly of D-mannose and D-galactose units. The polysaccharide is composed of (1→4)-β-D-glucan backbone substituted with side chains of α-D-xylopyranose and β-D-galactopyranosyl (1 to 2)-α-D-xylopyranose linked (1→ 6) to glucose residues. The molecular weight of the polysaccharide is reported to the range from 115,000 to 2,500,000 Daltons (www.ijpsdr.com).

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Tamarind seed polysaccharide can also be used in cosmetics, in pharmaceutical and insecticidal preparation. It forms a stiff gel and is used for thickening, stabilizing and gelling in food. It is commercially available as a food additive for improving the viscosity and texture of processed foods (Morton, 1987).

Pomelo – also called pummelo or pommel – is largest member of citrus (*Rutaceae*) family. This large, pale, green or yellow fruit has a pink, white or red inside that tastes like a slightly sweeter alternative to grapefruit. Pomelo is an excellent source of vitamin C and contains small amounts of thiamin, riboflavin, niacin, and vitamin B6. Minerals in pomelo include potassium and smaller amounts of phosphorus, copper, iron, calcium, magnesium, phosphorus, manganese and zinc (https://www.verywellfit.com).

The aim this research is the preparation and utilization of tamarind seed polysaccharide as stabilizing agent in pomelo juice. The specific objectives of this research are:

- to extract the tamarind seed polysaccharide from tamarind seeds
- to investigate the physico-chemical characteristics of prepared tamarind seed polysaccharide and
- to discover its utilization of the prepared tamarind seed polysaccharide as stabilizing agent in pomelo juice.

Materials and Methods

Materials

In this research, tamarind seeds were collected from Kyaukpadaung Township, Magway Region. Good, sound and matured pomelo fruits were purchased from Mingalar Market, Maha Aung Myay Township, Mandalay Region. 95% ethanol, distilled water, pectin, sodium carboxy methyl cellulose (SCMC) and potassium sorbate were purchased from Able Chemical Shop, 76th street, between 27^{th} x 28^{th}, Chan Aye Thar Zan Township, Mandalay Region.

Methods

Preparation of Tamarind Kernel Powder

The tamarind seeds were washed with water to remove the adhering dirt and impurities. Then, they were dried at room temperature. The tamarind seeds were weighed and roasted at 100ºC for 10 min on electric stove and then pounded. The tamarind kernel was ground and sieved through the screen (-40+60 mesh size) to obtain the tamarind kernel powder. The obtained tamarind kernel powder was stored in air-tight container at room temperature.

Preparation of Tamarind Seed Polysaccharide

(50) g of tamarind kernel powder (-40+60 mesh size) was soaked with (1000) ml of distilled water containing (1) g of citric acid at room temperature (25-30ºC) for 6 hours. Then, it was boiled in a water-bath at 95 ± 5ºC for an hour and was allowed to stand for 30 min to settle out most of the fiber and protein. The resulting mixture was filtered and the liquid tamarind seed polysaccharide was heated at 95±5ºC for 15 min to obtain the concentrated tamarind seed polysaccharide. The concentrated tamarind seed polysaccharide was precipitated with twice its volume of 95% ethanol and filtered to obtain better purity of tamarind seed polysaccharide. After that, the resulting tamarind seed polysaccharide was dried in hot air oven at 50ºC for 5 hours and then ground into powder. Finally, the tamarind seed polysaccharide was sieved through the screen (-80+100 mesh size), weighed and then stored in air-tight container at room temperature.

Determination of the Maximum Yield Percent of Tamarind Seed Polysaccharide

Effect of Amount of Citric Acid on the Yield Percent of Tamarind Seed Polysaccharide

Tamarind seed polysaccharides were prepared by using (50) g of tamarind kernel powder, (1000) ml of distilled water and different amounts of citric acid ranging from (1) g to (9) g while keeping the soaking temperature and time, drying temperature and time were constant as mentioned above procedure. The yield percent of tamarind seed polysaccharides was determined and the results are recorded.

Effect of Amount of Distilled Water on the Yield Percent of Tamarind Seed Polysaccharide

Tamarind seed polysaccharide were prepared by using (50) g of tamarind kernel powder, (5) g of citric acid and different amounts of distilled water ranging from (800) ml to (1600) ml while keeping the soaking temperature and time, drying temperature and time were constant as mentioned above procedure. The yield percent of tamarind seed polysaccharides was determined and the results are recorded.

Effect of Soaking Time on the Yield Percent of Tamarind Seed Polysaccharide

Tamarind seed polysaccharides were prepared by using (50) g of tamarind kernel powder, (5) g of citric acid, (1200) ml of distilled water and different soaking time ranging from (6) hours to (14) hours while keeping the soaking temperature, drying temperature and time were constant as mentioned above procedure. The yield percent of tamarind seed polysaccharides was determined and the results are recorded.

Properties of Tamarind Kernel Powder and Tamarind Seed Polysaccharide

The physico-chemical properties such as moisture content, ash content, crude fiber content, crude fat content, protein content, carbohydrate content, total solids content, acidity and yield percent of tamarind kernel powder and tamarind seed polysaccharide were determined by Association of Official Analytical Chemists (AOAC) method and pH by pH meter (Hanna, HI 98128, USA).

Identification of Tamarind Kernel Powder and Tamarind Seed Polysaccharide

Solubility Test

(1) g of tamarind kernel powder and 5 ml of distilled water / ethanol were added into a clean, dry and tarred 25 ml beaker. The beaker containing the sample was heated in water bath at 60ºC for 5 min and the appearance of tamarind kernel powder was observed and recorded.

Gel Formation Test

(1) g of tamarind kernel powder and 9 ml of distilled water were added into a clean, dry and tarred 50 ml beaker and heated in water bath until all the tamarind kernel powder was dissolved. Periodic addition of water was made to compensate for loss during evaporation. The beaker containing the sample was then cooled and the appearance of sample was observed and recorded. This procedure was carried out by using 1 ml of 2 M sodium hydroxide solution as solvent and its appearance was observed and recorded.

Precipitate Formation Test

(1) g of tamarind kernel powder and 10 ml of distilled water were added into a clean and dry 50 ml beaker and shaken well until the clear solution was obtained. Then, 5 ml of tamarind kernel powder solution was mixed with 5 ml of ethanol into 25 ml beaker and shaken well for 5 min. After shaking, the appearance of resulting precipitate was observed and

recorded. The resulting translucent opaque gel or semi-gel was acidified with 2 ml of 0.1 M hydrochloric acid solution and shaken well and its appearance was observed and recorded.

The solubility test, gel formation test and precipitate formation test of prepared tamarind seed polysaccharide were conducted the same procedure as mentioned above and the results were recorded.

Utilization of Tamarind Seed Polysaccharide as Stabilizer in Pomelo Juice

Preparation of Pomelo Juice

The good, sound and matured pomelo fruit was thoroughly washed with water, free from dirt and soil. The washed pomelo fruit was peeled and discarded the seeds. Then, edible, juicy inner flesh of pomelo was placed in juice extractor for 15 min. The extracted pomelo juice was then collected in the plastic cup provided in juice extractor. (50) g of pomelo juice and the prepared tamarind seed polysaccharide as stabilizer ranging from (0.5) g to (1.5) g was placed in a cleaned and dried stainless steel pan. Then, the juice was thoroughly mixed with 0.05% potassium sorbate as chemical preservative (based on weight of pomelo juice) and the mixture was heated in water bath at 94ºC for 5 min and stirred well. The foam was skimmed off during heating. After that the pomelo juice was filled into cleaned, sterilized glass bottle and pasteurized at 80ºC for 10 min. Finally, the pomelo juice containing bottle was tightly sealed with sterilized cap and allowed to cool at ambient temperature for 20 min and stored in room temperature.

The same procedures as described above were carried out by using sodium carboxy methyl cellulose and pectin as stabilizer ranging from 0.5 g to 1.5 g and fixed amount of extracted pomelo juice. The quality of pomelo juice was tested with the organoleptic properties.

Characterization of Prepared Pomelo Juice

The characteristics such as total solids content, soluble solids content and acidity of prepared pomelo juice were determined by Association of Official Analytical Chemists (AOAC) method and pH by pH meter (Hanna, HI 98128, USA).

The organoleptic properties, namely colour, flavour and texture of prepared pomelo juice were determined on the basis of 9 point Hedonic scale by a panel of 10 semi-trained judges. The overall acceptability of prepared pomelo juice was taken as the average score of all these organoleptic properties.

Figure (1) Tamarind Seeds

Figure (2) Tamarind Seeds Kernel

Results and Discussion

The physico-chemical characteristics of tamarind kernel powder were determined and the results are shown in Table (1). From the results in Table (1), it can be seen that the ash, crude fiber, crude fat, protein and carbohydrate content of tamarind kernel powder were found to be in agreement with those of literature values.

The identification tests such as solubility test, gel formation test and precipitate formation test of prepared tamarind kernel powder were conducted. The results from Table 2 revealed that tamarind kernel powder was soluble in water and gives yellowish white precipitate but it was insoluble in ethanol. According to the results of gel formation test of tamarind kernel powder, yellowish white solution was observed with water but in 2M NaOH solution, yellowish white curd was found. From the results of precipitate formation test of tamarind kernel powder showed that yellowish white gelatinous precipitate was found in both ethanol and 0.1M HCl solution.

In the preparation of tamarind seed polysaccharide, the effects of amount of citric acid, amount of distilled water and soaking time on the yield percent of tamarind seed polysaccharide were determined and the results are shown in Tables (3) to (5) respectively. According to the results in Tables (3) to (5), the maximum amount, (21.4) g of tamarind seed polysaccharide was obtained from (50) g of tamarind kernel powder by using 1200 ml of distilled water containing (5) g of citric acid and 12 hours soaking time.

The physico-chemical characteristics such as moisture content, ash content, crude fiber content, crude fat content, protein content, carbohydrate content, total solids content, acidity, pH and yield percent of prepared tamarind seed polysaccharide were determined and the results are shown in Table (6).

The prepared tamarind seed polysaccharide was identified by solubility test, gel formation test and precipitate formation test. The results from Table (7) indicated that tamarind seed polysaccharide was soluble in water and gives white translucent solution but it was insoluble in ethanol. From the results of gel formation test of tamarind seed polysaccharide, it was observed that white translucent solution occurred with water and white translucent semi-gel occurred with 2M NaOH solution. According to the results of precipitate formation test of tamarind seed polysaccharide, it can be seen that white semi-gelatinous precipitate was found in ethanol but in 0.1M HCl solution, white precipitate was observed.

The elemental compositions of prepared tamarind seed polysaccharide were determined by Energy Dispersive X-Ray Fluorescence (EDXRF) Spectroscopy as shown in Table (8). It can be seen that potassium, calcium, iron, sulphur, zinc and copper were present in prepared tamarind seed polysaccharide. Potassium is one of the seven essential macrominerals, along with calcium, magnesium, phosphorous, sodium, chloride and sulphur. High potassium intakes are associated with a lower blood pressure, protection against loss of muscle mass, preservation of bone material density and reduction of the formation of kidney stones. Calcium is very essential in blood clotting, muscles contraction and in certain enzymes in metabolic processes.

The prepared tamarind seed polysaccharide was utilized as a stabilizer in the preparation of pomelo juice. The effects of amount of different stabilizers on the properties of prepared pomelo juice were investigated and results are shown in Table (9). It was found that (1.5) g of tamarind seed polysaccharide was the most suitable stabilizer due to the properties of pomelo juice retained unchanged in their properties within 6 weeks of shelf-life period. The characteristics of prepared pomelo juice are shown in Table (10).

Table (1) Physico-chemical Characteristics of Tamarind Kernel Powder

Sr. No.	Parameters	Tamarind Kernel Powder (% w/w)	Literature Value (%w/w)*
1	Moisture content	8.52	11.4-22.7
2	Ash content	2.95	2.4-4.2
3	Crude fiber content	7.12	2.5-8.2
4.	Crude fat content	5.24	3.9-16.2
5	Protein	10.86	15.0-20.9
6	Carbohydrate	65.31	65.1-72.2
7	Total solids content	91.48	-
8	Acidity	0.04	-
9	pH	6.5	-

*Ref: (Bhattacharyya *et. al*. 1994).

Table (2) Identification of Prepared Tamarind Kernel Powder

Sr. No.	Tests	Solvents	Properties of Tamarind Kernel Powder
1	Solubility test	Water	Yellowish white solution
		Ethanol	Insoluble
2	Gel formation test	Water	Yellowish white solution
		NaOH solution (2M)	Yellowish white curd
3	Precipitate formation test	Ethanol	Yellowish white gelatinous precipitate
		HCl solution (0.1M)	Yellowish white gelatinous precipitate

Table (3) Effect of Amount of Citric Acid on Yield Percent of Tamarind Seed Polysaccharide
Soaking temp. and time = Room temperature (25-30ºC) for 6 hrs
Boiling temperature and time = 95±5 ºC for 1 hr
Drying temperature and time = 50 ºC for 5 hrs

Sample No.	Tamarind Kernel Powder (g)	Distilled Water (ml)	Citric Acid (g)	Tamarind Seed Polysaccharide	
				(g)	(%w/w)
I	50	1000	1	10.2	20.4
II	50	1000	3	11.8	23.6
III*	50	1000	5	12.6	25.2
IV	50	1000	7	10.7	21.4
V	50	1000	9	10.5	21.0

* Suitable amount of citric acid

Table (4) Effect of Amount of Distilled Water on Yield Percent of Tamarind Seed Polysaccharide
Soaking temp. and time = Room temperature (25-30ºC) for 6 hrs
Boiling temperature and time = 95±5 ºC for 1 hr
Drying temperature and time = 50 ºC for 5 hrs

Sample No.	Tamarind Kernel Powder (g)	Distilled Water (ml)	Citric Acid (g)	Tamarind Seed Polysaccharide	
				(g)	(%w/w)
I	50	800	5	12.0	24.0
II	50	1000	5	12.6	25.2
III*	50	1200	5	15.5	31.0
IV	50	1400	5	15.3	30.6
V	50	1600	5	14.9	29.8

* Suitable amount of distilled water

Table (5) Effect of Amount of Soaking Time on Yield Percent of Tamarind Seed Polysaccharide
Soaking temperature = Room temperature (25-30ºC)
Boiling temperature and time = 95±5 ºC for 1 hr
Drying temperature and time = 50 ºC for 5 hrs

Sample No.	Tamarind Kernel Powder (g)	Distilled Water (ml)	Citric Acid (g)	Soaking Time (hr)	Tamarind Seed Polysaccharide	
					(g)	(%w/w)
I	50	1200	5	6	15.5	31.0
II	50	1200	5	8	16.8	33.6
III	50	1200	5	10	18.3	36.6
IV*	50	1200	5	12	21.4	42.8
V	50	1200	5	14	21.5	43.0

* Suitable soaking time

Table (6) Physico-chemical Properties of Prepared Tamarind Seed Polysaccharide

Sr. No.	Properties	Tamarind Seed Polysaccharide (% w/w)
1	Moisture content	7.15
2	Ash content	1.07
3	Crude fiber content	2.18
4	Crude fat content	5.62
5	Protein	20.50
6	Carbohydrate	63.48
7	Total solids content	92.85
8	Acidity	0.072
9	pH	5.5
10	Yield percent	42.8

These data were determined at the Laboratory of Small Scale Industries Department, Ministry of Co-operative, North Okkalapa, Yangon Region.

Table (7) Identification of Prepared Tamarind Seed Polysaccharide

Sr. No.	Tests	Solvents	Properties of Tamarind Seed Polysaccharide
1	Solubility test	Water	White translucent solution
		Ethanol	Insoluble
2	Gel formation test	Water	White translucent solution
		NaOH solution (2M)	White translucent semi-gel
3	Precipitate formation test	Ethanol	White semi-gelatinous precipitate
		HCl solution (0.1M)	White precipitate

Table (8) Elemental Compositions of Tamarind Seed Polysaccharide Analyzed by Energy Dispersive X-Ray Fluorescence

Sr. No.	Elements	Compositions (%w/w)
1	Potassium, (K)	44.240
2	Calcium, (Ca)	36.071
3	Iron, (Fe)	8.515
4	Sulphur, (S)	8.401
5	Zinc, (Zn)	1.422
6	Copper, (Cu)	1.352

Table (9) Effect of Stabilizer on the Properties of Prepared Pomelo Juice

Shelf-life of pomelo juice = 6 weeks

Sample No.	Pomelo juice (ml)	Potassium Sorbate (g)	Amount of Stabilizer (%w/v)			Organoleptic Properties (Flavour)
			Pectin	TSP	SCMC	
I	100	0.05	1.5	-	-	Pleasant smell and taste, syrupy consistency
II	100	0.05	-	1.5	-	Pleasant smell and taste, syrupy consistency
III	100	0.05	-	-	1.5	Sour smell and taste, separate the layer of juice

Note: TSP = Tamarind seed polysaccharide
SCMC = Sodium carboxy methyl cellulose

Table (10) Characteristics of Prepared Pomelo Juice
Shelf-life of fruit juice = 6 weeks
Stabilizer = 1.5 g of tamarind seed polysaccharide

Sr. No.	Characteristics	Pomelo Juice
1	Total solids content, (%w/w)	42.85
2	Soluble solids content, (Brix)	8.0
3	Acidity, (%w/v)	0.25
4	pH	4.1

Figure (3) Tamarind Kernel Powder

Figure (4) Tamarind Seed Polysaccharide

Figure (5) Pomelo Fruit

Figure (6) Pomelo Juice

Figure (7) Pomelo Juice with Different Stabilizers

Conclusion

Nowadays, in the whole world there is turn to return towards the use of tamarind products and to adopt more natural way of life. In this research, in drying of tamarind seed polysaccharide, the colour became brown at higher temperature due to the oxidation of free sugars such as D-xylose, D-galactose and D-glucose. So, drying of prepared tamarind seed polysaccharide at a lower temperature 50˚C and at heating time 5 hours was used. The maximum yield percent 42.8 %w/w of tamarind seed polysaccharide was obtained using (50) g of tamarind seed kernel powder, (5) g of citric acid and 1200 ml of distilled water at 25-30˚C soaking temperature for (12) hours. The most important characteristics of tamarind seed polysaccharide were its ability to form jellies with sugar concentrates and citric acid over a wide pH range than fruit pectin. Therefore, the prepared tamarind seed polysaccharide could be used as a stabilizer in the preparation of fruit juice beverages.

Acknowledgements

We are grateful to Dr Thein Win, Rector, Dr Kathy Thin, Dr Myin Zu Minn and Dr Mi Mi Gyi, Pro-Rectors, University of Mandalay, for their permission to submit this article. We would like to express sincere thanks to Dr Nilar, Professor and Head of the Department of Industrial Chemistry, University of Mandalay for allowing us to submit this article.

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