ISSN: 2321-3132

Research Article

International Journal of Chemistry and Pharmaceutical Sciences

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Gum Isolation from the Seeds of *Brachystegia eurycoma* Harms and its Synergistic Studies with Cassava and Maize Starches

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Abstract

Gum was isolated from the seeds of *Brachystegia eurycoma* Harms with excess cold isopropanol. The gum constituted 38.1% of the seed endosperm. Proximate composition of the gum showed the presence of protein (2.88%), crude fat (0.62%), ash (1.26%), fibre (0.89%) and moisture (10.35%) but all in very low amounts suggesting high purity of the extracted gum. Cassava and maize starches were used in combination with the isolated gum to investigate certain physicochemical properties. Pasting behaviour of starch/*B. eurycoma* gum system was probed from its viscosity profiles by means of the Rapid Viscoanalyser. Peak viscosity of the starch/gum system was reduced which was attributed to tighter granular architectural structure. The gum also reduced paste clarity in both cassava and maize starches but cassava starch exhibited more clarity than maize starch. The gum also exhibited better freeze-thaw stability in maize than in cassava starch measured by the amount of water lost during a freeze-thaw cycle called syneresis. These investigations somewhat clarify the role and potential usefulness of *B. eurycoma* gum in modifying texture and functionalities of starch-based food products especially those of cassava and maize starches.

Keywords: *Brachystegia eurycoma*, Cassava starch, Maize starch, Rapid viscoanalyzer, pasting properties, Gum

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MS. ID: PRL2014-IJCPS1937

PAPER-QR CODE

1. Introduction

Starch contributes greatly to the textural properties of many foods. Starch, both native as well as various modified derivative forms offer a great scope to develop a variety of food products having varied texture and mouth feel [1]. The need to improve upon these and more properties gives rise to starch modification especially with gums. Gums are substances that swell in water to form gels or sticky or slimy solutions. Most plant gums consist of large open flexible polysaccharide molecules; they occur in some algae as well as in higher plants [2]. Plant gums are often produced in specialized secretory cells and may be released in response to injury, forming hard glassy exudates [2]. Some of the gums occur as storage material in the endosperm of certain leguminous trees and shrubs [3]. Certain dicotyledon woods contain gum ducts resembling the resin ducts of conifers. Gums are produced in slimy masses in

cells of succulent Xerophytic plants to increase their water-holding power [3]. In *Brachystegia eurycoma* tree, the gums are found as storage material in the endosperm of the seeds. When the endosperms are in powdery form, they have the ability to swell in water and thus, are able to influence the viscosity of the liquid [4]. These polysaccharides are commercially important mainly for their thickening properties. The foam (volume and stability) and emulsification properties of *B. eurycoma* seeds have been reported and were found to be better than that of *Afzelia africana* seeds suggesting that *B. eurycoma* seeds would be more efficient in food systems requiring the formation of stable foams and emulsions [5]. *B. eurycoma* belongs to the family *Fabaceae* and is genus of tree of the sub-family *caesalpinioidae* that is native to tropical Africa. Trees of the genus are commonly known as miombo, and are the predominant tree in the miombo woodlands of Central and Southern Africa [6]**.** *B. eurycoma* is commonly found along the river banks in Western and Eastern Nigeria and also in Cameroon. It is a large tree with irregular and twisted spreading branches. The seed has a roundish flat shape with brown colour and hard hull. The fruit ripens from September to January and is released by an explosive mechanism [4].

In Nigeria, the main culinary use of gums from *B. eurycoma* is in thickening soups. In the preparation of most soups, thickeners are normally added in order to make the soup to be thicker [7]. They are known to cause increased viscosity in soups, giving it a more acceptable mouth feel. Outside this culinary use, it may be possible for the gum, when used as additives in other foods, to impact desirable textural and functional properties to the finished food products, particularly the "convenience foods" which contain one or more gums [4]. The antifungal properties of ethanol and water extracts of the bark of *B. eurycoma* have been reported and the water extract was found to be more active than its ethanol extract [8]. The stem exudates, seeds and stem bark of *B. eurycoma* have been reported to possess antioxidant, anti-inflammatory and antibacterial activities [9, 10, 11]. The exudates from the stem bark is used in faster healing of wounds and in right combination with mucin and honey is used for wound healing, prevention of bacterial infections, scar formation and promotes regeneration of hair follicle [12]. We report herein, gum isolation from the seeds of *B. eurycoma* and evaluation of its pasting property interactions with cassava and maize starches which include the assessment of viscosity changes, paste clarity and freeze-thaw stability of these starches modified in *B. eurycoma* gum solution aimed at developing a category of starches that would have better specific applications in food and beverage industries.

2. Materials and Methods

Plant Material

B. eurycoma seeds were bought from Umuahia market in Abia State, Nigeria. Clean seeds were selected and decoated by roasting in an open pan, and cooled immediately by the addition of water. The cracked coats were washed off with several changes of water to give clean seed endosperms which were softened by soaking in water for 24 hours at room temperature. They were however milled by means of a manually driven attrition mill and the flour obtained by passing it through a 500 µm sieve. The flour was then transferred into a clean polythene bag and stored properly.

Gum Extraction

The flour of *B. eurycoma* seed endosperms were defatted by extracting with hexane. 10 g of sample was dispersed in 250 ml distilled water and hydrated continuously by means of a stirrer for 2 hours. This was poured into centrifuge tubes and centrifuged at 250 rpm for 30 minutes. The supernatant was poured into a large beaker. The residue was reconstituted repeatedly with fresh distilled water, stirred and centrifuged again. The supernatant was pooled together and treated with isopropanol, when the gum spooled out; the clear liquor was decanted while the trapped solvent was removed by filtration. The crude gum was re-precipitated with isopropanol. The gum sample was dried in a convention oven at 60˚C overnight and cooled in desiccators. This was pulverized using a blender and stored in a sealed container. The gum preparation was carried out in triplicate. Gum yield was then determined.

Proximate Determination

Moisture and ash contents were determined by the method of Association of Official Analytical Chemists [13]. Protein was determined by the method described by HACH [14]. Crude fibre and fat were determined by the method of James [15].

Rapid Viscoanalyser Analysis

Rapid visconalyzer model Rva-3D was used which was connected to a computer IBM compatible, capable of running RVA control software. The instrument also consisted RVA canister, stirrer, balance that can weigh up to 0.01 g, adjustable dispenser or pipette to deliver 25 ml of water or buffer and a laboratory mill with screen in case sample grinding is required. The samples (starches) were milled to fine powder and moisture content determined. 3.0 g (on 100 % dry matter basis) of sample was weighed into the canister. The paddle was placed into the canister and the canister inserted into the instrument. The measurement cycle was initiated by depressing the motor tower of the instrument when the computer said 'press down the tower'. The canister was removed on completion of the test.

Paste Clarity Determination

Weights of 0.05, 0.1, 0.2, 0.3 and 0.4 g starches were separately introduced into different test tubes. 10 ml of distilled water was added into each and stirred with a glass rod properly to give dispersion. The test tubes were

clamped and immersed in a boiling water bath for 30 minutes while making sure the starch was continuously stirred throughout the heating period to prevent settling. They were removed at the end of 30 minutes, left for about 10 minutes to cool and then the percentage light transmittance was measured at wavelength of 660 nm base line calibration). The experiment was repeated with 10 ml of 0.2% *B. eurycoma* gum solution. Light transmittance of the 0.2% gum solution was also measured. The percentage light transmittance was plotted against starch concentration as shown in Figure 1.

Freeze-thaw Stability Determination

A weight of 0.5 g starch was introduced into a conical flask and 50 ml distilled water added. The starch was dispersed by mixing properly and then pasted in boiling water for 30 minutes (with stirring to prevent the starch from settling at the bottom during the heating period). The flask was brought down with continued stirring to prevent formation of skin during cooling. 10 ml of the starch paste was transferred into 3 pre-weighed centrifuge tubes, the tubes containing the pastes were weighed and weight of pastes obtained by difference. The tubes were put in a freezer for 18 hours (overnight), brought out and left to thaw at room temperature for 3 hours and thereafter centrifuged for 10 minutes at 2,500 rpm. The water separated was carefully decanted and the tubes still containing the paste were weighed again and weight of water separated was calculated by difference. The procedure was repeated using 0.2% gum solution of *B. eurycoma*. Freeze-thaw stability = 100(weight of water separated divided by weight of paste). Triplicate results were obtained.

Statistical Analysis

Analyses were replicated three times and standard deviations obtained.

3. Results and Discussion

Proximate Composition

The proximate composition of the gum isolated from the seeds of *B. eurycoma* is shown in Table 1. The moisture and ash contents were found to be 10.35% and 1.26% respectively. The moisture content may depend on the extent of drying and storing conditions. On the other hand, the low level of ash suggests high level of gum purity. The crude fat content of the gum was 0.62% which was suspected to be the residual fat content since the seed flour was defatted prior to gum extraction. The protein and dietary fibre contents were 2.88% and 0.89% respectively, also indicating high level of gum purity.

Table 1: **Proximate composition of *Brachystegia eurycoma* gum**

Proximate Composition (%)	*Brachystegia eurycoma* Gum
Protein	2.88±0.03
Fat	0.62±0.04
Ash	1.26±0.001
Fibre	0.89±0.006
Moisture	10.35±0.02

Data are means ± standard deviations of triplicate determinations

RVA Analysis

The RVA pasting properties of the starches in distilled water and in *B. eurycoma* gum solution are shown in Tables 2 and 3. Rapid Viscoanalyser gives information about pasting properties and characterization of a particular starch. RVA is based on viscosity measurement or changes with time. The pasting temperature is the temperature at which starch slurry changes due to melting of crystallite starch granule on heating. The time difference between the time of heating and peak viscosity is the peak time. The temperature at peak viscosity indicates the maximum swelling of starch granule or the point of highest viscosity. The viscosity profiles of the cassava and maize starches were changed by the presence of *B. eurycoma* seed gum. The results showed that the gum in the two starches, reduced peak viscosity and breakdown but increased pasting temperature with increased in gum concentration. Retardation of granule pasting and leaching of amylose seemed to be the cause of the reduction in peak viscosity. This viscosity change suggests that the starch granules became more resistant to thermal pasting and mechanical shearing in the presence of the gum.

Table.2 RVA pasting properties of cassava starch in *Brachystegia eurycoma* gum solutions of different concentrations

Gum Concentration (%)	Peak	Trough	Break Down	Final Viscosity	Set Back	Peak Time	Pasting Temp. (˚C)
0	346.08	222.75	123.33	289.00	66.25	5.20	78.40
0.1	343.58	220.00	123.58	285.67	65.67	5.27	78.45
0.25	334.33	211.58	122.75	274.83	63.25	5.20	78.55
0.5	251.83	220.08	31.75	309.25	89.17	5.27	87.35

Table.3 RVA pasting properties of maize starch in *Brachystegia eurycoma* gum solutions of different concentrations

Gum Concentration (%)	Peak	Trough	Break Down	Final Viscosity	Set Back	Peak Time	Pasting Temp. (˚C)
0	346.08	222.75	123.33	289.00	66.25	5.20	78.40
0.1	337.25	214.58	122.67	278.08	63.50	5.13	78.45
0.25	295.00	227.75	67.25	388.08	160.33	4.73	85.70
0.5	237.17	187.83	49.33	283.92	96.08	5.20	86.70

Paste clarity

The paste clarity values of cassava and maize starches in distilled water and in 0.2% *B. eurycoma* gum solution are shown in Tables 4 and 5. However, paste clarity of 0.2% *B. eurycoma* gum solution gave 36.5%. Paste clarity values vary considerably with the starch source, the amylose/amylopectin ratio, chemical or enzymatic modifications and addition of solutes. The starch granules swelling and brittleness would affect the paste clarity [16]. There was a progressive decrease in paste clarity of the two starches in the control (distilled water) and in 0.2% *B. eurycoma* gum solution. However, the decrease in paste clarity was more in the gum solution. This observation is not unconnected to the fact that the association between the starch and the gum molecules could involve the formation of difficult-to-swell starch granules. These modified starch granules remain dense, thus, reflecting the maximum of light entering the medium. Consequently, pastes appeared turbid or opaque in agreement with literature [16]. The plots of % transmittance versus starch concentrations in distilled water and in 0.2% *B. eurycoma* gum is shown in Figure 1. There was a gradual change in slope as starch concentration increased. Addition of *B. eurycoma* gum in small concentration of 0.2% conferred a profound effect on the paste clarity of cassava and maize starches. This gum introduction no doubt involved a significant interaction with the starch chains that could have led to modified structure which would reflect light in a significant way and consequently would induce a reduction in the transmitted light. Starch paste clarity is drastically reduced in the presence of *B. eurycoma* gum and is hereby suggested to be useful in spoonable salad dressing and other food processing applications where opacity is required.

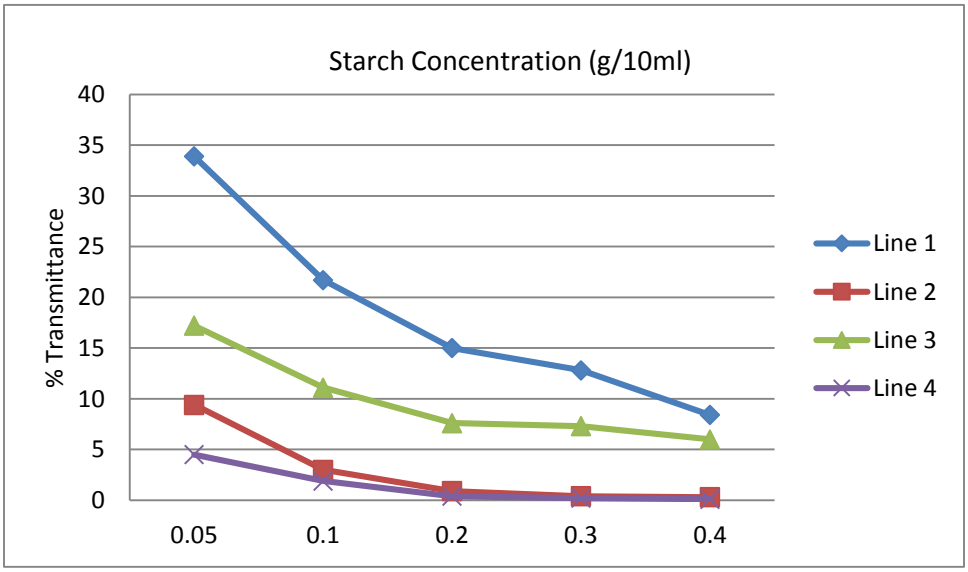

Fig.1 Plots of % transmittance versus cassava and maize starch concentrations in distilled water and in 0.2% *Brachystegia eurycoma* gum solution.

Line 1= cassava starch in distilled water; Line 2= cassava starch in gum solution

Line 3= maize starch in distilled water; Line 4= maize starch in gum solution.

Table 4: Paste clarity of cassava and maize starches in distilled water as control

Starch Concentration (g/10ml)	Paste Clarity (% Transmittance)	
	Cassava Starch	Maize Starch
0.05	33.9±0.02	9.4±0.01
0.1	21.7±0.01	3.0±0.002
0.2	15.0±0.03	0.9±0.001
0.3	12.8±0.02	0.4±0.001
0.4	8.4±0.02	0.3±0.001

Data are means ± standard deviations of triplicate determinations

Table 5: Paste clarity of cassava and maize starches in 0.2% *Brachystegia eurycoma* gum solution

Starch Concentration (g/10ml of 0.2% gum solution)	Paste Clarity (% Transmittance)	
	Cassava Starch	Maize Starch
0.05	17.2±0.01	4.5±0.005
0.1	11.1±0.02	1.9±0.03
0.2	7.6±0.01	0.4±0.03
0.3	7.3±0.005	0.2±0.002
0.4	6.0±0.01	0.1±0.001

Data are means ± standard deviations of triplicate determinations

Freeze-thaw stability

When a starch gel is frozen, starch-rich regions are created in the matrix, where water remains partially unfrozen. High solid concentration in the regions facilitates the starch chains to associate forming thick filaments, whereas water molecules coagulate into ice crystals forming a separate phase. Upon thawing, ice transforms to bulk phase water, which can be readily released from the polymeric network (syneresis) [17]. In the presence of *B. eurycoma* gum at 0.2%, syneresis of cassava starch reduced from 72% to 66% while that of maize starch reduced from 77% to 45%. It is evident from this result that *B. eurycoma* gum effectively and synergistically combined with cassava and maize starches to improve freeze-thaw stability. The addition of 0.2% solution of this gum perhaps retarded amylose retrogradation. The amylose chains leached during pasting were readily exposed to the hydrocolloid added, and the amylose would compete in the chain association between the hydrocolloid molecules and other amylose chains. The added gum had more effect on maize starch than on cassava starch. The difference could be because of the higher amylose proportion in maize starch than in cassava starch causing more leaching of amylose chains.

Table.6 Freeze-thaw stability of cassava and maize starches in distilled water and in 0.2% gum solution of *Brachystegia eurycoma*

Starches	Media	(%) Freeze-thaw
Cassava	Distilled water	71.891±1.33
cassava	Gum solution	65.626±0.42
Maize	Distilled water	77.070±0.64
Maize	Gum solution	45.038±1.51

Data are means ± standard deviations of triplicate determinations

4. Conclusion

Variations in the paste viscosity were used to study interactions between cassava and maize starches with *B. eurycoma* gum. When the temperature of the starches in the gum was raised, the gum increased the gelatinization temperature and reduced the peak viscosity of the starch granules as compared to the control without added gum. Peak viscosity of the starch/gum system was reduced which was attributed to tighter granular architectural structure. The gum also reduced paste clarity in both cassava and maize starches but cassava starch exhibited more clarity than maize starch which was traced to the higher amylose content of maize starch. The gum also exhibited better freeze-thaw stability in maize than in cassava starch measured by the amount of water lost during a freeze-thaw cycle. Expanded uses for starch may result from the emerging ability to modify starch structure and the relatively inexpensive character of the resulting products. This work clarified the role and potential usefulness of *B. eurycoma* gum in modifying texture and functionalities of starch-based food products with respect to cassava and maize starches.

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