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Isolation of gum from the seeds of *Delonix regia* and evaluation of its interactions with cassava and maize starches

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Abstract

Gum was isolated from the seeds of *Delonix regia* with excess cold isopropanol. Proximate composition of the gum showed the presence of protein (1.08%), crude fat (0.31%), ash (1.05%), fibre (0.37%) and moisture (10.42%) but all were in very low amounts suggesting high purity of the extracted gum. Cassava and maize starches were used in combination with gum to investigate certain physicochemical properties which included pasting viscosity changes, paste clarity and freeze-thaw stability. Pasting behaviour of starch/*D. regia* 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 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 called syneresis. These investigations somewhat clarify the role and potential usefulness of *D. regia* gum in modifying texture and functionalities of starch-based food products especially those of cassava and maize starches.

Key words: *Delonix regia*, Gum, Cassava starch, Maize starch, Pasting viscosity, Rapid viscoanalyzer.

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1. Introduction

The search for alternative novel sources of gums which are luxuriantly available but inconspicuous and consequently underutilized is crucial and invaluable. This is because many of the polysaccharides currently available as industrial gums were first used in an empirical way in domestic cookery. Obvious examples include pectin as the setting-agent in jam, carrageenan in milk desserts, and starch as a thickener in soups and sauces [1]. There are many others, however, that are not yet exploited commercially, but are used extensively in traditional local recipes, particularly in the less industrialized regions of the world. These gums usually come from plants that grow wild or are cultivated only on a very limited scale and their functional properties as food hydrocolloids remain largely unexplored [1]. *Delonix regia* gum is one of such gums. There exists published information on gums from plants other than *Delonix* species. Examples are galactomannans which are non-gelling polysaccharides also found in the seed endosperm of certain leguminous trees and shrubs. These polysaccharides consist of linear chain of (1,4)-linked β-D-mannose residues, substituted with C-D-galactose single units at the 0-6 position of the mannan main chain. The molecular weight, the mannose to galactose ratio (M/G) and the sequential distribution of the galactose substituents depend on their source [2].

Delonix regia also called flamboyant, flamboyant flame tree, flame of the forest, flame tree, etc, belongs to the family, *Fabaceae*. It originated from Madagascar, where it is now almost extinct but widespread in most tropical and subtropical areas of the world. The tree yields thick mucilage of water-soluble gum in yellowish or reddish-brown warty tears. The seeds contain gum as well, that may find use in textile and food industries [3]. The flowers are reputed to produce bee forage while large pods as well as the wood are used for fuel. The tree could provide timber. The bark has medicinal properties while the hard, elongated seeds are occasionally used as beads. *D. regia* is planted as a shade tree in dairy farms, tea plantations and compounds. It is mainly valued as a decorative tree, often being planted in avenues and gardens. It could also be planted as live fence posts (boundary or barrier or support) [3].

Efforts to improve the properties of native starches have focused on their low shear stress resistance, easy

thermal decomposition, high retrogradation and syneresis [4,5]. Native starches contain free hydroxyl groups in the 2,3 and 6 carbons of the glucose molecule, making them highly reactive. This allows them to be modified by different chemical treatments and thus regulate their properties [4,6]. The abundant hydroxyl groups on the starch molecules impart the characteristic hydrophilic properties. The polymer attracts water and is self-attractive through hydrogen bonding. The self-attraction and crystallization tendencies are most readily apparent for the amylose or straight-chain components [7,8]. Starches and gums are often used together in food systems to provide proper texture, control moisture and water mobility, improve overall product quality and/or stability, reduce costs, and/or facilitate processing [9]. In food and beverage preparations, starch is used as ingredient and provides texture to several foods. Thus, it can be used as thickeners, stabilizers, binders, adhesives, tonics, coagulants, gelling and forming agents, emulsions and foams stabilizers and water retention agents [10]. It is, therefore, important to understand interactions between starches and gums that are critical to the functionalities they impart to food products [9].

This research is concerned with the extraction of gum from the seeds of *Delonix regia* as well as investigating the physicochemical properties of the gum in association with cassava and maize starches in order to determine the trend in variation of the parameters analyzed for, which could show if this gum could be used as industrial gum on a large scale due to specific desirable functionalities.

2. Material and Methods

2.1. Plant Material

D. regia seeds were harvested from the tree plant at the University of Ibadan, Nigeria. Clean seeds were selected and dried in an oven at 45˚C for 24 hours for easy milling. The seeds were however milled by means of a hammer 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.

2.2. Gum Extraction

The flour of *D. regia* seeds 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.

2.3. Proximate Determination

Moisture and ash contents were determined by the method of Association of Official Analytical Chemists [11]. Protein was determined by the method described by HACH [12]. Crude fibre and fat were determined by the method of James [13].

2.4. 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.

2.5. 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 using distilled water in the reference cell (distilled water was used to get the base line calibration). The experiment was repeated with 10 ml of 0.2% *D. regia* 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.

2.6. 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 *Delonix regia*. Freeze-thaw stability = 100(weight of water separated divided by weight of paste). Triplicate results were obtained.

2.7. Statistical Analysis

Analyses were replicated three times and standard deviations obtained [14].

3. Results and Discussion

3.1. Proximate Composition

The proximate composition of the gum isolated from the seeds of D. regia is shown in Table 1. The moisture content was found to be 10.42%. The amount of moisture contained in the gum could depend on the conditions of drying, relative humidity as well as the condition in which it was stored. The ash content of the gum was 1.05%. The gum has low amount of ash suggesting that there was a low amount of the presence of inorganic constituents since the ash content is a measure of the presence of inorganic matter of the extract after the organic portion must have been burnt off [13]. The low value also shows that the gum has been extracted with a high level of purity. The ash content could among other factors depend on the mineral level of the soil and climate where the plant grows. A value of 0.31% for the crude fat only represented the residual fat content. This is because the seed flour was first defatted before the gum was extracted. The protein content was 1.08% while the dietary fibre content was 0.37%.These low values suggest that the gum was extracted with a high level of purity.

3.2. RVA Analysis

The rapid viscoanalyzer (RVA) can be used to assess the quality of any product where the cooked viscosity is important. The precise linear ramped heating and cooling abilities of the RVA, along with steady state temperature control, allow careful control of the cooking environment, whilst changes in viscosity are continuously recorded. The pasting properties of starch and starch-containing products are readily assessed in the RVA. During the test, the starch is gelatinized with consequent rise in viscosity, subject to high temperature and controlled shear during which its stability is revealed, and then cooled to provide an indication of setback. During gelation, samples can be assessed for pasting temperature, peak paste viscosity, peak time, temperature peak, hot and cold paste viscosity breakdown, set back and final viscosity. The RVA pasting properties of cassava and maize starches in distilled water and in D. regia gum solution are shown in Tables 2 and 3. The viscosity profiles of the cassava and maize starches were changed by the presence D. regia seed gum. The results showed that the gum in the two starches, reduced peak viscosity and breakdown but increased pasting temperature with increased 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 under the influence of the gum had tighter granular architecture and became more resistant to thermal pasting and mechanical shearing. The gum must have had a tendency to interact with the starch granules by way of competing for available water and prevented complete hydration, resulting in lower viscosity development.

3.3. Paste Clarity

The values of the paste clarity of cassava and maize starches in distilled water and in 0.2% *D. regia* gum solution are shown in Tables 4 and 5. These values depended mainly on the spectrophotometer type and starch concentration. Transmittances were improved by lower starch concentrations but were tremendously affected by *D. regia* gum. This observation is in agreement with a model proposed by Alphonse *et al*. [10] which explained that in a dilute and purified starch paste, the quasi totality of incident light passing through the media is transmitted, which made the medium clear. There was a progressive decrease in paste clarity of the two starches in distilled water. Distilled water was used as a control to study the effects of *D. regia* gum to the paste properties of cassava and maize starches. Also, in the presence of *D. regia* gum, the decrease became exponential. This observation could be as a result of the interaction or association of the gum molecules with the starch molecules. Starch paste degree of transmittance is directly affected by degree of swelling [15]. This association could involve suppressing starch swelling resulting in less transmittance of light. The light transmittance decreased with a weak slope as the starch concentration increased from 0.05 to 0.4 g (Figure 1). The reduction in transmittance with increase in the concentration of starch in the gum solution would be due to the effect of the gum on the amylose concentration giving rise to a more tightly granular structure that would show resistance to swelling causing opacity and more reflection of light thus reducing clarity. Although amylopectin can retrograde upon cooling, linear amylose molecules have a greater tendency to reassociate and form hydrogen bonds than the longer amylopectin molecules [16]. As the retrogradation process occurs, the starch paste becomes increasingly opaque. It has been reported that maize starch contains more amylose than cassava starch [16] and this explains why maize starch exhibited higher opacity than cassava starch and this opacity was shown to increase further in the presence of *D. Regia* gum. Paste clarity of 0.2% *Delonix regia* gum solution (without any starch) gave 73.6%.

3.4. Freeze-thaw Stability

The release of water by starch gel after some time is referred to as syneresis and is commonly found in food products such as sauces and dips that are formulated with unmodified amylose-containing starch [16]. In the presence of *D. regia* gum at 0.2% concentration, syneresis of cassava starch reduced from 72% to 68% while that of maize starch reduced from 77% to 61%. It is evident from this result that *D. regia* gum effectively and synergistically combined with cassava and maize starches to improve freeze-thaw stability. The hydrocolloid improved the water-holding capacity of the starches in aqueous system. It has been reported that the functions of hydrocolloid to starch, including the inhibition of retrogradation or the improvement of water-holding capacity for the starch system, should vary depending on the macromolecular characteristics of hydrocolloids [17]. Because the retrogradation process is amplified when heated starch is cooled, syneresis is most prevalent in refrigerated and frozen products [16]. Freeze-thaw stability, measured by the degree of syneresis, or water released, is an important consideration for food starches and should be kept in mind when refrigerated and frozen foods are formulated.

4. Conclusion

Food and technological applications have encouraged investigations on mixed systems particularly those involving gums and starches. The addition of *D. regia* gum to cassava and maize starches at a low concentration of 0.2%, caused a significant decrease in peak viscosity and

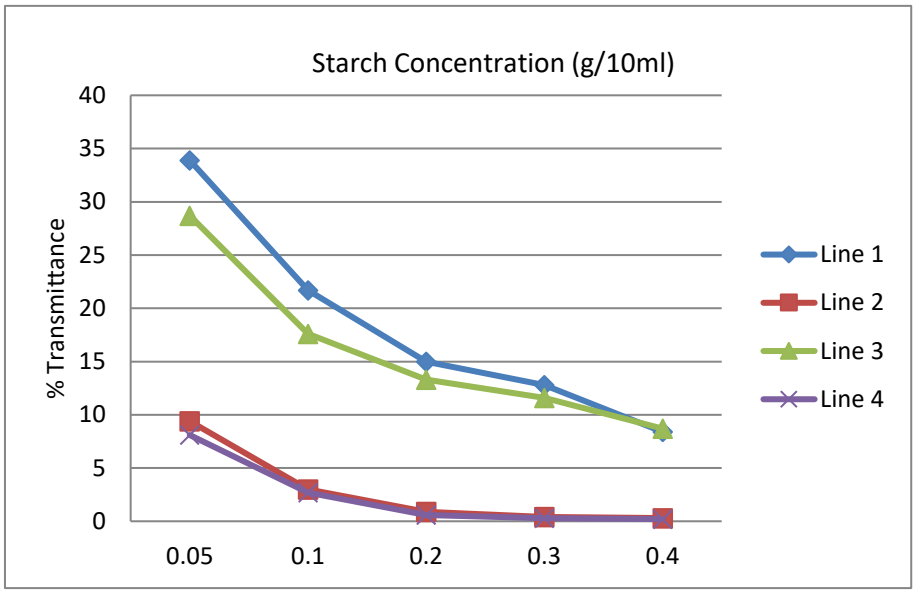

Fig. 1: Plots of % transmittance versus cassava and maize starch concentrations in distilled water and in 0.2% *Delonix regia* gum solution

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 1: Proximate composition of *Delonix regia* gum

Proximate Composition (%)	*Delonix regia* Gum
Protein	1.08±0.02
Fat	0.31±0.01
Ash	1.05±0.005
Fibre	0.37±0.03
Moisture	10.42±0.04

Data are means ± standard deviations of triplicate determinations

Table 2: RVA pasting properties of cassava starch in *Delonix regia* gum solution 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	278.67	199.83	78.83	391.58	191.75	5.00	85.80
0.25	195.75	156.67	39.08	246.83	90.17	5.27	89.80
0.5	194.25	148.75	45.50	237.58	88.83	5.00	87.25

Table 3: RVA pasting properties of maize starch in *Delonix regia* gum solution 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	220.92	196.58	24.33	290.75	94.17	5.53	89.05
0.25	206.75	174.75	32.00	265.33	90.58	5.20	87.20
0.5	189.83	158.92	30.92	245.67	86.75	5.27	88.30

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±0.5	9.4±0.05
0.1	21.7±0.2	3.0±0.02
0.2	15.0±0.2	0.9±0.005
0.3	12.8±0.02	0.4±0.002
0.4	8.4±0.1	0.3±0.001

Data are means ± standard deviations of triplicate determinations

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

Starch Concentration (g/10ml of 0.2% gum solution)	Paste Clarity (% Transmittance)	
	Cassava Starch	Maize Starch
0.05	28.7±0.1	8.1±0.01
0.1	17.6±0.005	2.7±0.03
0.2	13.3±0.1	0.6±0.01
0.3	11.6±0.02	0.3±0.005
0.4	8.7±0.03	0.2±0.005

Data are means ± standard deviations of triplicate determinations

Table 6: Freeze-thaw stability of cassava and maize starches in distilled water and in 0.2% gum solution of *Delonix regia*

Starches	Media	(%) Freeze-thaw
Cassava	Distilled water	71.891±1.33
Cassava	Gum solution	68.386±0.64
Maize	Distilled water	77.070±0.64
Maize	Gum solution	60.779±2.30

Data are means ± standard deviations of triplicate determinations

paste clarity and improved freeze-thaw stability of the starches. The gum was effective in stabilizing the starches against one freeze-thaw treatment. The gum also reduced peak viscosity through strong network formation with starch granules. The stabilizing effect of the gum on the starches and reduction in paste clarity resulted from the interactions of the gum with starch chains and with water in the system. Proximate compositions of the gum were very low suggesting high purity of the extracted gum. The objective of the starch modification with *D. Regia* gum has been realized which was to alter the physical and (maybe) chemical characteristics of the native cassava and maize starches to improve their functional characteristics for specific food and industrial application.

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