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Facile formation of caramel colours using the polysaccharide material that is extracted from the fruit of *Azanza garckeana*

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Abstract

The mucilage from *Azanza garckeana* fruit was extracted with water and the extract heated at 130°C in the presence of ammonium salts. When the mucilage, composed of galactose, glucose, arabinose and rhamnose units, was heated, a brown colour was formed. When water was not allowed to evaporate off, the extent of colour formation depended on the initial concentration of the mucilage. The amount of soluble colour that was initially formed decreased as the viscous mass was transformed into a friable insoluble mass. The presence of ammonium salts and pH had only a small effect on the development of colour.

1. Introduction

Caramel colours, the most widely used food colours, are brown powders or viscous liquids that are used to impart yellow to dark brown hues to carbonated beverages, distilled liquors, pharmaceutical flavouring extracts, candies, soups and bakery products (Chappel & Howell, 1992; Light et al., 1992). These ill-defined colours may be produced by controlled heating of sugars at up to 400°C until 10 to 15% of the initial mass is lost. Although glucose and fructose caramelise most readily, cane sugar, lactose, malt, starch hydrolysates, honey and molasses have been used to prepare caramel colours. Most caramels, however, are being prepared from corn syrup.

In the production of caramels from the usual carbohydrate materials, the formation of colour depends on the nature of the starting material, its concentration, temperature, pH and the nature of catalysts that may be used. In general, the formation of caramel involves condensation reactions in which water molecules are lost. Although, there is great variation in the nature of caramel colours, depending on the type of carbohydrate that is used and the experimental conditions, it appears that the brown colour arises from Maillard and caramelization reactions with both reactions producing high molecular weight aldehydes and dicarbonyl compounds. The degradation of carbohydrates involves a number of competing and consecutive reactions that are accelerated at high temperatures and are catalysed by acids and bases. Caramelization reactions include changes in the ring size of monosaccharides, breakage and reformation of glycosidic bonds, dehydration, introduction of double bonds and the formation of anhydro rings. The formation of colour could also arise from oxidation reactions that involve ascorbic acid (Greenshields & Macgillivray, 1972).

In general, the formation of caramel involves condensation reactions in which water molecules are lost. Caramel colours may be prepared from sugars in the presence of acids, alkali, salts, sulphite compounds or nitrogenous materials. The reactants may need to be heated under pressure for maximum formation of the desired coloured compounds.

Azanza garckeana is a small tree that grows to about 12 m in height in central Africa. In Zimbabwe, the tree is found in large numbers in the low rainfall areas in the west of the country.

The tree bears fruit, about the size of a peach, which turns from green to dark brown upon ripening between May and October. The ripe fruit bears yellowish hairs that contribute to the overall colour of the ripe fruit. Upon ripening the fruit splits into five segments, and a brown resinous material may be exuded from the segments. The fruit of *A. garckeana* is widely consumed in Southern Africa. The segments of the ripe fruit are

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worked apart, the hair covered seeds removed and individual segments chewed. During chewing, the sweet resinous mass is separated from a cellulosic mass that is discarded. When in season, the fruit is available in large quantities even in urban centres.

Care is required to ensure that fruit are properly ripe before consumption. The consumption of unripe fruit can lead to inability to control the exit of a slimy mass through the anus, a probable indication that the polysaccharide in the unripe fruit is not susceptible to attack by enzymes of the human digestive tract.

When attempting to purify the polysaccharide from *Azanza* fruit, we observed that it was not possible to dry the material by the usual procedures. Attempts to dry ethanol precipitates of extracts from *Azanza* fruit at 100°C easily resulted in the formation of a brown mass that produced golden colours when suspended in water. The formation of a caramel colour from extracts of the fruit of *A. garckeana*, under relatively mild conditions, is now reported.

2. Materials and methods

2.1. Preparation of the polysaccharide material

Ripe sound fruit from *A. garckeana* were split into the component segments and the seeds removed. Loose materials and infesting insects or their remains were removed before the fruit were steeped in tap water containing 0.5% benzoic acid as a preservative. Enough liquid was added to cover the fruit completely and the submerged fruit left at 4°C for up to 72 h. Extraction of polysaccharide material was encouraged by stirring the viscous mixture periodically. The mixture was poured over cheese cloth in order to separate the viscous solution from the fruit segments and solid debris.

A 50% stock solution was prepared by heating the extracts over a boiling water bath. The concentration of the solutions was determined by heating samples to dryness in an oven set at 100°C. The stock solution was used to prepare solutions of the desired concentrations.

2.2. Identification of component monosaccharides

Dried mucilage (100 mg) was added to 10% sulphuric acid (20 ml) in a round-bottomed flask and the mixture refluxed for 3 h. After refluxing, the sample was cooled to room temperature and neutralized with barium carbonate. The neutral sample was centrifuged at 20 000 rpm for 10 min in a BHG Hermle ZK-401 centrifuge to remove precipitated barium sulphate. The clear supernatant was separated and the water evaporated off in a rotary evaporator at 50°C until a syrup was obtained.

The syrup was dissolved in 40% methanol (10 ml) and stored at 4°C until required for analysis.

To identify the monosaccharides, the hydrolysed samples were analyzed by thin-layer chromatography on Whatman K5 silica gel plates previously impregnated with NaH_2PO_4. The plates were developed with ethyl acetate:pyridine:water (10:4:3). Developed plates were allowed to dry at room temperature and sprayed with diphenylamine reagent (Chaplin & Kennedy, 1986). Sprayed plates were air-dried and then heated in an oven set at 100°C for 10 min after which coloured spots appeared.

2.3. Determination of polysaccharide concentration

The concentration of the extracted material was determined by weighing solutions of the extract into weighed stainless steel dishes and evaporating off the water at 100°C. It was assumed that any chemical changes that might take place in the sample at this stage would have little effect on the mass. In the later stages of the work, dry samples of the mucilage were obtained by lyophilization in a Christ Alpha 2-4 freeze-drier.

2.4. Preparation of the caramel colour

Solutions of suitable concentration were dispensed into 2.5 ml vials, which were placed in a VWR Scientific heating block set at 130°C. Vials, containing solutions at each of the concentrations used, were left at room temperature as controls.

After the desired time of heating, distilled water was added to make up to the original volume. Samples were removed from the heating block and cooled to room temperature. The formation of caramel colour was monitored by measuring the absorbance of the samples at 290 nm in a Shimadzu UV/Vis spectrophotometer. Where necessary, the solutions were diluted before the measurement of absorbance.

To prepare large quantities of caramel extracts of *Azanza* fruit were heated in a stainless steel pot. Samples were removed periodically in order to assess the formation of caramel colour.

2.5. Monitoring of the formation of caramel colour

In order to monitor the progress of the formation of colour in the mass of mucilage that was being heated, a small sample of the caramelizing mass was removed, cooled to room temperature and suspended in water and, if possible, dissolved to make a 2% solution. To the solution, two volumes of ethanol were added and the mixture stirred thoroughly.

If the conversion of the polysaccharide to caramel was not complete, a precipitate formed upon the addition of ethanol. When no precipitate formed upon the addition of ethanol, the formation of a soluble caramel was regarded as complete. When, upon suspending a

portion of the caramelizing mixture in water, only part of the mass dissolved, the insoluble fraction was taken to be the insoluble form of *Azanza* caramel.

Spectra of preparations of caramelized *Azanza* mucilage were dissolved in suitable amounts of water and the spectra between 200 and 700 rim recorded using a Shimadzu UV-visible spectrophotometer. The spectrum of a sample of positive caramel from the Zimbabwe Sugar Refinery, Harare, Zimbabwe, was obtained for comparison.

2.6. Qualitative test for caramel

Testing for caramel was done using the Mathers test of the AOAC (1990). The method involved mixing solutions of the putative caramel with pectin and adding ethanol to precipitate a brown gelatinous material. When the precipitate, after being dissolved in water and precipitated with ethanol three times, was dissolved in water, a brown solution resulted. The presence of caramel was confirmed by adding 2,4-dinitrophenylhydrazine in sulphuric acid to the above solution upon which a brown fibrous non-gelatinous precipitate was formed.

2.7. Estimation of protein

Protein was determined by determining the total organic nitrogen in the caramel samples (2 g) using the Kjeldahl method. Protein content was obtained by multiplying the nitrogen content by a factor of 6.25.

2.8. Determination of moisture and ash content

Moisture content was determined by measuring the loss in weight when solid samples of caramel preparation (1.5 g) were heated at 100°C.

To determine ash, the sample used for the measurement of moisture was first charred at 200°C and then heated in a muffle furnace at 550°C for 5 hours or until there were no black particles and grey patches in the ash sample (Allen, 1989).

2.9. Effect of concentration of the polysaccharide on the formation of colour

The stock solution was diluted with distilled water to provide solutions of concentrations up to 10%. Solutions were dispensed into vials and heated as described above.

2.10. Effect of ammonium salts on the formation of colour

Solid ammonium salts were added to solutions of the polysaccharide to achieve the desired salt concentration. The solutions were dispensed into vials and heated as described above.

3. Results and discussion

When *Azanza* fruit were left immersed in water, the mixture became viscous as the polymer was extracted into the water to form a pale yellow liquid. The concentration of the polysaccharides in the extracts at the different stages of extraction is shown in Fig. 1. It was decided that after the fourth extraction, the amount of mucilage that was recovered in further extractions was not enough to justify the amount of work involved. If the polysaccharide extract of *Azanza* is to be commercially exploited, it will be necessary to optimize the method of extracting the polymer from the fruit. An advantage of the method of extraction that was adopted in this study is that the extract was clean, contaminated only by the loose particles that remained on the surface of the fruit after cleaning.

Some of the properties of the caramelized material that resulted when the mucilage from *A. garckeana* was heated are shown in Table 1. When samples of the dried extract were hydrolysed in acid, the sugars that are shown in Table 2 were identified. More work will be done in order to characterize the mucilage further.

The resinous material that exudes from *Azanza* fruit darkens with time even within the fruit itself so that very ripe fruit will appear almost black. It is evident that at least some of the reactions that are involved in the for-

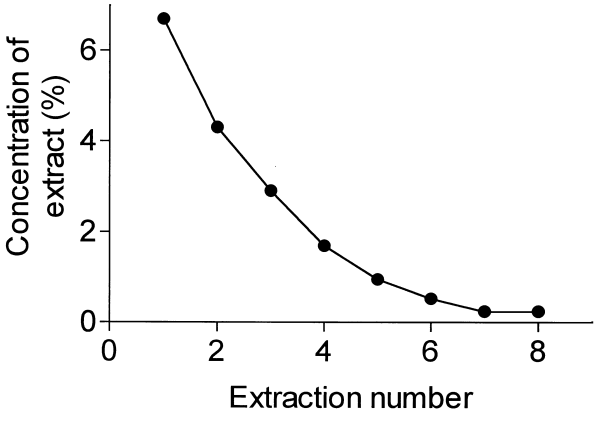

Fig. 1. Concentration of mucilage in extracts *A. garckeana* fruit during repeated extractions.

Table 1
Some properties of the caramelized material prepared from the polysaccharide extract of the fruit of *A. garckeana*

Moisture (%)	5.6
Protein (%)	0.26
Ash (%)	7.2
pH of 1% solution	4.8

Table 2
Monosaccharides that were identified in hydrolysates of the polysaccharide isolated from *A. garckeana*

Monosaccharide	R_f value for	
	Standard	Sample spot
Galactose	0.14	0.14
Glucose	0.2	0.20
Arabinose	0.27	0.28
Rhamnose	0.62	0.63

mation of brown coloured compounds from the mucilage of *Azanza* fruit begin during the ripening process.

The aqueous extract from ripe *A. garckeana* fruit has a very pale colour of honey. When the extract was heated to evaporate the water, the preparation gradually turned into a sticky viscous brown mass. Continued heating produced,a hard cake that eventually turned into a friable coffee coloured mass that was only slightly soluble in water. The maximum production of soluble caramel colour seems to occur just before the formation of the friable product.

It is likely that the formation of caramel colour from the polysaccharide extract of *A. garckeana* fruit follows other caramelization reactions. Under non-oxidative conditions, reactions that lead to the formation of colour by sugars depend on the presence of reducible carbonyl groups. In the case of polysaccharides, the quantity of the reducing forms of sugars is generally low but is regenerated as the carbonyl groups, blocked in acetal linkages are released by hydrolysis during the reaction. We postulate that the regeneration of carbonyl groups that are in acetal links, and from ring forms of monosaccharides, is easier than with other caramelizable carbohydrates. As the extract was heated and the brown colour developed, the proportion of extract that was precipitable with ethanol gradually decreased until no precipitate was formed at all. Continued heating eventually resulted in a mass that was practically insoluble. We also postulate that at the point when there was no material that was precipitable with ethanol, the polysaccharides would have been completely hydrolysed with only monosaccharides and their derivatives remaining. The original polysaccharide would have been destroyed. The insoluble mass that was finally formed probably represents a highly polymerized product in which only a small proportion of soluble low molecular weight species remains.

An example of the spectrum of a solution of the caramel colour from *Azanza* is shown in Fig. 2. Although the spectra of different batches showed some differences, most had absorption maxima between 270 and 300 nm. The properties of a given batch of colour would be expected to be influenced by the nature of the starting material and the conditions of processing.

When solutions of suitable concentrations of the polysaccharide from *Azanza* fruit were heated, a brown

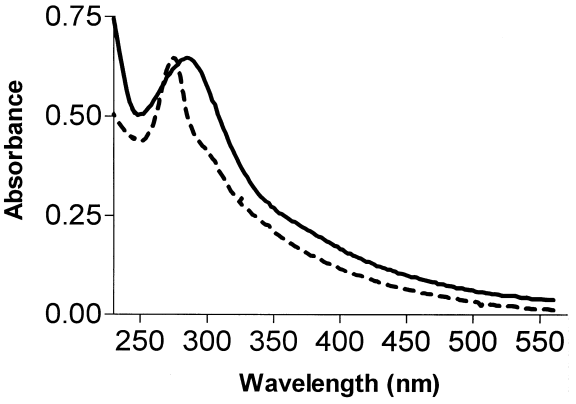

Fig. 2. Spectrum of a batch of caramel colour prepared from the polysaccharide extracted from *A. garckeana* fruit (–) and a commercial positive caramel (- - -).

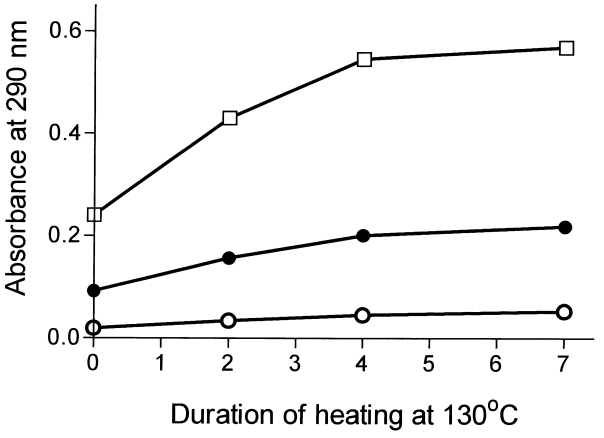

Fig. 3. Formation of colour when 1% (○), 5% (●), and 10% (■) solutions of *A. garckeana* mucilage were heated at 130°C.

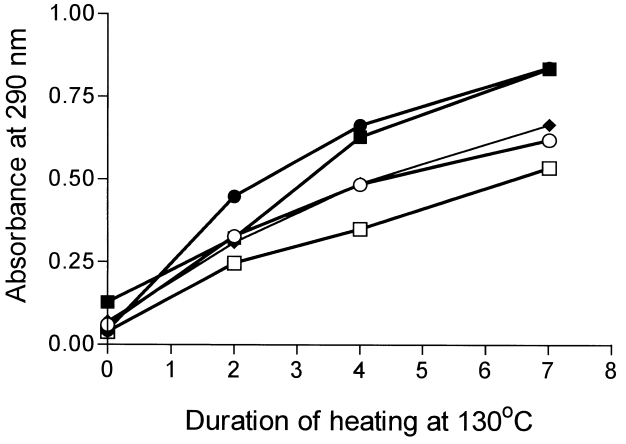

Fig. 4. Formation of colour in a 25% solution of *A. garckeana* mucilage containing ammonium acetate (○), ammonium chloride (●), ammonium carbonate (□) and ammonium sulphate (■) at 0.25% concentration at pH 4.01 during heating at 130°C. In the control (◆), no ammonium salt was added.

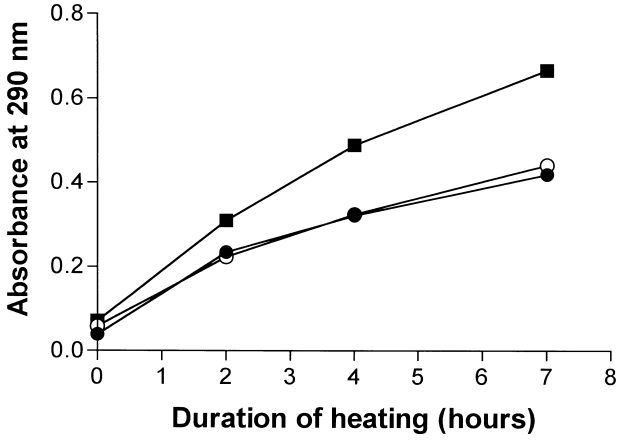

Fig. 5. Formation of colour in a 25% solution of *A. garckeana* mucilage at pH 4 (■), pH 7 (○), and pH 9 (●) during heating at 130°C.

colour was formed. In closed vials, where water was not allowed to evaporate off, the formation of colour depended on the initial concentration of the extract. As shown in Fig. 3, practically no colour formation was observed when solutions of 1% concentration were heated. When concentrations of the polysaccharide were increased to 5 and 10%, there was a corresponding increase in the formation of colour.

The formation of caramel colour from the polysaccharide extract of *Azanza* fruit in the presence of ammonium salts at pH 4 is shown in Fig. 4. In the presence of ammonium acetate and ammonium carbonate, the development of a brown colour was less than in the control. As shown in the same figure, the presence of ammonium chloride and ammonium sulphate resulted in some improvement in the development of colour.

As shown in Fig. 5, the development of colour at pH 4 was greater than that at pH 7 and pH 9. Low pH appeared to enhance the caramelization of the polysaccharide extract from *Azanza*.

Nitrogen compounds have been shown to enhance the formation of caramel colours in many situations. The observation that presence of ammonium salts had only a small effect on the formation of the caramel colour that is produced by *Azanza* extract does not necessarily indicate that the formation of colour was not influenced by nitrogen compounds. Instead, the observation may reflect the presence of endogenous nitrogenous material, including protein, that enhances colour formation and thus would render additional nitrogen compounds ineffectual.

We believe that the facile formation of a caramel colour by the extract of *A. garckeana* provides an opportunity to use the material as a renewable resource in the production of a product that can be used by modern technology. In preliminary investigations, we have shown that the soluble caramelized material can be used to impart colours ranging from pale golden to dark brown to liquids and semisolids. The insoluble material can be used to impart shades of brown colours to powders.

Acknowledgements

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