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An enzymatic method for assessing the degree of gelatinisation in plantain (*musa AAB*) foods *in situ*

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ABSTRACT

Gelatinisation temperature in excess moisture was determined on plantain starches and flours and the degree of starch gelatinisation in whole cooked foods was deduced. The gelatinisation temperature of plantain starch was determined using an enzymatic method (with confirmation from scanning electron microscopy). Starch gelatinisation temperature for plantain starch was observed at 75 ± 1 °C $n=3$, while it was 80 ± 1 °C, $n=3$, for starch in the flour. The enzymatic digestion method was then applied for the determination of the *in vitro* degree of starch digestion in ripe and unripe flours of plantain (*in situ*) at different treatment temperatures, and an attempt was made to deduce the equivalent treatment temperatures of cooked foods from the flours using three equations. One-way anova statistical analysis was used to determine the equation that gave the best fit, which was subsequently used to estimate values for the degree of starch gelatinisation in some cooked plantain foods. Treatment temperatures extrapolated from the equation of best fit ranged between 73.2 ± 0.3–79.5 ± 0.2 °C and 67.8 ± 0.5–80.1 ± 1.3 °C for ripe and unripe plantain foods respectively. Processing conditions are therefore important in preserving the nutritional quality of starch in plantain.

1. Introduction

Unripe plantain in its raw/uncooked form contains about 61 g/100 g native resistant starch respectively (Englyst & Cummings, 1985; Ovando-Martinez, Sayago-Ayerdi, Agama-Acevedo, Goni, & Bello-Perez, 2009; Pelissari, Andrade-Mahecha, Sobral, & Menegalli, 2012), while ripe plantain in its raw/uncooked forms contain about 32 g/100 g the resistant starch (Oladele E, unpublished work). Native resistant starches suffer poor enzyme digestibility but this quality is lost once they are gelatinised and they become easily and rapidly digested (Englyst, Kingman, & Cummings, 1992). When starch granules are heated in the presence of water, an order-to-disorder phase transition, referred to as gelatinisation, occurs. The determination of gelatinisation temperature and extent of starch gelatinisation becomes very important because most foods are usually processed before consumption. In the past, gelatinisation has been considered as affecting product quality through changes in physicochemical properties such as texture, viscosity, etc.; however, with the increasing incidence of diet-related health conditions such as diabetes and obesity, it has become important to consider the health implications of processing starch. The nutritional properties of starch are, to a large extent, related to its availability for digestion and/absorption in the gastrointestinal tract, hence starch bioavailability is also very much dependent on processing conditions (Qiang, 2005).

A considerable amount of work has been done on starch gelatinisation temperature as earlier reviewed by Shyam (2009), but it is not certain if this can be applied to domestic cooking which does not involve the use of pure starches or flours, but whole foods. In some studies, the degree of digestion had been determined but this has not been linked to the degree of gelatinisation (Baks, Ngene, Van Soest, Janssen, & Boom, 2007; Liu & Han, 2012). The fact that gelatinised starch is susceptible to enzyme hydrolysis has been a basis for the study of the gelatinisation process and proves to be one of the most sensitive methods for the measurement of this parameter, (Shyam, 2009; Tester & Sommerville, 2001). In this work, this principle has been exploited for the prediction of the degree of gelatinisation in some cooked plantain foods.

Domestic cooking temperatures by many individuals are not usually regulated so as to preserve food nutrients, because the need to satisfy hunger is sometimes given priority over the nutritional values that can be derived from the meal. The ability to introduce a system where domestic cooking can be optimised by effective monitoring of food processing temperatures to preserve the nutritional qualities of starch will be of great benefit to all. This is much more relevant in an age where there are a lot of campaigns regarding the consumption of more healthy foods.

Although it is known that most resistant starches do not

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survive hydrothermal treatment, especially under high moisture and high temperature treatments, it is not certain if some or all domestic cooking processes used for some foods actually retain significant quantities of resistant starch. This is because in domestic cooking it is quite difficult to ascertain the starch concentration of the sample/food. The determination of the degree of starch gelatinisation will help to maintain good levels of resistant starch in processed foods. Zhang, Whistler, Bemiller, & Hamaker (2005), indicated that a lot of questions still remain unanswered regarding cooked banana starch (in that review, cooked banana starch referred to starches of the *musa* family), especially regarding its digestion properties. The fate of starch in domestically processed plantain is therefore the focus of this work.

2. Materials and methods

2.1. Materials

Soluble starch from potato (product number: S2004, CAS-number: 9005-25-8), amylose from potato starch (product number: A05121-1G, CAS-number 9005-82-7, containing $\leq$10% water and $\leq$2% butanol as impurities) were purchased from Sigma Aldrich, UK. The enzymes used were pancreatin from porcine pancreas (EC 232-468-9, Sigma Cat. no. P-7545), with an activity of 8 $\times$ USP; (amylase activity $\geq$ 200 USP units/mg, lipase activity $\geq$ 16 USP units/mg and protease activity $\geq$ 200 USP units/mg); amyloglucosidase from Aspergillus niger (EC 3.2.1.3, Megazyme E-AMGDF, specific activity = 3260 U/ml on soluble starch; 1U = 1 μmol/min) from Megazyme international, Ireland. Glucose oxidase analysis kit was also purchased from Megazyme international, Ireland.

2.2. Sample preparation

Cultivars of ripe and unripe plantains from Columbia were purchased from Leeds city market. Unripe plantains selected were full green (stage 2) while ripe plantains used were in the fully ripe stage (yellow) in colour (stage 6) on the colour index scale (Aurore, Parfait, & Fahrasmane, 2009). The samples were cut into thin slices of about 2 mm thickness, freeze dried, blended into a fine flour and stored in clean plastic containers at ambient temperature. Starch was isolated by eliminating pigments and sugars by extraction and centrifugation with 50% ethanol/water mixture until the colour and sugars were removed (complete removal of sugars was ascertained by testing the supernatant for sugars using phenol-sulphuric acid method) (Fournier, 2001). Aqueous starch slurry (about 5% starch) was prepared from the residue and filtered through a 100 μm aperture sieve to remove fibre and other non-starch particles. The starch obtained was rinsed several times with water and subsequent sedimentation to obtain starch slurry that was subsequently freeze-dried to less than 1% moisture content and stored in plastic containers at room temperature for further analysis. The percentage purity and yield of the starch respectively was 90 $\pm$ 1.1% and 50 $\pm$ 3.2% for unripe plantain and 70 $\pm$ 2.2% and 21 $\pm$ 3.1 for ripe plantain; n = 3, respectively. The percentage yield for the starch was determined using the equation below while the percentage purity was obtained by determining the total starch content of the starch using the megazyme international total starch analysis kit.

$$\%\ \text{yield} = \frac{\text{weight of freeze dried starch isolate}}{\text{initial weight of flour}} \times 100$$

2.3. Domestically prepared foods

2.3.1. Boiling

One finger of plantain (ripe or unripe) was cut into five portions and cooked in 750 ml boiling water (ordinary/pre-salted by addition of 3 g of table salt). One finger each was removed from the cooking pot at 3, 6, 10, 15 and 20 min, cut into thin slices on petridishes and quenched in liquid nitrogen. Samples were then stored in the −80 °C freezer and freeze-dried, blended and sieved to obtain fine powder and stored in plastic containers for the digestion experiment.

2.3.2. Grilling

150 g of sample was grilled at high/medium heat in a grill for 30 min.

For each cooking experiment, 3 fingers were used and the experiment was repeated three times.

2.4. Heat treatment

500 mg starch/flour was treated with 15 ml de-ionised water and vortex-mixed for 5 min to produce a starch suspension. This was subsequently incubated in a shaking water bath for 30 min at temperatures ranging from 40 °C to 100 °C. After incubation, the sample tube was immediately transferred to another water bath maintained at 37 °C to prevent retrogradation from occurring. The tubes were allowed to equilibrate for about 15 min before commencement of the digestion process.

2.5. In vitro starch digestion

The procedure of Englyst et al. (1992) was used for *in vitro* starch digestion and subsequent the estimation of RDS, SDS and RS. Briefly, for 10 analysis tubes, 10 g of pancreatin was mixed with 60 ml deionised water and stirred on a magnetic stirrer for 10 min, the resulting suspension was subsequently centrifuged at 1500*g* for 10 min at 20 °C; 45 ml of the supernatant was taken and mixed with 5 ml amyloglucosidase. 5 ml of the mixture was used for the digestion. Incubation of sample with pancreatin and amyloglucosidase was done at pH 7 and 37 °C in capped tubes immersed horizontally in a shaking water bath. At all times a sample blank was prepared in duplicate. Aliquots were taken at 20 min and measured as the glucose released from the food at 20 min (G_{20}), of enzyme incubation and at 120 min (G_{120}) as glucose released at 120 min of enzyme incubation. For cooked foods and flours, an aliquot was taken before digestion as the free glucose (FG) after 30 min incubation at 37 °C. A value for total glucose (TG) was obtained by heating the starch/flour suspension at 100 °C and subsequent digestion at 37 °C with pancreatin amyloglucosidase for 120 min. Glucose was determined using the glucose oxidase analysis kit.

2.6. Determination of leached amylose

The quantity of amylose leached during heat treatment of starches has been used for monitoring gelatinisation for over three decades. Amylose chains released during starch gelatinisation can be measured as dissolved amylose which form a blue–black complex with iodine (Baks et al., 2007; Shyam, 2009). Total amylose was determined by the method of (Hoover & Ratnayake, 2001). Leached amylose was determined on heat treated samples at each temperature considered. A 0.2 ml aliquot of heat-treated sample suspension was taken in 2.67 ml water and centrifuged. Then 500 μl of I_2/KI reagent (0.5395 g KI plus 0.1575 g Iodine in 500 ml of water) was added to 250 μl aliquot of the supernatant in 2.5 ml water. Tubes were allowed to stand for 30 min in the dark