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Original Research Article

Effect of maturity on in vitro starch digestibility of Saba banana [*Musa 'saba' (Musa acuminata x Musa balbisiana)*]

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ABSTRACT

Saba banana [*Musa 'saba'(Musa acuminata x Musa balbisiana)*] is the most popular among the many banana cultivars in the Philippines in terms of production and trade because of its wide range of applications in the local food industry. It is one of the important food sources not only as a raw material for essentially starch-based products but also as an alternative food staple to rice in rural areas. The study involving simulated *in vitro* gastrointestinal digestion model was conducted to evaluate the effect of maturity on the digestibility of starch in Saba banana. The stage of ripeness of the pulp was determined using peel color (L*, Chroma, and hue) index, which was divided into 5 stages, 1, all green; 2, green but turning yellow; 3, greenish yellow; 4, yellow with green tips; and 5, yellow with brown flecks. Changes in the physicochemical properties such as moisture, resistant starch, total starch, and sugar (sucrose, glucose, and fructose) contents were also examined at different stages. During the ripening process, a decrease in the total starch was observed. More than 80% of resistant starch was degraded from stage 1 to stage 5 with a concomitant formation of sugars. This breakdown of starch preceded the increase in moisture content due to osmotic transfer. Among the stages, the highest starch hydrolysis (%) was determined in stage 1 after 4 hours of simulated intestinal digestion. Results showed that the variations on the physicochemical properties of different maturity stages may account for the decreasing rate of starch hydrolysis. These findings suggested that the maturity stages of Saba banana may influence the rate at which starch is digested due to the physicochemical changes accompanying maturation.

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INTRODUCTION

The degree of maturity and ripeness of fruits are of utmost importance in determining its suitability for processing since the quality characteristics of end products are dependent on the quality of horticultural produce (Singhal et al., 1997). Underlying the various noticeable physical changes during postharvest ripening are a series of biochemical processes in which the chemical composition of the unripe fruit is transformed (Rhodes, 1978). One reliable technique used to determine maturity at harvest in fruits is the measurement of starch content. Extensive research studies about the hydrolysis of starch during ripening were reported by Marriott et al. (1981), Cordenunsi and Lajolo (1995), Lustre et al. (1976), and Agravante et al. (1990). Banana, with its innumerable varieties, is the usual climacteric fruit when starch reserves accumulation and degradation during maturation and ripening, respectively, are particularly investigated.

The 'Saba' banana [*Musa 'saba'(Musa acuminata x Musa balbisiana)*] is one of the cultivars known to grow in Southeast Asia which has a potential to be a commodity starch. Other names of the fruit are giant *pisang kepok* (Indonesia), *pisang abu nepah* (Malaysia), *kluai hin* (Thailand), *chuoi mat* (Vietnam), and *cardaba* (Philippines) (Lim, 2012). In the Philippine food industry, this variety is the most popular among the many banana cultivars in terms of production and trade because of its wide range of applications in the local market. It is one of the important food sources in rural areas where it is often used as an alternative food staple to rice. Moreover, several products such as chips, ketchup, sauces, and sweetened boiled/fried banana can be made using Saba banana as raw material (Lustre et al., 1976). Due to its cheaper price when compared to dessert bananas and high possibility of formulation into wide range of products, an increase in both consumption and utilization of Saba banana has been observed over the year (Olawoye et al., 2017).

Jiang et al. (2015) reported that the starch from different banana cultivars shows differences in structural, physicochemical, and digestibility characteristics. To date, information on digestibility of food products using banana starch as raw material is extensive. However, little to no information is available on the raw Saba banana variety and the influence of maturation on its starch digestibility. Knowing the percentage of starch digestibility at different maturity stages of Saba banana is important as it is related to the blood glucose response. For this reason, this study aimed to determine the physicochemical changes during maturation of Saba banana and their effect on *in vitro* starch digestibility.

MATERIALS AND METHODS

Materials

Commercial unripe Saba bananas [*Musa 'saba'(Musa acuminata x Musa balbisiana)*] were purchased from Diamond Star Agro-Products Inc., Taguig City, Philippines and were allowed to ripen until 5 different maturity stages developed — all green (stage 1), green but turning yellow (stage 2), greenish yellow (stage 3), yellow with green tips (stage 4), and yellow with brown flecks (stage 5) (Figure 1). Enzymes used in *in vitro* digestion such as pepsin (porcine gastric mucosa, 800-2500 units/mg protein, pancreatin (hog pancreas, 4x USP), and invertase (invertase, grade VII from baker's yeast, 401 U mg^{-1} solid) were bought from Sigma-Aldrich Ltd. (St. Louis, USA) while amyloglucosidase (3260 U mL^{-1}) was purchased from Megazyme International Ltd. (Ireland). Ultrapure water used as mobile phase was from Wako Pure Chemical Corp. (Japan).

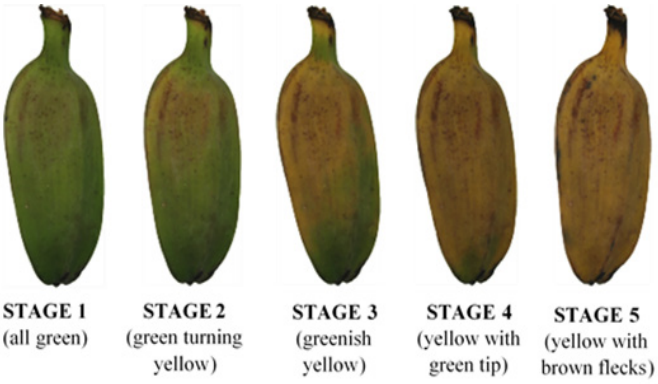

Figure 1. Five (5) stages of Saba banana used in the study.

Sample preparation

Seven (7) banana fingers were randomly selected every sampling period. The samples were peeled and cut into small round sections. Part of the sample was freeze-dried (Eyela FDU-1100, Tokyo Rikakikai Co. LTD., Japan) and half of the amount was homogenized using a household blender (NM200, Yamazen, China) for 2 minutes. The former dried sample was ground and passed through a 0.5 mm mesh sieve (Sanpo, Sanyo, Japan) before analysis of the total starch. The latter, on the other hand, was used for *in vitro* digestion.

Physicochemical analyses

Saba banana maturity was determined based on peel color image captured by a digital camera (E-510 Olympus, 1/500 shutter speed, F4.0 exposure, ISO 100). L*a*b* values were obtained using Adobe Photoshop CC. L* is lightness and a* (–greenness to +redness) and b* (–blueness to +yellowness) are the chromaticity coordinates. Chroma [$C = (a^{*2} + b^{*2})^{1/2}$] and hue angle [$h = \tan^{-1}(b^* / a^*)$] were calculated from a* and b* values. Before measurement, each captured image was calibrated by adjusting its RGB values. The peel color values of each stage of maturity are shown in Table 1. Moisture content of the bananas was analyzed by gravimetric heating using oven dyer (Oven 8150, Labserv, Ireland) following the method of AOAC (2000) with some modifications. Total starch and resistant starch content of the banana samples were determined using an assay kit (K-TSTA 07/11, Megazyme International, Ireland) following the company instructions. Absorbance was measured using UV-Vis spectrophotometer (V-630Bio, Jasco, USA).

Table 1. Lightness (L*), chroma (C), and hue (h) values of 5 stages of maturity of Saba banana (n = 7).

Stages	L*	C	h
1	34.40 ± 1.48^{b}	31.09 ± 1.26^{b}	114.85 ± 2.09^{a}
2	35.00 ± 0.53^{ab}	31.32 ± 1.25^{b}	102.59 ± 2.35^{b}
3	36.30 ± 2.79^{ab}	34.56 ± 2.15^{a}	83.79 ± 2.60^{c}
4	38.30 ± 1.75^{a}	35.17 ± 1.64^{a}	77.99 ± 1.96^{d}
5	37.30 ± 2.42^{ab}	33.03 ± 1.90^{ab}	76.21 ± 2.59^{d}

L*, from 0 for black to 100 for white; C is calculated as [$C = (a^{*2} + b^{*2})^{1/2}$]; and h is calculated from the arctangent of b/a. Mean values with different letters in the same column indicate significant differences (p<0.5).

In vitro digestion

The *in vitro* starch digestibility was evaluated based on the method described by Dartois et al. (2010) with slight modifications. One hundred seventy grams (170 g) of the sample, with approximately 4% of starch, was directly poured into the jacketed-glass reactor with a temperature of 37°C, and was continuously stirred using a magnetic stirrer. For the gastric digestion, the pH of the sample was adjusted to 1.20±0.01 using different molar concentrations of HCl before the simulated gastric fluid (SGF) containing pepsin was added. After 30 min of gastric digestion (G), the pH was changed to 6.00±0.01 by the addition of different concentrations of NaOH. The intestinal digestion (I) process began when the simulated intestinal fluid (SIF), containing pancreatin, invertase, and amyloglucosidase, was added. After the addition of SIF, the pH was adjusted to 6.80±0.01 using 0.2 M NaOH. Supernatants (0.5 ml) were collected from the following time periods: after 5 (G5) and 30 min (G30) of simulated gastric digestion; and after 5 (I5), 10 (I10), 15 (I15), 30 (I30), 60 (I60), 90 (I90), 120 (I120), 180 (I180), and 240 min (I240) of simulated small intestinal digestion. The digested fractions were mixed with 3 mL of 95% ethanol to stop the enzymatic reactions and were centrifuged at 1800×*g* for 10 min.

Sugar analysis

Reversed-phase high-performance liquid chromatography (RP-HPLC) was used to determine the amounts of sucrose, glucose, and fructose in the digestive fractions of different maturity stages of Saba banana. Chromatographic separation was performed using Shimadzu LC-20AD isocratic system consisting of refractive index detector (RID-20A) and Shim-pack SCR-101N column with ultrapure water as the mobile phase. The eluent was pumped at a flow rate of 0.8 mL min^{-1} with an oven temperature of 60°C. Quantification of sugars was achieved by comparison of the obtained data from the calibrated standard curves of glucose, sucrose, and fructose.

Total starch and percent starch hydrolysis

For freeze-dried sample, the total starch content is defined as follows:

Total starch (TS) = Resistant starch + [Non-resistant starch-(Initial Glucose + Initial Sucrose/2)]

The percent starch hydrolysis into glucose is defined as follows:

Starch hydrolysis (%) = ((Glucose-Glucose at G30)-(Fructose-Fructose at G30))/TS x 100

Statistical analysis

Results were calculated as means ± standard deviations. One-way analysis of variance (ANOVA) was used to determine the significant differences of all the data gathered. Tukey's Honest Significance Test was used to compare the treatment means at 5% level of significance using R software version 3.4.3 (R Development Core Team, 2017).

RESULTS AND DISCUSSION

Physicochemical properties

The ripening process of Saba banana brought substantial changes in its physicochemical properties. The significant decrease in hue values (Table 1) means a change in the fruit skin color from green to yellow. This accounts to the breakdown of chlorophyll, leaving the characteristic yellow color of xanthophyll and carotene (Kajuna et al., 1998; Von Loesecke, 1930). There was also a significant increase in moisture content from an initial 61.31% to 63.10% in the last stage of maturity (Table 2). This increase in moisture is expected as a result of osmotic transfer from peel to pulp which is reported to exceed the net water lost due to transpiration (Palmer, 1971; Von Loesecke, 1950). Conversely, the total starch decreased from 28.39% in stage 1 to 13.31% after 8 days of normal ripening. The significant decrease in starch content was primarily due to the breakdown of resistant starch to sugars as part of the ripening process. More than 80% of resistant starch was degraded from stage 1 to stage 5. Because banana naturally contains starch degrading enzymes such as alpha- and beta-amylases, glucosidase, phosphorylase, and invertase, which increase in activity during ripening, starch is hydrolyzed rapidly and converted into free sugars leading to accumulation of glucose, fructose, and sucrose (Tucker et al., 1993; Verma et al., 2017). The data revealed that the green stage stood out for having high starch and resistant starch contents, which is of industrial interest for developing new products (Bezerra et al., 2013).

Table 2. Moisture, resistant starch, and total starch contents (as % fresh weight) of Saba banana at different maturity stages.

Stages	Moisture content	Resistant starch	Total starch
1	61.31±0.36^{b}	15.25±0.59^{a}	28.39±0.20^{a}
2	61.32±0.37^{b}	8.63±0.28^{b}	26.18±0.09^{b}
3	61.69±0.68^{b}	3.73±0.33^{c}	20.96±0.51^{c}
4	61.98±0.49^{b}	2.96±0.23^{d}	17.05±0.20^{d}
5	63.10±0.56^{a}	2.22±0.35^{e}	13.31±0.05^{e}

Mean values with different letters in the same column indicate significant differences (p<0.5). Moisture content (n = 7); Resistant and Total starch percentage (n =7).

Sugar contents

Figure 2 shows the sugar contents during *in vitro* digestion of the different maturity stages of Saba banana. Sucrose, glucose, and fructose are the main sugars found in fruits (Wrolstad and Shallenberger, 1981). Sucrose content increased and preceded formation of glucose and fructose as a result of starch degradation during fruit ripening (Terra et al., 1983). Among the stages, 4 and 5 initially had the highest amounts of sugars especially sucrose. During gastric digestion, the presence of hydrochloric acid catalyzes the hydrolysis of sucrose chemically (Miloski et al., 2008). This continued until more than 90% of sucrose was hydrolyzed in all stages after 4 hours of simulated *in vitro* digestion. Increasing maturity showed increasing percent sucrose hydrolysis. The enzyme invertase plays an important role in cleaving of sucrose biochemically during intestinal digestion into glucose and fructose monosaccharides (White, 2014; Ladaniya, 2008), which concomitantly increased as sucrose was rapidly reduced. These monosaccharides showed an inverse relationship with sucrose hydrolysis in different stages. There was a decreasing trend of percent glucose and fructose accumulated after digestion while increasing maturity. The green stage showed the highest percentage gain of glucose and fructose after *in vitro* digestion. Aside from sucrose, the increase in glucose was accounted to the hydrolysis of starch whose principal end product is also glucose upon digestion (Goñi et al., 1997). The rate and extent of starch hydrolysis is influenced mainly by food characteristics and presence of other food constituents (Miller and Stanner, 2016). In this experiment, slurry sample, which regarded as structure-less, was used and its rate of glucose release may be higher than the non-homogenized sample (Thuengtung et al., 2017).

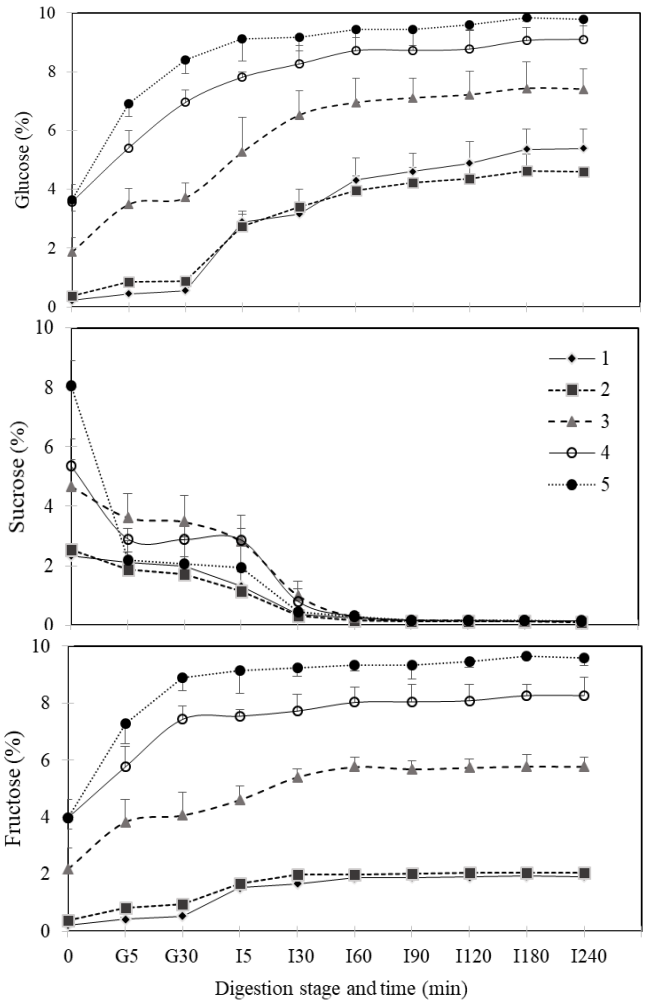

Figure 2. Glucose, sucrose, and fructose (%) release behavior of the different maturity stages of Saba banana during *in vitro* digestion. Error bars represent standard deviation (n = 3).

Starch hydrolysis

Figure 3 shows the starch hydrolysis rate of Saba banana at different maturity stages. The study used a two-stage *in vitro* digestion model with pepsin and pancreatin as enzymes for gastric and intestinal phases, respectively, since majority of the starch hydrolysis is carried out by pancreatic amylase. The salivary α-amylase in the oral phase was not incorporated in the process as it plays a very minor role in starch digestion (Singh et al., 2010). Among the maturity stages, the highest percent starch hydrolysis after 4 hours of intestinal digestion was observed in stage 1 (52%), followed by stage 2 (40%). As the Saba banana ripening proceeded, there was a significant decrease in starch hydrolysis. The stage 5 showed the lowest value of percent hydrolysis (16%) but was not significantly different from stages 3 (29%) and 4 (25%).

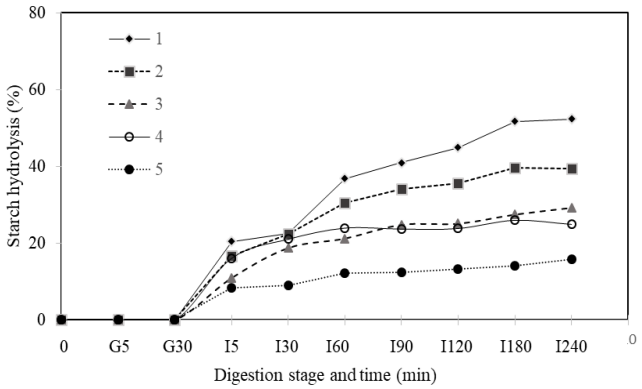

Figure 3. Rate of starch hydrolysis (%) of Saba banana at different maturity stages during *in vitro* digestion (n=3).

The variations on the physicochemical properties of different maturity stages of Saba banana may account for the decreasing rate of starch hydrolysis as the fruit ripens. One component that possibly has an effect on starch digestibility of the fruit is the pectin content. The study did not evaluate the pectin content of Saba banana at different stages. However, previous research studies reported that during ripening of banana the total pectin (protopectin and pectin) remains practically constant, but the pectin increases until the fruit becomes overripe (Von Loesecke, 1930). Kertesz (1951) showed a range of pectin contents (as calcium pectate) of banana with the lower range value of 0.59% from unripe stage to the higher range value of 1.28% after 5 days of storage in a ripening room. This was further confirmed by the study of Lustre et al. (1976) which compared pectin contents of Saba banana in normal and acetylene-induced ripening. Both conditions showed increasing pectin content during ripening. The pectin content (as calcium pectate) ranged from 0.10, value of the unripe stage, to 0.92 after 8 days of harvest.

The firmness of banana is accounted to the presence of pectic substances which act as cementing substance between cell walls. Prior to the formation of pectin, protopectin is the pectic substance in immature fruit. As the fruit ripens, enzymes convert protopectin to the more water-soluble pectin and the texture becomes soft. It is the pectin extracted from ripe fruit that is capable of swelling in water and form gel (Brown, 2017). Two effects can be considered with the increasing pectin content during ripening. First, the ability to form gel and viscosity are generally related as factors. The increase tendency to gel will also mean increase viscosity (Sundar Raj et al., 2012). According to Singh et al. (2010), the increase in viscosity of digesta can affect gastric function and inhibit propulsive and mixing effects. This result to less frequent interactions between substrates and digestive enzymes leading to low rate of starch digestion. In the study, the increasing sugar and moisture contents during ripening with a concomitant decrease in pH may contribute to the weak gel-like behavior of the feed slurry in stages 3, 4, and 5. Second, the inhibition of digestive enzymes by pectin can also cause digestion rate reduction. Bai et al. (2017) reported that there was an association between pectin and amyloglucosidase (AMG) during digestion which changed the latter's conformation and hindered its access to starch. The non-specific interaction between AMG and pectin may be electrostatic and/or hydrogen bonding. This complex formation between proteins and polysaccharide is of great interest because they may act as barrier towards starch digestibility.

CONCLUSION

The maturity of Saba banana may influence the *in vitro* digestibility of starch due to the physicochemical changes accompanying maturation. The weak-gel like behavior of feed slurry in ripe stages due to the interaction of pectin, water, sugar, and acid as the fruit proceeds ripening may increase the bulk viscosity and thus reduce the rate of hydrolysis of starch. Moreover, the possible interaction of non-starch components such as pectin with the digestive enzyme can account for this result. Thus, the analysis of pectin content at different maturity stages is of utmost importance to verify the results of this study. However, this may not only be the sole explanation for these findings and further study is needed. The possibility of the effects of starch morphological attributes and other components of Saba banana, which may have synergistic effect that can hinder and reduce the starch digestibility, should be also integrated for future studies.

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