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Effects of moisture content and storage time on starch digestibility of cooked banana

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Abstract

Unripe banana fruit has been claimed to be a source of resistant starch type 2 (RS2), but it is not heat stable during cooking. The retrogradation after cooking could promote resistant starch type 3 (RS3) development, and affect starch digestibility. The objective of the present work was to assess the effects of moisture content and storage time of cooked bananas on their starch digestibility and some functional properties. Unripe banana fruits were cooked in boiling water, then samples were stored at 4°C in the form of whole cooked fruit (RB-Fruit, 64% moisture content, mc) and as a dry powder, or cooked banana flour (RB-Flour, 7% mc) for three weeks. The properties determined were X-ray crystallinity, pasting and gel properties, and starch fractions (rapidly digestible starch, RDS; slowly digestible starch, SDS; and resistant starch, RS). These properties were compared to raw banana flour (Raw) and freshly cooked banana (Fresh cooked). Compared to Raw, RS content of Fresh cooked was much lower (80.55 ± 0.6 *vs* 7.14 ± 0.4), whereas SDS was higher (1.91 ± 0.0 *vs* 25.25 ± 0.7). Storage time had no significant effect on proportions of the starch fractions, nor on pasting or gel properties, but significantly affected these properties of RB-Fruit. This indicated that the high moisture in RB-Fruit enhanced re-association of starch molecules. In the RB-Fruit case, the cooked banana fruit after one-week storage had significantly higher RS and SDS fractions, and lower total starch digestibility (11.94 ± 0.0, 31.34 ± 0.2, and 72.68 ± 1.3% db, respectively) than the RB-Flour (7.75 ± 0.0, 22.58 ± 0.5, and 74.51 ± 0.9% db, respectively). In conclusion, the comparatively high moisture content (64% mc) in cooked banana (RB-Fruit) promoted starch retrogradation, giving high RS and SDS fractions (in total 43.28% db of cooked banana). Storage of cooked banana fruit at 4°C for only one week can be a simple approach to producing banana based functional foods or ingredients with desirable low glycaemic index.

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Introduction

Starch is an important energy source for humans. Based on digestibility, starch can be divided into three fractions: RDS, SDS, and RS. The RDS fraction is digested within 20 min, while SDS is digested in 20 - 120 min, and the undigested fraction after 120 min is considered as RS (Englyst *et al.*, 1992). The RS has been further categorised into RS1, RS2, RS3, RS4, or RS5 subtypes, which have been clearly defined in the literature (Englyst *et al.*, 1992; Raigond *et al.*, 2015; Liu *et al.*, 2020). RS1 and RS2 are found in raw starch and in natural food sources. RS3 is retrograded starch emerging after cooking and during storage. RS4 is obtained by chemical modification, while RS5 consists of amylose-lipid complexes.

Among the three starch fractions, RS has received the most attention since it benefits health (Aston, 2006; Raigond *et al.*, 2015). Various long-term health benefits of RS consumption have been reported (Higgins, 2004; Fuentes-Zaragoza *et al.*, 2010; Bojarczuk *et al.*, 2022); for example, decreased glycaemic and insulin responses, promotion of lipid metabolism, improved colon health, and increased mineral absorption. SDS is also currently receiving attention as a novel functional component in products. Various health benefits of the SDS have

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also been reported (Englyst *et al*., 1992; Hoover and Zhou, 2003; Raigond *et al*., 2019), such as prolonged glucose release when digested, and hence, a comparatively low glycaemic index (GI) (Miao *et al*., 2015).

RS is already formulated into many starch-based food products, for example snacks, breads, cookies, muffins, and breakfast cereals (Raigond *et al*., 2015; 2019). Knowhow and technologies for the production of RS have been developed. Hence, now there are commercially available RSs (RS2 - RS4) (Raigond *et al*., 2015). Although such commercial RSs are now in the food ingredient market, they are comparatively expensive to use.

RS2 is normally found in various natural sources such as raw banana, potato, and cereal grains, of which, the present work focussed on banana. However, RS2 is not stable when heated in thermal food processing. During gelatinisation, starch transforms, and its crystalline structure is converted to amorphous. Consequently, retrograded starch or RS3 develops after cooking. Along with RS3, SDS could emerge at the same time.

Raw or unripe banana has been reported as a good source of RS2, and as an alternative source of dietary fibre, ≈30 - 89% db (Vatanasuchart *et al*., 2012; Liao and Hung, 2015; Campuzano *et al*., 2018; Cahyana *et al*., 2019; Li *et al*., 2020; Yang *et al*., 2020).

RS2 can be converted and lost as it is not heat stable. When preparing food, the raw bananas are, in practice, cooked and the banana starch is gelatinised. Starch retrogradation occurs readily after cooking and during the storage of heat-processed starchy foods, as a spontaneous process reaching a metastable state of lower free energy. These phenomena could alter not only the commonly assessed starch functional properties, but also the digestibility as regards the proportions of RDS, SDS, and RS (Bello-Pérez *et al*., 2005). Retrogradation is the re-association of leached starch molecules, and the key process to obtain elevated SDS and RS contents in cooked starch. In fact, there are many factors contributing to this process, including type of starch, amylose/amylopectin contents and their structures, water content during gelatinisation, as well as storage time and temperature after cooking (Farhat *et al*., 2000).

It was found that in cooked banana, the combined amount of RS and SDS was 46%, which was higher than in cooked corn or potato starches, reducing susceptibility to enzyme digestion. In rice starch, amylose is the key component that plays an important role in retrogradation (Hsu *et al*., 2015b); while in banana starch, the long-chained amylopectin plays the dominant role (Zhang and Hamaker, 2012). Cahyana *et al*. (2019) reported that the thermal treatments (heat with moisture, annealing, and dual retrogradation treatments) changed the digestibility of starch from that of raw banana starch, decreasing the RS content, and increasing the RDS plus SDS content.

Most previous studies related to digestibility have been done on banana starch or flour, but not on whole banana fruit. Also, there is no prior report on how the moisture content in cooked banana, combined with storage time, affects digestibility and other properties. If low GI banana could be prepared using a simple method in households, this could inexpensively provide a healthy starch source to consumers, and this was the key motivation of the present work. Therefore, in the present work, unripe banana was used, cooked in boiling water as is common in households, and then stored at a refrigerated temperature to assess how these choices would affect starch digestibility. The objective of the present work was to investigate the effects of moisture content in cooked banana and its storage time, on starch digestibility and other functional properties.

Materials and methods

Banana sample

'Namwa' bananas [*Musa sapientum* (ABB)] were obtained from a local banana orchard in Pattani province, Thailand. Green banana was harvested at 110 - 120 days after flowering (85% maturity). The peel was totally green without any yellow part. The samples were tested and screened for the quality indicators firmness (0.92 ± 0.01 kg) and total soluble solids (TSS; 7.33 ± 0.01°brix). Chemical composition of the banana samples (tested as flour) was 2.23, 0.24, 2.20, 0.88, 87.98, and 14.95% db of protein, lipid, ash, crude fibre, carbohydrate, and amylose, respectively.

Retrograded banana flour preparation

The banana samples were washed and then cooked for 10 min in boiling water (banana:water = 1:3). Importantly, the size of sample was selected to be uniform for boiling (diameter: 3.10 ± 0.30 cm, ventral straight length: 13.00 ± 0.80 cm). The cooked samples were allowed to cool at room temperature. The cooked samples had a moisture content of 64 ± 1%. To test retrogradation during storage, the cooked samples were stored at 4°C in two forms: as flour (RB-Flour) and as fruit (RB-Fruit).

To prepare the RB-Flour samples, freshly cooked banana samples were peeled and sliced to a thickness of around 2 mm. These slices were then dried with a tray dryer for 5 h at 55°C. Using a blade grinder (DXM 2000, China), the dried slices were ground into powder that was passed through an 80-mesh sieve. Packaged in polypropylene bags, the powder (RB-Flour) was then kept in the refrigerator at 4°C. Over three weeks, samples were taken once a week to examine their properties.

Preparing the RB-Fruit samples involved packing the cooked banana fruit into polypropylene bags and storing in a refrigerator at 4°C. Over three weeks, samples were taken once a week to assess some key properties. To determine these properties, the cooked samples were also converted to flour form, as described earlier, to enable the measurements.

Analysis of retrograded banana samples

Both RB-Flour and RB-Fruit (after prepared to powder form) samples were analysed for functionality and for starch digestibility fractions, and compared to freshly cooked banana as flour (Fresh cooked) and raw banana as flour (RAW).

X-ray diffraction (XRD)

X-ray diffraction analysis was carried out on powder using an X-ray diffractometer (Empyrean, PANalytical, Netherlands) across the 2θ range 3 - 60° using a voltage of 40 kV and filament current of 30 mA. Crystallinity was calculated using SigmaPlot program, as reported in the literature. This method was modified from Liu *et al*. (1991).

Pasting characteristics

The pasting properties of all samples were determined using the Rapid Visco Analyser, RVA (Super-4, Newport Scientific, Australia), controlled by Thermocline for Windows software. A 3 g flour sample (14 g/100 g moisture on wet basis) was weighed directly into the RVA canister, along with 25 mL distilled water. For a total testing time of 20 min, the RVA was programmed to be held at 25°C for 2 min, heated to 95°C in 5 min, held at 95°C for 3 min, cooled to 25°C in 5 min, and finally held at 25°C for 5 min. For the first 10 s, the rotating speed was kept constant at 960 rpm, then reduced to 160 rpm for the remainder of the measurement. Cold viscosity, peak viscosity, trough (holding viscosity), and final viscosity, as well as breakdown, setback, and peak time, were calculated as the characterising pasting parameters. The viscosities were reported in cP.

Gel hardness

To prepare gel from banana flour, 250 mL of 10% (db) flour suspension was heated to 95°C for 30 min with constant stirring. Next, 40 g of the paste was poured into each plastic cup (5.4 cm diameter and 4.4 cm height). The cups were allowed to cool for 1 h at room temperature (30°C) before being stored at 4°C for 12 h. Gel hardness was analysed with a method modified from Wang *et al.* (2010), using a TA-XT2i (Texture Analyser TA-XT2i, Stable Micro Systems, USA) in return-to-start mode. The gel (without a plastic cup) was compressed at the speed of 0.5 mm/s for a 10 mm distance with a hemispherical probe (P/0.5). The trigger force was set as 5 g. The pre-speed and post-speed were 1.0 mm/s, and the data acquisition rate was 200 pps. The maximum force during the first compression was recorded as hardness (N). Five replications per sample type were performed.

Digestible starch contents and resistant starch (RS)

RDS, SDS, and RS contents were determined following the *in vitro* digestion method described by Englyst *et al*. (1992) to reflect the rate of *in vivo* starch digestion. Total digestible starch (TDS), RDS, SDS, and RS contents were determined using the digestible and resistant starch assay kit (K-DSTRS; Megazyme International Ireland Ltd., Wicklow, Ireland). In brief, a mixture of pancreatic-amylase and amyloglucosidase (PAA/AMG) in maleate buffer was added to the sample which was then incubated at 37°C with constant stirring for up to 4 h. Aliquots of the mixture were taken at 20, 120, and 240 min, and used to determine RDS, SDS, and TDS contents, respectively. Then the glucose released was measured with glucose oxidase/peroxidase (GOPOD) reagent. The RS (the starch fraction remaining after 240 min of digestion) content was the starch remaining after incubation of the sample with PAA/AMG for 4 h. To measure the RS, an aliquot sampled at 240 min was centrifuged, and the pellet was washed with aqueous ethanol to remove free glucose, and then suspended in sodium hydroxide to dissolve the RS. The solution was neutralised and hydrolysed with AMG. The glucose was measured with the GOPOD reagent.

Statistical analyses

The data were expressed as mean ± standard deviation from triplicate determinations. Statistical analysis was carried out using analysis of variance (ANOVA), and comparisons among treatments were carried out by Duncan's multiple range test (DMRT) at the significance level $p < 0.05$ using the SPSS software program, version 16 for Windows.

Results and discussion

X-ray diffractograms

The Raw flour showed peaks at 5.6°, 17.1°, and 22.2° (2θ) in the X-ray diffractogram (Figure 1), indicating a B-type starch. After pre-gelatinisation, the X-ray diffraction pattern of Fresh cooked showed only a peak at 17.1° (2θ). The Raw had 18.74% relative crystallinity while the Fresh cooked had 6.33% relative crystallinity (66% decrease). This indicated that starch in Fresh cooked immediately recrystallised/retrograded when cooled down (Yamaguchi *et al*., 2019). This agreed well with González-Soto *et al*. (2007) who reported that banana starch gel retrograded within 24 h after storage at 4°C, and the RS content increased.

The effects of storage time on crystallinities of RB-Fruit and RB-Flour were observed. Compared to a freshly cooked sample (Fresh cooked), the crystallinity of RB-Fruit1 (one-week stored sample) had increased from 6.63 to 8.97%. When stored longer for two and three weeks (RB-Fruit2 and RB-

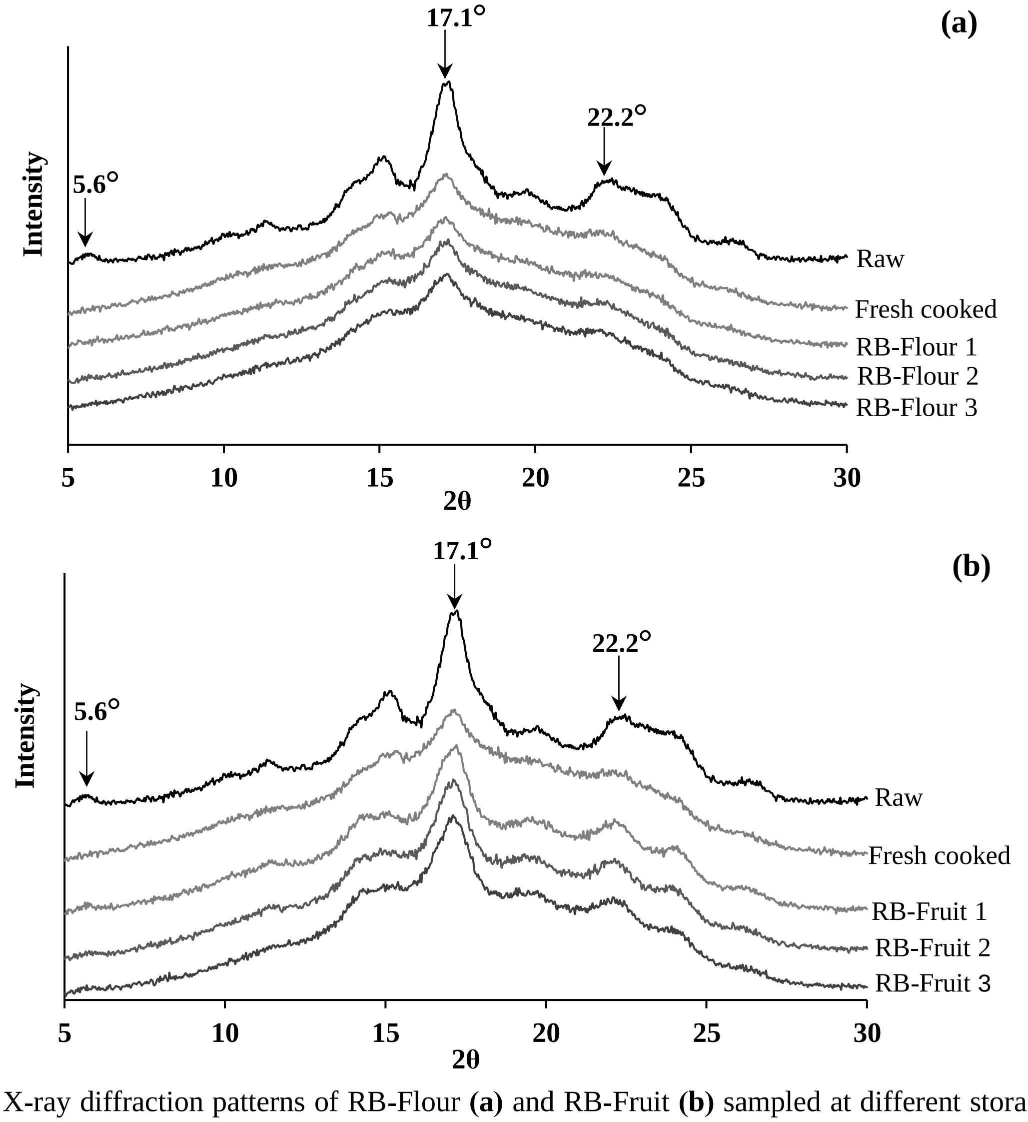

Figure 1. X-ray diffraction patterns of RB-Flour **(a)** and RB-Fruit **(b)** sampled at different storage times (1, 2, and 3 w). Raw: uncooked banana flour; Fresh cooked: freshly cooked banana flour; RB-Flour 1, 2, 3: cooked banana kept as dry flour for 1, 2, and 3 w, respectively; RB-Fruit 1, 2 and 3: cooked banana kept as whole cooked fruit, for 1, 2 and 3 w, respectively.

Fruit3, respectively), slight further changes in crystallinity were observed (to 8.84 and 8.05%, respectively). This indicated that the recrystallisation of cooked starch likely occurred immediately on cooling down after cooking, and at a high rate during early storage. In banana starch extrudates, retrogradation rapidly occurred, and reached its maximum at around 11 h of storage (Bello-Pérez *et al*., 2005). The rapid retrogradation of banana starch is attributed to the amylose component, and could be related to its high proportion (21.4%) of long amylopectin branched chains (DP > 36) (Zhang and Hamaker, 2012).

For RB-Flour samples, the storage time up to three weeks made no difference to crystallinity, which remained closely similar to that of the Fresh cooked. RB-Flour was in dry powder form, and had a similar moisture content as the Fresh cooked. These results indicated that moisture content influenced starch retrogradation in cooked banana. Since the moisture content of RB-Fruit (64% mc) was much higher than that of RB-Flour (7.37% mc), its retrogradation was stronger than that of RB-Flour. The higher moisture content was favourable for amylopectin recrystallisation, whereas the moisture content had little effect on amylose recrystallisation (Ding *et al*., 2019).

Pasting characteristics

The pasting properties and RVA pasting profiles of all banana samples are summarised in Table 1 and shown in Figure 2. In this testing, the temperature profile used was 25 - 95 - 25°C, in order to be able to observe changes in viscosity at comparatively low temperatures (cold peak viscosity). The cold peak viscosity of Raw and all cooked samples were not different, and all of them were very low in the range 22 - 30 cP.

Cooking strongly affected the viscosity parameters, except for cold peak viscosity. The viscosities (raw peak, hold, and final) of Fresh cooked were approximately half those of the Raw (Figure 2a and Table 1). This was because the thermal treatment contributed to starch gelatinisation, which degraded starch granules and molecules, resulting in a viscosity decrease (Huang *et al*., 2022).

The viscosity of Fresh cooked and all of RB-Flour 1 - 3 were similar (Figure 2b and Table 1). This indicated no clear effect of storage time on RVA viscosity of cooked banana in flour form (RB-Flour). Among the RB-Fruit samples, the RB-Fruit 1 showed significantly ($p < 0.05$) higher viscosity than the Fresh cooked. Among RB-Fruit 1, 2, and 3, only very slight changes of viscosity (especially of setback viscosity) were observed. So, there was no significance change after one week of storage. This in turn indicated that significant changes due to retrogradation in the starch occurred mainly right after cooking and during the first week of storage. This agreed well with crystallinity results in the previous section. After starch gelatinised and was cooled down, the re-association of linear amylose molecules initially

Table 1. Pasting properties of Raw, Fresh cooked, RB-Flour, and RB-Fruit at different storage times (1, 2, and 3 w) at 4°C.

Sample	Viscosity (cP)					
	Cold Peak	**Raw Peak**	**Hold**	**Breakdown**	**Final**	**Setback**
Raw	30 ± 3^{a}	5378 ± 21^{a}	3233 ± 26^{a}	2145 ± 9^{a}	6688 ± 100^{a}	3455 ± 108^{a}
Fresh cooked	26 ± 2^{bc}	2456 ± 17^{f}	1987 ± 27^{d}	468 ± 40^{d}	3856 ± 23^{d}	1869 ± 46^{c}
RB-Flour 1	32 ± 2^{a}	2459 ± 9^{f}	1923 ± 25^{e}	536 ± 31^{d}	3777 ± 5^{e}	1854 ± 25^{c}
RB-Flour 2	27 ± 3^{bc}	2500 ± 20^{e}	1986 ± 14^{d}	514 ± 33^{d}	3904 ± 39^{d}	1918 ± 50^{c}
RB-Flour 3	26 ± 2^{c}	2459 ± 17^{f}	1973 ± 40^{e}	486 ± 41^{d}	3787 ± 34^{e}	1815 ± 58^{c}
RB-Fruit 1	30 ± 2^{ab}	3833 ± 29^{b}	2942 ± 9^{b}	891 ± 37^{b}	5399 ± 61^{b}	2457 ± 70^{b}
RB-Fruit 2	24 ± 2^{d}	3568 ± 34^{d}	2841 ± 60^{c}	727 ± 94^{c}	5184 ± 53^{c}	2344 ± 102^{b}
RB-Fruit 3	22 ± 1^{cd}	3752 ± 13^{c}	2961 ± 37^{b}	791 ± 43^{c}	5396 ± 46^{b}	2435 ± 60^{b}

Data are expressed as mean ± standard deviation of triplicate determinations, and within the same column, different lowercase superscripts indicate significant differences ($p < 0.05$). Raw: uncooked banana flour; Fresh cooked: freshly cooked banana flour; RB-Flour 1, 2, 3: cooked banana kept as dry flour, for 1, 2, and 3 w, respectively; RB-Fruit 1, 2, and 3: cooked banana kept as whole cooked fruit, for 1, 2, and 3 w, respectively.

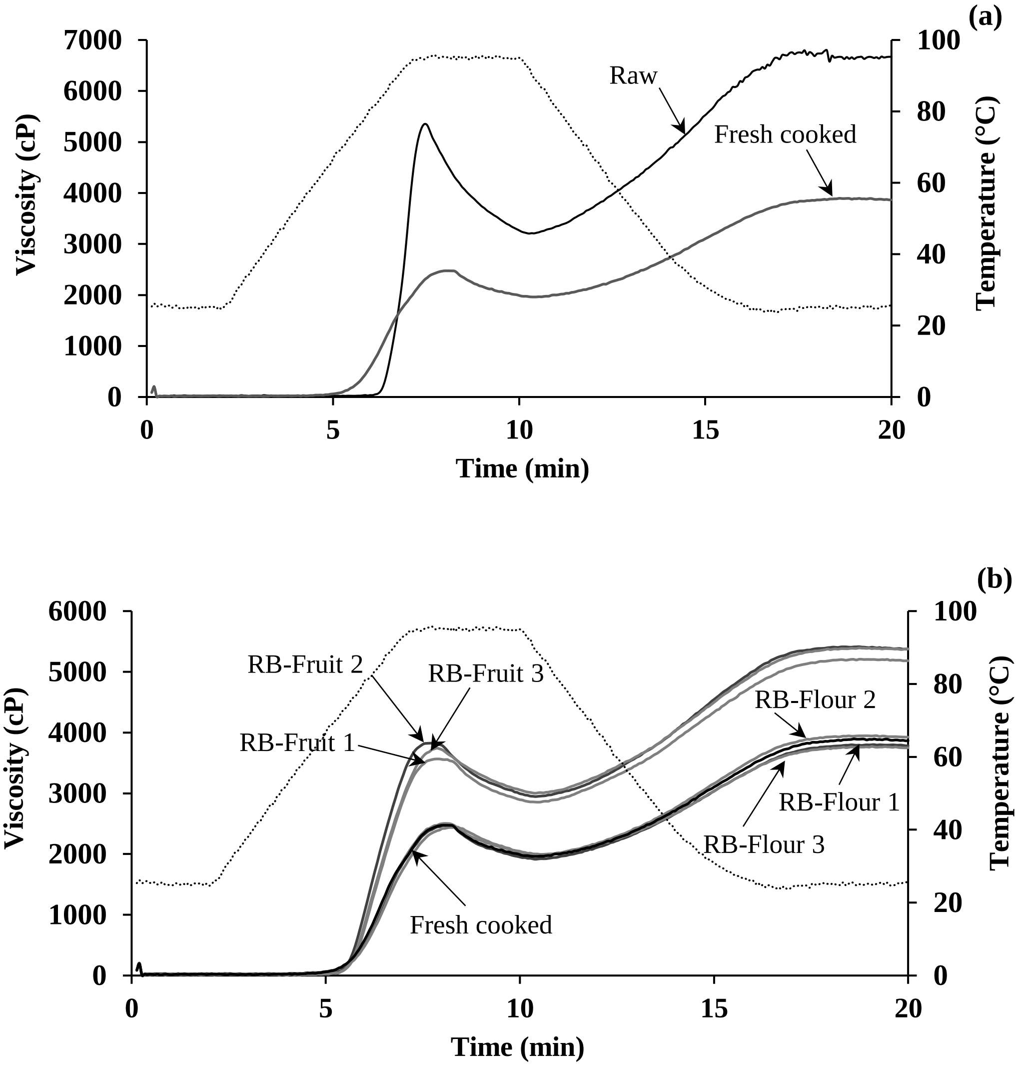

Figure 2. Comparison of RVA profiles of **(a)** Raw and Fresh cooked, and of **(b)** RB-Flour and RB-Fruit at different storage times (1, 2, and 3 w) at 4°C. Raw: uncooked banana flour; Fresh cooked: freshly cooked banana flour; RB-Flour 1, 2, 3: cooked banana kept as dry flour, for 1, 2, and 3 w, respectively; RB-Fruit 1, 2 and 3: cooked banana kept as whole cooked fruit, for 1, 2 and 3 w, respectively.

occurred rapidly. In contrast, the retrogradation of amylopectin occurred at a later time and as long-term retrogradation (Matalanis *et al.*, 2009; Wang *et al.*, 2015; Jiang *et al.*, 2024). Some physical changes due to starch retrogradation include gel formation, with increases in viscosity, syneresis, and degree of crystallinity. RVA setback viscosity (the difference between final and hold viscosities) indicates starch retrogradation, with a higher value signifying greater tendency for retrogradation (Zaidul *et al.*, 2007). Similar to indica rice starch gels, the retrogradation rapidly increased in the first seven days of storage (at 4°C), and decreased after that (Lu *et al.*, 2024).

Raw peak viscosity, final viscosity, and setback of RB-Fruit 1 were about 1.5, 1.4, and 1.3-fold those of the Fresh cooked, respectively. During its storage for one week, the viscosity of RB-Fruit 1 had rapidly increased from that of the Fresh cooked. High moisture content at the beginning of storage allowed final viscosity and setback to increase (Bello-Pérez *et al.*, 2005). This result was related to the higher crystallinity of the RB-Fruit. As final and setback viscosities reflect the re-association of starch molecules, it can be inferred that the retrogradation in RB-Fruit was stronger than that in RB-Flour. This, again, could be related to the moisture contents of the samples, before and during storage.

The changes in viscosities during three weeks of storage for RB-Flour and RB-Fruit were compared. All the viscosity parameters of RB-Fruit were significantly higher than those of RB-Flour at the same storage time over the three weeks, because the higher moisture content of the RB-Fruit induced higher crystallinity.

Gel hardness

Gel hardness of the Raw was 1.19 ± 0.01 N. After cooking, gel hardness of Fresh cooked was lower (0.74 ± 0.01 N) than that of the Raw (Figure 3). This is not surprising since the Fresh cooked was the pre-gelatinised sample, so its starch granules and molecules could have been degraded during gelatinisation process, and this would reduce gel network formation.

Comparing the gel hardness of RB-Flour and RB-Fruit during three weeks of storage, the RB-Flour gels were clearly stronger than the RB-Fruit gels (0.78 ± 0.01 - 0.84 ± 0.01 N *vs* 0.43 ± 0.00 - 0.51 ± 0.01 N). This could be an effect of retrogradation, and in this case would be influenced by moisture content of stored samples. RB-fruit, at a high moisture content (64%), could have higher retrogradation than RB-flour, as indicated by higher relative crystallinity.

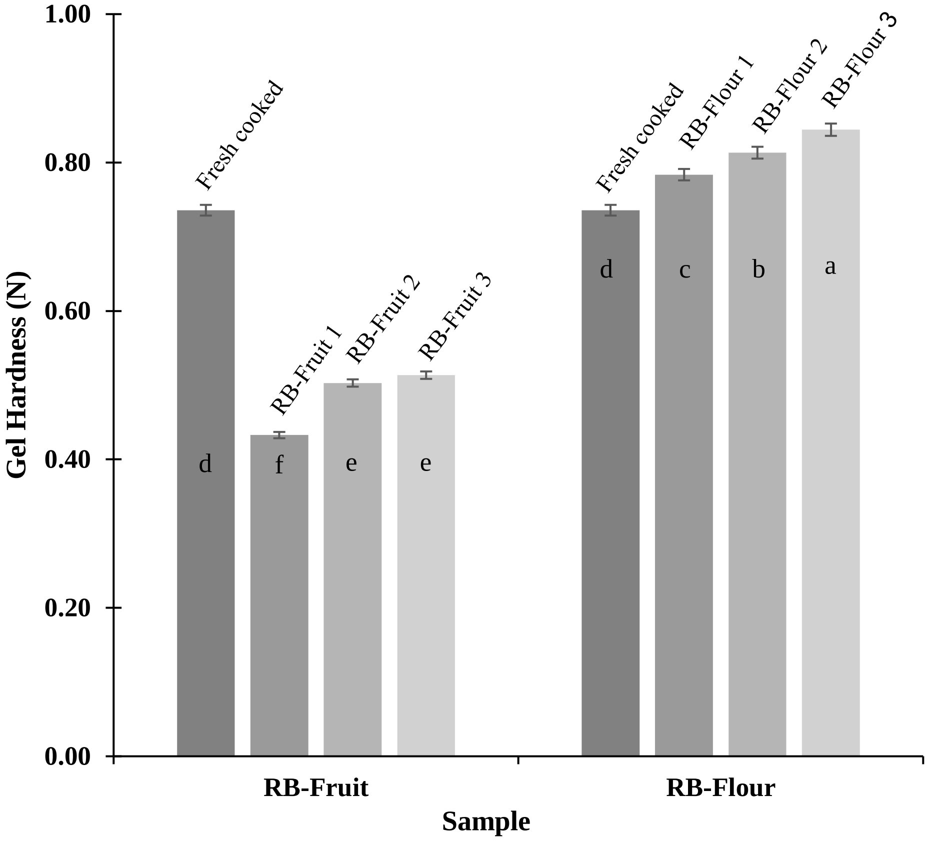

Figure 3. Gel hardness of Fresh cooked, RB-Flour, and RB-Fruit at different storage times (1, 2, and 3 w) at 4°C. Data are expressed as mean ± standard deviation from triplicate determinations. Different lowercase letters indicate significant differences ($p < 0.05$) in a cluster of histogram columns. Fresh cooked: freshly cooked banana flour; RB-Flour 1, 2, 3: cooked banana kept as dry flour, for 1, 2, and 3 w, respectively; RB-Fruit 1, 2, and 3: cooked banana kept as whole cooked fruit, for 1, 2, and 3 w, respectively.

Starch fractions

The starch fractions of RB-Flour and RB-Fruit are shown in Table 2. After cooking, the proportions of starch fractions had totally changed from those of native banana. Compared to Raw, RS of Fresh cooked had decreased, while TDS, RDS, and SDS had significantly increased. This confirmed that the RS2 in raw banana was not heat stable. RS2 consists of tightly packed starch granules in a radial pattern, and is relatively dehydrated. This compact structure limits the access by digestive enzymes, and accounts for the resistant nature of RS2 (Sajilata *et al*., 2006). When RS2 is heated, the compact structure will be destroyed, and it becomes easy to digest. This result

Table 2. Rapidly digestible starch (RDS), slowly digestible starch (SDS), total digestible starch (TDS), and resistant starch (RS) of Raw, Fresh cooked, RB-Flour, and RB-Fruit at different storage times (1, 2, and 3 w) at 4°C.

Sample	Starch fraction (g/100 g dry sample)			
	RDS	**SDS**	**TDS**	**RS**
Raw	0.34 ± 0.10	1.91 ± 0.00	5.80 ± 0.20	80.55 ± 0.60
Fresh cooked	43.13 ± 0.63^{a}	25.25 ± 0.68^{c}	76.43 ± 0.17^{a}	7.14 ± 0.35^{d}
RB-Flour 1	43.80 ± 0.78^{a}	22.58 ± 0.52^{d}	74.51 ± 0.94^{b}	7.75 ± 0.03^{c}
RB-Flour 2	43.13 ± 0.73^{a}	21.72 ± 0.81^{d}	75.30 ± 0.27^{ab}	7.76 ± 0.05^{c}
RB-Flour 3	42.63 ± 0.81^{a}	21.89 ± 1.69^{d}	72.73 ± 0.36^{c}	7.83 ± 0.01^{c}
RB-Fruit 1	34.98 ± 0.24^{b}	31.34 ± 0.21^{a}	72.68 ± 1.33^{c}	11.94 ± 0.01^{b}
RB-Fruit 2	34.21 ± 0.12^{b}	26.49 ± 1.34^{b}	69.82 ± 1.89^{d}	12.16 ± 0.01^{b}
RB-Fruit 3	30.97 ± 1.94^{c}	24.87 ± 0.44^{c}	60.25 ± 0.29^{e}	12.76 ± 0.41^{a}

Data are expressed as mean ± standard deviation of triplicate determinations, and within the same column, different lowercase superscripts indicate significant differences (p < 0.05). Raw: uncooked banana flour; Fresh cooked: freshly cooked banana flour; RB-Flour 1, 2, 3: cooked banana kept as dry flour, for 1, 2, and 3 w, respectively; RB-Fruit 1, 2, and 3: cooked banana kept as whole cooked fruit, for 1, 2, and 3 w, respectively.

agreed well with previous studies (Zhang and Hamaker, 2012; Hsu *et al*., 2015a). Zhang and Hamaker (2012) found that banana starch cooked for 20 min in excess water had a significantly lower resistant fraction (27%) than that in raw banana starch (84.5%).

After one week of storage at 4°C, RS content of RB-Flour 1 and RB-Fruit 1 had increased, while TDS had decreased significantly compared with the Fresh cooked. The RS content of RB-Fruit 1 was higher than that of the Fresh cooked by approximately 67%, and in the RB-Flour 1 it was higher by approximately 8%. SDS content of RB-Fruit 1 was also significantly higher than in Fresh cooked, while that of RB-Flour 1 was lower. Additionally, it was related to crystallinity; RB-Fruit 1 had more crystallinity than RB-Flour 1. Thus, storage time induced crystallinity, causing starch digestibility to decrease. Moreover, regarding the combination of RS and SDS fractions, RB-Fruit 1 contained 43.28% db., clearly exceeding its RDS fraction (34.96% db). The high content of RS plus SDS fraction in RB-Fruit 1 showed promise of relatively balanced energy release (Zhang and Hamaker, 2012).

Moisture content of a sample during storage affected its RS development, as well as starch digestibility overall. All RB-Fruit cases had significantly higher RS and SDS contents, but lower TDS than the RB-Flours. It is possible that RB-Fruit, with higher water content, developed RS and SDS better than RB-Flour. For RB-Flour (7% mc), the storage time (one to three weeks) did not significantly affect starch fraction proportions (*i.e.*, of RS, SDS, and TDS), while significant effects were observed in RB-Fruit (64% mc). The high-water content of RB-Fruit would induce mobility of the amylose and amylopectin chains that formed double helix structures of RS3 crystallites during storage. These results agreed with those of Rodríguez-Damian *et al*. (2013).

The RS content of RB-Fruit rapidly increased within a week, and the RS content in RB-Fruit 3 was further significantly higher than that of RB-Fruit 1, while its digestible starch fraction (TDS) had decreased. Thus, the TDS and RDS contents in RB-Fruit 3 were the lowest. Interestingly, the SDS content in RB-Fruit 1 was comparatively high, approximately three times its RS content. The RS increase indicated starch retrogradation in the sample (Bello-Pérez *et al*., 2005), and preservation of re-associated molecules (Babu *et al*., 2014). This also clearly confirmed that the moisture content in cooked starch strongly affected its retrogradation.

Conclusion

Unripe banana was cooked and thereafter stored at 4°C, as low moisture flour or alternatively as high moisture cooked fruit, for three weeks. The key results obtained from the present work were as follows: (1) raw banana fruit (before cooking) had 84% of RS2 but this was not heat stable, instead the proportion decreased on cooking, while the RDS and SDS contents increased. High moisture content (64%) of cooked banana significantly promoted starch retrogradation/re-crystallisation within one week of storage, thus altering the proportions of digestible starch fractions (RDS, SDS, and RS). (2) Storage of RB-Fruit (64% mc) increased its RS and SDS contents, as well as relative crystallinity more than storage in the form of RB-Flour (7% mc). Additionally, the changes affected the functional properties, namely the viscosity of RB-Fruit which was also higher than that of RB-Flour or Fresh cooked. This indicated that the cooked banana with high moisture content allowed starch retrogradation better than the pre-gelatinised banana flour stored at a comparatively low moisture content. High moisture cooked banana after one-week storage contained combined RS and SDS for up to 43.28% db, while the low moisture sample contained 30.33% db. (3) For RB-Flour (7% mc), storage time did not significantly affect viscosity, gel hardness, or RS and SDS contents. Those properties were similar to the freshly cooked banana. Since its functional properties only slightly changed during storage, the RB-Flour could potentially support long-term use of cooked banana powder for any purpose. The present work demonstrated a simple and low-cost method (cook banana fruit and store them at 4°C) to produce a high content of slowly digestible starch with controlled energy release in cooked unripe banana. This method could be applied in both household and industrial scales. It also has the potential to produce pre-gelatinised flour from cooked banana fruit in an industrial scale, for use as a functional food ingredient, which could be tested in pilot scale in further studies.

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