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Effects of ripening stage and cooking methods on available glucose, resistant starch and estimated glycemic index of bananas (*Musa sapientum;* Nam-wa variety)

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ABSTRACT

Introduction: Resistant starch (RS) has been associated with health benefits including reduced cholesterol absorption, and also been considered as a prebiotic. Little is known of the RS contents of bananas from Thailand. **Methods:** This study determined the digestibility of starch in bananas (Nam-wa variety) at different ripeness stages, based on roasting and boiling. *In vitro* glycemic response of the bananas was also investigated. Based on peel colour, banana maturity stages were classified into 8 stages namely, unripe (stages 3–5) and ripe (stages 6–8). Analysis methods used were *in-vitro* enzymatic digestion and HPLC analysis. **Results:** Unripened bananas contained less total sugar compared to ripened bananas. Rapidly Available Glucose (RAG) and Slowly Available Glucose (SAG) contents increased in tandem with progression of the ripening stage. However, there was no significant difference in the RS content with ripening stage (p>0.05). The RS content also did not show significant difference between the cooking methods. Boiling of banana at the same ripening stage considerably reduced the estimated glycemic index (eGI) (34-56), whilst roasting did not produce any marked changes in pGI (53-56). **Conclusion:** The RAG and SAG amounts in the bananas studied were found to be directly related to ripening stage. Boiling was shown to be a better cooking method for lowering the pGI of bananas compared to roasting.

Keyword: Banana, cooking, glycemic index, processing, resistant starch

INTRODUCTION

Bananas are a leading food crop worldwide, secondary to rice, wheat, corn and potatoes. They are a rich source of carbohydrates, vitamins and minerals. The high carbohydrate content of bananas makes them a staple calorie resource for over 500 million inhabitants of tropical countries (Aurore, Parfait & Fahrasmane, 2009). Banana is a rich source of vitamin B6, vitamin C and potassium.

Harvested bananas pass through several physiological development stages, namely the pre-climacteric (green) stage, the climacteric stage, the ripening stage and the ripe and senescence stage (Robinson & Sauco, 2011). Unripe

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bananas are a rich source of starch, accounting for 70–80% of the overall composition, whereas in ripe bananas, the amount of sugar is increased with the reduction on the starch content.

Different sources of carbohydrates affect the blood glucose response differently after consumption. Blood glucose response is normally estimated by the glycemic index, which compares the response of a test food to that of a reference food, usually glucose or white bread. The primary factor that affects the glycemic index is the rate or absorption of carbohydrates in the presence of food. The rapidly available glucose (RAG) and slowly available glucose (SAG), which are nutritionally classified according to the glucose availability, have been shown to influence the glycemic index (GI) (Englyst *et al.*, 1999). Foster-Powell, Holt & Brand-Miller (2002) reported that the addition of high fibre to food products reduced the food's GI value.

Many studies have reported that the GI of bananas ranges from 53 to 70, leading to bananas being considered to be a medium-GI food (Foster-Powell *et al.*, 2002; Yusof, Talib & Karim, 2005; Hettiaratchi, Ekanayake & Welihinda, 2011). In general, the different GI values of bananas could be due to differences in ripening stages and cultivars. Unripe (green) bananas can be cooked e.g. by roasting or boiling, like it is practiced for plantain and cooking bananas, depending on cultivar and preferences (Dufour *et al.*, 2011). The cooking method has been found to have an impact on the starch content, resistant starch (RS) and GI (Moongngarm *et al.*, 2014).

The starch digestion rate in releasing glucose into the bloodstream at a slower rate results in reduced glycemic and insulinemic postprandial responses from food products containing high amounts of dietary fibre (Yamada *et al.*, 2005). In addition, the RS acts as a fermentation substrate in the colon, similar to non-starch carbohydrates, with positive implications for the prevention of food-borne diseases, such as colon cancer and hypolipidemia. Odenigbo *et al.* (2013) showed that cooked bananas had a lower RS content than uncooked bananas. The RS content of bananas decreased during postharvest treatments (Wang *et al.*, 2014).

The RS of bananas has been studied primarily in the form of banana flour. Thus, the results from these studies do not represent the actual RS in bananas. There are limited studies that investigated the effects of processing, roasting and boiling on the RS content, SAG, RAG and GI of bananas available in Thailand. The objective of this study was to determine the digestibility of starch in bananas (the Nam-wa variety) at different ripeness stages using two cooking methods, roasting and boiling, and investigate the *in vitro* glycemic response. The studies were conducted for 2–3 different stages but not the completely ripe stage. The processing of bananas could influence their RS content and food microstructure. However, the slowly digested starch (SDS), rapidly digested starch (RDS), RS and GI of bananas at different ripening stages, as well as the effect of cooking on these compounds under the same experimental conditions, have not been previously investigated.

MATERIALS AND METHODS

Sample preparation

Freshly harvested bunches of green (unripe) bananas (*Musa sapientum*) were obtained from the Nonthaburi province, Thailand, in 2014. After species identification (as *Musa sapientum*, triploid hybrid banana), the fruits were classified as non-plantain cooking banana according to the dry matter content ranging between 30-33% (Dufour *et al.*, 2009), the ripening process

was conducted in house by leaving the bananas at room temperature. The ripening of the bananas was assessed by the peel colour and divided into 8 stages,: green (stage 1), green with a trace of yellow (stage 2), more green than yellow (stage 3), more yellow than green (stage 4), yellow with a green tip (stage 5), all yellow (stage 6), yellow with a few brown spots (stage 7) and yellow with many brown spots (stage 8). Fresh bananas at ripening stages 3-8 were used to study the RAG, SAG, RS and eGI, whereas bananas at ripening stages 3-5 were used to study the effect of cooking on the RAG, SAG, RS and eGI. Stage 1 and 2 were used for nutritional composition analysis and sugar but not for cooking effect.

Colour measurement

The peel colour was measured using a colorimeter (Minolta, CM 600d, US). The bananas' outer peels were cut into small pieces and placed in a glass dish, covering the entire base of the dish. Measurements were performed in triplicate.

Cooking methods

Roasting

Banana roasting was conducted as follows. A whole unpeeled banana was placed on a wire mesh (width 11.5 x 11.5 cm) over a preheated charcoal stove. The banana sample was frequently turned to prevent charring and burning. The banana was cooked for 40 min until a golden-brown colour was obtained. Roasting temperature was in between 100-110°C The sample was then divided into 2 lots for immediate analysis and left at room temperature prior to analysis.

Boiling

An unpeeled whole banana was boiled in water (gentle boil at 100°C) for 30 min. The ratio of water to banana was 1:3 (v/w). The banana sample had a soft texture and became purple.

Both roasted and boiled bananas were removed from the heat source and left at room temperature (25-30°C) for 0 min and 120 min to study the effect of cooling on RS formation. Sample was then divided into 2 lots for immediately analysis and left at room temperature prior analysis.

Proximate analysis

All of the nutritional components were analyzed according to the AOAC (2012) method, including the carbohydrate, protein (991.20), fat (932.06), dietary fibre (991.43), sugar (980.13), ash (930.30) and moisture content (926.12).

Sugar analysis

The method used for sugar analysis was adopted from the AOAC method (977.20). Banana samples of approximately 5 g were ground. Then, 85% EtOH was added and placed in a water bath at 60°C for 1 h. The extraction was performed in triplicate. The solution was evaporated using a rotary evaporator (Buchi, Switzerland) until completely dry. The residue was redissolved with 3 ml of distilled water, filtered through a 0.22 µm PTFE prior HPLC analysis.

RAG, SAG and estimated glycemic index analysis

The *in vitro* starch digestibility was assessed according to the protocol developed by Goni *et al.* (1996). In brief, approximately 100 mg of a sample were weighed in a 50 ml tube. Potassium chloride buffer was added to the sample, and 0.2 ml of a pepsin solution was then added. The sample was then incubated in a water bath at 40°C for 60 min with constant shaking. Then, the pH was adjusted to 6.9 using a 0.1 M Tris-maleate buffer. After 1 ml of the α-amylase solution was added,

the mixture was incubated at 37°C for 2 hours with constant shaking. The samples that were removed at 30 and 120 min were considered the RAG and SAG, respectively. (Goni, Garcia-Alonso & Saura-Calixto, 1997).

Measurement of RS

The RS measurement was performed according to Goni, Garcia-Alonso & Saura-Calixto (1997). In brief, a digested sample was incubated at 37°C for 16 h. The sample was then centrifuged the pellets were washed with l0 ml of distilled water. Before the addition of the enzyme, the pellets were washed with distilled water and this was followed by the addition of 3 ml of a 0.4 M sodium acetate buffer and 80 µL of amyloglucosidase. The mixture was incubated in a water bath at 60°C for 45 min with constant shaking. The glucose was converted into starch by applying a factor of 0.9, which included the conversion of RAG and SAG into RDS and SDS, respectively.

Tannin screening method

The screening of tannin was performed according to Geetha & Geetha (2014). In brief, banana samples (peel or pulp) were ground with a blender and diluted with DI water. The aliquot was then made to react with lead acetate anhydrous (1% solution). The formation of a red colour solution indicated the presence of tannin. Qualitative test was performed by comparing with a tanning standard as a positive control.

Statistical analysis

All of the values shown are the mean averages of triplicate determinations. The glycemic index and starch fraction after hydrolysis were analyzed by one-way analysis of variance using SPSS version 19, Mahidol University at a 95% confidence interval. All of the data are reported as the means and standard errors of the mean (mean±SEM). The area under the curve associated with a change in the glucose level was calculated using GraphPad Prism version 5.01 (GraphPad software, CA, USA).

RESULTS AND DISCUSSION

Proximate analysis and ripening stages

The strong correlation between fruit colour and ripening makes it feasible to evaluate the ripening level based on colour (Zhang *et al.*, 2014). In this experiment, bananas at 8 different ripening stages were classified into 2 main groups, unripe (stages 3-5) and ripe (stages 6-8). This classification was different in comparison with other studies that divided ripening stage into either 5 stages (Khawas *et al.*, 2014) or 7 stages (Chiun *et al.*, 2015). The banana pulp was composed of carbohydrates, proteins, lipids, ash, and dietary fibre (Table 1). With regards to energy (kcal), no significant difference was noted between fresh bananas and processed bananas (roasted and boiled) at different ripening stages. The moisture content of bananas at different ripening stages varied from 66-69% DW in fresh bananas. It must be noted that the water percentage increases in the pulp during ripening due to the respiratory breakdown of starch and the osmotic movement of water from peel to pulp. In roasted bananas, water constituted approximately 56-59% DW, compared with 67-70% DW in boiled. During boiling, excessive water was used, whereas during roasting, most of the moisture content originated from intracellular water.

Changes in the carbohydrate content in banana pulp during ripening were due to conversion of starch to sugars. However, the total carbohydrate content was not significantly different among the fresh, roasted and boiled bananas. The lipid content remained constant during the ripening process. Lipids

Table 1. Proximate analysis of fresh, roasted and boiled bananas at different maturity stages (g/100 g DW)

Sample	*Energy*^{ns}	*Mo*	*P*^{ns}	*TF*^{ns}	*CHO*^{ns}	*TDF*	*IDF*^{ns}	*SDF*	*Ash*^{ns}
Raw banana									
Stage 3	388.64±0.13	66.54±0.40^{a}	3.08±0.04	nd	94.08±0.07	6.47±0.03^{a}	2.94±0.03	3.53±0.00^{a}	2.84±0.03
Stage 4	387.02±0.06	67.49±0.08^{a}	3.04±0.08	nd	93.71±0.09	7.47±0.15^{a}	2.83±0.14	4.64±0.01^{a}	3.24±0.01
Stage 5	386.79±0.06	66.08±0.27^{a}	3.07±0.06	nd	93.63±0.07	6.38±0.07^{a}	1.98±0.03	4.41±0.10^{a}	3.30±0.01
Stage 6	388.46±0.63	69.03±0.15^{a}	3.37±0.13	0.27±0.11	93.12±0.03	7.75±0.04^{a}	2.66±0.17	5.08±0.14^{b}	3.23±0.02
Stage 7	387.92±0.49	69.24±0.15^{a}	3.27±0.05	0.15±0.07	93.39±0.08	7.83±0.05^{a}	2.52±0.10	5.31±0.05^{b}	3.20±0.04
Stage 8	388.80±0.19	69.21±0.26^{a}	3.28±0.12	0.219±0.00	93.26±0.17	8.80±0.12^{b}	2.14±0.16	6.66±0.04^{c}	3.17±0.05
Roasted banana									
Stage 3	388.75±0.17	56.55±0.36^{b}	3.24±0.04	0.30±0.07	93.27±0.07	12.53±0.41^{c}	3.77±0.13	8.76±0.54^{d}	3.19±0.04
Stage 4	388.44±0.04	58.18±0.20^{b}	3.11±0.05	0.30±0.02	93.33±0.10	9.60±0.17^{b}	3.13±0.08	6.47±0.26^{c}	3.26±0.03
Stage 5	389.31±0.38	58.64±0.11^{b}	3.16±0.01	0.34±0.14	93.41±0.21	8.44±0.02^{b}	2.77±0.06	5.67±0.04^{b}	3.09±0.08
Boiled banana									
Stage 3	388.86±0.37	67.52±1.27^{a}	3.85±0.28	0.38±0.01	92.50±0.36	7.28±0.09^{a}	1.22±0.03	6.07±0.06^{c}	3.26±0.08
Stage 4	385.91±0.24	71.11±0.11^{c}	3.98±0.16	0.38±0.00	91.64±0.11	9.66±0.23^{b}	3.10±0.06	6.56±0.29^{c}	3.99±0.06
Stage 5	386.93±0.08	70.33±0.25^{c}	3.52±0.15	nd	93.21±0.13	8.76±0.03^{b}	3.05±0.19	5.71±0.17^{b}	3.27±0.02

Values expressed are mean±standard deviation of triplicates analysis

$^{a, b, c}$ Different alphabets within the same column indicate a significant difference at $p<0.05$

Mo = Moisture, P = Protein, TF = Total fat, CHO = Carbohydrate, TDF = Total Dietary Fibre, SDF = Soluble Dietary Fibre, IDF = Insoluble Dietary Fibre, and nd = Not detected. ns = not significantly different at $p<0.05$

were not detected in fresh bananas at stages 3-5 but appeared subsequently in stages 6-8. Only 3% protein content was detected, and this value did not markedly change during ripening, (Robinson & Sauco, 2011). As ripening progresses, the water-insoluble fibre decreases, and the soluble fibre increases significantly (3-6%) except at stage 5. This agreed with the softer in texture as they ripen. The cell wall of the less-mature fruit is generally more compact due to the pectin molecules being tightly bound in the cell wall, which could contribute to the firmness of the fruits Table 1shows that banana contains high soluble fibre which is likely to be pectin.

Effect of ripening stage on glucose availability and RS

The RAG, SAG and RS contents are shown in Figure 1, in terms of glucose units formed by starch hydrolysis. An increase in the RAG content was observed with advancing ripening stage. The RAG contents of stages 3 (12.5 g/100 g DW) and 4 (12 g/100 g DW) were not very different compared with that obtained in stage 5 (19 g/100 g DW). A marked change was observed during the transition from stage 5 to stage 6 (32 g/100g DW). In stage 6, the entire banana turned yellow (as indicated in the methods section). By comparison, bananas contain a lower RAG (40%) compared to cornflakes (70%) and biscuits (50%) (Bhavya & Prakash, 2012).

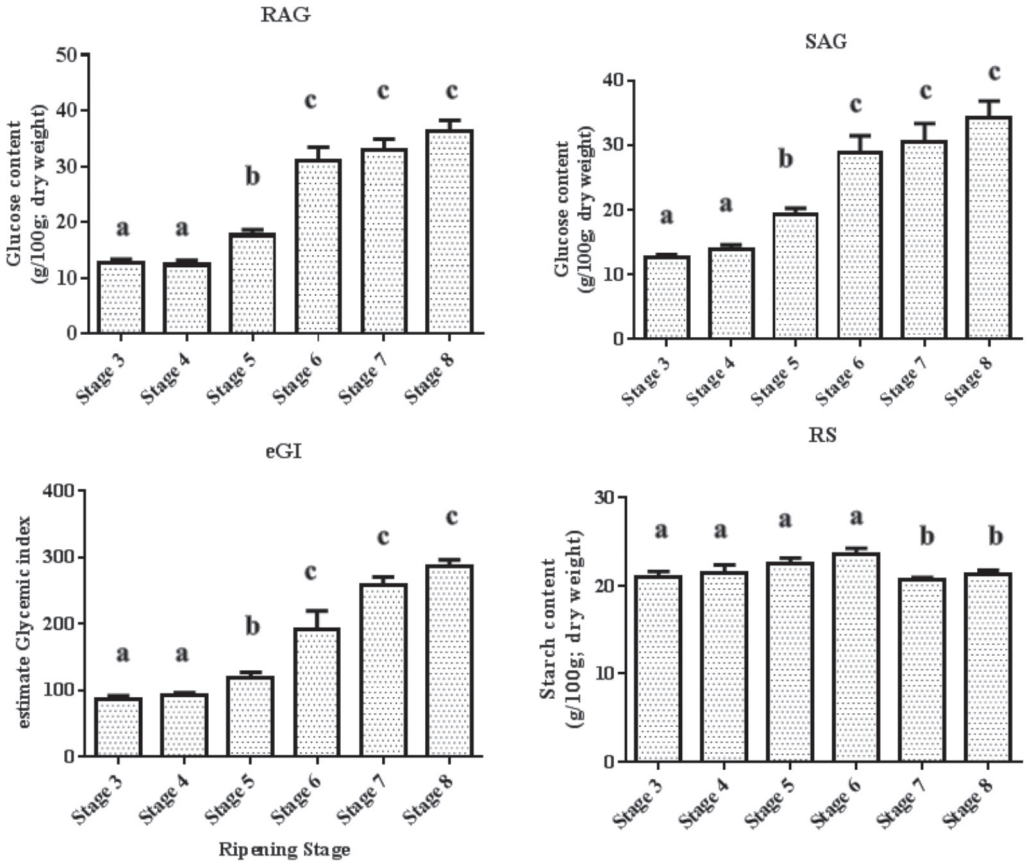

Figure 1. *In vitro* starch hydrolysis of banana at different ripening stages (g/100 g DW).
[a,b, c] Different alphabets indicate significant difference at $p<0.05$

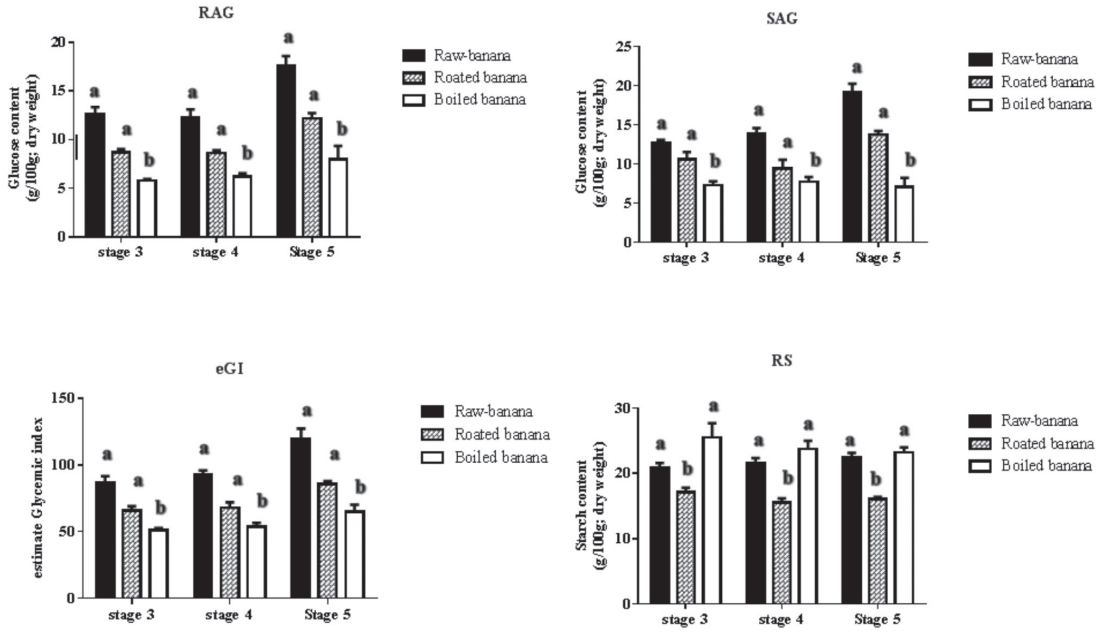

Figure 2. RAG, SAG, RS content and pGI of banana stage 4, 5, 6 cooked by different methods. [a, b, c] Different alphabets indicate significant difference at $p<0.05$

Figure 2 shows that ripe bananas have a high sugar content. This finding indicates a direct correlation between the RAG and SAG and eGI, which is in agreement with the results of several studies (Bhavya & Prakash, 2012; Englyst & Englyst, 2005). The estimated GI was quite high when was compared with sucrose of the same weight. RS was detected in all stage of bananas. This finding implies that most raw banana starch is resistant to enzymes found in nature. This type of starch known as RS2 represents starch that is in a certain granular form and resistant to enzyme digestion according to the Englyst classification, RS type 1-5.

In raw starch granules; starch is tightly packed in a radial pattern and is relatively dehydrated. This compact structure limits the accessibility of digestive enzymes, various amylases, and accounts for the resistant nature of RS2. The RS2 has slightly increased at the beginning from stage 3 to stage 6 but then declined from stage 7 to stage 8. This shows that RS2 formed progressively as banana was ripening and declined after the peel colour turned entirely into yellow. The decreased of RS3 at stage 7 and 8 (Figure 1) can be explained by the increase in moisture content (Table 1) hence RS2 was rehydrated and therefore became less resistant. Moreover, as ripening progresses, pectin esterase, α- and α-amylase activities increase which loosens starch that is tightly packed, results in available starch (Soares *et al.*, 2011).

Three types of sugars were detected in fresh bananas, namely sucrose, fructose and glucose (Table 2). However, the concentration varied with the ripening stage. Ripe bananas had a higher sucrose content compared with unripe bananas. The glucose and fructose concentrations exhibited a similar trend, increasing from stage 3 to stage 8 with a slightly lower concentration at stage 8.

Table 2. Fructose, glucose and sucrose of fresh and cooked banana of different maturity stages (g/100g DW)

Maturity stages	*Fructose*	*Glucose*	*Sucrose*
Fresh banana			
Stage 3	4.68±0.40[b]	3.73±0.03[b]	16.71±0.50[a]
Stage 4	6.49±0.50[b]	5.68±0.72[b]	17.65±0.29[a]
Stage 5	5.49±0.28[b]	4.63±0.40[b]	27.32±0.46[b]
Stage 6	8.68±0.30[c]	7.81±0.25[c]	36.75±0.15[c]
Stage 7	10.14±0.50[c]	9.12±0.40[c]	40.91±0.23[c]
Stage 8	9.77±0.42[c]	8.76±0.30[c]	44.07±0.33[c]
Roasted banana			
Stage 3	2.00±0.47[a]	0.63±0.75[a]	20.58±0.49[a]
Stage 4	2.39±0.70[a]	0.67±0.44[a]	26.45±0.19[b]
Stage 5	2.31±0.12[a]	0.89±0.39[a]	30.72±0.18[b]
Boiled banana			
Stage 3	1.25±0.26[a]	0.24±0.16[a]	17.78±0.34[a]
Stage 4	1.40±0.70[a]	0.44±0.22[a]	26.25±0.04[b]
Stage 5	3.10±0.21[b]	1.17±0.31[a]	32.55±0.40[b]

Values are mean±SD
[a, b, c] Different alphabets indicate significant difference at $p<0.05$

Effect of cooking on glucose availability, RS content and eGI

Table 2 shows higher content of sucrose in roasted and boiled bananas. This may be explained by cell wall degradation by high heat and starch degradation. Higher content of sucrose is also due partly to lower moisture content in cooked banana. The reduction of glucose and fructose was caused by their participation in the Maillard reaction, as evidenced by the brown colour of the roasted and boiled bananas.

Reduction of glucose and fructose varied at each stage, indicating that cooking method influences the release of glucose molecules differently, as shown in Figure 2. Roasting and boiling significantly reduced the RAG of bananas at stages 3, 4 and 5. These stages were chosen for their cooking applications. Bananas at stages 1-2 were very green and hard, whereas those are stages 7-8 were overripe and very soft; thus, none of these four stages are suitable for cooking. Roasting relatively reduced the RAG via the Maillard reaction by producing a Maillard reaction byproduct (MRP), which is the chemical reaction of reducing sugar and protein present in banana. In addition, the MRP was found to exhibit α-amylase activity (Chung, Lee & Rhee, 2011).

Boiling yielded the lowest RAG and SAG contents detected, which may be due to the leaching of sugar into the boiling water, compared to starch, which is less mobilized. The RS content level was ranked highest in boiling, followed by roasting. Increase in the RS content by boiling whole bananas was more likely due to the migration of starch from the banana peel into the pulp, while roasting led to significant reduction of RS (Figure 2). Boiling provided moist heat enabling starch to be gelatinized prior to recrystallization to form RS (specifically RS3). Therefore, conditions that could fully gelatinize banana starch favor the formation of RS3.

The eGI of boiled bananas was lower than that of the roasted sample due to

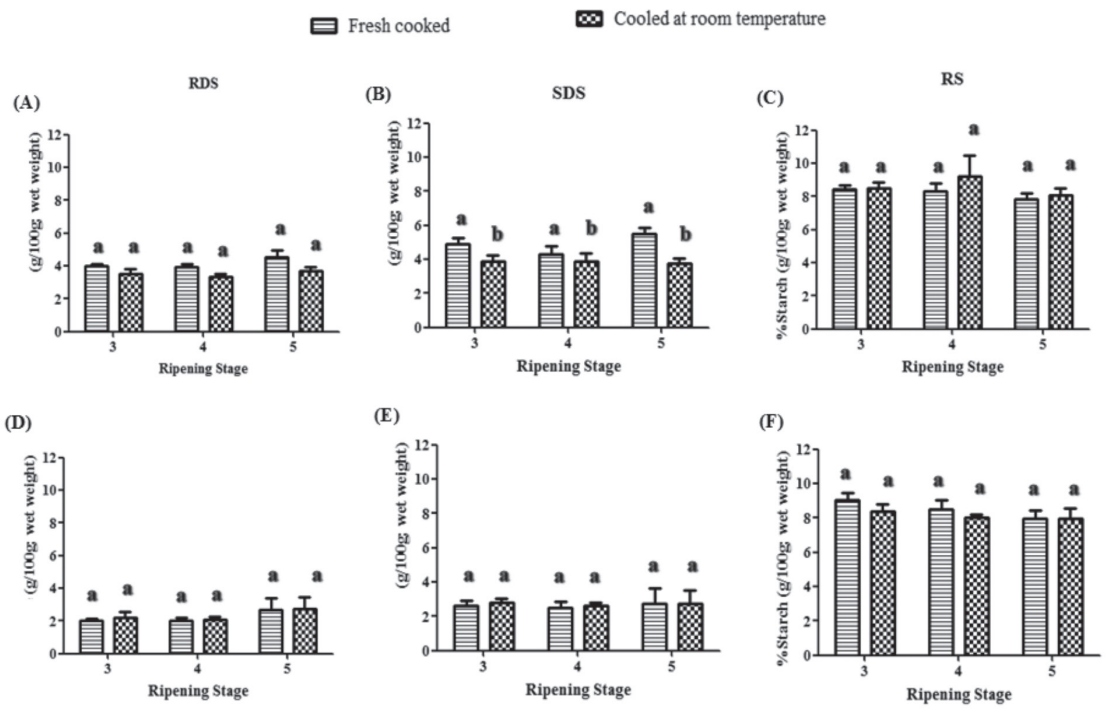

Figure 2. RAG, SAG, RS content and pGI of banana stage 4, 5, 6 cooked by different methods. [a, b, c] Different alphabets indicate significant difference at $p<0.05$

the whole, unpeeled banana being boiled and the tannins in the banana exhibiting α-amylase inhibition; therefore, the reduction of the GI of boiled bananas maybe due not only to a low sugar concentration but also other factors that inhibit α-amylase activity. Moreover, the consumption of RS proved an improvement in the glucose metabolism (Tabibloghmany & Ehsandoost, 2014). Thus, the presence of RS could provide health benefits, including an increase in the total dietary fibre. Boiling is a better method compared with roasting because it results in lower RAG and SAG contents, which determines the GI, as well as a high RS content.

Effect of storage conditions on RAG, SAG, RS and eGI

Several studies have shown that retrogradation results in RS formation. Thus, cooking and cooling have been found to increase the RS content (Arcila, Weier & Rose, 2015).

As shown in Figure 3, the RS increased after the roasted bananas were maintained at room temperature for 120 mins, compared with 0 min. This finding may be explained by the recrystallization of complex carbohydrates during cooling. This result is in agreement with the hardening of the outer banana pulp, whereas in boiled bananas, the trend was not apparent. In addition, stages 3 and 4 showed the reduction of RS after the bananas were left to cool at room temperature. After being cooled at room temperature, the final temperature of the roasted and boiled bananas was 30°C and 29°C, respectively. The heat transfer rate of the roasted and boiled bananas was 0.6°C/min and 0.5°C/min, respectively. These heating rates

were not different; therefore, it was postulated that the differences in the RS formation were due to the differences in the moisture content, type of heat and microstructure. Consequently, a different rearrangement of amylose and amylopectin might have occurred.

Roasting resulted in starch hydrolysis due to the decrease in the RDS and DSD content (Figure 3) because heat was involved in the process and bananas contain both reducing sugars and proteins (Tables 1 and 2). It is assumed that the Maillard reaction would occur in both boiled and roasted bananas. In terms of the Maillard reaction and its properties, eating cooked bananas could result in increased blood sugar via α-amylase inhibition. Furthermore, a MRP has been associated with impairments in glucose metabolism. However, the active dose of the MRP has not yet been defined. From the experiment, the reduction in temperature was not an effective procedure for improving RS formation in both roasted and boiled bananas. The formation of RS was limited by the hydrolysis of starch during heating, leading to a low amylose and amylopectin content and thereby reducing retrogradation. Thus, no significant difference was observed. Therefore, it is hypothesized that a rapid reduction of temperature could assist in RS formation through the retrogradation process. In addition, RS is not only chemically distinct, but its physiological properties, including fermentation characteristics and crystal formation, need to be studied to fully understand the function of RS2 which is naturally found in banana and RS3 that is formed in bananas (*Musa sapientum*) after cooking.

CONCLUSION

This study showed that the RS content range of 20-25 g/100 g DW in the bananas studied (Nam-wa variety) did not vary with maturity. The RAG and SAG amounts increased with progression of maturity. The RS content of the bananas increased with boiling compared to roasting. The beneficial properties of RS in bananas should be further investigated.

Authors' contributions

SC performed the experiment, collected data and performed data evaluation. WS designed experiment, performed experiment, interpreted the result, prepare manuscript, corrected manuscript. WK designed experiment.

Conflict of interest

The authors declared no conflict of interest.

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