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The effect of maturity stages of banana on the formation of acrylamide in banana fritters

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ABSTRACT

The effect of maturity stages of two varieties of banana (*Musaparadisiaca* variety *Awak* and *Abu*) on the formation of acrylamide was obtained in banana fritters, the most mature banana had significantly ($p < 0.05$) higher concentrations of reducing sugars; however, the concentrations of free amino acids at different maturity stages were relatively similar ($p > 0.05$). The study indicated that reducing sugars had a significant ($p < 0.05$) and strong correlation ($R^2 = 0.92$ for Abu, $R^2 = 0.82$ for Awak) with the formation of acrylamide compared to asparagine. The formation of acrylamide in both banana varieties was enhanced with an increase in both reducing sugars (glucose and fructose). This research demonstrated that the formation of acrylamide was strongly dependent on the concentration of, both glucose and fructose.

1. Introduction

Acrylamide is formed in carbohydrate and protein rich foods during heat processing such as frying, baking and roasting at high temperatures; even microwave heating provides a suitable medium for the occurrence of acrylamide and probably affects the formation and kinetics of acrylamide distinctively due to its extraordinary heating style (Tareke, Rydberg, Karlsson, Eriksson, & Tornqvist, 2002). The most important factors for the formation of acrylamide are the occurrence of precursors, such as reducing sugars (glucose (Glc) and fructose (Fru)), an amino acid, such as asparagine, and the magnitude of the combined temperature and time (Brathen & Knutsen, 2005). Several reactions concerning the formation of acrylamide in cooked foods have been considered. These reactions are common thermally allowed reactions in food (Yaylayan, Wnorowski, & Locas, 2003).

The reducing sugars and asparagine are believed to be the major reactants in food, and that are responsible for the of acrylamide formation (Stadler et al., 2003; Zyzak et al., 2003). The potential of generating acrylamide from suitable precursors has mainly been attributed to the concentration of asparagine, when its concentration is lower than reducing sugars (Stadler et al., 2002; Zyzak et al., 2003), however, there is an indication in the literature that the type of sugars may significantly affect the final concentration of acrylamide produced through the Maillard reaction (Pollien, Lindinger, Yeretzian, & Blank, 2003; Stadler et al., 2002; Stadler et al., 2004; Zyzak et al., 2003). Asparagine has been identified as a possible source of acrylamide because decarboxylation and deamination of this amino acid, in principle can produce acrylamide. The other mechanism was also found in other study which revealed that some oxidised lipids are able to convert asparagine into acrylamide to a high extent (Zamora & Hidalgo, 2008).

The presence rather than the concentration of asparagine, i.e. the amount/concentration of acrylamide formed would depend on whether asparagine or glucose/fructose is limiting. Although there are differences between the major mechanisms for the formation of acrylamide in plant-derived foods, all involve the reaction of asparagine with glucose/fructose present in these foods. According to Surdyk, Rosean, Andersson, and Aman (2004) append asparagine caused increase concentration of acrylamide in crusts of wheat bread while added fructose did not affect the content of acrylamide. Even though the limiting factor in acrylamide formation in potato products is expected to be the concentration of reducing sugar (glucose/fructose) due to the higher amount of asparagine in potatoes (one-third of the total free-amino acid pool) (Nigel et al., 2012), Elmore et al. (2005) found a linear relationship between

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acrylamide formation and the residual levels of asparagine and reducing sugars for potato, wheat, and rye. Other researchers reported concentration of free asparagine as the major determinant of acrylamide-forming potential in wheat and rye flour (Curtis et al., 2010; Granvogl, Wieser, Koehler, Von Tucher, & Schieberle, 2007). The objective of this research was to determine the effect of the maturity stages of banana on the formation of acrylamide in banana fritters. Banana of different degrees of ripeness was presumed to contain different compositions and concentrations of sugars, which could act as precursors for acrylamide formation. The banana fritter, which has the highest acrylamide concentration of tested foods according to a previous study, was chosen to be investigated for the acrylamide formation in this study (Daniali, Jinap, Zaidul, & Hanifah, 2010).

2. Materials and methods

2.1. Materials

Ammonium acetate, phenyl isothiocyanate (PITC), cuprum sulphate, selenium dioxide methyl red, methylene blue, zinc pellet, glass ball, hydrochloric acid, boric acid, sulphric acid and sodium hydroxide were purchased from Merck (Darmstadt, Germany) and were of analytical grade. Triethylamine (TEA) and ethyl acetate were purchased from Fluka (Buchs, Switzerland), whereas sucrose, D–(−)–fructose, D–(+)–glucose and the amino acid standards were purchased from Sigma Chemical Co. (St Louis, MO, USA). Bromine was obtained from Merck (Darmstadt, Germany). Tepung goring pisang (banana fritter flour) containing rice flour and conditioner (Adabi consumer industries Serdang, Kuala lumpur, Selangor, Malaysia), salt (pure dry vacuum salt, 99.9%, ES food industries, Kajang, Malaysia), and palm olein (Buruh, Lam Soon, Selangor, Malaysia) were purchased from a local market.

2.2. Samples

The two varieties of banana used in the study, Abu and Awak are commonly used to produce banana fritter. Two mature branches (stage 1; green stage) of each variety was purchased from an open market in Serdang, Malaysia, and stored at room temperature for 2 weeks to allow for ripening. The maturity of both banana varieties (stage 1–4) was determined based on peel colour using a colorimeter (Minolta CR-10, Konica Minolta, Tokyo, Japan); the colorimeter readings for each stage of maturity are shown in Table 1. Banana of four different maturity stages for both varieties were also analysed for amino acid, reducing sugar, sucrose and acrylamide concentrations (with 3 replications).

2.3. Sample preparation

Six bananas were picked randomly from three different parts of each branch of the Awak and Abu for each maturity stage (2 from the top, 2 from the middle and 2 from the end of the branch). Three bananas were peeled and crushed with a crusher (Braun, Frankfurt, Germany) for 3 min before the samples were used for the determination of reducing sugars and amino acids. The other 3 banana samples were used for frying, to produce banana fritters as food samples for acrylamide determination. The experiment was replicated twice.

The preparation of the banana fritter samples was as follows. First, the banana was peeled and soaked in a dough (30 min, 21 °C), which consisted of banana fritter flour (42.8%), salt (0.2%) and distillated water (57%). The banana and dough were mixed for 2 min. The samples were deep fried in palm olein using an electrical fryer (Philips HD 6153, Beijing, China) at 170 °C. The temperature was monitored by the use of a thermometer 51 series II coupled with a temperature probe (Fluke Corporation, Everett, USA) for 10 min. After cooling, the acrylamide concentration was determined. The same dough was used for all bananas with different maturity degrees.

2.4. Instrumentation

A high performance liquid chromatography system (HPLC) (JASCO 2080 Tokyo, Japan) equipped with a fluorescence detector (MD–2010 plus FP–2020), pump (PU–2080) degasser (DG–2080–54) and wavelength (254 nm) was used for amino acid analysis. A Lichrocart analytical column (250 × 4.6 mm i.d. and 5 μm particle size) from Merck (Darmstadt, Germany) was used. The flow rate was 1 mL/min.

The HPLC used for sugar analysis was consisted of a gradient unit (Model LG–1580–04), a pump (PU–1580) and a refractive index detector (RI–1350, JASCO Corp. Tokyo, Japan). Manual injections were carried out using a Rheodyne injector (Model 7125 Tokyo, Japan) with a 20 μL sample loop. The analytical column used was NH_2 μBondapak (Waters Corporation, Massachusetts, USA) with a 125Å pore size, 300 × 3.9 mm i.d and 10 μm particle size. The flow rate was 1 mL/min.

Gas chromatography–mass spectrometry (GC–MS) used for acrylamide analysis was HP 5890 gas chromatograph, connected to an HP 5973 mass spectrometer and HP 7683 auto sampler (Hewlett–Packard, Avondale, PA, Pennsylvania, USA). The GC column used was an Innowax capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies, Palo Alto, CA, USA). The MS conditions were a follows: ionization by EI mode at 70 eV with ion source temperature as high as 230 °C, ion analysis by TOF (time of flight) analyser with scan range at 20–500 *m/z*, and using SIM (selected ion monitoring) mode for monitoring ions at *m/z* 149 for 2–bromopropenamide, and *m/z* 154 for 2–bromo ($^{13}C_3$) propenamide.

2.5. Determination of sugars by HPLC

The HPLC method used to determine the sugars in Awak and Abu varieties at the four maturity stages were performed according to the method of Hunt, Jackson, Mortlock, and Kirtc (1977) and AOAC (2000). The sugar standards used were glucose, fructose and sucrose in the concentration range of 0.5, 1, 1.5, 2 and 2.5% (w/v). The extraction of samples performed prior to injection into HPLC was carried out according to Wills, Balmer, and Greenfield (1980) and AOAC (2000).

Table 1
Peel colour of 4 stages of maturity of two varieties of commercial banana (Awak and Abu) ($n = 3$).

Banana variety	Stage 1 green			Stage 2 yellow green			Stage 3 yellow			Stage 4 yellow with black spots		
	L	*C*	*h*	*L*	*C*	*h*	*L*	*C*	*h*	*L*	*C*	*h*
Awak	55.0	37.4	119.2	55.4	35.9	117.4	66.3	45.5	90.2	69.3	56.3	90.1
Abu	55.5	38.3	119.3	55.9	37.4	117.3	70.8	48.2	90.7	73.5	56.0	90.9

L = lightness ranged from 0 for black to 100 for white, *C* = Chroma indicate a hue of red/purple, bluish/green and also indicate yellow and blue, Chroma is calculated as $(a2 + b2)^{½}$ and *h* = hue is calculated from the arctangent of *b/a*.

Sample preparation for sugar analysis was as follows: ten grams of the crushed banana was heated with 150 mL of methanol on a steam bath for 30 min. The mixture was filtered using Whatman No. 1 filter paper (Kent, UK) into a round bottom flask. The residue was re-extracted twice in 100 mL portions of methanol, and then filtered. The filtrate was evaporated to less than 10 mL under vacuum at 50 °C in a rotary evaporator (Rotavapor R–210, Buchi Labror technik AG, Flawil, Switzerland). The volume was increased to 10 mL in a volumetric flask. The solution was then filtered through a SEP–PAK cartridge and a 0.45 μm membrane filter. Ten micro litres of this solution were injected to HPLC equipped with refractive index detector. The mobile phase used in this HPLC was acetonitrile and distilled water (80:20, v/v).

2.6. Determination of amino acids by HPLC

The samples used for amino acid analysis were the same as those used for sugar analysis. The determination of amino acids by HPLC was carried out according to Khan, Kuo, Kebede, and Lambein (1994) and AOAC (2002). One gram of the crushed banana was weighted, transferred into a tube, combined with 15 mL of 6 N HCl and stirred by autovortex mixer (Stuart scientific, Manchester, England) for 2 min. The tube was heated in an oven at 60 °C for 24 h and then combined with 10 mL of an internal standard and a sufficient volume of deionized water to reach a final volume of 50 mL. The upper layer was filtered into a tube and was stored at −20 °C until use in HPLC analysis.

Ten-microlitre aliquots of extract or 10 μL of the amino acid standards mixture for preparing standard was dried under vacuum (37 °C, 20 mmHg). A 20 μL aliquot of the first coupling reagent [methanol, water, triethylamine (TEA) (2:2:1; v/v)] was added, and the mixture was swirled after mixing, the sample was dried under vacuum (Rhino Pump, Ningbo, China) for 10 min and then reacted with 30 μL of phenyl isothiocyanate (PITC) reagent (methanol, PITC, TEA, water (7:1:1:1; v/v)) at room temperature for 20 min before drying under a vacuum to remove the PITC. The derivatized samples were then redissolved in 500 μL of buffer A, which is used as mobile phase for HPLC and filtered through a Millipore membrane (0.22 μm). A 20 μL sample of this mixture was injected in to an HPLC system. Buffer A (0.1 M ammonium acetate, pH 6.5) and buffer B (0.1 M ammonium acetate containing acetonitrile and methanol, 44:46:10, v/v, pH 6.5) were used as a mobile phase (80:20).

3. Statistical analysis

Analysis of variance (ANOVA) was used to analyse the significant difference between acrylamide, reducing sugar and amino acids concentrations. The descriptive statistics and ANOVA were performed using Minitab (Release 14 for Windows, Pennsylvania, USA). Tukey's test was used for the statistical analysis; a p-value of less than 0.05 was considered to indicate statistical significance. The strength of the association between acrylamide, reducing sugar and amino acids concentrations in the samples was measured using Pearson's correlation coefficient.

4. Results and discussion

The amino acids compositions of Awak and Abu banana varieties are presented in Table 2 and Table 3, respectively. The amino acids composition of Abu and Awak bananas were different at different maturity stages. Asparagine did not show any significant difference in concentration ($p > 0.05$) between different maturity stages and different banana varieties. Asparagine was present in Abu variety at a range of 1.37–2.16 mg/g (fresh wt.), and in Awak range of 0.92–1.37 mg/g (fresh wt.). This results showed that the presence of asparagine and reducing sugar are determining factors for acrylamide formation, but correlation between acrylamide formation and asparagine concentration is extremely weak.

As shown in Tables 2 and 3, both varieties showed no significant difference ($p > 0.05$) in asparagine concentrations at different maturity stages; however, for glutamine concentrations, there were significant differences ($p < 0.05$) between stages 1 and 2 with 3 and 4 in Awak variety. The concentrations of asparagine and glutamine in the Abu variety were significantly higher ($p < 0.05$) than those in the Awak variety.

Table 4 shows the concentrations of fructose, glucose and sucrose in the two banana varieties at different maturity stages. The calibration curves obtained were all in the acceptable linearity range ($R^2 > 0.990$). Each calibration curve was used to calculate each corresponding sugar concentration. The sugars in the samples were quantified by comparing the peak areas of samples with those of the standards. Fructose and glucose are reducing sugars, which are important for Maillard reaction. Although sucrose is not a reducing sugar, it is a possible reaction partner, considering its potential hydrolysis into an equimolar mixture of glucose and fructose upon heating.

Table 2
Concentration of free amino acids (mg/g of fresh wt) in commercial banana (Awak) after hydrolysis at 4 stages of maturity after acid hydrolysis and pre-column derivatization by phenyl isothiocyanate ($n = 3$).

Amino acids	Stage 1 green	Stage 2 yellow green	Stage 3 yellow	Stage 4 yellow with black spots
Asparagine (Asn)	0.92 ± 0.54[a*]	1.22 ± 0.41[a]	1.37 ± 0.07[a]	1.04 ± 0.19[a]
Glutamine (Gln)	0.80 ± 0.28[b]	0.78 ± 0.064[b]	1.29 ± 0.27[a]	1.05 ± 0.15[a]
Serine (Ser)	0.42 ± 0.07[a]	0.34 ± 0.016[a]	0.68 ± 0.01[a]	0.68 ± 0.08[a]
Glycine (Gly)	0.26 ± 0.12[b]	0.34 ± 0.02[b]	0.50 ± 0.05[a]	0.50 ± 0.04[a]
Histidine (His)	0.15 ± 0.01[b]	0.15 ± 0.05[b]	0.36 ± 0.04[a]	0.49 ± 0.32[a]
Arginine (Arg)	1.10 ± 1.55[b]	ND	0.41 ± 0.06[a]	0.42 ± 0.05[a]
Threonine (Thr)	2.00 ± 0.40[a]	1.02 ± 0.01[b]	0.74 ± 0.21[b]	0.64 ± 0.07[b]
Alanine (Ala)	0.60 ± 0.04[a]	0.57 ± 0.02[a]	0.57 ± 0.01[a]	0.55 ± 0.08[a]
Proline (Pro)	0.20 ± 0.17[b]	0.32 ± 0.01[b]	0.43 ± 0.04[a]	0.40 ± 0.01[a]
Tyrosine (Tyr)	0.03 ± 0.04[a]	0.08 ± 0.01[a]	0.13 ± 0.01[b]	0.16 ± 0.02[b]
Valine (Val)	0.27 ± 0.16[a]	0.42 ± 0.01[a]	0.32 ± 0.02[b]	0.37 ± 0.10[b]
Isoleucine (Ile)	0.07 ± 0.10[a]	0.26 ± 0.01[b]	ND	0.26 ± 0.01[b]
Leucine (Leu)	2.4 ± 1.58[a]	1.41 ± 0.06[a]	4.79 ± 0.79[b]	4.51 ± 0.44[b]
Phenylalanine (Phe)	0.34 ± 0.48[a]	0.52 ± 0.06[a]	0.33 ± 0.01[b]	0.28 ± 0.03[a]
Lysine (Lys)	0.43 ± 0.02[a]	0.41 ± 0.01[a]	0.43 ± 0.02[a]	0.40 ± 0.03[a]

*Similar letter in each row shows insignificant difference ($p > 0.05$).

Table 3
Concentration of free amino acids (mg/g of fresh wt) in commercial banana (Abu) after hydrolysis at 4 stages of maturity (1.green, 2. yellow green, 3. yellow, 4. yellow with black spot), after acid hydrolysis and pre–column derivatization by phenyl isothiocyanate ($n = 3$).

Amino acids	Stage 1 green	Stage 2 yellow green	Stage 3 yellow	Stage 4 yellow with black spots
Asparagine (Asn)	$1.37 \pm 0.01^{a*}$	2.16 ± 0.60^{a}	1.97 ± 0.42^{a}	1.97 ± 0.51^{a}
Glutamine (Gln)	1.08 ± 0.01^{a}	1.26 ± 0.02^{a}	1.44 ± 0.22^{a}	1.45 ± 0.18^{a}
Serine (Ser)	0.38 ± 0.01^{a}	0.64 ± 0.01^{b}	0.69 ± 0.06^{b}	0.69 ± 0.07^{b}
Glycine (Gly)	0.30 ± 0.03^{a}	0.55 ± 0.01^{b}	0.61 ± 0.04^{b}	0.61 ± 0.01^{b}
Histidine (His)	0.23 ± 0.05^{a}	0.53 ± 0.01^{b}	0.59 ± 0.04^{b}	0.59 ± 0.12^{b}
Arginine (Arg)	0.01 ± 0.03^{a}	0.48 ± 0.07^{b}	0.51 ± 0.08^{b}	0.51 ± 0.01^{b}
Threonine (Thr)	1.39 ± 0.02^{a}	1.10 ± 0.71^{a}	1.15 ± 0.49^{a}	1.14 ± 0.48^{a}
Alanine (Ala)	0.62 ± 0.02^{a}	0.65 ± 0.02^{a}	0.70 ± 0.01^{a}	0.70 ± 0.01^{a}
Proline (Pro)	1.37 ± 0.01^{a}	0.48 ± 0.01^{b}	0.50 ± 0.06^{b}	0.51 ± 0.02^{b}
Tyrosine (Tyr)	0.08 ± 0.02^{a}	0.18 ± 0.03^{b}	0.21 ± 0.05^{b}	0.21 ± 0.01^{b}
Valine (Val)	0.34 ± 0.04^{a}	0.27 ± 0.02^{a}	0.36 ± 0.13^{a}	0.36 ± 0.01^{a}
Isoleucine (Ile)	0.21 ± 0.01^{a}	0.07 ± 0.00^{b}	0.14 ± 0.13^{b}	0.15 ± 0.12^{b}
Leucine (Leu)	1.36 ± 0.01^{a}	4.00 ± 1.18^{b}	6.16 ± 0.55^{b}	6.16 ± 2.00^{b}
Phenylalanine (Phe)	0.64 ± 0.01^{a}	0.28 ± 0.05^{b}	0.38 ± 0.07^{b}	0.38 ± 0.01^{b}
Lysine (Lys)	0.39 ± 0.01^{a}	0.51 ± 0.01^{b}	0.56 ± 0.059^{b}	0.56 ± 0.01^{b}

*Similar letter in each row shows insignificant difference ($p > 0.05$).

From a chemical point of view, glucose as an aldohexose sugar was expected to generate more acrylamide from asparagine monohydrate, due to its higher chemical reactivity provided by the more reactive aldehyde group compared to the ketohexose fructose (Robert et al., 2004). However, results from our study are in agreement with those of Robert et al. (2004) showed that the fructose generated acrylamide at an earlier time than glucose, although, fructose has been shown to be more efficient than glucose in the formation of acrylamide in a potato system (Rydberg et al., 2005). The glucose content is often higher than fructose in potatoes resulting in the two similarly influencing the formation of acrylamide.

Based on results presented in Table 4 there was a significant difference ($p < 0.05$) in the Abu variety; however, there was no significant difference ($p > 0.05$) between the concentrations of the total reducing sugars in the Awak variety, except in stage 1. The values were found to be much higher in the Abu variety ($p < 0.05$) at stage 4. However, the glucose and fructose concentrations in the Abu variety were not significantly different ($p > 0.05$). Glucose concentration in the Abu variety ranged from 0.57 to 6.48 mg/g (fresh wt.), and the corresponding values for fructose were from 0.62 to 7.61 mg/g. There was a significant difference between the fructose and glucose concentrations ($p < 0.01$) in the Awak variety in stage 1 and in the other 3 stages. Their concentrations were higher in the other stages. The corresponding values for fructose ranged from 0.70 to 4.39 mg/g for the Awak variety, whereas glucose ranged from 0.80 to 4.20 mg/g fresh wt. The sucrose concentration in the Awak variety was 1.33–6.57 mg/g fresh wt, and in the Abu variety, it ranged from not detected to 7.87 mg/g fresh wt.

The results obtained from the analysis of acrylamide in the banana varieties are shown in Table 5. The acrylamide concentration in the banana fritter made from the Abu variety ranged from 173.43 to 879.92 μg/kg and those from the Awak variety ranged from 30.07 to 201.18 μg/kg. The acrylamide concentrations were significantly different ($p < 0.05$) between different maturity stages of the Abu variety. The mean acrylamide concentrations in the Abu variety samples from stage 4 were approximately two times higher than those from stage 2. This result supports the results related to sugar concentrations (Table 4). Table 5 shows that the acrylamide concentration in both varieties corresponded to those of the reducing sugar in both varieties. This finding

Table 4
Concentration of reducing sugar (mg/g of fresh wt) in two varieties of commercial banana at 4 stages of maturity.

Sugar	Variety of banana	Stage 1	Stage 2	Stage 3	Stage 4
Reducing sugars					
Fructose	Abu	$0.62 \pm 0.25^{d*}$	2.50 ± 0.11^{c}	4.95 ± 0.11^{b}	7.61 ± 0.28^{a}
	Awak	0.70 ± 0.37^{b}	4.13 ± 0.68^{a}	3.98 ± 0.94^{a}	4.39 ± 0.11^{a}
Glucose	Abu	0.57 ± 0.32^{d}	2.23 ± 0.63^{c}	3.97 ± 0.13^{b}	6.48 ± 0.47^{a}
	Awak	0.80 ± 0.19^{b}	4.20 ± 0.64^{a}	3.95 ± 0.95^{a}	3.96 ± 0.60^{a}
Non reducing sugar					
Sucrose	Abu	1.03 ± 0.17^{b}	5.00 ± 0.83^{a}	ND**	7.87 ± 0.32^{a}
	Awak	1.33 ± 1.44^{d}	3.70 ± 1.00^{b}	6.57 ± 0.01^{a}	1.66 ± 0.48^{c}

*Similar letter in each row shows insignificant difference ($p > 0.05$).
**ND = non detected.

Table 5
Concentration of acrylamide (μg/kg) in banana fritters from two varieties of commercial banana (Awak and Abu) at 4 stages of maturities ($n = 3$).

Parameter	Banana variety	Stage 1 green	Stage 2 yellow green	Stage 3 yellow	Stage 4 yellow with black spot
	Awak	67.3 ± 37^{bB}	157.2 ± 36.1^{aB}	208.2 ± 40^{aB}	187.3 ± 19.6^{aB}
	Abu	$201.6 \pm 28^{dA*}$	368 ± 40^{cA}	502 ± 42^{bA}	809.2 ± 41^{aA}

*Similar capital letter in each column shows insignificant difference ($p > 0.05$).
*Similar letter in each row shows insignificant difference ($p > 0.05$).

Table 6
Pearson's correlation between acrylamide and sugars concentration of two varieties of commercial banana (Awak and Abu).

Banana variety	Sugar	Pearson's correlation (r)	P-Value	R^2
Awak	Glucose	0.810	0.004	0.796
	Fructose	0.803	0.005	0.782
	Sucrose	0.368	0.416	0.094
	Reducing sugar	0.849	0.002	0.829
Abu	Glucose	0.956	0.001	0.914
	Fructose	0.948	0.001	0.899
	Sucrose	−0.067	0.85	0.023
	Reducing sugar	0.968	0.001	0.920

demonstrates that the increase in acrylamide concentration in the Abu variety corresponds with the increases in glucose and fructose concentration in each stage. These results also show that in the Awak variety, the formation of acrylamide presented a strong dependence on the concentration of reducing sugar. These findings are in agreement with those of Silva and Simon (2005). Their study showed that glucose and fructose concentrations in the tubers were significantly and positively correlated with subsequent acrylamide formation in the products. Tuber sucrose and asparagine concentrations did not have an effect on acrylamide levels.

The acrylamide concentrations in samples of Awak variety (Table 5) parallelled the sugar concentrations (Table 4). Moreover, a significant difference ($p < 0.05$) was found between acrylamide concentrations of stage 1 and the other maturity stages, which supports the results found for the sugar concentrations. These results also indicated that the formation of acrylamide in banana fritters was dependent on the concentration of reducing sugar in banana varieties.

The correlations between acrylamide and sugars concentration for the Awak and Abu varieties were examined using pearson's correlation (r) and the results are shown in Table 6. The data showed a highly significant correlation ($R^2 = 0.83–0.92$; $p < 0.01$) between reducing sugar and acrylamide concentration. It is noted that R^2 value of more than 0.7 shows strong correlation (Damez, Clerjon, Abouelkaram, & Lepetit, 2008; Mustonen, Hissa, Huotilainen, Miettinen, & Tuorila, 2007). These data indicate that concentrations of reducing sugars are more important than those of amino acids for formation of acrylamide in banana fritters. This finding corresponds to the results from other researchers who found that acrylamide formation was preliminarily related to sugar concentration (Amrein et al., 2003; Becalski et al., 2004; Grob et al., 2003). The correlation between glucose and acrylamide concentration was higher than that for fructose. Claeys, de Vleeschouwer, and Hendrickx (2005) found that glucose reacted more effectively in generating acrylamide than fructose when the heating temperature was higher than 140 °C in a model system. This is in good agreement with our findings for the Awak variety, which presented a correlation between glucose and acrylamide concentration ($R^2 = 0.796$; $p < 0.01$) that was higher than that for fructose ($R^2 = 0.782$; $p < 0.01$). The correlation between glucose and acrylamide concentration for the Abu variety ($R^2 = 0.914$; $p < 0.01$) was higher than that for fructose ($R^2 = 0.899$; $p < 0.01$). The result showed that glucose is more reactive in acrylamide formation than fructose when both of them are present in the banana fritter. Although Rydberg et al. (2005) found that fructose is more efficient precursor than glucose in forming acrylamide in a potato system, these two substrates, especially fructose can be involved in other reactions.

Similar results were observed for the correlations of reducing sugars with the acrylamide formation in the banana fritters made from the Abu and Awak varieties. Fructose and glucose had higher correlations compared to other sugars contained in the two banana varieties, i.e. sucrose ($R^2 = 0.023$ and 0.094). The correlations between these two reducing sugars and acrylamide formation found in this study are in agreement with those found in other studies (Chuda et al., 2003; Ohara–Takada et al., 2005; Williams, 2005). In a related study on the influence of sugars and amino acids on acrylamide formation, Becalski et al. (2004) found that the acrylamide levels of French fries can be minimized by using potatoes with low levels of sugars (fructose, glucose, and sucrose). By means of labelling experiments it has been shown that the entire carbon skeleton of acrylamide originates from asparagines (Zyzak et al., 2003). Although thermal decarboxylation and deamidation of asparagine alone, in principle, can lead to acrylamide generation, the presence of sugars is necessary to effect the conversion of asparagine into acrylamide (Yaylayan et al., 2003; Zyzak et al., 2003).

For both varieties, there was no correlation found between acrylamide and asparagine concentrations (Table 7). However, it seems that as long as asparagine is present, the concentration of reducing sugars are important role for information of acrylamide

Table 7
Pearson's correlation between acrylamide and amino acids concentration of two varieties of commercial banana (Awak and Abu).

	Awak			Abu		
Amino acids	Pearson's correlation (r)	P-value	R^2	Pearson's correlation (r)	P-value	R^2
Asparagine (Asn)	0.147	0.686	0.021	0.415	0.180	0.172
Glutamine (Gln)	0.581	0.078	0.337	0.486	0.109	0.236
Serine (Ser)	0.442	0.201	0.195	0.474	0.119	0.225
Glycine (Gly)	0.513	0.129	0.263	0.386	0.216	0.148
Histidine (His)	0.288	0.420	0.082	0.735	0.006	0.540
Arginine (Arg)	−0.111	0.760	0.012	0.525	0.080	0.275
Threonine (Thr)	−0.709	0.022	0.502	0.181	0.574	0.032
Alanine (Ala)	−0.520	0.123	0.270	0.462	0.130	0.213
Proline (Pro)	0.507	0.135	0.256	0.400	0.198	0.159
Tyrosine (Tyr)	0.535	0.111	0.286	0.503	0.096	0.252
Valine (Val)	0.071	0.846	0.005	0.265	0.405	0.070
Isoleucine (Ile)	−0.268	0.454	0.07	0.51	0.914	0.122
Leucine (Leu)	0.533	0.112	0.284	0.235	0.462	0.093
Phenylalanine (phe)	−0.303	0.395	0.091	−0.541	0.069	0.292
Lysine (Lys)	−0.206	0.568	0.042	0.305	0.334	0.093
Total amino acids	0.380	0.279	0.144	0188	0.558	0.144

(Amrein et al., 2003; Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002; Williams, 2005; Yaylayan et al., 2003).

5. Conclusions

The concentration of acrylamide in banana fritter of both banana varieties was enhanced by the increase in the concentration of the reducing sugars, glucose and fructose with the increase of the maturity stages of banana. This finding demonstrated that the acrylamide concentration is strongly dependent on the concentration of reducing sugars and the reducing sugars are the limiting factor for the formation of acrylamide in banana fritters.

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