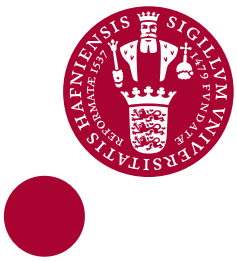

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Lasekan, Ola ; Khatib, Alfi; Juhari, Nurul Hanisah Binti; Pattiram, Parveen; Lasekan, Seye

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High-performance liquid chromatographic determination of 5-hydroxymethyl furfural in roasted plantain cultivars

Ola Lasekan [1]*, Alfi Khatib [2], Hanisah Juhari [3], Parveen Patiram [1] and Seye Lasekan [2]

[1] Department of Food Technology, Faculty of Food Science and Technology, University Putra Malaysia, UPM 43400, Serdang, Selangor, Malaysia. [2] Department of Food Science, Faculty of Food Science and Technology, University Putra Malaysia, UPM 43400, Serdang, Selangor, Malaysia. [3] Department of Food Service and Management, Faculty of Food Science and Technology, Universiti Putra Malaysia, UPM 43400, Serdang, Selangor, Malaysia.
e-mail: nurulhanisah.juhari@gmail.com, lasekan@food.upm.edu.my

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Abstract

The extent of Maillard reaction and the development of 5-(hydroxymethyl)-2-furaldehyde (HMF) were evaluated in two roasted plantain cultivars (French sombre and Dwarf kalapua), at different ripening stages. Roasting at high temperature (210°C) and roasting duration were shown to increase the development of HMF in the roasted samples. Similarly, ripening stage had significant influence on the development of HMF. The colour and fluorescence intensities were significantly ($P > 0.05$) increased by the roasting conditions and the degree of ripening. In addition, the plantain cultivars showed positive correlations with HMF, fluorescence, colour index and the sugars, respectively.

Key words: Roasted plantains, hydroxymethyl furfural, fluorescence, colour index, Maillard reaction.

Introduction

Plantain, a member of the banana family, is native to India and grown most widely in tropical climates [1]. Plantains tend to be firmer and lower in sugar content than the dessert bananas. Bananas are most often eaten raw, while plantains tend to be cooked, fried or roasted. Plantains are used in many savoury dishes somewhat like a potato would be used and is very popular in Western Africa and the Caribbean countries [2]. In Nigeria, plantain is eaten boiled, fried or roasted. Roasted plantain ('booli') is produced by roasting matured unripe or ripe plantain on hot charcoal with constant turning until a uniformly dark brown colour is obtained. The roasted plantain is usually served with roasted fish, peanuts and hot palm oil sauce. It is a popular lunch snack in Southern and Western Nigeria.

Plantains and bananas have high nutritional value as they contain many essential nutrients, including potassium and vitamin A and C [3]. In addition, they are high in dietary fibre and carbohydrates. In the area of quality analysis and, more specifically, processing and flavour analyses, banana has been the subject of numerous studies with respect to (1) differences in chemical composition due to difference in origin [4]; (2) the impact of ripening and storage procedures on flavour [5]; (3) losses in β-carotene and vitamin C due to frying [6] and (4) effect of processing on micronutrient contents of chips produced from some plantain and banana hybrids [7]. However, there has been little or no report on the effect of heat treatment on the formation of Maillard reaction products, more specifically, hydroxymethyl furfural.

Hydroxymethyl furfural or 5-hydroxymethyl-2-furaldehyde (HMF) is a cyclic aldehyde that is produced in the acidic decomposition of monosaccharide, so it appears naturally in all products where water coexists with monosaccharide in an acidic medium [8]. Also, HMF can be formed through the condensation of carbohydrates that have free amine groups, in line with the well-known Maillard reaction [9]. This produces amino acid destruction together with the appearance of some anti-nutritive products, which are sometimes toxic. Therefore, attention has been focused on the study of the reaction products, including HMF and related compounds [10].

Several analytical methods have been developed for the determination of HMF in various food products. Spectrophotometric methods have been used for many years and are often the official method for the determination of HMF in foods. However, the original Winkler method involves the use of the toxic compound p-toluidine and is rather complicated by uncertainties in the colour measurement. In recent times, several HPLC methods have been published using UV detection at 250 – 285 nm. This method allows the simultaneous analysis of other compounds, whereas other methods such as gas chromatography require a previous derivatization [11].

This study aimed at investigating the presence and content of HMF in roasted plantains and to observe differences in the profile with respect to ripening.

Materials and Methods

Sample: The plantain fruits (French sombre, AAB and Dwarf kalapua, ABB) were obtained from Serdang, Malaysia. The Dwarf kalapua was chosen because the pulp is similar to that of the French sombre. All the cultivars were harvested at fully green matured stage. Samples were separated individually, randomized and stored in cartoons at room temperature (30°C, 75% relative humidity). Thirty fingers per cultivar at stages 1, 3 and 5 of ripening were used: stage 1= fruits are dark green; stage 3 = fruits are pale green with yellow tips, and stage 5 = fruits are more yellow than green [2]. Samples (30 fingers per cultivar for each treatment) were roasted at 150, 180 and 210°C for 25 min (Fig. 1) in a Salva downdraft gas roasting double deck oven (Model E-2006-2, Hicksville, NY, USA).

Figure 1. Roasted and unroasted green and yellow plantain fruits.

Chemicals and standards: The following chemicals and standards were obtained from the suppliers shown: HPLC grade acetonitrile, methanol (Merck, Germany), ultra-pure water (milli-Q), HMF (Sigma, USA), 0.45 µm micro filter (nylon), D-glucose (GLU), D-fructose (FRU) and sorbitol (Sigma, USA). Other chemicals were of analytical grade.

Extraction procedure: A longitudinal section cut (10 g of each roasted samples) obtained from the central part was pulverized and a sample (500 mg) was suspended in 5 ml of 0.1% formic acid in a 10 ml centrifuge tube. The tube was shaken for 10 s by a vortex mixer and clarified with 0.25 ml of potassium ferrocyanide (15%, w/v) and zinc acetate (30%, w/v) solutions. The resulting mixture was centrifuged at 2000 g for 10 min at 4°C. The supernatant was collected in a 10 ml volumetric flask and two further extractions were performed using 2 ml of 0.1% formic acid. The supernatants were mixed and centrifuged again. Analysis for HMF was made by HPLC in the filtered (0.45 µm) solution [12].

Instruments and HMF measurement: Agilent 1200 HPLC system equipped with a JP-73069224 solvent degasser, a quaternary pump, auto sampler, a thermostated oven column (Pickering Laboratories, USA), a DEG-4260468 UV detector, and a Diamonsil C18 column (250 mm x 4.6 mm x 5 µm; Dikma) with an ODS guard column (4 mm x 3 mm i.d. 5 µm; Phenomenex) and Agilent HP1100 chromatography workstation was used for analyses. A mixture of acetonitrile in 0.1% formic acid (5%, v/v) delivered at a flow rate of 1.0 ml/min under isocratic conditions through the analytical column at 37°C was used as a mobile phase. The UV detector was set at 280 nm and 20 µl of extract was injected. HMF was quantified using the external standard method.

Preparation of calibration curve: Stock solution of HMF was prepared by dissolving 13.62 mg of HMF in a 50 ml volumetric flask with ultra-pure water and stored at 4°C. Seven concentrations of HMF standard solutions (2.72×10^{-2}, 5.45×10^{-2}, 8.17×10^{-2}, 1.09×10^{-1}, 1.36×10^{-1}, $1.63 \times 10^{-1}$and 2.72×10^{-1} mg/ml) were prepared by appropriately diluting the stock solution with mobile phase and used to evaluate the linearity of HPLC. The calibration curve was established by plotting the peak areas against the concentrations of HMF standard solutions.

Fluorescence measurement: Free fluorescent intermediate compounds of roasted sample extracts in 0.1% formic acid (from HMF analysis) were measured at the excitation wavelength of 347 nm and the emission wavelength of 415 nm. The procedure described by Morales and Jimenez-Perez [13] was used. The linearity of fluorescence response was checked with quinine sulphate solution dissolved in 0.1 M H_2SO_4. The results were expressed as quinine sulphate (mg/l). A fluorescence detector DEG 4260468 (Agilent Technologies, Waldbronn, Germany) was used.

Colour measurement: Colour of the roasted and pulverized samples was evaluated as reflectance using a spectrophotometer CR-300 series (Minolta, Osaka, Japan) according to the L* a* b* colour space also referred to as CIELab defined by CIE (Commission Internationale de l'Eclairage, the International organization concerned with light and colour). The system gives the values of three colour components; the luminosity L* (-black to + white component), the chromaticity coordinates, a* (+ red to – green component) and b* (+ yellow t – blue component). A powdered sample (10 g) was added into a glass Petri dish (diameter, 5 cm) according to Morales and Van Boekel [14]. The sample was illuminated with D65 artificial daylight (standard angle, 10°) under conditions provided by the manufacturer. Each colour value reported was the average of three determinations. The colour difference between the processed and unprocessed sample (ΔE index) was calculated from the equation:

$$\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2} \quad (1)$$

where ΔL* being the brightness difference, Δa* the redness difference, and Δb* the yellowness difference.

Sugar determination: Contents of glucose, fructose and sucrose were determined using HPLC with a refractive index (RI) detector PU 4003 (Pye Unicam, Cambridge, United Kingdom) on a Polymer IEX Ca^{2+} form column (250 mm x 8 mm, 8 µm particle size; Watrex,

Berlin, Germany) at 90° C. Deionised water was used as a mobile phase with a flow rate of 0.5 ml/min. Sample volumes of 20 µl were injected. Each sample of 1.0 g was twice extracted with 10 ml of deionised water. Sorbitol was used as an internal standard. The mixture was mixed by a vortex mixer for 2 min and centrifuged at 1910 g for 10 min. The extract was filtered through a 0.45 µm pore size nylon syringe filter.

Statistical analysis: Analysis of variance (ANOVA) and correlation coefficients were calculated according to Blank [16].

Results and Discussion

The effective separation, acceptable sensitivity, and symmetric peak shapes were attained in a short analytical period. The column efficiency, which is also known as the number of theoretical plates (N) was determined using the following equation in which W_h is the peak width at half height (expressed in unit of time) and t_R is the retention time.

$$N = 5.545 (t_R/W_h)^2 \quad (2)$$

Also, theoretical plate numbers are indirect measurement of peak width for a peak at a specific retention time. Columns with high plate numbers are considered to be more efficient, that is, have higher column efficiency than columns with a lower plate count. Similarly, it will have a narrower peak at a given retention time than a column with a lower number. In this study, the number of theoretical plates of column (N) and the tailing factor (T) for analysing HMF were about 151, 200 and 0.95, respectively. Also, the retention time of HMF was about 11.40 min (Fig. 2) with an average recovery of 98.70% and a relative standard deviation of recoveries of 2.45%, respectively.

Effect of roasting temperature and ripening on the development of HMF in plantains: The development of HMF in the different plantain cultivars during roasting is presented in Table 1. Results revealed that roasting temperature exhibited a direct relationship with the development of HMF. The highest concentrations were found in samples roasted at 210°C. Increasing the roasting time beyond 25 min also led to further increase in HMF (data not shown). HMF is one of the major degradation products of carbohydrates that have been studied extensively as an indicator of heat damage [15, 16]. HMF has been used successfully as a chemical index in ensuring adequate heat processing or for monitoring storage conditions for fruit juices [17], vinegar [18], honey [8], cereal products and cookies and jams [19]. Formation of HMF from carbohydrate has been found to depend on several factors, e. g. temperature [17, 20]. Other factors include time, water activity and amount and type of catalyst and sugar [21]. In the current study, HMF development increased significantly (P>0.05) with roasting temperature and ripening stages. Plantain cultivars which were at the fifth stage (stage 5) of ripening produced the highest concentration of HMF when roasted at 210°C. The reason for this observation is not farfetched since these samples were shown to have the highest sugar concentrations (Table 2). Earlier studies have revealed that during normal plantain ripening, starch is degraded rapidly to sucrose followed by the accumulation of glucose and fructose [22]. Sucrose is hydrolysed to glucose and fructose by acid invertase [23].

A recent study has indicated that fructose is the most reactive sugar relative to sucrose and glucose in the formation of HMF under acidic conditions [24]. Similarly, in the absence of acid catalysis and at 250°C, the conversion rate of glucose into HMF was 24% and for fructose the rate was 36% [25]. Recently, the formation mechanism of HMF from sucrose has been suggested [24]. At high temperatures and in dry systems, sucrose easily cleaves the glycosidic bond of the fructose moiety with the assistance of lone pair electrons of the fructofuranosyl ring oxygen to release a free glucose and a fructofuranosyl cation as a reactive intermediate. This intermediate is quickly converted into HMF. At low temperatures, it can be trapped as methyl fructofuranoside and therefore only free glucose moiety can be converted into HMF

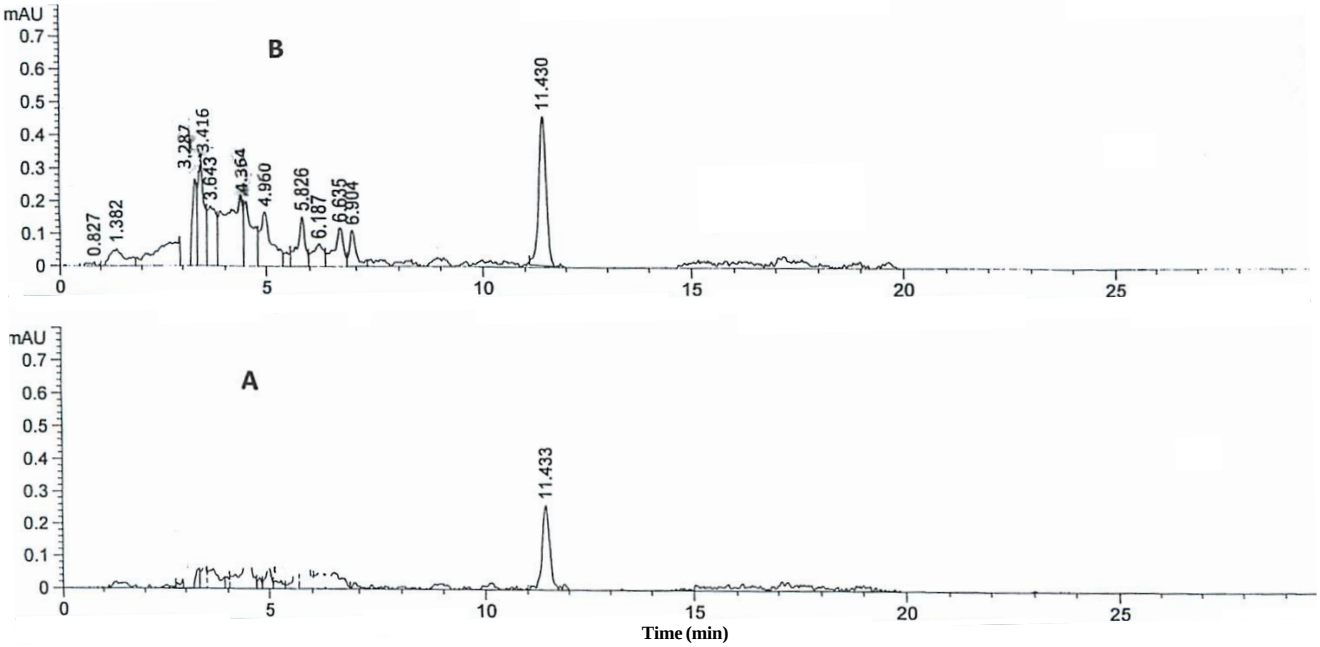

Figure 2. Chromatograms of 5-HMF standard (A) and roasted plantain sample (B).

Table 1. Hydroxymethyl furfural concentrations in roasted plantain cultivars (mg/kg).

Roasting temp (°C)	French sombre			Dwarf kalpua		
	Stage 1	Stage 3	Stage 5	Stage 1	Stage 3	Stage 5
150	76.11±5.7^{c}	97.36±7.2^{c}	99.40±6.9$_{c}$	81.18±8.1^{c}	91.54±7.0^{c}	107.80±9.2^{c}
180	81.20±4.8^{b}	109.00±6.2^{b}	141.00±10^{b}	96.32±3.9^{b}	114.60±9.3^{b}	149.21±16.2^{b}
210	90.30±6.3^{a}	116.10±9.8^{a}	153.10±7.3^{a}	102.12±8.3^{a}	124.00±5.7^{a}	203.52±9.4^{a}
Δ E	52.1	50.3	44.3	43.6	42.0	40.5

Stages 1, 3, and 5: The stages of ripening in roasted plantain cultivars. ΔE: Colour index between processed and unprocessed samples.
Means with the same superscript are not significantly different (P>0.05).

Table 2. Sugar concentration (mg/100 g) in plantain cultivars at different stages of ripening.

	French sombre			Dwarf kalapua		
	Stage 1	Stage 3	Stage 5	Stage 1	Stage 3	Stage 5
Sucrose	0.90±0.0^{a}	7.67±0.1^{a}	11.25±0.2^{a}	1.69±0.0^{a}	10.4±1.5^{a}	14.95±1.5^{a}
Glucose	0.21±0.0^{b}	1.80±0.0^{c}	2.60±0.1^{c}	0.39±0.0^{c}	2.4±0.0^{c}	3.45±0.1^{c}
Fructose	0.30±0.0^{b}	2.36±0.0^{b}	3.70±0.0^{b}	0.52±0.0^{b}	3.2±0.1^{b}	4.6±0.1^{b}

Means with the same superscript are not significantly different (P>0.05).

through 3-deoxyglucosone pathway following ring opening and enolization steps. This probably accounted for the high concentrations of HMF in fully ripened plantains roasted at 210°C (Table 1), while those roasted at much lower temperatures generated much lower concentrations of HMF. Statistical analysis also showed that the roasted plantain cultivars were positively correlated for HMF (r = 0.978), fructose (r = 0.997), glucose (r = 0.996) and sucrose (r = 0.999), respectively (Table 3).

Table 3. Correlations matrix among HMF, fluorescence (FI), ΔE and sugar concentrations in roasted plantains.

		Statistical analysis					
		Correlation (r)					
		French sombre (at stage 5 ripening)					
		HMF	FI.	ΔE	Fru.	Glu.	Suc.
Dwarf kalapua (Stage 5)	HMF	0.978					
	FI.		0.989				
	ΔE			0.949			
	Fru.				0.997		
	Glu.					0.996	
	Suc.						0.999

HMF: Hyroxymethyl furfural; Fru: Fructose; Glu: Glucose; Suc: Sucrose; FI: Fluorescence intensity; ΔE: Colour index between roasted and unroasted samples.

Fluorescence and colour development in roasted plantain cultivars: The fluorescence intensity (expressed as sulphate quinine amounts) in roasted (210°C) plantains is as shown in Fig. 3. Increasing the roasting time of samples led to significant increases (P>0.05) in fluorescence intensity most especially in the French sombre. Interestingly, the development of fluorescence in the roasted plantains was similar to browning or colour appearance

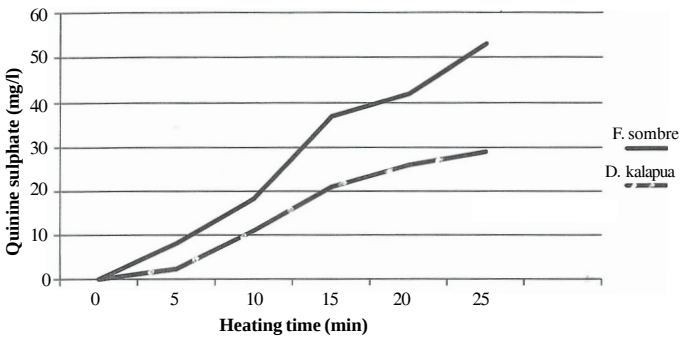

Figure 3. Fluorescence instensity (expressed as sulphate quinine amounts) in roasted (210°C) plantains (F. sombre and D.kalapua) at the 5th stage of ripening.

(Table 1). However, previously published studies have shown that fluorescence measurement is a more effective method of following formation of Maillard reaction product with free radical-scavenging activities [26]. Development of colour is an important and obvious consequence of the Maillard reaction. Indeed, depending on the production conditions and final temperatures, different concentrations and types of Maillard reaction products occur in roasted foods. The degree of non-enzymatic browning is a key factor in roasted food quality, as it not only involves the formation of colour but also the generation of flavour-active volatiles [15, 27]. For instance, in model system, Maillard reaction products responsible for colour perception are low molecular weight chromophores (< 1 kDa), and high molecular weight melanoidins (> 100 kDa) [28].

In the present study, the colour index (ΔE) between processed and unprocessed samples decreased significantly with ripening. Since the colour index is mainly influenced by the colour lightness (L*), a decrease in colour index (ΔE) is related to a loss of lightness in the sample. Plantain cultivars at the 5th stage of ripening produced a much darker colour than samples at stages 1 and 3, respectively. In a recent study in our laboratory, it was revealed that roasted plantain samples at this stage of ripening generated significant numbers of flavour active volatiles than unripe samples [29]. Strong positive correlations were exhibited by the roasted plantains for fluorescence (r = 0.989) and colour index (r = 0.949) (Table 3).

Conclusions

Although heat treatment of foods is related to formation of possibly harmful 5-hydroxymethyl-2-furfural, desirable properties such as colour, browning and flavour are developed during Maillard reaction. Roasting at high temperature (210°C) and long roasting time were shown to increase HMF in roasted plantain cultivars. Similarly, ripening stage had significant influence on the development of HMF. The colour and fluorescence intensities were significantly (P>0.05) increased by the roasting conditions. In addition, positive correlations were exhibited by the plantain cultivars for HMF, fluorescence, colour index (ΔE), and the sugars.

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