

Smoking Treatment in Enhancing Banana Ripening and its Effects on Physical and Biochemical Attributes of Ambul Banana (*Musa acuminata*, AAB)

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ABSTRACT

Smoking is a traditional practice used in enhancing the ripening process of banana. This study was conducted to analyze the effects of smoking treatment on different physical and biochemical parameters of Ambul banana (*Musa acuminata*, AAB). Bananas were screened by peel colour and fruit firmness to obtain samples at similar maturity stage. A sample was treated with smoke and stored at 20 °C and 80-85% relative humidity and the control sample was allowed to ripe naturally under the same conditions. Physical (peel colour, firmness) and biochemical parameters (moisture %, pulp pH, TSS, titratable acidity, total sugar, total starch, organic acid profile, sugar profile, aroma profile) were tested at fully yellow stage (stage 6). Results revealed that smoking treatment can reduce the quality of banana by reducing its peel yellowness (a*) significantly at stage 6 and making black scars on peel. Further fruit firmness, moisture % in peel and pulp pH were significantly ($p < 0.05$) affected by smoking. Total sugar showed nearly a twofold decrease in treated fruits (10.26 g/100g)

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while there was no appreciable difference in starch content. Fructose, glucose, malic acid, citric acid and oxalic acid were found in considerably lower levels in treated samples than in non-treated. Sucrose was completely unavailable in treated banana flesh. Smoked banana reported a poor aroma profile with few numbers of aroma compounds (19) where some esters such as ethyl acetate, butyl acetate, butyl pentanoate, 3-methylbutyl 2-methylbutanoate, 2-methylpropylhexanoate, 2-methylpropyl acetate, 3-methylbutyl hexanoate and [(E)-hex-4-enyl] butanoate were not found.

Keywords: Ambul banana, Aroma volatiles, Induced ripening, Organic acids, Sugars

INTRODUCTION

Banana is the term used to represent many cultivars or species under genus *Musa*. It is known as one of the most popular fruits in the world. It has been ranked as the fourth most important food crop in the world (Maureen *et al.* 2016). There are two main types of banana; dessert types which are eaten raw and plantains which are eaten in cooked form. Annually 130 million tons of banana are being produced across the world while 50% of this production is consumed in the cooked form (Salunkhe and Kadam, 1995). Banana is one of the most important fruit crops in Sri Lanka as well. Annual banana production in Sri Lanka is around 62549 t. It is reported that

there are 55 cultivars available in Sri Lanka where the most popular species are Ambul (*Musaacuminata*, AAB), Seeni (*Musaacuminata*, ABB), Kolikuttu (*Silk*, AAB) and Anamalalu (Gross Michel, AAA).

Banana fruit is made with a peel and an edible pulp. Banana is rich in nutritional profile. Levels of nutrients vary with the type of cultivar and the maturity stage. Typically, the energy provided by banana (dessert type) lies between 80-120 kcal/100g. Ripe banana contains 1-2% starch and 70-80% free sugars including sucrose, fructose and glucose. Banana contains a very low amount (0.3g/100g) of fat (Chandler, 1995; Marriott and Palmer, 1980). It is also rich with minerals such as phosphorous, sodium, potassium, calcium, magnesium, iron, copper, zinc, and manganese. Further it contains vitamin A, B6 and vitamin C. Banana is rich with bioactive compounds such as phenolics, flavonoids, carotenoids and biogenic amines (Singh *et al.* 2016).

In natural conditions, banana ripens slowly, leading to high weight loss, desiccation, uneven maturity levels and fails to develop good colour and aroma (Subbaiah *et al.* 2013). Therefore, to meet consumer expectations bananas are harvested when green but mature, and artificially ripened using ripening agents (Sogo-Temi *et al.* 2014). The ripening agents include artificial agents such as ethylene gas, ethephon, ethrel and calcium carbide. In developing countries inexpensive traditional and biological

methods such as smoking, keeping with hay and banana leaves are practiced.

Smoking is popular in Sri Lanka as a traditional practice used to instigate ripening of banana which is still being used in some regions of the country. In traditional practice, green bananas are laid in a pit, covered with leaves and smoke is directed in to the pit for several hours. It is believed that unsaturated compounds such as acetylene and ethylene present in the smoke enhance the ripening process. (Maduwanthi and Marapana, 2019a). As well increased temperature in the smoke accelerates the de-greening of the peel (Ranaweera *et al.* 2020).

Many studies have been done to analyze the effect of artificial ripening on physicochemical properties and sensory properties of banana. Many of them have revealed that artificial ripening can significantly affect biochemical and sensory attributes of banana (Kulkarni *et al.* 2011; Lustre *et al.* 1976; Sogo-Temi *et al.* 2014; Hakim *et al.* 2012; Maduwanthi and Marapana, 2019b). This study was done to analyze the effect of smoking on different physical and biochemical properties of Ambul banana (*Musa acuminata*, AAB).

MATERIAL AND METHODS

Plant Material

Ambul banana was procured from an orchard in Dankotuwa, Sri Lanka. Green mature bananas were tested for their peel

colour and firmness using a Chroma meter (Lovibond LC 100, China) and a texture analyzer (CT3 Brookfield USA). Bananas which showed significantly similar ($p < 0.05$) levels of peel colour and firmness were taken for the analysis.

Chemicals

Standards of malic acid, oxalic acid, citric acid, tartaric acid, fructose, glucose, sucrose, maltose and ascorbic acid were obtained from Sigma, USA. All the other reagents and chemicals were analytical grade.

Smoke Treatment

Banana samples were kept in a smoking chamber covered with Bilimbi (*Averrhoa bilimbi*) leaves and exposed to wood smoke for 20 minutes. Then the samples were kept in a closed chamber under 20 °C and 80-85% relative humidity (RH) until fruits become fully ripe.

Control samples were kept in a closed chamber under 20 °C and 80-85% RH until fruits become fully ripe.

General Analysis

The peel colour was measured using a Chroma meter (Lovibond LC 100, China) according to the L*a*b* colour scale with six replicate measurements. Texture analyzer (CT3, Brookfield USA) was used to measure the firmness of banana fruit. The test was done with TA44 probe,

1mm/s test speed at 5 mm distance using 5g trigger load.

Moisture content was analyzed using AOAC 925.10 method in banana peel and flesh. A solution was prepared by homogenizing the flesh with distilled water at 1:3 (w/w) ratio. The solution was used to measure TSS (Total Soluble Solids), pH and titratable acidity % (TA%). TSS of the solution was measured using digital refractometer (ATAGO PAL-1, Japan). The pH of the solution was measured using pH meter (Consort, C6010). TA% of the banana pulp was determined by a titration according to Hossain *et al.* (2014). Above solution was titrated with 0.1 N NaOH in the presence of phenolphthalein. TA% was calculated using equation 1 and expressed as % of malic acid.

$$TA\% = \left(\frac{\text{Normality of NaOH} \times \text{Volume of NaOH} \times 0.067 \times \text{Dilution factor}}{\text{Volume of the sample}} \right) \times 100 \quad (\text{Eq. 1})$$

Total Sugar Analysis

Total sugar analysis was done according to Hossain *et al.* 2014. Exactly 2 g of banana flesh was homogenized well and boiled with 20 ml of 80% ethanol for 10 minutes. Further, extraction was done twice with 80% hot ethanol. Then the solution was filtered through a filter paper (Whatman 41). The extract was evaporated to dryness

in a water bath and the residue was dissolved in 100 ml of distilled water. Aliquot of 1 ml of the prepared extract was mixed with 4 ml of 0.2% Anthrone reagent (Sigma, Switzerland). Then the mixture was heated in a boiling water bath for 10 min and allowed to cool. The absorbance was measured at 680 nm using a spectrophotometer (SHIMADZU, UVmini-1240). Total sugar was calculated using a standard curve prepared with five different concentrations of glucose standard (Supelco, Germany).

Total Starch Analysis

Total starch was analyzed according to the method described by Rose *et al.* (1991). Exactly 0.5g of banana flesh was crushed with 80% hot ethanol twice to remove sugars. Extract was centrifuged at 6000 rpm for 10 minutes. The residue was dried in a water bath. Then 5 ml of distilled water and 6.5 ml of perchloric acid were added. The sample was mixed well and kept at 0°C for 20 minutes. Then the sample was centrifuged and the supernatant was separated. Then it was diluted up to 100 ml with distilled water. Exactly 1 ml of the extract was mixed with 4 ml of 0.2% anthrone reagent (Sigma, Switzerland) and incubated in a boiling water bath for 10 minutes. After cooling, the absorbance was measured at 680 nm using a spectrophotometer (SHIMADZU, UVmini-1240). The amount of glucose present in the extracts was determined from the standard curve prepared. Starch content was calculated using the equation 2.

$$\text{Starch} = \text{Glucose} \times 0.9 \quad (\text{Eq. 2})$$

Organic Acid Analysis

Malic acid, oxalic acid, tartaric acid and citric acid in banana flesh at fully yellow stage were analyzed by following the method described by Maduwanthi and Marapana, 2019b. Exactly, 5 g of banana flesh was homogenized with 10 ml of deionized water. Then the mixture was centrifuged at 6000 rpm for 10 minutes. The supernatant was filtered through 0.45 µm filters and filled in to 1.5 ml sample vials. The organic acids were separated in the HPLC system (Agilent-1260-Infinity, Germany) using C18 column (Agilent Eclipse Plus C18, 5 µm 4.6 x 250 mm) and UV detector. A mixture of Dipotassium Hydrogen Phosphate buffered at pH 2.6 with orthophosphoric acid and 100 % Methanol in the ratio 97:3 (v/v) was used as the mobile phase. For the quantification, external standards of malic acid, oxalic acid, tartaric acid and citric acid (Sigma, USA) were used.

Sugar Analysis

Sucrose, glucose, fructose and maltose in banana flesh at fully yellow stage were analyzed under this analysis. The procedure was developed and optimized based on Ma *et al.* (2014) with a few modifications. Five grams of fruit flesh was homogenized with 10 ml of deionized water and centrifuged at 6000 rpm for 10 minutes. The supernatant was filtered through 0.45 µm filters and filled into 1.5

ml sample vials. HPLC analysis was carried out using the Agilent-1260-Infinity HPLC system (Agilent, Germany) equipped with Refractive Index (RI) detector and Zorbax NH2 (4.6 mm x 250 mm, 5 µm, Agilent USA) analytical column. The mobile phase consisted of the ratio of 70: 30 v/v acetonitrile: water. External standards of sucrose, maltose, fructose and glucose (Sigma, USA) were used for the quantifications.

Aroma Profile Analysis

Solid Phase Micro Extraction (SPME) technique was used to analyze aroma profile of banana at fully yellow stage. The method was developed based on the method given in Bugaud and Alter (2016) with some modifications. Five grams of banana flesh was homogenized with 5 ml of distilled water. Exactly, 1 g from the above mixture was transferred into a 20 ml sample vial and it was heated at 60 °C for 10 min in a water bath. SPME fiber (Supelco, 50/30 µm divinylbenzene/ Carboxen on polydimethylsiloxane on a 2 cm stable flex fiber) was exposed into the headspace of the sample for 10 min at 60

°C. Volatile compounds were separated and identified using the GC-MS system under the conditions stated in Table 1.

The reference mass spectra (NIST 08) was used to identify the compounds. Relative Abundance % (RA%) of each aroma compound was calculated using the following equation 3.

$$RA\% = \frac{\text{Area of the peak}}{\text{Total sum of areas of all peaks}} \times 100\% \quad (\text{Eq. 3})$$

Vitamin C Analysis

Vitamin C analysis was done based on the method given by Kapur *et al.* (2012) with few modifications. Five grams of banana flesh were homogenized with 25 ml of metaphosphoric acid - acetic acid solution and diluted up to 50 ml with metaphosphoric acid - acetic acid solution. Then the mixture was centrifuged at 6000 rpm for 15 minutes. Four ml of the supernatant was mixed with 230 µl of 3% bromine, 130 µl of 10 % thiourea solution and 1 ml of 2, 4 DNPH solution.

Table 1. Operational conditions of GC-MS used for aroma analysis

GC system	Agilent 7890A coupled with 5975C Mass Spectrophotometer
Column	DB-225MS (Agilent) capillary column (30 m x 250 µm x 0.25 µm)
Carrier gas	Helium
Gas flow rate	1 ml/min
Temperature program	Started at 40 °C, 2 °C/min to 70 °C, held at 70 °C for 5 min, up to 170 °C by 5°C/min, then 50 °C/min up to 220 °C and finally maintained at 220 °C for 5 min
Run time	51 min

Then the solutions were kept in a water bath at 37 °C for 3 hours. The samples were cooled in ice for 30 minutes and 5 ml chilled 85 % H₂SO₄ was added. Absorbance was read at 521 nm in the UV-VIS spectrophotometer (SHIMADZU, UV mini- 1240). The standard curve prepared with different concentrations of Vitamin C standard (Supelco, Germany) was used for the quantification.

Statistical Analysis

Data obtained were analyzed using two-sample t-test and Mann-Whitney U test where appropriate using MINITAB 17. Anderson-Darling test was used to test the normality of data. All the tests were done under 95% confidence level.

RESULTS AND DISCUSSION

When considering the efficiency of smoke treatment, banana treated with smoke was ripen at 6th day while it took 8 days for the natural ripening process. Table 2 shows change in the ripening stage determined according to the Von Loesecke ripening scale (Von Loesecke, 1950).

Table 2. Ripening stage with time

Treatment	No of days									
	1	2	3	4	5	6	7	8	9	10
Natural ripening	S1	S2	S3	S3	S3	S3	S4	S6	S6	S7
Smoke	S1	S2	S3	S3	S5	S6	S6	S7		

S1- Green, S2-Colour Break, S3- More green than yellow, S4- More yellow than green, S5- Yellow with green tip, S6-Fully yellow, S7- Over ripe.

However, black scars on peel were observed at stage 6 on the peel of smoked banana. A similar observation was noticed by Adane *et al.* (2015) where banana treated with kerosene smoke exhibited off ripening black scars on peel giving lower sensory quality.

Peel colour is the most important ripening index in banana fruit. In L*a*b* colour scale L* represents lightness of the peel while a* and b* represent “Redness Vs Greenness” and “Yellowness Vs Blueness” respectively. Figure 1 shows change in L*, a*, b* and C* in banana peel with time during the period of ripening.

Lightness (L*) of the peel increased from 53.92±2.27 at stage 1 to 67.48±0.95 at stage 6 in natural ripening. L* at stage 6 in smoked banana was reported as 70.75±1.43 which was significantly high (p<0.05) compared to the corresponding value in natural ripening. When the fruit ripens gradually greenness reduces.

Accordingly, a* increased from negative values to positive values. Green banana (Stage 1) showed -11.93±0.71 a* value. It was

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increased up to 2.6 ± 0.66 in natural ripening and 5.13 ± 1.59 in smoked bananas. Yellowness (b^*) increased during ripening up to stage 6 in natural ripening. It decreased when fruit reached to stage 7. However, in smoked banana yellowness at stage 6 was 46.80 ± 0.80 which was lower than yellowness of naturally ripened samples at stage 6.

Reduction in flesh firmness during banana ripening is mainly associated with cell pectin degradation and the conversion of starch into sugar (Khanbarad *et al.* 2012). The firmness of banana flesh was 414.85 ± 53.45 g at the unripe stage and it showed a continuous reduction during the ripening process (Figure 2).

Flesh firmness at stage 6 in naturally ripened samples was 42.6 ± 2.9 g while it was 31.3 ± 4.8 g in smoked banana. It indicates that smoke treatment can significantly reduce flesh firmness at fully yellow stage which can lead to poor quality in banana.

When considering moisture % in peel, smoked banana showed reduced content of moisture. However, moisture content in flesh did not show any appreciable difference between treated and non-treated samples (Table 3).

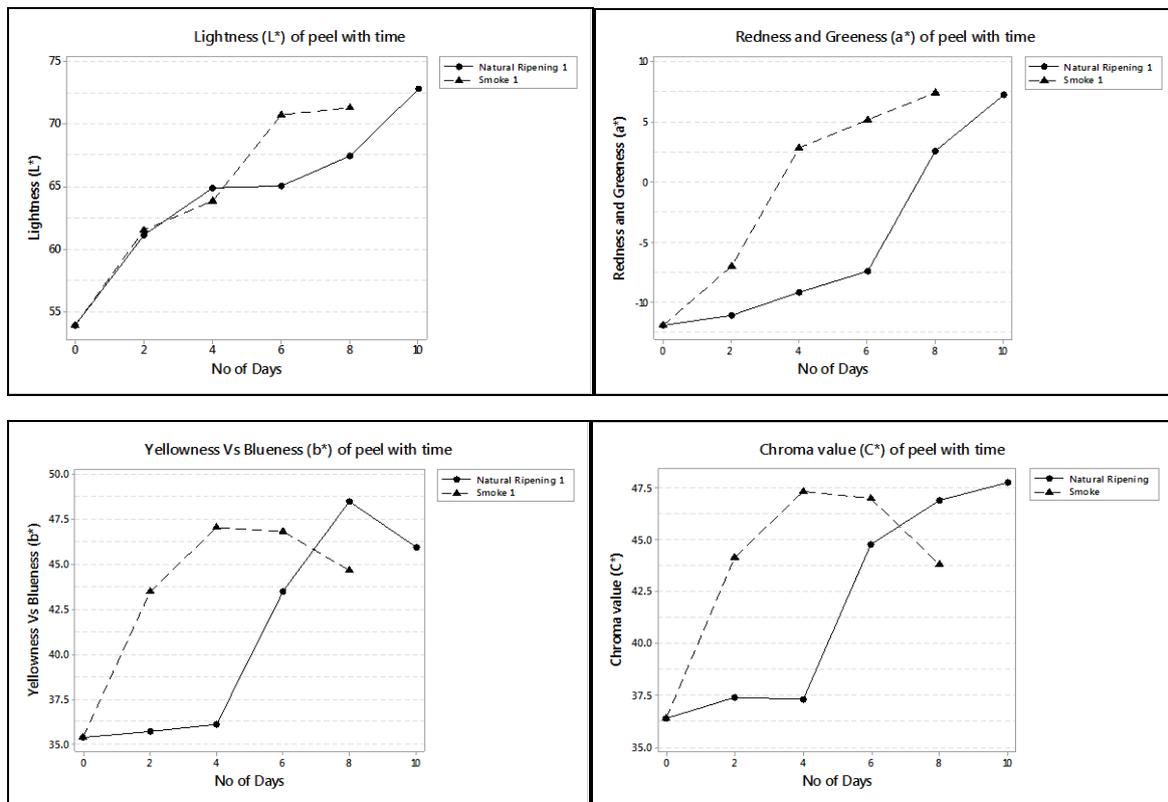
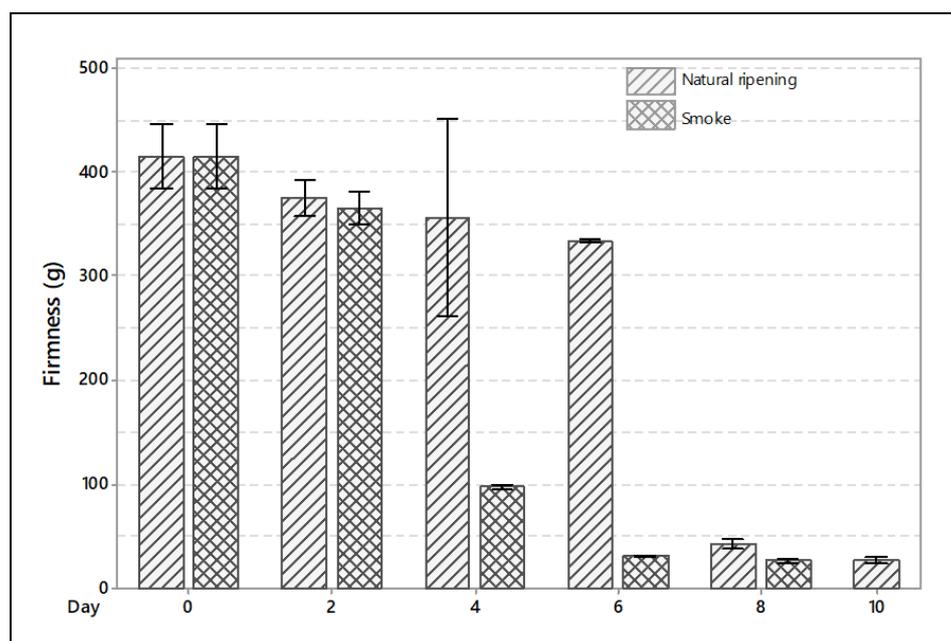


Figure 1. Variation of L^* , a^* , b^* and C^* in banana peel during ripening of banana



Each bar represents mean \pm SD of 6 independent samples

Figure 2. Variation of firmness in banana flesh during ripening in treated and non-treated banana.

Further, TA% and TSS in banana flesh was not significantly affected by smoke treatment. Anyhow, it was noted that pH in flesh was considerably low in smoked

banana indicating that smoking can reduce the sensory quality of banana. During ripening sugar content increases as starch converts to sugar.

Table 3. Some of the physicochemical parameters of banana treated with smoke against non-treated banana at stage 6

Parameter	Fully yellow stage (Stage 6)	
	Natural Ripening	Smoke
Moisture % in peel	86.0 \pm 0.9% ^a	83.6 \pm 0.5% ^b
Moisture % in flesh	74.0 \pm 0.5% ^a	73.8 \pm 0.9% ^a
Titrateable Acidity %	0.104 \pm 0.069% ^a	0.114 \pm 0.009% ^a
pH in flesh	5.130 \pm 0.140 ^a	4.265 \pm 0.064 ^b
TSS in flesh (^o Brix)	4.9 \pm 0.0 ^a	5.0 \pm 0.1 ^a
Total sugar (g/100g)	19.91 \pm 0.74 ^a	10.26 \pm 0.09 ^b
Total starch (g/100g)	1.22 \pm 0.19 ^a	1.12 \pm 0.02 ^a
Vitamin C (mg/ 100g FW)	30.15 \pm 0.81 ^a	7.34 \pm 0.39 ^b

The table consists of mean \pm SD of 4 independent samples. Means bearing different superscripts, row-wise differ significantly at 95% confidence level.

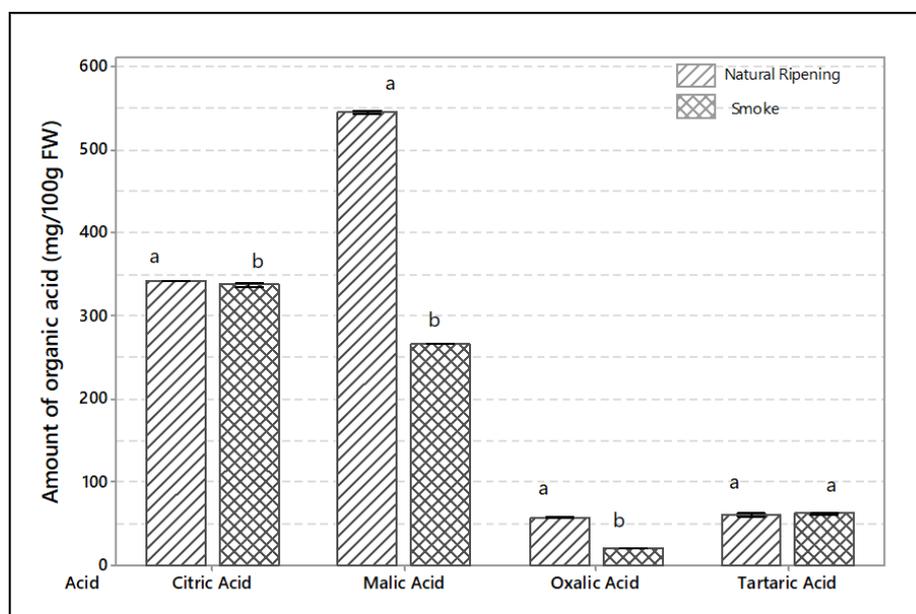
Total sugar content in naturally ripened banana at stage 6 was 19.91 ± 0.74 g/100g. Total sugar at stage 6 was significantly low in smoked banana as it was nearly a twofold reduction. This may be due to the incompleteness of the sugar metabolism in smoked samples due to early activation of climacteric rise. Starch remaining at stage 6 was similar in both treated and non-treated samples.

As represented in Table 3, vitamin C is considerably affected by smoke treatment as there was 7.34 ± 0.39 mg/100g of vitamin C in treated banana samples. However, the content of vitamin C was 30.15 ± 0.81 mg/100g in naturally ripened banana samples.

Figure 3 shows the variations of organic acids; citric, malic, oxalic and

tartaric acid flesh of banana treated with smoke. Organic acids play a major role in flavour of banana as well as in many fruits. Many researchers have reported that malic acid is the major acid in ripe banana (Kheng *et al.* 2012).

A similar observation was noted under this analysis as malic acid was the most abundant acid in ripe banana flesh which was naturally ripened. According to the data obtained, citric and malic acids were the most abundant acids in both treated and non-treated banana at stage 6. Smoked banana contained considerably less amount of malic acid (265.74 ± 0.93 mg/100g of Fresh Weight/FW) comparing to that of naturally ripened samples. Further, citric acid and oxalic acid contents



Each bar represents mean \pm SD of 3 independent samples. Bars bearing same letter within a group are not significantly different at 95% confidence level

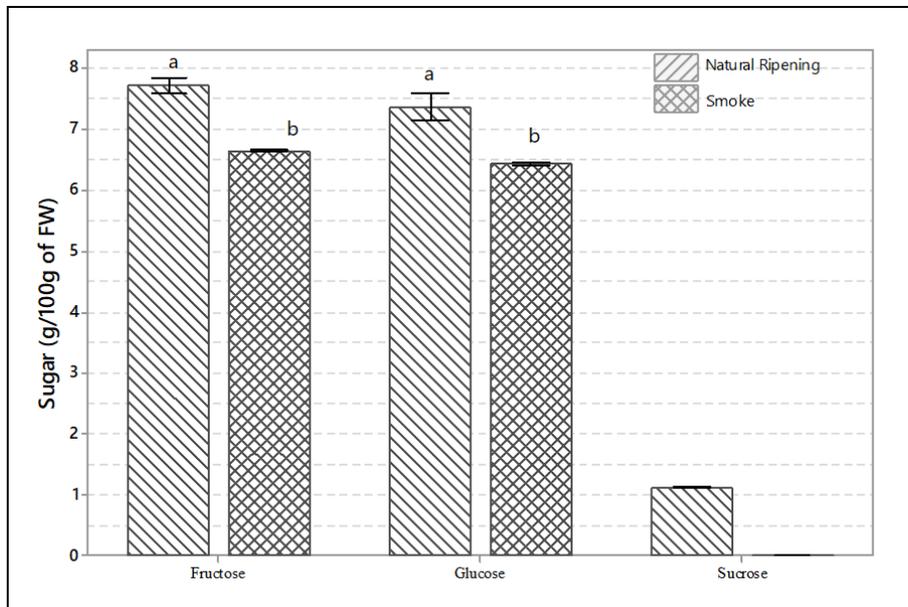
Figure 3. Organic acids in banana flesh at stage 6 in treated and non-treated banana

were also significantly low in smoke treated samples. There was no significant difference in tartaric acid content between treated and non-treated banana.

During ripening, starch degrades and sugar accumulates in flesh. Under this analysis sucrose, fructose, glucose and maltose were analyzed. However, maltose was not present in banana flesh at any ripening stage. Fructose and glucose were the most available free sugars in banana flesh at the fully yellow stage (Figure 4). Fructose and glucose contents in banana flesh under natural ripening were 7.72 and 7.36 g/100g of FW respectively. The amounts of fructose and glucose in smoked banana reported as 6.64 and 6.43 g/100g of FW respectively which were significantly

lower than that of naturally ripened samples. Sucrose was present only in naturally ripened samples where the content was very less (1.11 g/100g of FW). Sucrose was not detected in smoked fruits.

Based on the results of aroma profile analysis, 27 aroma compounds were identified in naturally ripened banana whereas only 19 compounds were detected in smoked samples. Some esters such as ethyl acetate, butyl acetate, butyl pentanoate, 3-methylbutyl 2-methylbutanoate, 2-methylpropyl hexanoate, 2-methylpropyl acetate, 3-methylbutyl hexanoate and [(*E*)-hex-4-enyl] butanoate were not detected in smoked banana samples.



Each bar represents mean \pm SD of 3 independent samples. Bars bearing same letter within a group are not significantly different at 95% confidence level.

Figure 4. Sugars in banana flesh at stage 6 in treated and non-treated banana

Considering the numbers of esters present in the aroma profile (Figure 5), 17 aroma compounds were detected in naturally ripened samples where 13 esters were available in smoked samples. Sonmezdag *et al.* (2014) reported that artificially ripened banana has a poor aroma profile compared to naturally ripened banana. The number of alcohols, aldehydes and benzenic derivatives was also less in number in smoked samples. Some esters namely heptan-2-yl butanoate, 2-methylpropyl 2-methylbut-2-enoate and hexyl butanoate were only found in smoked banana.

CONCLUSION

Smoke treatment can enhance banana ripening successfully as smoked banana

samples were fully yellow within the first 6 days while the natural process took 8 days. Black scars on peel were noticed in smoked samples at stage 6. As per peel colour measurements, yellowness (a^*) was significantly low in banana treated with smoke. Flesh firmness was also affected by smoking treatment indicating that smoked banana is less hard than naturally ripened samples. Moisture % in peel and pH in flesh was considerably low in treated samples whereas TA%, TSS in flesh and pulp moisture % did not show any significant difference between the samples. There was a huge reduction in total sugar in treated banana flesh. Contents of individual free sugars such as fructose and glucose were reported to be low in smoked samples. Sucrose was found only in naturally ripened samples.

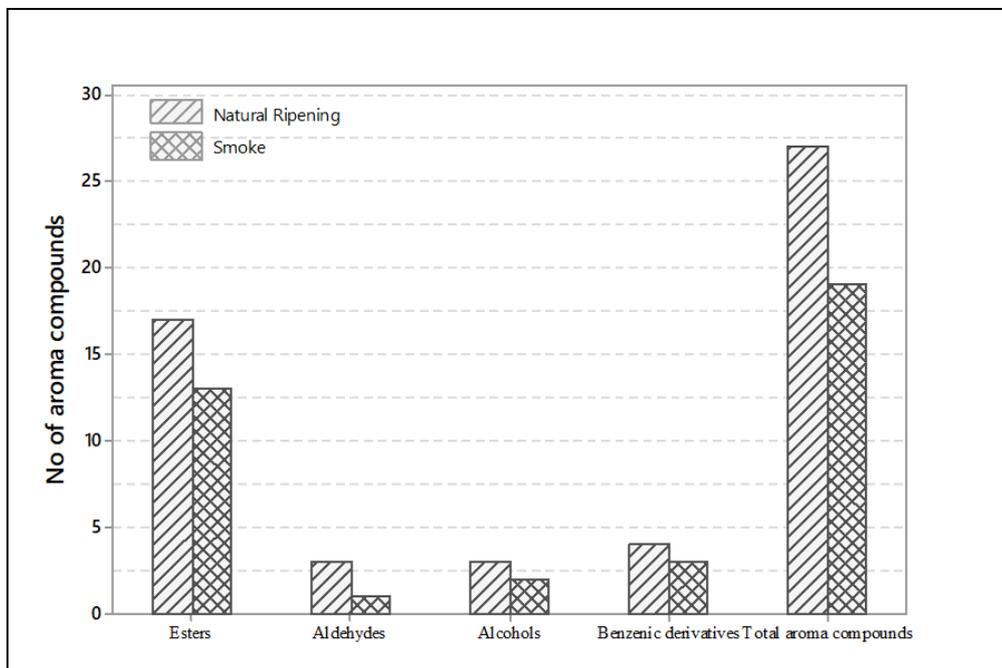


Figure 5. Number of esters, aldehydes, alcohols and benzenic derivatives in the aroma profile of treated and non-treated banana.

Malic acid was the major acid available in ripe banana flesh under natural ripening. Anyhow, the content of malic acid was considerably affected by smoke treatment. Most abundant organic acid in smoked banana flesh was citric acid. There was no considerable variation in starch content between two samples. However, smoked banana reported a nearly fourfold reduction in vitamin C content. Aroma profile of smoked banana was reported to be poor having less number of total aroma compounds and less number of esters. Some of the esters were completely unavailable in the aroma of smoked samples.

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