Changes in volatile compounds of palm sap (*Arenga pinnata*) during the heating process for production of palm sugar

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Abstract

This study was carried out to determine the changes of volatile compounds composition during palm sugar production. The samples were collected at every 30 min interval for 4 h of heating process at 150 °C from a local traditional manufacturer in Kuala Pilah, Malaysia. The analyses were performed by gas chromatography–mass spectrometry after headspace solid phase micro extraction. The results showed that *N*-heterocyclic chemical class possessed the highest relative percentage area (RPA) 83.69%, followed by *O*-heterocyclic group with RPA of 14.5%. Main volatile compounds the determined were 5-methyl-6,7-dihydro-5H-cyclopenta pyrazine and 4-hydroxy-2,5-dimethyl-3(2H) furanone which were responsible for roasty and sweet caramel-like aroma notes, respectively. The pyrazine compounds increase exponentially with heating time but furan derivatives compounds were formed at a later stage, i.e. at 180 min of heating time and above 105 °C.
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1. Introduction

Palm sugar which is one of the local delicacy widely consumed by Asians is used for making cakes, desserts, food coating or mixed with drinks and is produced by heating the sap derived from the tropical coconut tree (*Cocos nucifera*) or a kind of palm tree called *Arenga pinnata* (Panyakul, 2001). For the traditional production of palm sugar, a large volume of filtered palm sap is transferred into a big wok, where the filtered palm saps are heated on the wood fired stove for a few hours at >100 °C until it becomes concentrated to obtain a typical aroma. Mainly, two major reactions occur during the heating process of palm sap: Maillard reaction and caramelisation. After the heating process, the palm sap liquid is poured into bamboo moulds to form pure solid palm sugar which is ready for consumption.

Palm sap contains abundant sucrose and polar side chain amino acids especially asparagine and glutamine. Sohn and Ho (1995) reported that these polar side chain amino acids were essential precursors to the volatile compound formation by releasing more free amino groups during the Maillard reaction than other amino acids. The released amino group substrate will catalyzes sucrose conversion into their reactive component of monosaccharides or can directly undergo retro-aldol reactions producing more reactive C2, C3, C4 and C5 dicarbonyl compounds, depending on the environment conditions (Carline & van Boekel, 2003). All these compounds are highly reactive and take part in further reactions. In essence, dicarbonyl compounds can react with amino acids to form aldehydes and α-aminoketones. This reaction is known as the Strecker degradation. Subsequently, in the advanced stage, a range of reactions takes place, including cyclisation, dehydrations, retro-aldolisations, rearrangements, isomerisations and further condensations, which ultimately, in the final stage, lead to the formation of volatile compounds

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and melanoidins. The relative proportions of these compounds influenced by the cooking temperature, heating time and whether the conditions are acidic or basic are important to the Maillard reaction (Martins, Jongen, & van Boekel, 2001).

Till date, volatile compounds produced by the heating of palm sap have not been investigated. The origin of the volatile compounds responsible for the flavour of palm sugar is still relatively difficult to determine, due to their multiple origin. Therefore, this study was carried out to determine the volatile compounds produced during traditional palm sugar process.

2. Materials and methods

2.1. Materials

A sample of the sweet liquid generated during the heating of palm sap for production of palm sugar was collected from the traditional palm sugar producer in Kuala Pilah, Negeri Sembilan, Malaysia. The samples were collected at every 30 min interval for 4 h. Each time approximately 1 ml of the sample was collected. An internal standard, tridecane (1 ppm) was spiked into the sample. The mixture was then placed into a 12 ml headspace vials and sealed tightly with a crimp cap and a PTFE/silicone septum. The initial temperature of each sample was measured using an infra-red thermometer (Quicktemp 826 T4. Testo, Vienna, Austria). The samples were then immediately chilled in an ice bath and were kept frozen at −18 °C.

The standard solutions 2-methyl pyrazine, 2,5-dimethyl pyrazine, 2,3-dimethyl pyrazine, 2-ethyl pyrazine, 2-ethyl-5-methyl pyrazine, 2-ethyl-3,5-dimethyl pyrazine, 2,3-diethyl-5-methyl pyrazine, 5H-5-methyl-6, 7-dihydrocyclopentapyrazine, 2-furfural, 2,5-dimethyl-4-hydroxy-3(2H)-furanone and 5-methyl furfural, as well as tridecane (internal standard) were purchased from Sigma–Aldrich, USA. The purity of the standard solutions was 97–99.9%.

2.2. Extraction of volatile compounds

The volatile compounds of each sample were extracted using solid phase micro extraction (SPME). A 50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane SPME fibre was used (Supelco, Bellefonte, PA, USA). This fiber was chosen because it was reported by Ho, Wan Aida, Maskat, and Osman (2006) to be the most efficient for trapping N- and O-heterocyclic group volatile compounds of palm sugar. The vial was equilibrated at 50 °C for 10 min in a water bath. A manual SPME holder containing fibre was inserted into the vial and was exposed to the sample headspace for 10 min at 50 °C. The fiber was then transferred directly into the injector port of the GC–MS system. Thermal desorption of the analytes from the fiber in the GC injector port was carried out with an SPME inlet liner (0.75 mm i.d., Supelco) in the splitless mode at a desorption

temperature of 250 °C. The SPME fibre was conditioned at 250 °C for 30 min before the first measurement and left in the injection port for re-conditioning during the whole GC run before it was exposed to the headspace of the next sample.

2.3. Gas chromatography–mass spectrometry analysis

All samples were analyzed using gas chromatography–mass spectrometry (Hewlett–Packard). The separation was achieved on a nonpolar HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) purchased from J & W Scientific, Agilent Technologies. The injector and detector temperatures were 240 °C and 280 °C, respectively. Carrier gas helium was used at a flow rate of 2 ml/min and was held at a constant pressure of 117.5 kPa. The column temperature was programmed from 50 °C (held for 2 min), at 20 °C/min to 80 °C (held for 1 min), at 20 °C/min to 100 °C (held for 1 min), then at 30 °C/min to 230 °C (held for 2 min). Other conditions were as follows: scanning mass range (m/z) 50–550 a.m.u at a rate of 1.5 scan/s; electron ionization energy, 70 eV.

2.4. Identification and quantification

The measured mass spectra were compared with those obtained from reference compound if available, as well as with data found in the literature (the respective references are given in Table 2) and also from a commercially available mass spectra database (Wiley 275.1). Additionally, the volatile compounds were identified by matching the retention indices (RI), calculated according to the equation of Van den Dool and Kratz (1963) and based on a series of alkanes (C6–C22) (i.e., RI of hexane is 600). The retention indices for the compounds of interest are given in Table 2.

$$\text{Retention Indices (RI)} = 100Z + 100 \left[\frac{\ln\ t_{R,A} - \ln\ t_{R,Z}}{\ln\ t_{R,Z+1} - \ln\ t_{R,Z}} \right]$$

where retention time (t_R) of $t_{R,Z} < t_{R,A} < t_{R,Z+1}$; Z = number of atom carbon.

Tridecane (TDC) was used as an internal standard, as this compound was not found in the palm sugar. It is well known that discrimination of compounds with high or low volatility occurs in SPME. Consequently, quantification cannot be carried out seriously by the use of one single internal standard. We used the internal standard, not for quantification, but for correction of variations of the capacity of the SPME fibre, as well as the variations of the sensitivity of the GC–MS system. In addition, it was not the aim of this study to quantify volatile compounds, but to determine the relative concentration changes of volatile compounds during the traditional production of palm sugar. Therefore, given concentration values are not noted as absolute concentration values but as equivalents to the internal standard. The relative concentrations of the investigated compounds were calculated by relating the areas of

the internal standard (tridecane) to the areas of the compounds of interest.

$$\text{Relative Conc.} = \left[\frac{\text{Peak area of particular compound}}{\text{Peak area of internal standard (IS)}}\right]$$
$$\times \text{Concentration of IS}$$

3. Results and discussion

3.1. Time–temperature curve

Fig. 1 shows the time–temperature curve during the heating process of palm sap. For the first 30 min, the temperature of the palm sap increased drastically up to 76.9 °C. Then, the temperature of the sample rose to 100 ± 5 °C at 60 min heating time for 2 h before continuously increasing to the final temperature of about 150 °C at 240 min. From these results, it seems clear that there were two drastic increments of the palm sap temperature which occurred during the whole period of the heating process. Firstly, an increase slope of the temperature for the first 60 min of the heating time was believed partly for
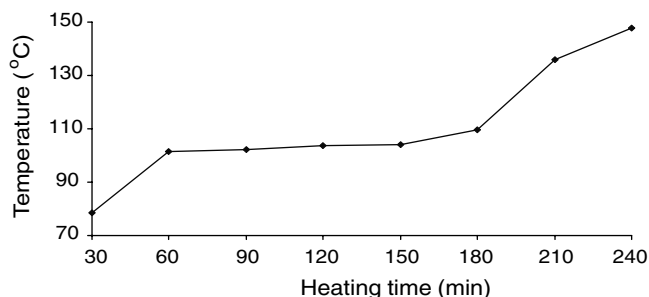

Fig. 1. Temperature changes during the traditional heating process for production of the palm sugar.

the supporting of the dehydration of water from the palm sap. The evaporation of water from the palm sap to an appropriate level is important as it could create a suitable environment for enhancing further reaction, for example moderate water activity (A_w). Meanwhile the increment of temperature at the later stage of the heating time was expected to be related to the formation of a particular compound that gave a roasted nut and sweet caramel-like aroma note which were the characteristics of a palm sugar product.

3.2. Volatile compounds

Table 2 lists 30 volatile compounds which grouped into 4 major chemical classes were used to follow the concentration changes at different heating times. The four major chemical classes were N-heterocyclic (17 pyrazines compound), O-heterocyclic (7 furan derivatives compound), as well as non-heterocyclic: aldehydes (4) and ketones (2). Odour threshold in water and flavour attributes of particular compounds are given in Table 1.

3.2.1. N-Heterocyclic

A rather high number of compounds identified in the headspace of the heated palm sap belonged to the N-heterocyclic chemical classes. Seventeen pyrazines which consisted of 83.69% relative percentage area (RPA) were the highest contributors to the aroma profile of palm sugar when compared to the other chemical classes of volatile compounds as shown in Table 2. Among all the pyrazines, 2-ethyl-3,5-dimethyl pyrazine (1.1% RPA) and 2,3-diethyl-5-methyl pyrazine (0.3% RPA) were respectively reported as potent odorants in cheese products (Rychilik & Bosset, 2001a, 2001b) and as aroma impact compounds that intensified the roasty flavour in coffee and roasted beef flavour (Czerny & Grosch, 2000; Czerny & Grosch, 1994). These

Table 1
Mean relative concentrations (ppb), relative percentage area (RPA), odour threshold and odour descriptions[a] of selected compounds identified using headspace-solid phase microextraction and gas chromatography–mass spectrometry (HS-SPME and GC–MS) of palm sugar during the traditional heating process for production of palm sugar

Compound	Mean relative concentration (ppb)	RPA (%)	Odour threshold in water (ppb)[b]	Odour description
2-Methyl pyrazine	1190	13.2	60000	Nutty[c]
2,5(6)-Dimethyl pyrazine	2323	25.8	1700	Nutty, sweet, roasty[c]
2-Ethyl pyrazine	343	3.8	6000	Roasty, sweet[c]
2,3-Dimethyl pyrazine	684	7.6	2500	Roasted nut, coffee, peanut, nutty, sweet[c,d]
2-Ethyl-5-methyl pyrazine	1386	15.4	100	Roasty, sweet[c]
2-Ethyl-3,5-dimethyl pyrazine	100	1.1	0.04	Roasty, earthy[e]
2,3-Diethyl-5-methyl pyrazine	24	0.3	0.5 (SF oil)[e]	Roasty, earthy[e]
2-Furfural	545	6.1	3000	Smoky, burnt[c]
5-Methyl furfural	114	1.3	500	Nutty[c]
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	9	0.1	60	Caramel, sweet, burnt sugar, strawberry-like[d]

[a] Odour threshold and description are obtained from the literature. The source is given for each compound in the list.
[b] Buttery et al. (1995,1997).
[c] Lasekan et al. (1997).
[d] Moio and Addeo (1998).
[e] Rychilik and Bosset (2001a, 2001b).

Table 2
Volatile compounds and relative concentrations (ppb) identified using headspace-solid phase microextraction and gas chromatography–mass spectrometry (HS-SPME and GC–MS) of palm sugar during the traditional heating process for production of palm sugar

Compound[a]	RI[b] (HP5)	Concentration (ppb)[c]							
		Heating time (min)							
		30	60	90	120	150	180	210	240
N-Heterocyclic (pyrazines)		2	94	325	1487	1560	3260	5474	7523
Pyrazine	672	0	0	0	45	33	63	95	129
2-Methyl pyrazine	823	0	0	79	161	114	295	791	1190
2,5(6)-Dimethyl pyrazine	912	0	86	211	908	957	1781	2117	2323
2-Ethyl pyrazine	917	0	0	0	0	0	0	0	343
2,3-Dimethyl pyrazine	919	0	0	13	63	64	108	540	684
2-Ethyl-6-methyl pyrazine	992	0	0	0	24	16	21	66	432
2-Ethyl-5-methyl pyrazine	1001	2	8	21	260	303	637	1089	1386
2-Ethenyl-6-methyl pyrazine	1016	0	0	0	7	6	17	58	56
3-Ethyl-2,5-dimethyl pyrazine	1082	0	0	1	9	25	143	369	523
2-Ethyl-3,5-dimethyl pyrazine	1088	0	0	0	3	7	24	68	100
5-Ethyl-2,3-dimethyl pyrazine	1089	0	0	0	3	11	26	33	56
2,5-Diethyl pyrazine	1091	0	0	0	4	7	10	11	14
Dimethyl-2-vinyl pyrazine	1098	0	0	0	0	14	125	160	152
5-Methyl-6,7-dihydro-5H-cyclopenta pyrazine	1147	0	0	0	0	0	0	1	14
3,5-Diethyl-2-methyl pyrazine	1162	0	0	0	0	2	8	71	89
2,3-Diethyl-5-methyl pyrazine	1171	0	0	0	0	1	2	3	24
3-Furfuryl-2,5-dimethyl pyrazine	1413	0	0	0	0	0	0	2	8
O-Heterocyclic (furan derivatives)		0	0	0	0	3	8	322	1299
2-Methyl furan	598	0	0	0	0	3	7	13	46
2-Methyl tetrahydrofuran-3-one	806	0	0	0	0	0	1	22	41
2-Furfural	835	0	0	0	0	0	0	119	545
2-Furan methanol	860	0	0	0	0	0	0	139	518
5-Methyl furfural	964	0	0	0	0	0	0	24	114
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	1060	0	0	0	0	0	0	2	9
3-Fenyl furan	1224	0	0	0	0	0	0	3	26
Aldehydes		52	369	298	442	143	61	95	140
Benzaldehyde	956	26	263	207	234	69	0	0	0
Phenylacetaldehyde	1046	0	0	0	3	14	11	16	32
Nonanal	1103	14	48	40	72	33	28	49	57
Decanal	1202	12	58	51	133	27	22	30	51
Ketones		12	51	45	51	7	22	20	27
6-Methyl-5-hepten-2-one	984	4	22	18	27	2	9	9	13
Geranyl acetone	1421	8	29	27	24	5	13	11	14

[a] The compounds were identified by comparison of the measured mass spectra with mass spectra obtained from reference compounds if available as well as by comparison of the mass spectra from a mass spectra library (Wiley 275).

[b] Retention indices (RI) were based on a series of alkane (C6–C22).

[c] Integration of the peak was performed by choosing the characteristic ion (m/z) for the respective compounds with the highest matching index (>80%) as to avoid possible interference by other compounds. The concentrations are given in terms of equivalents to the internal standard tridecane.

statements are well supported due to their low odour threshold value possessed by 2-ethyl-3,5-dimethyl pyrazine (0.04 ppb) and 2,3-diethyl-5-methyl pyrazine (0.5 ppb) as shown in Table 1.

In addition, 5-methyl-6,7-dihydro-5H-cyclopentapyrazine, the only alkyl substituted dihydrocyclopentapyrazines identified in palm sugar was reported in the aromas of fried beef, roasted nuts, cocoa and coffee (Maga, 1992). With the exception of 5-methyl-6,7-dihydro-5H-cyclopentapyrazine (0.2% RPA), which was reported to have a sweet, crust-like odour, most of the cyclopentapyrazines possess an earthy, baked potato-like flavour (Maga, 1992). Surprisingly, we noticed that both the 2,3-diethyl-5-methyl pyrazine and 5-methyl-6,7-dihydro-5H-cyclopentapyrazine volatile compounds were concurrently formed at

180 min of heating time. Therefore, we believed that these volatile compounds could be potential aroma impact compounds that were responsible for the palm sugar aroma notes even though the RPA was below 0.5%.

Fig. 2 shows the concentration changes of some pyrazines. All of these pyrazines are correlated with sensory attributes, such as roasty, nutty, earthy and potato-like which consequently contribute significantly to the typical aroma notes of palm sugar. Fig. 2 shows the formation of pyrazine compound from a quantitative point of view which requires a minimum reaction time of 120 min, corresponding to a temperature of above 100 °C. Reaction temperatures higher than 110 °C, corresponding to 180 min of heating time led to a nearly exponential increase in the formation of pyrazine compounds. Numerous studies
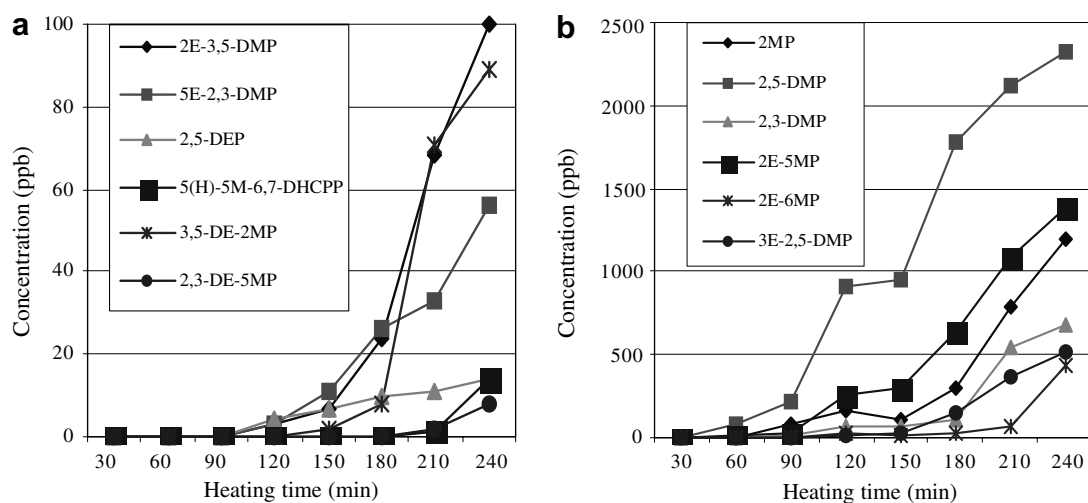
Fig. 2. Formation of pyrazine compounds during the traditional heating process for production of the palm sugar (DMP: dimethyl pyrazine; DEP: diethyl pyrazine; DHCCP: dihydrocyclopenta[b] pyrazine; MP: methyl pyrazine; EP: ethyl pyrazine; E: ethyl).

concerning the thermal treatment on the formation of volatile compound on solid food system such as perilla seed oil (Kim, Yoon, & Rhee, 2000), Arabica coffee (Czerny & Grosch, 2000) and sorghum (Lasekan, Lasekan, & Idowu, 1997) indicated the presence of various pyrazines which was the determining factor for the typical nutty, roasty and sweet aroma of this type of product. However, a study conducted on palm sap which itself was an aqueous food system is never reported and based on our results, it was suggested that thermal processing of palm sugar at temperatures higher than 110 °C is necessary for the formation of various pyrazines compound responsible for the typical roasted nut aroma of palm sugar. The results from the roasting of pumpkin seeds (Barbara & Michael, 2004) indicate that temperatures higher than 110 °C are necessary for the formation of reasonable amounts, of not only pyrazines, but also of other flavour compounds confirm our findings.

3.2.2. O-Heterocyclic

As shown in Table 2, O-heterocyclic chemical classes consisted of seven furan derivatives with an RPA of almost 14.5% were identified in the headspace of the sample. Their occurrence in various nuts and oilseeds products has been previously reported (Maga, 1979) and play an important role in the contribution of thermally processed food flavour. Surprisingly, 4-hydroxy-2,5-dimethyl-3(2H)-furanone (Furaneol^TM) with an RPA of only 0.1%, the lowest among all furan derivatives was known to be a powerful flavour compound and an important aroma constituent of many fruits (e.g. strawberry) (Rychilik & Bosset, 2001a; Larsen, Poll, & Olsen, 1992) and processed foods (e.g. Cheddar cheese) (Milo & Reineccius, 1997). Furaneol had also been reported to possess a sweet caramel, burnt sugar flavour with appreciable fruitiness and occurs in beer, Arabica coffee and white bread crust (Schieberle, 1995). However, little is known about the occurrence and influence of furaneol on palm sugar flavour properties. In processed food, furaneol is probably formed in the course

of cooking. Model experiments have revealed that this compound is produced by thermal degradation of fructose or 6-deoxyhexoses, in the presence of amines and amino acids via the Maillard reaction (Hecquet, Sancelme, Bolte, & Demuynck, 1994; Pischetsrieder & Severin, 1994). Hence it was believed that such mechanisms could occur during the production of palm sugar. For this reason and on the basis of its low odour threshold value (Table 1), furaneol may be responsible for the sweet caramel-like attributes of palm sugar.

Fig. 3 shows the concentration changes of some furan derivatives. All the furan derivatives showed significant increases. The increased concentration could be determined for furan derivatives compounds after 210 min of heating time except for 2-methyl furan. For the formation of furan derivatives, two formation pathways are possible: (i) lipid peroxidation and (ii) degradation of carbohydrates. In our present study, all these furan derivatives (Table 2) reported are known to be formed by the degradation of carbohydrates (Maga, 1979). Coincidently, the temperature of more than 130 °C at 210 min of heating time further support the possibility of the occurrence of the carbohydrate degradation reaction.

3.2.3. Non-heterocyclic

The non-heterocyclic chemical classes consisted of four aldehdye and two ketones volatile compounds represent less than 2% RPA. Overall, the aldehyde group show different sensory attributes. Phenylacetaldehyde, formed by phenylalanine degradation, had been reported as a major aroma odour active compound of Gruyere cheese (Rychilik & Bosset, 2001a, 2001b) and explicit honey-like aroma notes. Other two straight chain aldehdyes, nonanal and decanal were expected to be responsible for the fresh and slightly green notes of the palm sap.

From Fig. 4, no phenylacetaldehyde compound was observed but the concentration increases obviously after 120 min of heating time. Therefore, we believe that primary
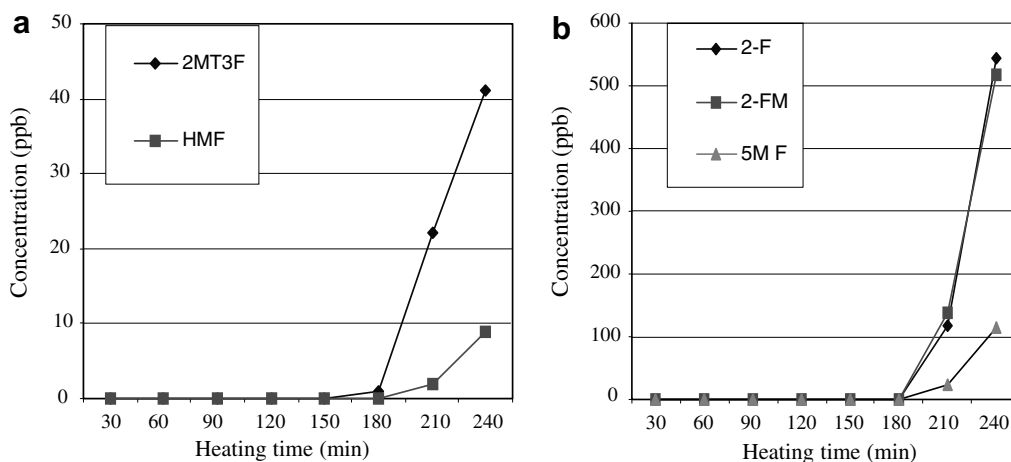
Fig. 3. Formation of furan derivative compounds during the traditional heating process for production of the palm sugar (2MT3F: 2-methyl tetrahydrofuran-3-one; HMF: 4-hydroxy-2,5-dimethyl-3(2H)-furanone; 2-F: 2-furfural; 2-FM: 2-furan methanol; 5MF: 5-methyl furfural).
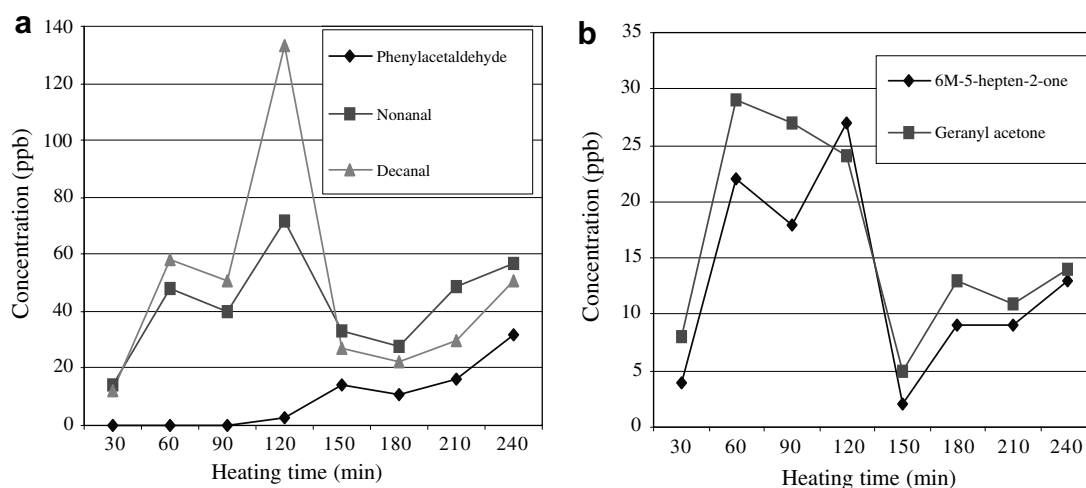

Fig. 4. Formation of Strecker degradation (a) and lipid oxidation (b) products during the traditional heating process for production of the palm sugar.

heating of the palm sap will lead to the degradation of phenylalanine amino acid. Further heating of the palm sap will definitely lead to acceleration of the Strecker degradation reaction and formation of the compounds in relatively high concentrations. Benzaldehyde, which is supposed to be a degradation product of the amino acid phenylalanine (Adamiec, Rossner, Velisek, Cejpek, & Savel, 2001), showed a contrast behaviour. Other two straight chain aldehydes, nonanal and decanal show a very large increase in concentrations in course of the heating process up to 120 min (Fig. 4). Meanwhile, the ketones which were identified at all stages of the heating process probably intensify the slightly fruity attributes of palm sugar.

4. Conclusion

The results of this study clearly show that the heating process of the palm sap is necessary to obtain the characteristic aroma of the final product, the palm sugar. Several flavour compounds are formed during the heating process, including compounds from Maillard reaction, Strecker degradation, as well as lipid oxidation. It is also demonstrated that for the formation of the typical roasty, nutty and sweet caramel-like aroma notes, heating temperatures significantly higher than 110 °C are necessary.

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