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Use of Gas Chromatography–Olfactometry in combination with Solid Phase Micro Extraction for cocoa liquor aroma analysis

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Abstract: Aroma of cocoa liquor extracted by using Solid Phase Micro Extraction with polydimethylsyloxane–divinylbenzene polymer was analyzed in Gas Chromatography-Olfactometry. Headspace extraction of cocoa odour-active compounds for 30 min at temperature of 60°C followed by GC separation elaborated with column oven initial temperature at 60°C and ramped at 5°C/min to reach 220°C identified most of the odour-active compounds including wide range of alcohols, carboxylic acids, aldehydes, ketons, esters, pyrazines, and amines. Cocoa specific aroma attributed in terms of sweet, nutty, caramel and chocolate-like notes were associated with trimethylpyrazine, tetramethylpyrazine, 2,3-butanediol, dodecanoic acid, phenylethyl alcohol, ethanone, benzeneacetaldehyde and 1,4-bis(morpholinoacetyl)piperazine. Meanwhile defective cocoa aroma such as smoky and rancid were generated by benzeneacetaldehyde, alphaethylidene; trimethylpyrazine, and trans-2,3-dimethyloxirane.

Keywords: Cocoa, flavour, aroma, gas chromathography, olfactometry, Solid Phase Micro Extraction, headspace, pyrazine, ester, alcohol

Introduction

Flavour is one of the most important properties of cocoa products and its precursors are developed during fermentation and drying of cocoa beans. Aroma precursors in cocoa beans, which include the free amino acids, peptides and reducing sugars, develop into cocoa specific aroma through Maillard reactions during roasting process (Barel *et al.*, 1985; Granvogl *et al.*, 2006). Through the Maillard reactions, all of the cocoa aroma precursors interact to produce cocoa flavor components, such as alcohols, ethers, furans, thiazoles, pyrones, acids, esters, aldehydes, imines, amines, oxazoles, pyrazines and pyrroles (Hoskin and Dimick,1994; Jinap *et al.*, 1998; Puziah *et al.*, 1998).

Sensory evaluation method has been extensively used in cocoa flavour analysis (Luna *et al.*, 2002; Jinap *et al.*, 1995; Baker *et al.*, 1994). According to ADM Cocoa (2005) sensory analysis is an applicable and reliable tool for cocoa flavor characterization in industrial uses. Sensory analysis examines overall flavour impression of the cocoa sample, although it could not determine a specific component that contributes to the perceived sense accordingly.

Gas chromatography (GC) has been employed to improve consistency and sensibility of aroma analysis and to obtain specific components contributing to an aroma character. Modification of GC conditions such as extraction method, column, separation condition and detector have been applied to improve the GC performance for aroma identification (Fraundorfer and Schieberle, 2008; Kratzer, *et al.*, 2008; Krings *et al.*, 2006; Ohashi, *et al.*, 2006; and Perego *et al.*, 2004). GC-Mass Spectrophotometry (MS) detector has been developed to detect components with further interpretation (Afoakwa, 2008). By development of recent MS-library, GC-MS analysis gets more reliable to be applied for cocoa quality determination.

Combination of GC and MS detector has been used widely in detection of varied vapour component (Choi, 2003; Goodner *et al.*, 2000, Weenen *et al.*, 1996). However, their combination with olfactory instrument as well as with aroma extraction method for cocoa aroma detection is still limited, Misnawi *et al.* (2009) developed combination of GC-MS and SPME for pyrazines analysis in cocoa liquor.

Extraction method of volatile compounds contributes to the validity of flavor analysis by using GC. The conventional extraction method is by using solvent extraction (Fraundorfer and Schieberle, 2008; Fraundorfer and Schieberle, 2006; Didzbalis *et al.*, 2004), however headspace extraction may improve volatile component selection (Counet *et al.*, 2004; Bucking and Steinhart, 2002). SPME is a method developed to elaborate entrapment of volatile compounds presence in the atmosphere around materials (Akiyama, *et al.*, 2005; Vazques-Landaverde *et al.*, 2005, Bonvehi & Coll,1998). Fibers used in SPME have different ability to trap certain components, based on their polarity (Shirey and Sidisky, 1999).

This research aimed to study the use of GC-MS combined with olfactory instrument in cocoa

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liquor aroma analysis in combination of SPME Polydimethylsyloxane–Divinylbenzene (PDMS-DVB) polymer. The compounds that contribute to cocoa-recognized odour were investigated as well those that contribute to cocoa defect odour.

Materials and Methods

Extraction of cocoa liquor aroma and GC-MS condition

Cocoa beans used in this study were fermented bulk cocoa beans from three different production areas of Indonesia and further labeled as Cocoa Bean 1, 2 and 3 respectively. Cocoa beans were deshelled and roasted at temperature of 140^{o}C for 45 min. Roasted beans then were ground to obtain cocoa liquor and subsequently poured into capped glass vial. SPME fiber used in this study was made of Polydimethylsyloxane–Divinylbenzene (PDMS-DVB) polymer (Supelco, USA). The extraction was held for 30 min at temperature of 60°C.

Separation and detection of cocoa liquor odour compounds were conducted by using GC system consisted of Agilent 7890A equipped with Mass Spectrometer Detector (MS Agilent 5975 C Triple Axis, USA). GC operation was obtained from several preliminary experiments which recommended the condition of splitless injection at 260°C, column oven at initial temperature of 60°C and ramped at 5°C/min to reach 220°C then hold for 5 minutes and carrier gas of helium flowed at 1 ml/min. Nitroterphthalic acid modified polyethylene glycol column with ID of 0.25 mm, length of 30 m and film thickness of 0.25 µm (J&W Scientific DB-FFAP) was used for the separation.

Odour identification by using Olfactometry

Odour identification was conducted by two panelists of Indonesian Coffee and Cocoa Research Institute (ICCRI) with at more than two years experience. Selection of the panelists was based on their sensitivity in distinguishing individual basic tastes including sweet, salty, sour, bitter, and alkali, followed by combination of the two or three basic tastes. Succeeded panelist continued to training on cocoa aroma and taste terminology prior to cocoa aroma analysis.

The two trained panelists sniffed the outflowing gas from Olfactometry Detection Port (ODP) of the GC apparatus. The Sniffing was carried out simultaneously during the GC analysis. Panelists described their perception of the sniffed odour and scored their intensity with 3, 5, and 7 representing weak, moderate and strong, respectively.

Results and Discussion

Detection and identification in GC-MS

Complete separation of cocoa aroma (Figure 1) was obtained during running time for total 36 minutes, while shorter running time resulted in less peaks. Identification by using GC-MS library showed that the major peaks were acetic acid, tetramethyl pyrazine, 3-methyl pentanoic acid and 2,3-dimethyl oxirane exposed at retention time (RT) of 17.54, 18.26, 22.52 and 25.99 min, respectively. Other volatile compound such as dodecanoic acid (RT 26.12 min), benzeneacetaldehyde (RT 28.59 min) and 1,4-bis(morpholinoacetyl)piperazine (RT 32.70 min) were detected in small peak areas.

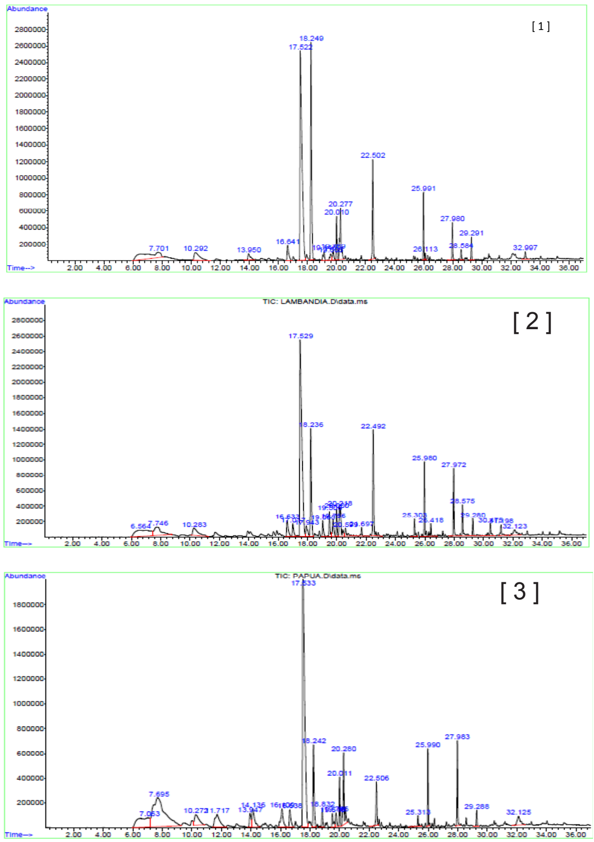

Figure 1. Gas chromatography separation of cocoa volatile compounds originated from three different cocoa liquors extracted by using solid phase micro extraction

There are nine compounds that consistently appeared in all samples including acetic acid, tetramethylpyrazine, phenyl ethyl alcohol, 2-phenylethyl ester acetic acid, propanol methoxy-acetate, trimethylpyrazine, benzaldehyde and 1-(H-pyrrol-2-yl)-ethanone. Those compounds are suggested as key compounds of cocoa aroma as they persist in further cocoa bean processing (Counet *et al.*, 2002). Two of those compounds i.e. acetic acid and pyrazine have been investigated to affect overall cocoa taste and aroma. Study by Krings *et al.* (2006) showed that acetic acid and pyrazine were found in cocoa powder where acetic acid brought significant aroma.

Acetic acid is a product of microbiological activity during fermentation, where the concentration is increasing as the day of fermentation. Acetic acid concentrates in bean more than 20 times higher after fermentation (Ardhana and Fleet, 2003). Jinap *et al*. (1995) reported that acetic acid in cocoa bean ranged from 0.38 to 0.92% and affects noticeable acid taste in final product. Ghanaian cocoa bean that renowned as high quality cocoa bean has lower in acid taste.

Tetramethyl pyrazine and other pyrazines mainly appeared during Maillard reaction, involving sugar and protein under heat treatment (Ferretti and Flanagan, 1971). Pyrazines are also formed in fermentation of cocoa bean (Jinap *et al*., 1994) and contribute to a pleasant aroma of chocolate. Pyrazines are ultimate components in cocoa flavour (Bonvehi and Coll, 2002; Barel *et al*., 1985; Chaveron *et al*., 1989; Hashim and Chaveron, 1994; Maga, 1992).

Successful pyrazine extraction by SPME also supported research by Pini *et al*. (1994) suggested that Head Space SPME is an appropriate tool for pyrazine analysis, comparable to other method employing solvent and distillation. In addition, SPME extraction that requires lower temperature could prevent further pyrazine formation through heating during distillation.

Compounds such as 3,7-dimethyl-1,6-Octadien-3-ol, 2,3-dimethyl-oxirane, ethyl ester benzeneacetic acid, alpha ethylidene benzeneacetaldehyde and n-hexadecanoic acid were found occasionally in two of three cocoa bean samples. 3,7-dimethyl-1,6-Octadien-3-ol (linalool), ethyl ester benzeneacetic acid and alpha ethylidene benzeneacetaldehyde which contribute to pleasant aroma are already exist in unroasted cocoa bean (Fraundorfer and Schieberle, 2008).

On the other hand, there are compounds found in particular cocoa bean sampel. Compounds of 1,4-Bis(morpholinoacetyl)piperazine, Dodecanoic acid, ethyl ester, Pentanoic acid, 3-methyl-, 2,3-Butanediol, and 2,3-Butanedioldiacetate detected particularly in Cocoa Bean 1, while Cocoa Bean 2 had compounds of Cyclobutanol, Octanoic acid, ethyl ester, 2,3-Dimethyl-5-ethylpyrazine, 2,3,5-Trimethyl-6-ethylpyrazine, Propanoic acid, 2-methyl-, 7H-Pyrrolo[2,3-d]pyrimidin-4-amine, Decanoic acid, ethyl ester, Hydrazine, 1,1-dimethyl-, n-Propyl benzoate, Octanoic Acid and 5-Methyl-2-phenyl-2-hexenal. Cocoa Bean 3 also had compounds consisted of 1,2-Propanediamine, Propane, 2-(ethenyloxy)-, Cyclobutanone, 2-ethyl-, 2-Butanone, 3-hydroxy-, 2-Heptanol, 2-Nonanone, 2-Nonanol and 3-methyl-Butanoic acid. The discovery of specific compounds which differs one bean sample to another, indicated the adequacy of SPME extraction for identification of cocoa aroma.

Sensory atributes identification

Cocoa aroma is complex compounds produced from its precursors and developed during roasting. Aroma precursors in cocoa bean, which include the free amino acids, peptides and reducing sugars, develop into cocoa specific aroma through Maillard reactions during roasting (Barel *et al*., 1985; Frauendorfer and Schieberle, 2006). These precursors are known to be produced through proteolysis of protein and hydrolysis of sucrose in cocoa bean during fermentation (Ziegleder and Biehl, 1988; Rohan and Stewart, 1966; Rohan, 1963). Proteolysis gives rise to amino acids and oligopeptides (Biehl *et al*., 1982a, 1982b) which depends on death of bean, pH and temperature raised (Biehl *et al*., 1993). The process of roasting understandably deserves considerable attentions (Hoskin and Dimick, 1994). The Maillard or non-enzymatic browning reactions is a very important process for the development of cocoa flavor, which occurs primarily during the roasting process (Maga, 1992; Bonvehi and Coll, 2002).

Results of the identification showed that cocoa aroma is mixture of varied odourants. Not all odourant produced specific smell, some of them were even odourless. Corresponding odourants with the cocoa volatile compounds identification (Table 1) showed that various compounds perform similar odourants as well certain compound could produce various odourants. For instance, we found that the term of sweet was presented by pyrazines, butanediol and ethyl ester compounds, meanwhile nutty odour presented in pyrazines, benzeneacetaldeyde and ethanone compounds. In contrast, certain compounds were also produced two odours at the same time i,e. trimethylpyrazine was perceived as either sweet or nutty. However, an odourant typically corresponds to other odourants. Sweet odour often comes with caramel-like, nutty and creamy or in exceptional term it comes with rancid as produced by the 3-methyl-pentanoic acid.

Frauendorfer and Schieberle (2006) identified 35 most active compounds from cocoa powder extract. Data presented in Table 2 shows that 36 compounds were detected in fermented cocoa liquor volatile compounds. They are representing alcohols, carboxylic acids, aldehydes, ketons, esters, pyrazines, amines and other volatile compounds. They were most volatile compounds associated in fermented cocoa aroma. The analysis covers main

Table 1. Cocoa volatile compounds detected and identified in GC-MS resulted from solid phase micro extraction

Cocoa Bean 1			Cocoa Bean 2			Cocoa Bean 3		
RT	Area %	Components	RT	Area %	Components	RT	Area %	Components
			7.74	4.6255	Cyclobutanol	7.06	6.6079	1,2-Propanediamine
			8.22		unknown	7.7	20.061	Propane, 2-(ethenyloxy)-
			9.49		unknown	10.27	2.767	1-Butanol, 3-methyl-, acetate
10.29	3.9134	1-Butanol, 3-methyl-, acetate	10.27	3.0639	1-Butanol, 3-methyl-, acetate	11.71	3.5234	Cyclobutanone, 2-ethyl-
10.65		unknown	13.32		unknown	13.96	1.2923	2-Butanone, 3-hydroxy-
16.65	2.3446	Pyrazine, trimethyl-	16.63	2.0672	Pyrazine, trimethyl-	14.14	2.6213	2-Heptanol
16.89		unknown	17.02	1.4191	Octanoic acid, ethyl ester	16.12	2.5966	2-Nonanone
17.39		unknown	17.54	32.8967	Acetic acid	16.63	1.617	Pyrazine, trimethyl-
17.54	35.6461	Acetic acid	17.95	1.4591	2,3-Dimethyl-5-ethylpyrazine	17.54	32.5032	Acetic acid
18.26	21.7023	Pyrazine, tetramethyl-	18.24	9.088	Pyrazine, tetramethyl-	18.24	5.1245	Pyrazine, tetramethyl-
18.61		unknown	19.06	1.3422	2,3,5-Trimethyl-6-ethylpyrazine	18.83	1.0741	2-Nonanol
18.96		unknown	19.51	1.7345	1,6-Octadien-3-ol, 3,7-dimethyl-	19.51	0.9721	1,6-Octadien-3-ol, 3,7-dimethyl-
19.14	1.1668	2,3-Butanedioldiacetate	19.76	1.8494	Benzaldehyde	19.76	0.9374	Benzaldehyde
19.59	1.6399	2,3-Butanediol	20.01	1.6761	Propanol, methoxy-, acetate	20.01	2.2024	Propanol, methoxy-, acetate
19.78	1.2361	Benzaldehyde	20.21	4.4555	Propanoic acid, 2-methyl-	20.27	4.7529	Oxirane, 2,3-dimethyl-, trans-
20.01	3.5215	Propanol, methoxy-, acetate	20.6	1.0655	7H-Pyrrolo[2,3-d] pyrimidin-4-amine	22.52	2.1938	Butanoic acid, 3-methyl-
20.27	6.9748	Oxirane, 2,3-dimethyl-, trans-	21.69	0.6903	Decanoic acid, ethyl ester	25.31	0.5333	Benzeneacetic acid, ethyl ester
22.52	8.1037	Pentanoic acid, 3-methyl-	22.5	7.734	Hydrazine, 1,1-dimethyl-	25.99	2.9526	Acetic acid, 2-phenylethyl ester
25.99	3.9217	Acetic acid, 2-phenylethyl ester	22.6		unknown	27.99	3.4658	Phenylethyl Alcohol
26.12	0.3484	Dodecanoic acid, ethyl ester	25.29	1.1633	Benzeneacetic acid, ethyl ester	29.29	0.6264	Ethanone, 1-(1H-pyrrol-2-yl)-
27.99	3.1267	Phenylethyl Alcohol	25.97	3.8683	Acetic acid, 2-phenylethyl ester	32.12	1.575	n-Hexadecanoic acid
28.59	0.7474	Benzeneacetaldehyde, .alpha.-ethylidene-	26.43	1.0822	n-Propyl benzoate			
29.31	2.5007	Ethanone, 1-(1H-pyrrol-2-yl)-	27.97	3.97	Phenylethyl Alcohol			
32.7	0.468	1,4-Bis(morpholinoacetyl) piperazine	28.56	2.3611	Benzeneacetaldehyde, .alpha.-ethylidene-			
			29.29	0.9754	Ethanone, 1-(1H-pyrrol-2-yl)-			
			30.48	1.4964	Octanoic Acid			
			31.2	1.0168	5-Methyl-2-phenyl-2-hexenal			
			31.7		unknown			
			32.12	1.4873	n-Hexadecanoic acid			
			35.54		unknown			

chocolate aroma compounds suggested by Jinap *et al.* (1998) including pyrazine, carbonyl, ester, alcohol, hydrocarbon and phenol.

In a series of intensive studies, Voigt *et al.* (1994) have successfully generated cocoa aroma *in-vitro* from its precursors in the presence of cocoa butter, although the real chocolate flavor remained obscured. The result also implies that cocoa bean aroma is characterized by presence of sweet, caramel-like, nutty and bean-like odour. This study revealed that those odours are expressed by pyrazines, ethyl ester and alcoholic compounds, particularly trimethylpyrazine, tetramethylpyrazine, 2,3-butanediol, dodecanoic acid, phenylethyl alcohol, ethanone, benzeneacetaldehyde and 1,4-bis(morpholinoacetyl)piperazine.

Corresponding odour identification showed that related cocoa specific odour in terms of caramel-like, roasted cocoa, chocolate and smoky was detected in all samples. Caramel-like was associated with phenylethyl alcohol; benzaldehyde; 1-(1H-pyrrol-2-yl)ethanone; 1,4-bis(morpholinoacetyl) piperazine; 7H-pyrrolo[2,3-d]pyrimidin-4-amine and 2-(ethenyloxy)-propane. Roasted cocoa note was associated with cyclobutanol; 5-Methyl-2-phenyl-2-hexenal; acetic acid, 2-phenylethyl ester; 2,3-dimethyl-5-ethylpyrazine and 1,1-dimethyl-hydrazine. Chocolate note was associated with octanoic acid and tetramethylpyrazine. Fruity notes

Table 2. Identified odour of cocoa volatile compounds obtained from SPME extraction and GC separation

Volatile compounds	Coresponding odour	Volatile compounds	Coresponding odour
Alcohol		**Ester**	
2,3-Butanediol	sweet, creamy	1-Butanol, 3-methyl-, acetate	lemon-like, flowery, sweet, fruity
Phenylethyl Alcohol	caramel-like, alcohol-like, sweet	Acetic acid, 2-phenylethyl ester	cereal-like, roasted cocoa
Cyclobutanol	roasted cocoa	Propanol, methoxy-, acetate	flowery, green
2-Nonanol	no smell	Benzeneacetic acid, ethyl ester	nutty, bean-like
2-Heptanol	no smell	Decanoic acid, ethyl ester	nutty
1,6-Octadien-3-ol, 3,7-dimethyl-	bean-like	Dodecanoic acid, ethyl ester	sweet, creamy
		Propanol, methoxy-, acetate	no smell
Carboxylic acid		Octanoic acid, ethyl ester	alcohol-like
Acetic acid	sour, nutty		
Pentanoic acid, 3-methyl-	sweet, rancid	**Pyrazines**	
Octanoic Acid	sweet, chocolate-like	Pyrazine, trimethyl-	sweet,nutty, bean-like, smoky
n-Hexadecanoic acid	no smell	Pyrazine, tetramethyl-	bean-like, chocolate, rancid
Butanoic acid, 3-methyl-	sour	2,3-Dimethyl-5-ethylpyrazine	roasted cocoa
		2,3,5-Trimethyl-6-ethylpyrazine	no smell
Aldehyde			
Benzaldehyde	bean-like	**Amines**	
Benzeneacetaldehyde, alpha.-ethylidene-	caramel-like, smooky, nutty	1,4-Bis(morpholinoacetyl)piperazine	caramel-like, sweet
5-Methyl-2-phenyl-2-hexenal	roasted cocoa	7H-Pyrrolo[2,3-d]pyrimidin-4-amine	caramel-like
		1,2-Propanediamine	citrus-like
Keton			
Ethanone, 1-(1H-pyrrol-2-yl)-	sweet, caramel-like, honey-like, nutty	**Other volatiles**	
Cyclobutanone, 2-ethyl-	no smell	Oxirane, 2,3-dimethyl-, trans-	smoky
2-Butanone, 3-hydroxy-	no smell	Propane, 2-(ethenyloxy)-	caramel-like
2-Nonanone	no smell	Hydrazine, 1,1-dimethyl-	roasted cocoa

in cocoa bean which typically perceived as lemon-like were brought by 1-butanol-3-methylacetate.

Taints or defective attribute of cocoa and cocoa products include smoky, sour and rancid odour. Smoky odour was obtained to be associated with benzeneacetaldehyde, alphaethylidene; trimethyl pyrazine, and 2,3-dimethyl-transoxirane. This odour was generated during drying and storage (Bohvehi and Coll, 1998). Meanwhile, rancid odour as product of fat oxidation was expressed by alkyl-pentanoic acid.

Pyrazine (1,4 diazines) are heterocyclic nitrogen-containing compounds that contribute to the unique aroma and cocoa flavor. A large number of pyrazines have been identified in cocoa and are considered extremely important for cocoa flavor (Bonvehi and Coll, 2002; Barel *et al*., 1985; Chaveron *et al*., 1989; Hashim and Chaveron, 1994; Maga, 1992). Approximately 95 pyrazines have been identified in cocoa aroma and their concentrations varied depending on time and temperature of thermal treatment (Jinap *et al*., 1998; Hashim and Chaveron, 1994). Bonvehi and Coll (2002) identified eight pyrazine compounds significantly presence in roasted fermented cocoa beans, namely Pyrazine, 2-Methylpyrazine; 2,5-Dimethylpyrazine;2,6-Dimethylpyrazine; 2-Ethylpyrazine;2,3-Dimethylpyrazine;2,3,5-Trimethylpyrazine and 2,3,5,6-tetramethyl-pyrazine. Their sensory attributes were varied from pungent, sweet, nutty, chocolate, cocoa, roasted nuts, green, peanut butter, musty, caramel and coffee. This study presented sensory attributes for pyrazines as sweet, nutty, bean-like, bean-like, chocolate, rancid, roasted chocolate, caramel-like and smoky.

Various research groups have reported the thermal processing i.e. cocoa bean roasting is a vital step for the formation of pyrazines and other volatile compounds via the Maillard reactions. The reactions are involving amino acids and reducing sugars (Hofmann and Schieberle, 2000; Hofmann *et al*., 2000; Hofmann, 1998; Davies *et al*., 1997). By using a model system, it was found reaction between ammonia and reducing sugar under heat treatment was able to produce pyrazine compounds (Maga, 1992; Rizzi, 1972, 1988; Weenen *et al*., 1994). It has also been demonstrated that time, temperature, pH, reactant concentration and water activity are important variables in determining the nature and quantity of the products from the reactions (Shibamoto and Bernhard, 1977). These results inferred that extraction of cocoa volatile compounds by using SPME with Polydimethylsyloxane–Divinylbenzene (PDMS-DVB) polymer, followed by GC-MS separation and identification covered most odour-active compound in cocoa liquor.

Conclusion

Gas Chromatography-Olfactometry in combination with Solid Phase Micro Extraction usingpolydimethylsyloxane–divinylbenzene polymer was able to identify most of the odour-active compounds from cocoa liquor. The identified cocoa odourants were originated from wide range of alcohols, carboxylic acids, aldehydes, ketons, esters, pyrazines, and amines. These compounds were generating cocoa aroma attributed in terms of sweet, nutty, caramel and chocolate-like notes as well as defective cocoa aroma such as smoky and rancid.

References

ADM Cocoa. 2005. Sensory Evaluation Of Cocoa Products. ADM Cocoa B.V. Holland.

Afoakwa, E.O., Paterson, A., Fowler, M. and Ryan, A. 2008. Matrix effects on flavor volatiles release in dark chocolates varying in particle size distribution and fat content using GC-Mass Spectrometry and GC-Olfactometry. Food Chemistry 113: 208-215.

Akiyama M., Murakami, K., Ikeda,M., Iwatsuki, K., Kokubo,S., Wada, A., Tokuno,K., Onishi, M., Iwabuchi, H. and Tanaka, K. 2005. Characterization of flavor compounds released during grinding of roasted robusta coffee beans. Food Science and Technology Research 11: 298-307.

Ardhana, M. and Fleet, G. H. 2003. The microbial ecology of cocoa bean fermentation in Indonesia. International Journal of Food Microbiology 86: 87-99.

Baker, D. M., Tomlins, K. I. and Gay, C. 1994. Survey of Ghanaian cocoa farmer fermentation practices and their influence on cocoa flavor. Food Chemistry, 51: 425-431.

Barel, M., Leon, D. and Vincent, J.C. 1985. Influence of cocoa fermentation time on the production of pyrazines in chocolate. The Café Cacao 4: 277–286.

Bonvehi, J.S. and Coll, F.V. 2002. Factors affecting the formation of alkylpyrazines during roasting treatment in natural and alkalized cocoa powder. Journal of Agricultural and Food Chemistry 50: 3743–3750.

Bonhevi, J. S. and Coll, F.V. 1998. Evaluation of smoky taste in cocoa powder. Journal of Agricultural and Food Chemistry 46: 620-624.

Bucking, M. and Steinhart, H. 2002. Headspace GC and sensory analysis characterization of the influence of different milk additives on the flavor release of coffee beverages. Journal of Agricultural and Food Chemistry 50: 1529-1534.

Chaveron, H., Guyot, B., Hashim, L., Pezoa, H. and Pontillon, J. 1989. Formation and evaluation of methylpyrazines during cocoa roasting.study of methylpyrazines extraction method. In Charalambous, G. Flavors and Off-flavor. Proceeding in the 6th International Flavor Conference, p 305–319. Greece.

Choi, H.S. 2003. Character impact odorants of Citrus hallabong [(C. unshiu Marcov x C. sinensis Osbeck) x C. reticulate Blanco] cold-pressed oil. Journal of Agricultural and Food Chemistry, 51: 2687-2692.

Counet, C., Ouwerx, C.C., Rosoux, D.D. and Collin, S.S. 2004. Relationship between procyanidin and flavor contents of cocoa liquors from different origins. Journal of Agricultural and Food Chemistry 52: 6243-6249.

Davies, C.G.A., Wedzicha, B.L. and Gillard, C. 1997. Kinetic model of the glucose-glycine reaction. Food Chemistry 3: 323–329.

Didzbalis, J., Ritter, K.A., Trail, A.C., and Plog, F.J. 2004. Identification of fruity/fermented odorants in high-temperature-cured rosted peanuts. Journal of Agricultural and Food Chemistry 52: 4828-4833.

Ferretti, A. and Flanagan, V.P. 1971. The lactose-casein. maillard browning system: Volatile components. Journal of Agricultural and Food Chemistry 19: 245-249.

Fraundorfer, F. and Schieberle, P. 2008. Changes in key aroma compounds of criollo cocoa beans during roasting. Journal of Agricultural and Food Chemistry 56: 10244-10251.

Frauendorfer, F. and Schieberle, P. 2006. Identification of the key aroma compounds in cocoa powder based on molecular sensory correlations. Journal of Agricultural and Food Chemistry 54: 5521-5529.

Goodner, K.L., Jella, P. and Rouseff, R.L. 2000. Determination of vanillin in orange, grapefruit, tangerine, lemon and lime juices using GC-olfactometry and GC-MS/MS. Journal of Agricultural and Food Chemistry 48: 2882-2886.

Granvogl, M., Bugan, S. and Schieberle, P. 2006. Formation of amines and aldehydes from parent amino acids during thermal processing of cocoa and model systems: new insights into pathways of the strecker reaction. Journal of Agricultural and Food Chemistry 54: 1730-1739.

Hashim, I. and Chaveron, H. 1994. Extraction and determination of methylpyrazines in cocoa beans using coupled steam distillation-microdistillator. Food Research International 27: 537–544.

Hofmann, T. 1998. Studies on melanoidin-type colorants generated from the maillard reaction of protein-bound lysine and furan-2-carboxaldehyde-chemical characterization of a red coloured domaine. Zeitschrift für Lebensmittel-untersuchung und -Forchung A 251-258.

Hofmann, T. and Schieberle, P. 2000. Formation of aroma-active strecker-aldehydes by direct oxidative degradation of amadori compounds. Journal of Agricultural and Food Chemistry 48: 4301–4305.

Hofmann, T., Munch, P. and Schieberle, P. 2000. Quantitave model studies on the formation of aroma-active aldehydes and acids by strecker-type reactions. Journal of Agricultural and Food Chemistry 48: 3761–3766.

Hoskin, J.C. and Dimick, P.S. 1994. Chemistry of flavour development in chocolate. In Beckett, S.T. Industrial Chocolate Manufacture and Use. 2nd ed. Glasgow: Chapman & Hall.

Jinap S., Dimick, P.S. and Hollender, R. 1995. Flavour evaluation of chocolate formulated from cocoa beans from different countries. Food Control 6: 105-110.

Jinap, S., Wan Rosli, W.I., Russly, A.R. and Nurdin, L.M. 1998. Effect of roasting time and temperature on volatile components profile during nib roasting of cocoa beans (*Theobroma cacao*). Journal of the Science of Food and Agriculture 77: 441-448.

Koehler, P.E. and Odell, G.V. 1970. Factor affecting the formation of pyrazine compounds in sugar-amine reactions. Journal of Agricultural and Food Chemistry 18: 895–898.

Kratzer U., Frank, R., Kalbacher, H., Biehl, B., Wostemeyer, J. and Voigt, J. 2008. Subunit structure of the vicilin-like globular storage protein of cocoa seeds and the origin of cocoa and chocolate specific aroma precursors. Food Chemistry 113: 903-913.

Krings, U., Zelena, K., Wu, S., Berger, R.G. 2006. Thin-layer high-vacuum distillation to isolate volatile flavor compounds of cocoa powder. European Food Research and Technology 223: 675-681.

Luna F., Crouzillat, D., Cirou, L. and Bucheli, P. 2002. Chemical composition and flavor of ecuadorian cocoa liquor. Journal of Agricultural and Food Chemistry 50: 3527-3532.

Maga, J.A. 1992. Contribution of phenolic compounds to smoke flavor. In Ho, C.T., Lee, C.Y. and Huang, M.T. Phenolic compounds in food and their effects on health I: analysis, occurrence and chemistry. ACS Symposium Series 506: 170-179.

Ohashi, M., Omae, H., Hashida, M., Sowa, Y. and Imai, S. 2006. Determination of vanillin and related flavor compounds in cocoa drink by capillary electrophoresis. Journal of Chromatography 1138: 262-267.

Perego P., Fabiano, B., Caviccioli, M. and Del Borghi, M. 2004. Cocoa quality and processing: a study by solid phase microextraction and gas chromatography analysis of methyl pyrazines. Journal of Food and Bioproducts Processing 82: 291-297.

Pini, G.F., de Brito, E.S., Garcia, N.H.P., Valente, A.L.P. and Augusto, F. 2004. A Headspace solid phase microextraction (HS-SPME) method for the chromatographic determination in cocoa samples. Journal of the Brazilian Chemical Society 15: 267-271.

Puziah, H., Jinap, S., Sharifah, K.S.M. and Asbi, A. 1998. Effect of mass and turning time on free amino acid, peptide-n, sugar and pyrazine concentration during cocoa fermentation. Journal of the Science of Food and Agriculture 78: 543–550.

Rizzi, G.P. 1972. A mechanistic study of alkylpyrazine formation in model systems. Journal of Agricultural and Food Chemistry 20: 1081–1085.

Rizzi, G.P. 1988. Formation of pyrazines from acycloin precursors under mild condition. Journal of Agricultural and Food Chemistry 36: 349–352.

Rohan, T.A. 1963. Precursors of chocolate aroma. Journal of the Science of Food and Agriculture 14: 799-805

Rohan, T.A and Stewart, T. 1966. The precursor of chocolate aroma: changes in the sugars during the roasting of cocoa beans. Journal of Food Science 31: 206-209.

Shibamoto, T. and Bernhard, R.A. 1977. Investigation of pyrazine formation pathways in sugar-ammonia model systems. Journal of Agricultural and Food Chemistry 25: 609–614.

Shirey, R.E. and Sidisky, L.M. 1999. Analysis of Flavors and Off-flavors in Foods and Beverages using SPME. US: Supelco.

Vazques-Landaverde, P.A., Velazquez, G., Torres, J.A. and Qian, M.C. 2005. Quantitative determination of thermally derived off-flavor compounds in milk using solid-phase microextraction and gas chromatography. Journal of Dairy Science 88: 3764-3772.

Voigt, J., Biehl, B., Heinrichs, H., Kamaruddin S., Marsoner, G. and A. Hugi. 1994. *In-vitro* formation of cocoa-specific aroma precursors aroma-related peptides generated from cocoa-seed protein by co-operation of an aspartic endoprotease and a corboxypeptidase. Food Chemistry 49: 173-180.

Weenen, H., Koolhaas, W. E. and Apriyantono, A. 1996. Sulfur containing volatiles of durian fruits (*Durio zibethinus* Murr.). Journal of Agricultural and Food Chemistry 44: 3291-3293.

Weenen, H., Tjan, S.B., deValois, P.J., Bouter, N., Pos, A. and Vonk, H. 1994. Mechanism of Pyrazine Formation. Thermally Generated Flavour, p.143–157. American Chemical Society.

Ziegleder, G. and Biehl, B. 1988. Analysis of cocoa flavour components and flavour precursors. In Lickens, H.F. and Jackson, J.F., Analysis of Non Alcoholic Beverages, Methods of Plant Analysis, Vol. 8. Pp. 321–393. Springer Verlag, Heidelberg, Germany.