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Matrix effects on flavour volatiles release in dark chocolates varying in particle size distribution and fat content using GC–mass spectrometry and GC–olfactometry

Emmanuel Ohene Afoakwa [a,b,*], Alistair Paterson [a], Mark Fowler [b], Angela Ryan [b]

[a] Centre for Food Quality, SIPBS Royal College Building, University of Strathclyde, 204 George Street, Glasgow G1 1XW, Scotland, UK
[b] Nestlé Product Technology Centre York, P.O. Box 204, Haxby Road, York YO91 1XY, England, UK

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ABSTRACT

Influences of matrix particle size distribution (PSD) (18, 25, 35 and 50 μm) and fat content (25%, 30% and 35%) on flavour release of dark chocolate volatiles were quantified by static headspace gas chromatography using GC–MS. Sixty-eight (68) flavour compounds were identified, comprising alcohols, aldehydes, esters, ketones, furans, pyrans, pyrazines, pyridines, pyroles, phenols, pyrones and thiozoles. From GC–olfactometry, 2-methylpropanal, 2-methylbutanal and 3-methylbutanal had chocolate notes. With cocoa/roasted/nutty notes were trimethyl-, tetramethyl-, 2,3-dimethyl-, 2,5-dimethyl-, 3(or 2),5-dimethyl-2(or 3)-ethyl- and 3,5(or 6)-diethyl-2-methylpyrazine and furfuralpyrrole. Compounds with fruity/floral notes included 3,7-dimethyl-1,6-octadien-3-ol and 5-ethenyltetrahydro-*R*,*R*,5-trimethyl-*cis*-2-furanmethanol. Caramel-like, sweet and honey notes were conferred by 2-phenylethanol, phenylacetaldehyde, 2-phenylethylacetate, 2,3,5-trimethyl-6-ethylpyrazine, 2-carboxaldehyde-1*H*-pyrrole, furancarboxaldehyde, furfuryl alcohol and 2,5-dimethyl-4-hydroxy-3(2*H*)furanone. There were direct relationships of fat content with 3-methylbutanal and branched pyrazines but inverse ones with 2-phenylethanol, furfuryl alcohol, methylpyrazine, phenylacetaldehyde, 2, 3, 5-trimethyl-6-ethylpyrazine and 2-carboxaldehyde-1-*H*-pyrrole. Particle size influenced higher alcohol, aldehyde, ester, ketone and pyrazine concentrations at all fat contents. A multivariate product space suggested flavour effects of the interacting factors.

1. Introduction

Flavour is central to acceptability in chocolate and is influenced, not only by volatile aroma compounds, but also by non-volatiles and behaviour of the continuous fat phase, influencing release of volatiles into the mouth headspace and taste perception. Precursor composition depends on bean genotype and environmental effects, particularly on contents of storage proteins and polyphenols (Afoakwa, Paterson, Fowler, & Ryan, 2008a; Kim & Keeney, 1984; Schwan & Wheals, 2004). Cocoa beans are rich in antioxidants, including catechins, epicatechin and procyanidins, polyphenols similar to those found in wine and tea (Carnesecchia et al., 2002; Grassi, Lippi, Necozione, Desideri, & Ferri, 2005; Hatano et al., 2002; Hermann et al., 2006; Lamuela-Raventos, Romero-Perez, Andres-Lacueva, & Tornero, 2005). Chocolate manufacture involves complex physical and chemical processes, determining rheological characteristics, flavour development, melting properties and ultimately sensory perceptions of character (Afoakwa, Paterson, & Fowler, 2007; Afoakwa, Paterson, & Fowler, 2008b; Do, Hargreaves, Wolf, Hort, & Mitchell, 2007; Ziegler & Hogg, 1999; Ziegler, Mongia, & Hollender, 2001). There are several studies of precursors for flavour formation in cocoa and chocolate (Counet, Ouwerx, Rosoux, & Collin, 2004; Kyi et al., 2005; Misnawi, Jinap, Jamilah, & Nazamid, 2003).

An appropriate cocoa bean composition can be converted, through controlled post-harvest treatments and subsequent processing technologies, to a high quality chocolate flavour character (Clapperton, 1994). Fermentation is crucial, not only to the formation of key volatile fractions (alcohols, esters and fatty acids) but also provision of Maillard flavour precursors (amino acids and reducing sugars) (Buyukpamukcu et al., 2001; Kyi et al., 2005; Luna, Crouzillat, Cirou, & Bucheli, 2002). Drying reduces levels of acidity and astringency in cocoa nibs, decreasing volatile acids and total polyphenols. Maillard reactions during roasting convert these flavour precursors into two main classes of flavour-active component – pyrazines and aldehydes (Dimick & Hoskin, 1999; Gill, MacLeod, & Moreau, 1984; Granvogl, Bugan, & Schieberle, 2006; Oberparlaiter & Ziegleder, 1997; Ramli, Hassan, Said, Samsudin, & Idris, 2006; Stark, Bareuther, & Hofmann, 2005). Flavour development continues during conching, following the elimination of volatile acids and moisture, with associated viscosity changes,

* Corresponding author. Address: Centre for Food Quality, SIPBS Royal College Building, University of Strathclyde, 204 George Street, Glasgow G1 1XW, Scotland, UK. Tel.: +44 (0) 7984288727.
E-mail address: e.afoakwa@strath.ac.uk (E.O. Afoakwa).

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due to emulsification and tannin oxidation (Mermet, Cros, & Georges, 1992; Plumas, Hashim, & Chaveron, 1996; Reineccius, 2006). Afoakwa et al. (2008a) reviewed relationships between initial composition and post-harvest treatments of cocoa beans and subsequent processing (roasting and conching) and technological effects on final flavour character in chocolate.

Particle size distribution influences dark chocolate structure – specifically inter-particle interactions and network microstructure, rheology and texture. Specific surface area and mean particle size influence yield stress, plastic viscosity, product spread and hardness (Afoakwa et al., 2008b; Afoakwa, Paterson, Fowler, & Vieira, 2008c; Beckett, 2008; Chevalley, 1999). Genovese, Lozano, and Rao (2007) suggested that non-hydrodynamic parameters, such as particle shape, particle size and size distribution, particle deformability and liquid polarity, influence food structure and flow behaviours. Such factors dictate the space dimension of a suspension, whether strongly or weakly flocculated, with influence on yield stress and plastic viscosity. Although key flavour compounds of milk and dark chocolates have been reported (Cerny & Fay, 1995; Counet, Callemien, Ouwerx, & Collin, 2002; Reineccius, 2006; Schieberle & Pfnuer, 1999; Schnermann & Schieberle, 1997; Taylor, 2002; Taylor & Roberts, 2004), their abundancy, release and contribution to product character and matrix effects remain unclear.

Modern healthier foods (less fat and low sugar products) require modifications in ingredients and recipe formulation with impacts on flavour release and product rheology, structure and texture**.** Knowledge of how variations in PSD and continuous phase fat content would influence flavour is useful for product development and manufacture. The objectives of this study were to characterise and quantify volatile flavour constituents in dark chocolates, and to evaluate matrix effects from varying PSD and fat content on release of flavour volatiles using headspace HRGC, identifying components with GC–MS and flavour notes by GC–olfactometry.

2. Materials and methods

2.1. Materials

Cocoa liquor of central west African origin was obtained from Cargill Cocoa Processing Company (York, UK), sucrose (pure cane extra fine) from British Sugar Company (Peterborough, UK) and pure prime pressed cocoa butter and soy lecithin from ADM Cocoa Limited (Koog aan de Zaan, Netherlands) and Unitechem Company Ltd. (Tianjin, China), respectively.

Recipe (Table 1) and sample formulations have been described previously (Afoakwa et al., 2008b). Chocolates were formulated with total fat of 25–35% (w/w) from cocoa liquor and cocoa butter with >34% total cocoa: composition as specified for dark chocolate (Codex Revised Standard, 2003; European Commission Directive, 2000). Sucrose and cocoa liquor (5 kg per formulation) were mixed in a Crypto Peerless Mixer (Model K175, Crypto Peerless Ltd., Birmingham, UK) at low speed for 2 min and then at high for 3 min then, using a 3-roll refiner (Model SDX 600, Buhler Ltd., CH-9240 Uzwil, Switzerland) to a specified particle size (D_{90}:18 ± 1 μm, 25 ± 1 μm, 35 ± 1 μm and 50 ± 1 μm), conducting particle size analysis, during refining, to ensure D_{90} values. Refined chocolate flakes were placed in plastic containers and conditioned at 50–55 °C for 24 h to ensure melting of fat prior to conching in a Lipp Conche (Model IMC-E10, Boveristr 40–42, D-68309, Mannhein, Germany) at low speed for 3.5 h at 60 °C. Lecithin and cocoa butter were added and the mixtures conched at high speed for 30 min to effect adequate mixing and liquefaction. Samples were stored in sealed plastic containers at ambient temperature (20–22 °C) and moisture and fat contents determined using Karl Fischer and Soxhlet methods (ICA, 1988; ICA, 1990), respectively.

Table 1
Recipes used for the formulation of the dark chocolate

Ingredient	25% Fat (% (w/w))	30% Fat (% (w/w))	35% Fat (% (w/w))
Sucrose (%)	59.0	49.9	40.8
Cocoa liquor (%)	35.5	44.6	53.7
Cocoa butter (%)	5.0	5.0	5.0
Lecithin (%)	0.5	0.5	0.5

2.2. Tempering procedure

Samples were melted at 50 °C for 4 h and tempered using a continuous three-stage tempering unit (Model AMK 10, Aasted Mikroverk A/S, Farum, Denmark), pumping chocolate through multistage units with a worm screw, driving product through heat exchangers. Sensors in equipment measured temperature of both chocolate and coolant fluid at each stage. Based on our previous work on modelling temperature controls to study tempering behaviour (Afoakwa, Paterson, Fowler, & Vieira, 2008d), the temperatures of each of the coolant fluids were thus set and controlled independently to obtain a final chocolate at ~27 °C to promote crystal growth of the desired triacylglyceride fractions. Pre-crystallization was measured with a computerised tempermeter (Exotherm 7400, Systech Analytics, SA, Switzerland), using a built-in algorithm to ensure an optimal temper regime of slope 0 ± 0.3 (Afoakwa et al., 2008d; Nelson, 1999). Tempered chocolate was formed using plastic moulds, 80 by 20 by 8 mm, allowed to cool at 10 ± 2 °C for 2 h before de-moulding onto plastic trays and conditioned at 20 ± 2 °C for 14 days before analysis.

2.3. Determination of particle size distribution

A MasterSizer® Laser Diffraction Particle Size Analyzer equipped with MS 15 Sample Presentation Unit (refractive index 1.590) (Malvern Instrument Ltd., Malvern, England) was used. About 0.2 g of refined dark chocolate was dispersed in vegetable oil (refractive index 1.450) at ambient (20 ± 2 °C) until an obscuration of 0.2 was obtained. Samples were placed under ultrasonic dispersion for 2 min to ensure that particles were independently dispersed and suspensions thereafter maintained by stirring. Size distribution was quantified as the relative volume of particles in size bands presented as size distribution curves (Malvern MasterSizer® Micro Software v 2.19). PSD parameters obtained included specific surface area, largest particle size (D_{90}), mean particle volume (D_{50}), smallest particle size (D_{10}) and Sauter mean diameter (D[3,2]).

2.4. Quantitation of flavour volatiles by gas chromatography

Static headspace isolation of volatile compounds was performed using solid phase micro extraction (SPME) for 30 min at 55 °C onto a polydimethylsiloxane-divinylbenzene, 65 μm fibre (Supelco, Bellafonte, PA, USA). Chocolate (~4 g) was previously heated to 55 °C and intermittently stirred for 60 min for headspace equilibration. Each experiment had a system control sample, made by stirring an empty vial under the same conditions. Volatile compounds were desorbed (5 min) into the splitless injector (220 °C) of an Agilent Technologies 6890N-5793 Network GC–MS system (Agilent Technologies, Santa Clara, CA, USA) and separated on a J&W 60 m DB-Wax capillary column (0.22 mm i.d., 0.25 μm film thickness). The temperature programme was: 5 min at 40 °C; 3 °C min^{-1} to 230 °C; finally 15 min at 230 °C. Compounds were fragmented using electron-impact ionisation (70 eV), with a source temperature of 200 °C, a scan range of 30–300 amu and a scan rate

of 5 s^{-1}. Components were identified based on comparison of mass spectra with those of spectral libraries NIST 05 and Wiley 7N Registry of GC Mass Spectral Data, (John Wiley, New York, USA).

2.5. Gas chromatography-olfactometry analytical conditions

The GC–O analyses were conducted using an Agilent Technologies instrument (6890N Network Systems, CA, USA) with analyses as before, diverting the effluent to a humidified sniffing port. Two chromatographic runs were assessed by two trained assessors (alternating for 20 min periods). Only matching descriptors for an aroma attribute were retained.

2.6. Experimental design and statistical analysis

Two experimental variables, comprising PSD and fat contents, were used with other variables, including refiner temperature and pressure, conching time and temperature held constant. A 4×3 factorial experimental design was used with PSD (D_{90}): 18, 25, 35 and 50 μm; fat was 25%, 30% and 35% (w/w). A Statgraphics Plus 4.1 (Graphics Software System, STCC, Inc., Rockville, USA) examined quantitative data, using two-way analysis of variance (ANOVA) and multiple range tests to determine effects of factors and interactions. Multivariate techniques, comprising principal component analysis and multiple regression analysis, were used to evaluate relationships between selected flavour volatiles obtained by quantification of GC–FID data and influential factors. Tukey multiple comparisons at 95% significance level were conducted to determine differences between factor levels. All process treatments and analyses were conducted in duplicate and the mean values reported.

3. Results and discussion

3.1. Particle size distribution of dark chocolates

Particle size distributions (Fig. 1), previously reported (Afoakwa et al., 2008b), show volume histograms consisting of narrow (18 μm PS) and wide (25 μm PS) bimodal and narrow (35 μm PS) and wide (50 μm PS) multimodal size distributions. This PSD range 18–50 μm, using D_{90} values (>90% finer), covers optimum minimum and maximum sizes with direct effects on texture and sensory character in manufacture (Afoakwa et al., 2007; Beckett, 2008; Ziegler & Hogg, 1999). Data from PSD showed variations in specific surface area, mean particle volume D(v,50), Sauter mean (D[3,2]) and mean particle diameter (D[4,3]) with increasing D_{90} particle sizes. Beckett (1999) concluded that largest particle size and solids specific surface area were two key parameters in manufacture. The former determines chocolate coarseness and textural character, the latter desirable flow properties. Specific surface area was inversely correlated with the different component of PSD (Afoakwa et al., 2008b; Beckett, 1999; Sokmen & Gunes, 2006; Ziegler & Hogg, 1999). Fat contents were 25%, 30% and 35 ± 1% (each) and moisture was in the range 0.90–0.98%.

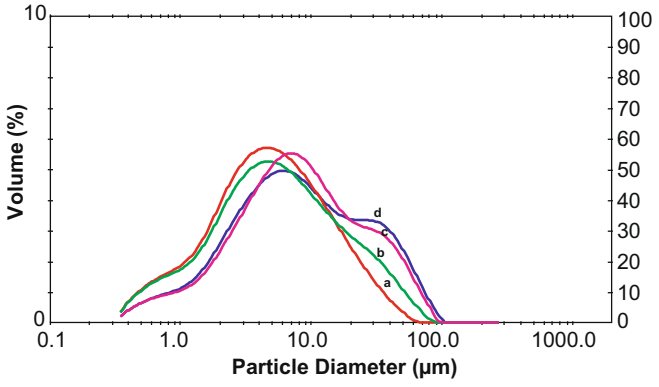

Fig. 1. Particle size distribution in dark chocolate with D_{90} of (a) 18 μm (b) 25 μm (c) 35 μm and (d) 50 μm.

3.2. Characterisation of flavour compounds in dark chocolates

Criteria for selection of the key volatiles were presence in headspaces at $>10^6$ abundant units) quantified by GC–FID and also detection and intensities by the GC–olfactometric techniques. In all, 68 flavour compounds (Table 2), comprising nitrogen and oxygen heterocycles, aldehydes and ketones, esters, alcohols, hydrocarbons, nitriles and sulphides, were identified by GC–MS in dark chocolates. A typical chromatogram is shown in Fig. 2.

Compounds quantified included: 1-pentanol (1), 3-(methylthiol)-propionaldehyde (12), methylbenzene (38), methylpyrazine (41), ethenylpyrazine (47), pyridine (55), 2-methylpyridine (56), 1-(2-furanylmethyl)-1*H*-pyrrole (62), 1*H*-indole (63) and dimethyl disulphide (67) (Table 1). Two others, benzyl alcohol (5) and dihydro-2-methyl-3(2*H*)-furanone (30) were only recently reported in dark chocolates (Counet et al., 2002). Specific nitrogen heterocycles from Maillard reactions included: 3(or 2),5-dimethyl-2(or 3)-ethylpyrazine (50), 3,5-(or 6)-diethyl-2-methylpyrazine (53), 2,3-dimethyl-1*H*-pyrrole (59), 3-ethyl-2,5-dimethyl-1*H*-pyrrole (61) and 10(2-furanylmethyl)-1*H*-pyrrole (furfurylpyrrole) (62) (Table 1). All had cocoa, praline, chocolate and roasted notes identified as important. The ethyl group in two pyrazine compounds suggests key roles for alanine and/or its Strecker aldehyde, acetaldehyde, in dark chocolate flavour (Cerny & Fay, 1995).

Flavour-active compounds identified as having strong chocolate characters included 2-methylpropanal (8), 2-methylbutanal (9) and 3-methylbutanal (10). Compounds derived from Maillard reactions were 2,3-dimethylpyrazine (45), 2,5-dimethylpyrazine (42), 2,6-dimethylpyrazine (43), trimethylpyrazine (47), tetramethylpyrazine (51), 3(or 2),5-dimethyl-2(or 3)-ethylpyrazine (50), 3,5(or 6)-diethyl-2-methylpyrazine (53) and furfurylpyrrole (60), exhibiting cocoa/roasted/nutty/cooked notes. Counet et al. (2002) identified such flavour volatiles in dark chocolates after conching, suggesting that these are formed during cocoa processing.

Volatiles, such as 2-phenylethanol (7), phenylacetaldehyde (15), 2-phenylethylacetate (22), 2,3,5-trimethyl-6-ethylpyrazine (54) and 2-carboxaldehyde-1*H*-pyrrole (60), were characterised by sweet, candy and honey flavours. Furancarboxaldehyde (furfural) (31), furfuryl alcohol (furfurol) (32) and 2,5-dimethyl-4-hydroxy-3(2*H*)furanone (furaneol) (35) were also characterised by caramel-like, sweet and honey notes, likely derivatives of Strecker degradation and caramelization reactions developed during cocoa processing and transformed during chocolate flavour synthesis in conching (Afoakwa et al., 2008a; Cerny & Fay, 1995).

Eight heterocyclic compounds, including 2,3-dimethylpyrazine (45), 2,5-dimethylpyrazine (42), 2,6-dimethylpyrazine (43), trimethylpyrazine (47), tetramethylpyrazine (51), 3(or 2),5-dimethyl-2-(3)-ethylpyrazine (50), 3,5(or 6)-diethyl-2-methylpyrazine (53) and 2,3,5-trimethyl-6-ethylpyrazine (54) were identified (Table 1). Characteristic key chocolate flavours, such as fruity and floral, likely derived from cocoa, were found in 3,7-dimethyl-1,6-octadien-3-ol (linalool) (6) and 5-ethenyltetrahydro-*R*,*R*,5-trimethyl-*cis*-2-furanmethanol (linalool oxide) (36). Ethyl cinnamate (23) and acetic acid (24), not previously reported important in dark chocolates, were characterised by fruity-spicy and astringent-vinegar notes, respectively. Tetramethylpyrazine (51), the most abundant flavour compound in dark chocolate, exhibited milk

Table 2
Key flavour volatiles identified in dark chocolate

No.	Flavour compound	Odour description[a]
Alcohols		
1	1-Pentanol	
2	2,4-Hexadien-1-ol	
3	3-Methyl pentanol	
4	2-Heptanol	
5	Benzyl alcohol	
6	3,7-Dimethyl-1,6-octadien-3-ol (linalool)	Flowery, floral, fruity (low)
7	2-Phenylethanol	Caramel-like, sweet, honey
Aldehydes		
8	2-Methylpropanal (isobutanal)	Chocolate
9	2-Methylbutanal	Chocolate
10	3-Methylbutanal	Chocolate
11	2-Methyl-2-butenal	
12	3-(Methylthio)propionaldehyde (methional)	Potato
13	Heptanal	
14	Benzaldehyde	Nutty
15	Phenylacetaldehyde	Flowery, sweet, honey
16	Nonanal	
17	2-Phenyl-2-butenal	Cocoa, roasted
18	2-Phenyl-5-methyl-2-hexenal	Roasted
Esters		
19	Ethylbenzoylformate	
20	Ethylbenzoate	
21	Ethyloctanoate	
22	2-Phenylethylacetate	Honey, sweet
23	Ethyl cinnamate	Fruity, floral (low)
24	Acetate (acetic acid)	Astringent, vinegar
Ketones		
25	2,3-Butanedione (diacetyl)	Buttery (low)
26	2-Heptanone	
27	4-Methylcyclohexanone	
28	3-Hydroxy-2-butanone	
29	3,4,4-Trimethyl-2-cyclopenten-1-one	
Furans		
30	Dihydro-2-methyl-3(2*H*)-furanone	
31	Furancarboxaldehyde (furfural)	Caramel-like, sweet
32	Furfuryl alcohol (furfurol)	Caramel-like, sweet
33	1-(2-Furanyl)ethanone (acetylfuran)	
34	5-Methyl-2-furancarboxaldehyde	
35	2,5-Dimethyl-4-hydroxy-3(2*H*)furanone (Furaneol)	Caramel-like, sweet
36	5-Ethenyltetrahydro-*R*,*R*,5-trimethyl-*cis*-2-furanmethanol (linalool oxide)	Fruity, floral/ flowery (low)
37	3-Phenylfuran	Cocoa, green, nutty
Hydrocarbons		
38	Methylbenzene (toluene)	
Nitrogen compounds		
39	Benzonitrile	
Pyrans		
40	3,4-Dihydro-8-hydroxy-3-methyl-1*H*-2-benzopyran-1-one	
Pyrazines		
41	Methylpyrazine	Nutty, green
42	2,5-Dimethylpyrazine	Roasted, cooked
43	2,6-Dimethylpyrazine	Roasted, cooked
44	Ethylpyrazine	Nutty, roasted
45	2,3-Dimethylpyrazine	Cooked, nutty
46	2-Ethyl-5(or 6)-methylpyrazine	Cocoa, roasted, green
47	Trimethylpyrazine	Cocoa, roasted, cooked
48	2-Ethyl-3-methylpyrazine	Hazelnut, roasted
49	2-Ethenyl-6-methylpyrazine	Roasted, smoky
50	3(or 2),5-Dimethyl-2(or 3)-ethylpyrazine	
51	Tetramethylpyrazine	Milk-coffee, roasted,
52	2,3-Dimethyl-5-ethylpyrazine	Cocoa, chocolate
53	3,5(or 6)-Dimethyl-2-ethylpyrazine	Cocoa, praline, chocolate
54	2,3,5-Trimethyl-6-ethylpyrazine	Candy, sweet,
Pyridines		
55	Pyridine	
56	2-Methylpyridine	Caramel-like, sweet
57	2-Pyridinamine	
58	1-(2-Pyridinyl)-1-propanone	
Pyrroles		
59	2,3-Dimethyl-1*H*-pyrrole	Cocoa, praline, chocolate
60	2-Carboxaldehyde-1*H*-pyrrole	Honey, candy (low)
61	3-Ethyl-2,5-dimethyl-1*H*-pyrrole	Cocoa, coffee
62	1-(2-Furanylmethyl)-1*H*-pyrrole (furfurylpyrrole)	Cocoa, roasted (low)
63	1*H*-Indole	Chocolate, green (low)
Phenols		
64	Phenol	
65	4-Methylphenol	
Pyrones		
66	3-Hydroxy-2-methyl-4-pyrone (maltol)	
Sulphur compounds		
67	Dimethyl disulphide	Meaty (low)
Thiozoles		
68	4,5-Dihydro-2-methylthiazole	

[a] Odour quality and intensity at GC–O outlet.

coffee-roasted-cooked notes, and trimethylpyrazine (47) had cocoa-roasted-cooked characters (Table 2).

3.3. Effects of particle size distribution (PSD) on flavour volatile release

Effects of PSD and fat content on the release of selected abundant (>10^6 U) flavour volatiles, characterised by distinct aroma, were evaluated using SPME-HRGC with FID detection. Data from ANOVA indicated that, with the exception of 3,7-dimethyl-1,6-octadien-3-ol (linalool) and 2-carboxaldehyde-1-*H*-pyrrole ($P = 0.965$ and 0.854, respectively), increasing particle size (PS) caused significant reduction in the release of all selected compounds measured in the sample headspace, with $P < 0.001$ for 3-methylbutanal, 2-phenylethanol, furfuryl alcohol (furfurol), acetic acid, methylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, trimethylpyrazine, tetramethylpyrazine and 2,3,5-trimethyl-6-ethylpyrazine, and $P < 0.05$ for 2-phenylethylacetate, 2-methylbutanal and 5-ethenyltetrahydro-*R*,*R*,5-trimethyl-*cis*-2-furanmethanol (linalool oxide), with significant interactions noted with fat content (Table 5).

The decreasing flavour volatiles release with increasing PS could be related to increased matrix retention through structural, rheological and textural differences (Afoakwa et al., 2008b; Afoakwa et al., 2008c; Afoakwa, Paterson, Fowler, & Vieira, 2008e). Beckett (2008) noted that movement of volatiles was related to an initial concentration gradient between phases, and refining (degree of particle sizes) in production may influence release during manufacture. Beckett (2008) also correlated compositional and sensory analyses for differences in flavour profile, with preference for lower PS (thick and pasty) chocolates, rather than the higher PS (thin and runny). These results suggest that dark chocolates with finer PS (18 and 25 μm) would release more cocoa-chocolate-praline and caramel-like-sweet-honey notes than would those with larger PS (35 and 50 μm), predicting perceived differences in flavour with varying PS. The increase in surface area with deceasing PS (D_{90}) would be predicted to facilitate volatiles release. The lack of significant effects of 3,7-dimethyl-1,6-octadien-3-ol (linalool) and 2-carboxaldehyde-1-*H*-pyrrole ($P = 0.965$ and 0.854, respectively) would not be expected to influence flavour character from headspace

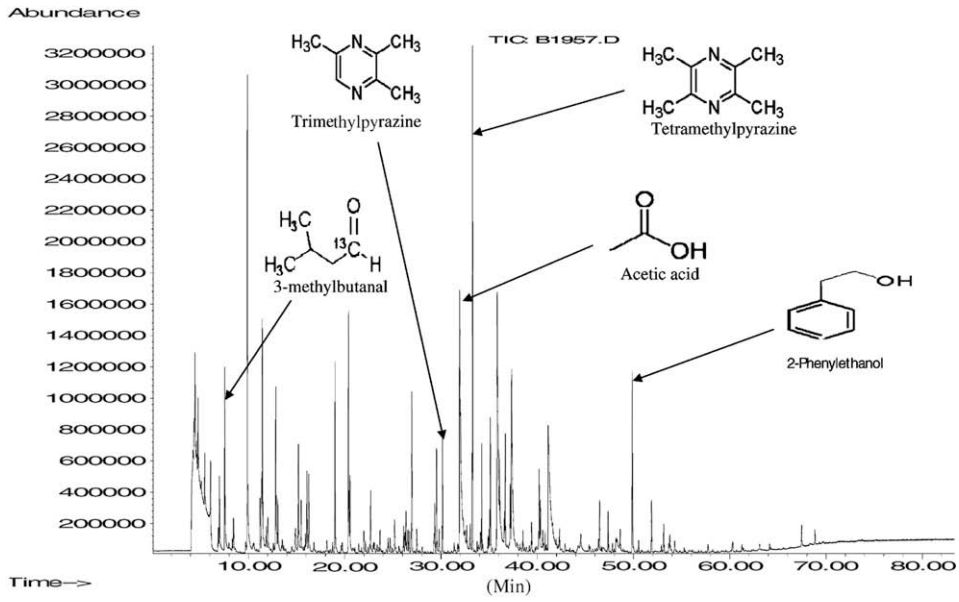

Fig. 2. Typical GC–MS chromatogram used to identify flavour volatiles.

contents and odour intensities (Tables 2–4). Voltz and Beckett (1997) and Ziegler et al. (2001) reported that finer (smaller PS) chocolates tended to be sweeter in taste than coarser (larger PS) ones, attributed to relative crystal sizes and melting behaviour. Particle size influences perceptions of creaminess and flavour release in soft model systems (Engelen & Van der Bilt, 2008; Engelen et al., 2005; Kilcast & Clegg, 2002). Concentration of flavour volatiles in headspaces has been reported as a function of diffusion in the solid phase (Carr et al., 1996; Engelen et al., 2003; Guinard & Marty, 1995; Kersiene, Adams, Dubra, De Kimpe, & Leskauskaite, 2008).

3.4. Effects of fat content on flavour volatile release

Fat content influenced the headspace concentration of volatiles, independently of PSD (Table 3). ANOVA showed that 3,7-dimethyl-1,6-octadien-3-ol (linalool), 2-methylbutanal, 2-phenylethylacetate and 2-carboxaldehyde-1-*H*-pyrrole lacked significant effects ($P > 0.05$). Fat content significantly influenced headspace concentrations of all other quantified volatiles ($P < 0.001$) at all PSD with significant interactions amongst factors studied (Table 5). Volatiles characterised by cocoa, chocolate, praline, fruity and roasted notes included: trimethypyrazine, 3-methylbutanal, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, tetramethylpyrazine, linalool oxide and 2,3,5-triethyl-5-methylpyrazine. All showed a direct relationship with fat content at all PS (Tables 3 and 4). Volatiles release data suggested that chocolates of higher fat content would exhibit greater release of components with cocoa-chocolate-praline notes than would those with lower fat. This decreased matrix retention, could be related to differences in (micro)structure as inter-particle flocculation and aggregates are reduced with higher fat contents (Afoakwa et al., 2008c), releasing more Strecker degradation compounds with cocoa-chocolate notes. Concentrations of less volatile heterocyclic compounds were increased, notably polysubstituted ethyl- and isobutylpyrazines, tri- and tetramethylpyrazine and furans (linalool oxide), suggesting structural and rheological effects as major determinants of chocolate character (Afoakwa et al., 2008b; Afoakwa et al., 2008c; Afoakwa et al., 2008e; Do et al., 2007).

By contrast, volatiles with caramel-like, sweet, honey and candy notes included: 2-phenylethanol, furfuryl alcohol (furfurol), methylpyrazine, phenylacetaldehyde, 2, 3, 5-trimethyl-6-ethylpyrazine and 2-carboxaldehyde-1-*H*-pyrrole. All showed an inverse relationship with fat content at all PS (Tables 3 and 4), primarily due to lipophilic matrix–flavour interactions. The major influence of fat content was observed with the most lipophilic compounds (Tables 3 and 4), particularly with fat contents above 25%. These results are consistent with earlier reports (Doyen, Carey, Linforth, Marin, & Taylor, 2001; Jo & Ahn, 1999) and are also consistent with

Table 3
Flavour volatiles in dark chocolates varying in PSD and fat content[a]

Volatile compound (abundance × 10^6)	25%				30%				35%			
	18 μm	25 μm	35 μm	50 μm	18 μm	25 μm	35 μm	50 μm	18 μm	25 μm	35 μm	50 μm
3,7-Dimethyl-1,6-octadien-3-ol (linalool)	1.93	1.74	1.24	1.03	2.07	1.72	1.37	9.99	2.66	1.91	1.85	1.26
2-Phenylethanol	46.8	32.4	26.2	25.2	27.8	24.5	22.3	22.0	26.4	24.0	22.0	17.1
2-Methylbutanal	2.64	2.24	2.02	2.01	2.92	2.24	2.14	1.99	3.04	2.23	2.25	1.99
3-Methylbutanal	8.12	8.17	7.24	7.11	8.82	8.26	7.46	7.16	9.42	9.19	8.86	7.14
Phenylacetaldehyde	20.9	12.8	10.2	10.0	9.78	9.44	7.99	7.86	6.70	6.35	6.37	3.99
2-Phenylethylacetate	11.9	8.75	7.09	6.82	6.74	6.08	5.86	5.56	6.14	6.01	5.80	5.32
Furfuryl alcohol (furfurol)	4.29	3.21	2.44	2.24	4.08	2.84	2.17	2.05	2.89	2.67	1.99	1.82
5-Ethenyltetrahydro-*R*,*R*,5-trimethyl-*cis*-2-furanmethanol (linalool oxide)	5.58	5.22	4.63	4.60	5.45	5.05	4.73	4.52	5.87	5.32	5.16	5.01
2-Carboxaldehyde-1-*H*-pyrrole	2.74	1.53	1.02	1.02	1.21	0.89	0.75	0.68	1.01	0.68	0.54	0.40
Acetic acid	130	78.6	58.8	56.1	112	65.3	48.8	42.0	30.8	28.7	28.1	27.2

[a] Quantification was by GC–MS, expressed as mean peak area.

Table 4
Abundant pyrazines in dark chocolates varying in PSD and fat content[a]

Volatile compound (abundance × 10^6)	25%				30%				35%			
	18 μm	25 μm	35 μm	50 μm	18 μm	25 μm	35 μm	50 μm	18 μm	25 μm	35 μm	50 μm
Methylpyrazine	5.48	5.08	3.78	2.92	4.25	3.30	2.94	2.60	3.51	3.09	2.78	2.46
2,3-Dimethylpyrazine	5.77	6.20	6.16	5.57	6.56	5.88	5.49	5.39	6.89	7.10	7.02	5.46
2,5-Dimethylpyrazine	9.27	9.04	8.43	8.43	10.09	8.95	7.43	6.60	10.24	9.65	9.43	8.88
Trimethylpyrazine	28.6	28.9	28.6	28.5	29.5	29.1	28.8	28.8	33.1	32.8	32.1	30.4
Tetramethylpyrazine	110	105	96.7	96.4	98.9	96.9	96.8	96.1	113	107	107	96.9
2,3-Diethyl-5-methylpyrazine	4.66	4.59	4.29	3.79	4.80	4.54	4.51	3.89	4.89	4.64	4.59	3.89
2,3,5-Trimethyl-6-ethylpyrazine	3.86	3.05	2.68	1.93	2.25	2.11	1.82	1.78	1.89	1.90	1.78	1.56

[a] Quantification was by GC–MS, expressed as mean peak area.

Table 5
ANOVA summary, showing *F*-values and regression coefficients of flavour compounds identified in dark chocolates with varying PSD and fat content

Volatile compound	PSD (D_{90}): A	Fat: B	Interactions: A × B	R^{2a}
3,7-Dimethyl-1,6-octadien-3-ol (linalool)	1.81	2.64	1.03	7.11
2-Phenylethanol	1305.56***	1906.11***	265.68***	75.21***
2-Methylbutanal	5.83*	0.48	4.76*	21.2
3-Methylbutanal	32.36***	20.79***	27.92***	84.3***
Phenylacetaldehyde	8.62***	29.46***	10.28**	81.8***
2-Phenylethylacetate	3.23*	1.67	3.28*	9.47
Furfuryl alcohol (furfurol)	70.57***	19.16***	27.82***	87.7***
5-Ethenyltetrahydro-*R*,*R*,5-trimethyl-*cis*-2-furanmethanol (linalool oxide)	4.89*	5.34*	5.71*	7.86
2-Carboxaldehyde-1-*H*-pyrrole	1.15	1.77	0.95	17.9
Acetic acid	13.67***	26.31***	12.62***	75.0***
Methylpyrazine	30.15***	34.81***	28.63***	86.4***
2,3-Dimethylpyrazine	8.93***	11.26***	10.56**	51.6*
2,5-Dimethylpyrazine	15.62***	12.32***	18.63***	61.4*
Trimethylpyrazine	13.01***	795.09***	18.52***	81.9***
Tetramethylpyrazine	13.68***	17.20***	12.29***	51.0*
2,3-Diethyl-5-methylpyrazine	312.88***	19.24***	48.78**	86.9***
2,3,5-Trimethyl-6-ethylpyrazine	9.67***	29.59***	13.36**	76.6***

Significant *F*-ratios at $^{*}P \leqslant 0.05$, $^{**}P \leqslant 0.01$, $^{***}P \leqslant 0.001$.
[a] *R*-squares from multiple regression.

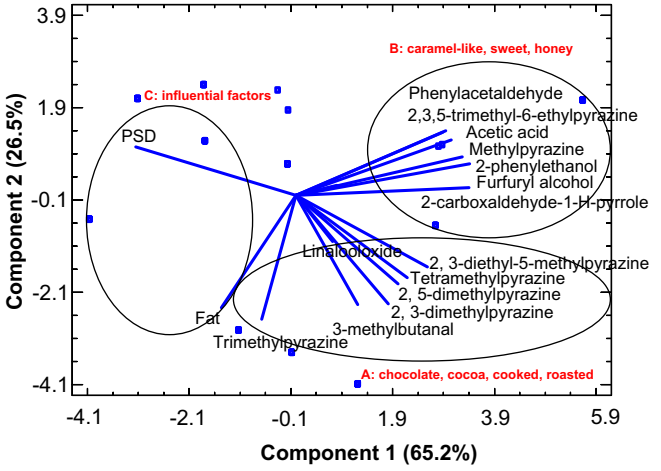

Fig. 3. PCA biplots of dark chocolate flavour volatiles as influenced by PSD and fat content.

the suggestion that the more lipophilic the volatile, the less lipid is needed to reduce its headspace concentration (Roberts, Pollien, & Watzke, 2003). More lipid generally reduces volatility of lipophilic components such as long-chain aldehydes and esters (Kersiene et al., 2008). Lack of significant effect on overall flavour character from 3,7-dimethyl-1,6-octadien-3-ol (linalool), 2-methylbutanal, 2-phenylethylacetate and 2-carboxaldehyde-1-*H*-pyrrole would be predicted (Tables 2 and 3). Studies from emulsions showed that release of lipophilic compounds is decreased with limited amounts of lipid (Carey et al., 2002; Roberts et al., 2003). Factors, such as lipophilicity or hydrophobicity of compounds could modulate the effect of fat content on release, specifically in confectionery (Barylko-Pikielna & Szczesniak, 1994; Hyvönen et al., 2003), as well as mouth-feel (De Wijk, Terpstra, Janssen, & Prinz, 2006) and thermal perceptions (Engelen et al., 2002).

A further key finding was related to headspace acetic acid contents with high value for products with 25% and 30% fat at lower (18 and 25 μm) PS, inversely related to fat content. Greater reduction in acetic acid (~4-fold) was noted with 35% fat at all PS, than with 25% and 30% fat (Table 3). Similarly, increasing PS from 25–50 μm reduced contents by ~2 to 3-fold with 25% and 30% fat, whereas only minimal (5%) reductions were noted with 35% fat. From ANOVA there were highly significant effects of PSD and fat content ($P = 0.001$) on acetic acid release, with significant interactions (Table 5). Acetic acid in 25% and 30% fat chocolate headspaces may be related to higher plastic viscosity and yield values (Afoakwa et al., 2008b), and greater flocculation and aggregation of inter-particle network structure (Afoakwa et al., 2008c), influencing release and volatilisation in conching. High acetic acid levels in low (25%) fat chocolates may reduce acceptability scores: effective elimination of volatile free fatty acids (e.g. acetic acid) and moisture during conching is crucial for development of final flavour character and texture in chocolates (Beckett, 2008; Kealey et al., 2001; Mermet et al., 1992; Plumas et al., 1996; Pontillon, 1995). As demand for healthier (low fat) chocolate has increased in recent years, good process optimisation to effect adequate release of acetic acid during manufacture of low (~25%) fat dark chocolates would be necessary to obtain well-balanced flavour characters.

3.5. Relating flavour volatiles release to PSD and fat content: product spaces

Multivariate principal component analysis (PCA) generated a product space exploring influence of PSD and fat content on headspace volatiles data of dark chocolates. The PCA space (Fig. 3) explained >91% variance in two factors, and showed two flavour volatiles clusters with loadings for PSD and fat content as influential factors. Fat content had polar influences on PC1 (65.2% variance) score while particle size had marginal influence on PC2 (25.6% variance) score. The PCA loading showed distinct relationships. Two components were extracted with eigenvalues $\geqslant 1$, and volatiles segregated into two groups, labelled A and B. Group A volatiles were: trimethypyrazine, 3-methylbutanal, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, tetramethylpyrazine, linalool oxide and 2,3,5-triethyl-5-methylpyrazine, all characterised by cocoa, chocolate, praline and roasted notes, possibly originating in cocoa. Group B consisted of: 2-phenylethanol, furfuryl alcohol (furfurol),

acetic acid, methylpyrazine, phenylacetaldehyde, 2, 3, 5-trimethyl-6-ethylpyrazine and 2-carboxaldehyde-1-*H*-pyrrole, characterised by caramel-like, sweet, honey and candy notes, developed during chocolate manufacture. PC1, and to some extent PC2, differentiated chocolate samples, with groupings clearly characterised by specific flavour notes from GC olfactometry (Table 2).

The PCA loadings on PC1 showed that flavour volatiles within Group A, characterised by cocoa, chocolate, praline and roasted notes, were highly related in abundancy to fat content. Our working hypothesis is that Group A flavour volatiles are primary origins of cocoa and chocolate notes in dark chocolates, with trimethylpyrazine and 3-methylbutanal central to these characters (Fig. 3). By contrast, the PCA loading showed a polar relationship of fat content with Group B, observed to have caramel-like, sweet, honey and candy notes (Table 2), suggesting that increasing fat content reduces the influence of such notes on flavour character in dark chocolates.

Regression models were developed to predict contribution of specific flavour volatiles to overall flavour character. One Strecker aldehyde and two nitrogen heterocycles derived from Maillard reactions had high regression coefficients, respectively, 3-methylbutanal, $R^2 = 0.843$, $P = 0.001$, trimethylpyrazine, $R^2 = 0.819$, $P = 0.001$ and 2,3-diethyl-5-methylpyrazines, $R^2 = 0.869$, $P = 0.001$ (Table 4). These emerged as probably the most interesting compounds in dark chocolates, providing cocoa, praline-chocolate and nutty flavours. Three other heterocycles, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine and tetramethylpyrazine, showed less but still significant effects with $R^2 = 0.516$, 0.614 and 0.510 ($P < 0.05$), respectively, contributing cocoa-chocolate notes. Others, 2-methylbutanal, 5-ethenyltetrahydro-*R*,*R*,5-trimethyl-*cis*-2-furan-methanol (linalool oxide) and 3,7-dimethyl-1,6-octadien-3-ol (linalool), had no significant influence ($P > 0.05$) (Table 4), possibly due to low contents in the central west African cocoa (Tables 3 and 4).

On the other hand, the regression models developed showed high and significant regression coefficients ($R^2 = 0.75 - 0.88$, $P = 0.001$) for Group B compounds. These predict likely contributions of 2-phenylethanol, furfuryl alcohol (furfurol), methylpyrazine, phenylacetaldehyde and 2, 3, 5-trimethyl-6-ethylpyrazine of caramel-like, sweet, honey and candy notes. Acetic acid had high regression coefficient ($R^2 = 0.75$, $P = 0.001$), and likely contributed astringent-sour characters to dark chocolates. Others, 2-phenylacetate and 2-carboxaldehyde-1-*H*-pyrrole, showed no significant effect ($R^2 = 0.095$ and 0.179, $P > 0.05$, respectively), indicating minimal impacts on flavour character in dark chocolate. This product space from PCA (Fig. 3), demonstrated the importance and relationships of the different flavour volatiles and likely effect on flavour character and, furthermore, the influence of solids PSD and continuous phase matrix fat content on the overall volatiles release into the headspace in dark chocolates.

4. Conclusion

Variations in flavour volatile release in dark chocolate matrices varying in particle size distribution and fat content were noted, suggesting potential effects of matrix structure and lipophilic-flavour interactions. Increasing PS significantly reduced the release of 3-methylbutanal, 2-phenylethanol, furfuryl alcohol (furfurol), acetic acid, methylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, trimethylpyrazine, tetramethylpyrazine and 2,3,5-trimethyl-6-ethylpyrazine, 2-phenylethylacetate, 2-methylbutanal and 5-ethenyltetrahydro-*R*,*R*,5-trimethyl-*cis*-2-furanmethanol (linalool oxide). Fat content was directly related to headspace concentrations of compounds characterised by cocoa, chocolate, praline, fruity and roasted notes: trimethypyrazine, 3-methylbutanal, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, tetramethylpyrazine, linalool oxide and 2,3,5-triethyl-5-methylpyrazine, at all particle size distributions. By contrast, there was an inverse relationship between matrix fat content and headspace concentrations of 2-phenylethanol, furfuryl alcohol (furfurol), methylpyrazine, phenylacetaldehyde, 2, 3, 5-trimethyl-6-ethylpyrazine and 2-carboxaldehyde-1-*H*-pyrrole, likely due to lipophilic matrix–flavour interactions.

The PCA and regression models predicted contribution of volatiles to overall flavour character. One Strecker aldehyde, 3-methylbutanal, and two nitrogen heterocycles derived from Maillard reactions, trimethylpyrazine and 2,3-diethyl-5-methylpyrazine, provided cocoa, praline-chocolate and nutty notes, with three others, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine and tetramethylpyrazine, likely making little contribution to cocoa-chocolate flavour. Ethyl groups in pyrazine compounds suggest the key role of alanine and its Strecker aldehyde and acetaldehyde in dark chocolate flavour formation. Others, 2-methylbutanal, 5-ethenyltetrahydro-*R*,*R*,5-trimethyl-*cis*-2-furanmethanol (linalool oxide) and 3,7-dimethyl-1,6-octadien-3-ol (linalool), probably had no effect on dark chocolate flavour character. Likewise, 2-phenylethanol, furfuryl alcohol (furfurol), methylpyrazine, phenylacetaldehyde and 2, 3, 5-trimethyl-6-ethylpyrazine emerged as compounds contributing caramel-like, sweet, honey and candy notes, with acetic acid contributing to acid-sour sensations. Matrix effects on flavour release in dark chocolate merit attention as new product development and consumers demand a wider range of origins and defined products, and their influence on sensory effects with PSD and fat content remains unclear.

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