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ORIGINAL ARTICLE

Variation of total aroma and polyphenol content of dark chocolate during three phase of conching

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Abstract Variation in the volatiles, total polyphenol, theobromine and caffein was investigated both qualitatively and quantitatively for all phases of conching with GC/MS/SPME, HPLC, GC/O, and UV-visible spectrophotometry. The volatile compounds being identified during the three phases consisted of aldehydes, ketones, pyrazines, acids, alcohols and esters. The number and concentration of these compounds were observed to be 31–25,681 ppb, 44–34,838 ppb and 44–29,809 ppb in the dry, pasty, and liquid phases respectively. The odor of dark chocolate was described as nutty, sweet, caramel, green and chocolate using olfactometry. The percent decrease in the concentration of total polyphenol, caffein and theobromine was observed to be only 3.0, 11.0, and 32.0 respectively.

Keywords Phases of conching · Dark chocolate · Aroma · Caffein · Polyphenol and theobromine

Introduction

Chocolate is a unique food due to its attractive flavour. Conching is regarded as essential stage for final flavor development and appropriate texture. This is the final stage in chocolate manufacture.Residual volatile acids and moisture are removed, angular sugar crytals and viscosity are modified, and the color changed due to emulsification and oxidation of tannins (Awua 2002; Beckett 2003; Reineccius 2006; Afoakwa et al. 2007).

Conching is a process of mixing, vigorously agitating and aerating chocolate while heating it. The process is basically undertaken with the addition of cocoa butter and lecithin to coat the nonfat particles, reduce viscosity and give a smother and more rounded mouthfeel. The conching process occurs in two main phases: dry conch during which the chocolate is crumbly and removal of moisture takes place, and wet conch where the addition of extra cocoa butter and emulsifier ensure coating of all solid particles with fat, thereby giving the chocolate a smooth mouthfeel (Beckett 2009).

Conching conditions show interaction between time and temperature so that higher temperatures reduce the processing time. (Beckett 2000; Whitefield 2005). Dark chocolate are typically conched at higer temperatures, 70 °C or up to 82 °C (Minifie 1989; Awua 2002).

Despite its importance, there are conflicting reports on the effect of conching on chocolate flavor. Counet et al. (2002) showed although no key aroma compounds were formed during conching, the levels of branched pyrazines increased significantly while most stecker aldeydes were lost through evaporation. Hoskin and Dimick (1983) reported that amino acid content of dark chocolate does not change during conching.

Although the conching process has been studied considerably as given above, only few articles has been published on the effect of conching on the theobromine, caffeine and total polyphenol content; as this was apparent from a literature survey on the related subject. This work was therefore carried out to contribute to the insufficient data in the literature.

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Materials and method

Conching process

Refined chocolate mix, containing 39.14 % sugar, 45.07 % cocoa mass, lecithin and cocoa butter were supplied from a local plant. A 5 kg-capacity, laboratory-scale conching machine (ELK'olino single-shaft conche, Bühler, AG, Switzerland) with temperature and speed control was utilized for the conching process, which was made up of three phases; dry phase: 2 h at 50 °C, pasty phase: 4 h at 80 °C and final phase: 1 h with linear decrease in temperature from 80 °C to 45 °C. The rotation speed of the conching machine was in the range of 600–1500 rpm. The formulations of dark chocolates used in this study with and without additives are shown in Table 1.

Composition analysis

The moisture, fat, protein and ash contents were analyzed according to the standard methods (AOAC 1990). The percent carbohydrate was then determined by difference. The analyses were carried out in triplicate and the values were averaged as shown in Table 2.

SPME extraction

Volatiles from samples were extracted by using 75 μm divinylbenzene/carboxen on polydimethylsiloxane on a Stable/Flex fiber (CAR/PDMS). Extractions were carried out in the vials. A 2 g-sample together with 1 μL toluene, as internal standard, was placed in a 20-mL vial. After tightly plugging its lid and inserting the SPME fiber, it was equilibrated for 60 min at 60 °C. The desorption time was 5 min and the temperature in the GC liner was 250 °C.

GC/MS

The volatiles extracted by fibers were thermally desorped and introduced in the capillary column (EQUITY™-5 FUSED SILICA Capillary Column 30 m × 0.32 mm × 0.25 μm film thickness Supelco). The GC (Perkin Elmer Clarus 500)-MS (Clarus 500 MS Perkinelmer) was set up with constant flow of 2 mL/min (helium), the oven temperature was programmed starting at 80 °C (5 min)-(10 °C/min) 150 °C-150 °C(10 min) -(10 °C/min) 200 °C-200 °C(5 min). The injector temperature was maintained as 250 °C. The analysis was carried out by using gas chromatography coupled with mass spectrometry. The ionization voltage was 70 eV, mass range m/z 40–300.

Table 1 Formulations of dark chocolate, all figures are in %

Ingredients	Dark chocolate
Cocoa mass	45.07
Sugar	39.14
Added cocoa butter during conching	15.45
Lecithin	0.34

Table 2 Composition of dark chocolate

Moisture	0.54 %
Protein	8.42 %
Ash	1.73 %
Carbohydrate	55.31 %
Fat	34 %

Odour identification by olfactometry

Two trained panelists sniffed the outflowing gas from the olfactometer's detection port. The sniffing was carried out simultaneously during GC analysis. Panelists described their perception of the sniffed odour.

Cafein and theobromine determination

Two grams of chocolate was weighed and placed in a beaker. 25 mL of petroleum ether was added and mixed for 5 min. The solution was centrifuged at 15Xg (2000 rpm) for 5 min. After decanting the solvent, the procedure was repeated. Following the second wash, the residue was dried at room temperature overnight. 0.5 g of the residue was weighed and 40 g of deionized water was added. The solution was heated in boiling water for 30 min followed by cooling to room temperature. The sample was then centrifuged at 2000 rpm for 5 min. The supernatent (2 ml) was filtered through a 0.45 μm nylon filter. Theobromine and caffeine were analyzed by high liquid chromatography (LC -2A AB made in Japan). The separation was accomplished on an Inertsil ODS-3 5 μ 4,6X250 mm column (made in Japan) using methanol/water (85/15) as mobile phase. Flow rate was 1 mL/min and detection occured at 280 nm in a UV (SPD-20A 228–45,003–38 L2013481410-AE Shimadzu Made in Japan) Stock standard solution of theobromine (400 ppm) and cafeine (1000 ppm) were prepared by dissolving the appropriate amounts in water and subsequently stored at 4 °C. The working solutions for external standard curve were prepared by diluting the corresponding stock standard solutions in water.

Total polyphenol determination

Total polyphenol was determined spectrophotometrically using the modified method of Singleton and Rossi (1965). 250 mg of defatted chocolate was dissolved in 40 mL of 80 % acetone solution. The solution was sonificated in a beaker fitted inside an ultrasound bath (Model B-2200 E4 Blason, Power output 60 W, Frequency 47 Hz, Danbury, CT) for 30 min at 0 °C. Sonification was preferred over shearing as an aid in solubilizing polyphenol since shearing promotes browning of the polyphenol extract by oxidation (Shamsuddin and Dimick 1986). The solution was then filtered through Whatman no.1 filter paper under vacuum and thus a clear solution was obtanied. The residue(cake) was washed with 80 % acetone solution and the volume was completed to 100 mL. 1 mL of the extract was placed in a flask and diluted with 70 mL of distilled water. 5 mL of 2 N Folin-Ciocalteau reagent was added and kept for 2 min. 15 mL of saturated Na_2CO_3 solution was then added to stabilize the color within 2 h. The absorbance was measured at 765 nm spectrophotometrically. Catechin of nine known concentrations, ranging from 100 to 900 mg/L, was used to prepare the standard curve. The results were expressed as ppm catechin.

Results and discussion

Conching is the agitation of chocolate coupled with aeration and heat. This is a time and energy consuming process but it has remarkable effects on the final chocolate flavor. The escape of undesirable volatiles derived from oxidative and carbonly-amino browning reaction catalyzed by heat and aeration. The conched chocolate flavor can be differentiated organoleptically from that of the unconched chocolate but the knowledge related with components responsible for the sensory chance is still lacking. The nature of flavor change during conching process is not exactly understood at the chemical level. However, the level of volatile compounds such as acids, pyrazines, phenols, aldehydes and ketons have been shown to decrease; at the same time gas chromatographic head space analyses with SPME was observed to have different flavor profiles during the three phases of conching (Keeney 1971).

GC-MS data showed that there is large difference in number and level of aroma compounds during conching process in which these volatiles decreased by about 57 % by mass. Table 3 shows this drastic change in the chocolate aroma profile for the three phases of conching. The main compounds making up the dark chocolate volatiles in the dry phase were observed to be nonanal, acetic acid, tetramethyl- pyrazine, benzaldeyde, 2-methyl-heptadecane, and n-octane. Beckett (2000) stated that the air space surrounding conching machine has acidic odor and acids whose concentration decrease firstly are the short chain volatile acids such as acetic acid known to be end product of fermentation. In the dry phase, the concentration of some of these compounds, such as nonanal and tetramethyl-pyrazine decreases while some of them such as acetic acid, increases. Also new compounds have been formed during this phase probably due to increase in temperature and agitation. 2-decen-1-ol, hexadecanoic acid, 2-methyl-heptadecane, and 2-oxo-ethly ester-propanoic acids are some of them. The total number of volatile compounds decreased from 35 to 31 in this phase; increased from 31 to 39 in the pasty phase and decreased again from 39 to 31 in liquid phase. The amount of volatiles was observed to decrease mostly in the pasty phase of conching. This decrease may be attributed to the relatively high temperature (80 °C) and long time (4 h).

As seen from the Table 3, the number of acids in the dry phase is 10; it increased to 16 in the pasty phase and decreased again to 11 in the liquid phase. The highest decrease of acids in both quantity and number is therefore in the liquid phase. This shows that conching are of vital importance for producing a chocolate with a mild flavor. Decrease in the amount of acids, tetramethyl-pyrazine, benzaldehyde and increase in the amount of 2-methyl propanol and 2-methyl-heptadecane are considered to be an indication of quality of conching (Minifie 1989).

3-methyl-butanal, nonanal, benzaldeyde, isopropanal, octanal, phenylacetaldeyde, decanal,hexanal and propanal were the identified aldehydes during the three phases of conching. Amount of these aldehydes was observed to vary from phase to phase; 2588 ppb in dry phase, 4156 ppb in pasty phase and 4458 ppb in liquid phase. Counet et al. (2002) pointed out that the concentration of Strecker aldehydes decreased partially during conching. In the present study 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, trimethyl-pyrazine and tetramethylpyrazine were the major detected pyrazines in all three phases. The number of these compounds did not change but their concentrations change.

Schnermann and Schieberle (1997) stated that the concentration of isopentylpyrazine, tri-or tetramethylpyrazine decreased during conching of dark chocolate.

2-furanone, 1,1-oxybis-2- praponol, 1,6-heptadien, 2-oxo-ethylester-propanoic acid were some of the compounds which were detected only in dry phase but in the detection limit in the pasty and liquid phases. Phenylacetaldeyde, 1-phenylethanone, 2-phenoxyethanol, and phenylethyl acetate were identified just in pasty phase but did not appear in the liquid phase.

The total amount of volatiles decreased from 37,606 to 27, 848 ppb in dry phase, increased to 38,073 ppb in pasty phase and finally decreased to 30,197 ppb in liquid phase. It was observed that there was remarkable change in the total amount of alcohols, acids, pyrazines, hydrocarbons in all phases. Total amount of acids was 7755 ppb in dry phase, 11,991 ppb in pasty phase and 6765 ppb in liquid phase. Change in alcohol concentration was 977 ppb in the dry phase, 5618 ppb in pasty phase and 2958 ppb in the liquid phase. Amount of pyrazine decreased during all phases of conching; 7442 ppb in the dry phase, 5901 ppb in the pasty phase and 3525 ppb in liquid phase. Concentration of hydrocarbon increased during conching; 3029 ppb in the dry phase, 5009 in the pasty phase and 10,777 ppb in liquid phase. Concentration of ketons was

Table 3 Change in the amount dark chocolate volatiles during three phase of conching

Compound	Before conching (ppb)	Dry-pahse conching (ppb)	Pasty-phase conching (ppb)	Liquid phase conching (ppb)
Butanal, 3-methyl	902			527
1-propen-2-ol-acetate	51			
Pyrazine, 2,5,dimethyl	1004			
Pyrazine, 2,3, dimethyl	307			
2-Isopropyl-5-methyhex-2-enal	215			
Nonanal	2575	2349	858	669
Pyrazine, trimethyl	1977	11		
Acetic acid	2471	6581	9797	5353
Pyrazine, tetramethyl	16,599	7431	5901	3525
Benzaldeyde	4774	239	478	124
1,6-octadien-3-ol,3,7-dimethyl-acetate	149	27		
Cyclobutane, 1,2,diethyl-trans	110			
2-butanone,3-hydroxy	178	34		81
Propanoic acid, 2-methyl	200			76
2-Furancarboxaldehyde, 5-methyl	119			
2-Decanone	209			
Cyclohexanol, 5-methyl-2-(1-methylethy)	413			
Butanoic acid, 4-hydroxy	89			75
Benzeneacetaldehyde	949			
Butanoic acid, 3-methyl	907			
Carboxylic acid, benzyl	76			
Acetic acid, 2-phenylethyl ester	982	105	78	67
1-butanol, 3-methyl-benzoate	577			
Hexanoic acid	156	310	56	35
Phenol, 2-methoxy	203			
Benzyl alcohol	193			148
Hexanoic acid	152	68		124
5-methyl-2-phenyl-2-hexenal	350			
Nonanoic acid	231			
2-Furanmethanol				211
Pentanoic acid	186			66
Isoproponal				896
Cyclohexene,1-methyl	302	298	168	141
Decane			310	615
3-methylpentane				999
Ethanone		260	676	94
2-methyl-1-propanol (Isobutabol)				1786
1,1-diflouoro-dodecane		404	578	1806
2-Furanone		235		
Octanal		–		
1,1-oxybis-2-praponol		204	153	131
1,6-heptadien		188		
Pentan-1,3-dioldisobutyrate		26		
2-propyl-2-benzoyloxy-3,3,3-trifluroproponoate		75		
Benzeneethanol		353	3300	395
2,3-dihydro-3,5-dihydroxy-6-methyl-4 H-pyran-one		1185	3031	1648
2-decen-1-ol		420	298	32
1-hexadecene		25	34	

Table 3 (continued)

Compound	Before conching (ppb)	Dry-pahse conching (ppb)	Pasty-phase conching (ppb)	Liquid phase conching (ppb)
Hexadecanoic acid		5	53	
2-metyhy-heptadecane		2706	3600	5748
2-hexanone		50	350	48
Propanoic acid, 2-oxo-ethyl ester		139		
5,6-dihyro-1 H-pyridin-2-one		112		
4-hydroxy-3-methoxy-benzoic acid		506	622	
1,2-benzenedicarboxylic acid	18	293	38	
Heneicosanoic acid, methyl ester	128	126	99	
N-Octane	1189			
1-methyl-cyclohexene		17		
Phenylacetaldehyde		1113		
Ethanone, 1-phenyl		104		
3-hexen-1-ol-benzoate		92		
Octanoic acid		135		
5-methyl-2-(1-methylethyl)-cyclohexanol		374		
cyclopentacycloheptene		235		
Heptanoic acid, ethyl ester		4		
Decanal		161		
2-phenoxy-ethanol		1493		
Phenylethy acetate		535		
Nonanoic acid		317		
Benzeneacetaldeyde		268	149	
1-methoxy-4-benzene		67		
2-Undecanone		27		
Butanoic acid, 1,1-dimethylethylester		31		
2,3-dimethyl-5-propylpyrazine		157		
Triacetin		570		
Decanoic acid		25		
5-methyl-2-phenyl-2-hexanal		1229		
Phenol,2,6-bis-(1,1-dimethylethyl)		397		
Octadecanoic acid		299		
Hexadecanoic acid, ethyl ester		155		
3-hexene			683	
2-methylene-1,3-diphenyl-1,3-propanediol			21	
1-methyl-2-phenylethanol			165	
Octadecane			80	
3,4-dimethylpentanol			69	
1-(5-hexenyl-1-methyl)-hyrazine			9	
9-Octadecenoic acid			45	
3-phenyl-2-propenal			91	
Propanoic acid			104	
Benzocyclo buten			429	
2-cyclobutene			705	
4-methyl-hexanal			373	
4-tridecene-2-decenal			1629	

Table 4 Odor qualities of chocolate volatile compounds before and after conching

Retention time (min.)	Compound (before conching)	Odour description
0.79	Butanal, 3-methyl	Chocolate
0.47	1-propen-2-ol-acetate	Fatty
4.78	Pyrazine, 2,5,dimethyl	Nutty, green
5.10	Pyrazine, 2,3, dimethyl	Cooked, nutty
5.20	2-Isopropyl-5-methylhex-2-enal	Fruity
5.61	Nonanal	Floral
5.82	Pyrazine, trimethyl	Cocoa, roasted
6.41	Acetic acid	Sour
6.69	Pyrazine, tetramethyl	Bean like
7.37	Benzaldehyde	Nutty
7.44	1,6-octadien-3-ol,3,7-dimethyl-acetate	Flowery
7.59	Cyclobutane, 1,2,diethyl-trans	Like laurel
7.70	2-butanone,3-hydroxy	Fruity
7.83	Propanoic acid, 2-methyl	Rancid
7.97	2-Furancarboxaldehyde, 5-methyl	Sweet, caramel like
8.11	2-Decanone	No smell
8.15	Cyclohexanol, 5-methyl-2-(1methylethy)	Like laurel
8.70	Butanoic acid, 4-hydroxy	Fruity
8.75	Benzeneacetaldehyde	Pungent
8.99	Butanoic acid, 3-methyl	Cheese
10.26	Carboxylic acid, benzyl	Sweety
10.60	Acetic acid, 2-phenylethyl ester	Vinegar
10.82	1-butanol, 3-methyl-benzoate	Fruity
10.90	Hexanoic acid	No smell
11.11	Phenol, 2-methoxy	Woody
11.27	Benzyl alcohol	Almond like
11.46	Phenylethanol	Sweet,honey
11.95	Benzeneacetaldehyde,α-ethyl-diene	Fruity
12.50	Ethanone	Fruity
12.99	Phenol	**Spicy**
13.12	4-Propyl-2-hydroxycyclopent-2en-1-one	Careme
13.38	Carbonic acid, decyl pheny ester	sour
13.66	2-propenal, 3-phenyl	Potato like
13.80	Hexanoic acid	Fatty
14.10	5-methyl-2-phenyl-2-hexenal	Cocoa
16.12	Nonanoic acid	Rancid
0.23	3-methylpentane	Odourless
0.47	2-methyl-1-propanol (isobutanol)	Sweet
0.77	Butanal, 3-methyl	Fruity
1.14	Decane	Sharp, bad
1.58	2-methyl-heptadecane	No smell
1.75	3-hexene	Green
2.57	Hexanoic acid	Sour
2.86	1,1-difluoro-dodecane	Camphor like
3.16	Cyclohexene, 1-methyl	Spicy
3.20	2-propanol	Sweet

Table 4 (continued)

Retention time (min.)	Compound (before conching)	Odour description
3.35	Cyclohexene	Sweet
3.80	Ethanone	Fruity
3.92	1-methyl-2-phenylethanol	Rose
4.21	Tetramethyl-pyrazine	Bean like
4.45	Nonanal	Floral
4.39	Octadecane	No smell
4.66	Benzeneethanol	Citrus
5.58	2-Decen-1-ol	No smell
5.79	3,4-dimethylpentanol	Sharp
6.24	2-hexanone	Fatty
6.40	Acetic acid	Sour, vinegar
6.66	Pyrazine, tetramethyl	Beanlike
6.80	2-phenylethyl ester-acetic acid	Vinegar
6.95	9-octanoic acid	Rancid
7.37	Benzaldehyde	Nutty
7.40	2-methyl-heptadecane	No smell
7.48	Isopropanol	No smell
7.69	2-butanone, 3-hydroxy	Creamy
7.83	Propanoic acid, 2-methyl	Fruity
8.07	Propanoic acid	Rancid
8.68	Butanoic acid, 3-methyl	Banana
10.59	Benzenebutanol	Floral
10.90	Pentanoic acid	Rancid
11.11	Phenol, 2-methoxy	Smoky
11.37	Phenylethanol	Sweet, honey
12.49	Ethanone	Fruity
13.65	2-propenol, 3-phenyl	Potato like
22.28	Octane	Gasoline
22.79	1,2-benzenedicarboxylic acid	Floral
24.20	2-decenal	Fatty

579 in the dry pahse, 1053 in the pasty phase and 142 in the liquid phase.

Odor identifications

The odor qualities of the volatile compounds were identified by GC-MS-olfactometer before and after conching as shown in Table 4.

Frauendorfer and Schieberle 2006 reported that acetic acid is an important compound which has a significant effect on chocolate aroma since it may provide undesired flavor, vinegar like odor. Akhtar et al. 2010 assumed that the acetic acid was produced from oxidation of aldehydes and alcohols and released from esters by mechanical and/or thermal mechanisms during the long conching process.

Ramli et al. 2006 claimed that aldehydes are one of the main groups formed from the roasting process in chocolate manufacture. It is believed that some aldehydes, such as 2-methylbutanal, 2-methylpropanal,benzaldehyde and phenylacetaldehyde, are important compounds in chocolate flavor (Counet et al. 2002). Schnermann and Schieberle 1997 reported that phenylacetaldehyde was an odor-active compound in dark chocolate. Pyrazine is one of the major groups formed in the Maillard reaction during roasting. Among 525 identified volatiles in roasted cocoa bean, one fifth of them were predominate pyrazine fractions (Schnermann and Schieberle 1997).

In the present work 2,5 dimethylpyrazine, 2,3 dimethylpyrazine, trimethyl and, tetramethyl pyrazines were observed to have a nutty odor while nonanal, 3-hydroxy-2-butanone, 4-hydroxy-butanoic acid, 3-methyl-1-butanol, and α-ethyl-benzeneacetaldeyhde, have fruity odour. Odour of acetic acid, 2-methyl-propanoic acid, 2-phenylethyl ester-acetic acid, nonanoic acid, pentanoic acid was rancid and sour.

Quantities of total polyphenols, caffeine, and theobromine

Arora et al. (2000), stated that the flavonoids represent a ubiquitous and abundant group of polyphenol consumed in diet, primarily from fruits and vegetables, derived from plant and act as antioxidants due to their free radical scavenging properties, their ability to reduce the formation of free radicals and their ability to stabilise membranes by decreasing membrane fluidity. Cocoa and its derived products are rich in flavonoids, characterised as flavan-3-ols or flavanols and include the monomoric forms, (−) epicatechin and (−) catechin and the oligomeric form of the monomeric units (Wollgast and Anklam 2000). Steinberg et al. (2003) declared, most of antioxidant activity in the chocolate to come from the polyphenol content. All fractions of cocoa bean polyphenols have been identified to have an antioxidant property.

Benowitz (1990) stated that the methylxanthines such as cafein and theobromine are regulary consumed from a variety of foods. Consumers want to know the methylxanthines content of foods because of their widespread consumption. Methylxanthines have been reported to have physiological effect on various body systems, including the central nervous, cardiovascular and respiratory systems (Nehlig et al. 1992; Spiller 1998).

As seen in Table 5, there is a negative effect of conching on the cafein, theobrimine and total polyphenol. Amount of these compound decreased durig conching. Cafein, theobrimine, and totaly polyphenol reduced by 10.51, 31.62 and 2.97 % during conching. This may be due to high temperature and shear effect of conching process.

Table 5 Change of cafein, theobrimine and total polyphenol of dark chocolate after conching

	Before conching	After conching
Cafeein (ppm)	59.93	53.63
Theobrimine (ppm)	8.38	5.73
Total polyphenol (ppm)	292	283.3

Conclusion

During three phase of conching concentration of some components decrease whereas some of them increase. The greatest impact of conching was observed pasty pahse. 42, 31, 44 and 44 components were identified before conching, dry, pasty and liquid phase respectively. Amount of volatile was high in the pasty phase compared to other phase. Unconched chocolate mass have green, smoky odour meanwhile conched chocolate mass have nuty, sweet and caramel odour. Concentration of cafein, theobromine and polyphenol decreases during conching.

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