The impact of pod storage on the formation of different alkylpyrazines from Ghanaian cocoa roasted at four roasting temperatures

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Abstract

The typical flavour characteristics of cocoa are related to the cocoa bean genotype and the growing environmental conditions. However, the flavour does not exist in freshly harvested beans. Flavour is generated by a series of procedures that begins with occasional pod storage (PS) after harvesting, followed by fermentation of the beans, and roasting. PS implies storing harvested cocoa pods for a period of time before opening. The effect of PS is believed to be beneficial for the subsequent development of cocoa flavour in the cocoa beans [1]. During roasting, several volatile heterocyclic compounds are formed, among them alkylpyrazines. These newly formed compounds are considered to be key odour components. Among alkylpyrazines, tetramethylpyrazine and trimethylpyrazine, are the most abundant ones. Other alkylpyrazines with different substituents also contribute to the aroma profile. Hence, monitoring pyrazines can be helpful in optimizing roasting conditions of cocoa beans for attaining the desired aroma of the cocoa liquors. In several studies, cocoa volatiles have been measured using gas chromatography mass spectrometry (GC-MS), frequently using headspace solid-phase microextraction (HS-SPME) to concentrate the volatiles.

The purpose of this study was to investigate the effect of PS (0, 3, 7 days PS) and roasting temperature (100°C, 120°C, 140°C and 160°C) on the formation of alkylpyrazines in Ghanaian cocoa liquors. HS-SPME extraction of the alkylpyrazines was carried out with a DVB/CAR/PDMS fibre. The identified compounds were measured semi-quantitatively and the results were statistically processed by multivariate analysis. In total, 18 different alkylpyrazines were determined. Higher roasting temperatures resulted in the formation of more complex alkylpyrazine profiles, compared to lower roasting temperatures. Moreover, an extended PS time of 7 days leads to highest formation of alkylpyrazines.

Introduction

During roasting, the typical roasty and chocolate flavours are developed and undesired flavours are eliminated, at least to some extent. Flavour precursors (free amino acids, oligopeptides and reducing sugars) participate in non-enzymatic browning (Maillard) reactions. An important route is the Strecker degradation, which leads to volatile aldehydes, pyrazines and other heterocyclic compounds. Pyrazines are the main class of nitrogenous heterocyclic volatiles and they are also key odour components in cocoa aroma. Several pyrazines contribute to the overall cocoa flavour, especially the alkylpyrazines with different substituents, of which tetramethylpyrazine and trimethylpyrazine are the most abundant ones. Roasting temperature is a critical factor

B. Siegmund & E. Leitner (Eds): Flavour Sci., 2018, Verlag der Technischen Universität Graz
DOI: 10.3217/978-3-85125-593-5-107, CC BY-NC-ND 4.0

that influences the concentration of pyrazines. The aim of this study was to investigate the effect of pod storage (0, 3, 7 days of PS) of Ghanaian cocoa liquor, followed by roasting at 4 different temperatures (100°C, 120°C, 140°C and 160°C), on the formation of alkylpyrazines.

Experimental

Ghanaian cocoa beans from 31-year old trees (hybrid type *Forastero)* were obtained from a farm in Jachere farming community (Brong Ahafo region). The beans were harvested in September-October; the cocoa beans have different times of PS: 0, 3 and 7 days. After PS, the beans were fermented for 6 days, and sun-dried for two weeks. 1 kg of cocoa beans was weighed and roasted in a conventional oven (Termarks, Lien 79, N-5057 Bergen, Norway) at 100°C, 120°C, 140°C and 160°C for 35 min. After cooling to room temperature, the cocoa beans were wrapped in aluminium foil and stored in odourless buckets. Prior to sample preparation, the beans were manually deshelled. For grinding the de-shelled cocoa beans to liquor an ECGC-12SLTA Cocoa T melanger (CocoaTown, Roswell, USA) was used.

The volatile aroma profiles were recorded using a Multi-Purpose Sampler (Gerstel, Mülheim an der Ruhr, Germany) equipped with a HS-SPME unit. Cocoa liquor (2 g) was weighed in a 20 ml vial and 0.792µg of 1-octen-3-ol was added as internal standard. Afterwards, the vials were sealed airtight with a magnetic cap equipped with a PTFE septum. Prior to extraction, each vial was heated at 60°C for 10 min for headspace equilibration in a thermostatic agitator. Next, the volatiles compounds were extracted for 25 min at 60°C using a 50/30µm DVB/CAR/PDMS fiber (1 cm) (Supelco, Sigma-Aldrich N.V., Bornem). Volatile components were desorbed (5 min) into the splitless injector (250°C) of an Agilent Technologies 6890-5793 GC-MS system (Agilent Technologies, Santa Clara, CA, USA) and separated on a Phenomenex 30m ZB-Wax plus capillary column (0.25 mm i.d.; 0.25 µm film thickness). The temperature program was 5 min at 35°C; heating at 4°C/min to 182°C and heating at 7°C/min to 240°C. Compounds were fragmented using electron-impact ionization (70eV), with a source temperature of 230°C, a scan range of 40-230 amu and a scan rate of $2s^{-1}$. After injection, each time the fiber was baked out for 7 min at 270°C. Components were identified based on comparison of mass spectra with those of spectral libraries of Wiley 7N Registry of GC Mass spectral Data (John Wiley, NY, USA). Additionally, confirmation of identified compounds was done by determination of Kovats indices (KI), determined after injection of series of n-alkane homologues using the analytical configuration as described above. The calculated values were compared to KI values found in literature obtained on polar columns and are inserted in Table 1.

Statistical analysis was performed using SPSS 22 (SPSS Inc., Chicago, USA). One-way analysis of variance (ANOVA) was used to investigate any significant differences between the samples (significance level at 0.05). Significant differences were identified with the Tukey's multiple range test. Principal Component Analysis (PCA) was performed (using Unscrambler 6.1., Camo, Norway) to visualize complex data matrix and the relationship between the different cocoa beans on their volatile composition.

Results and discussion

Formation of different pyrazines at different roasting temperatures

In total, 18 different pyrazines, derived from Maillard reactions, were identified in the cocoa liquors. In Table 1, all semi-quantitative concentrations (ng/g liquor) of the

Table 2: Semi-quantitative data (expressed as ng/g liquor) of different alkylpyrazines at 3 pod storage periods (0PS, 3PS, 7PS) as a function of roasting temperature (100°C, 120°C, 140°C, 160°C) (data are expressed as mean values of 3 replicates ± standard deviation, $^{A-H}$ different letters are significantly different at a significance level of $P < 0.05$ according to Tukey's test)(n.d. not detected).

RT	*Pyrazine*	*KI*	*KI(lit)*	*0PS100*	*0PS120*	*0PS140*	*0PS160*	*3PS100*	*3PS120*	*3PS140*	*3PS160*	*7PS100*	*7PS120*	*7PS140*	*7PS160*
15.25	**methyl-pyrazine**	1257	1251	18.7 ± 5.1^{D}	181.0 ± 9.1^{CD}	573.1 ± 69.3^{B}	1259.9 ± 143.3^{A}	51.1 ± 2.6^{D}	287.9 ± 30.7^{C}	629.0 ± 55.2^{B}	1243.1 ± 42.5^{A}	35.0 ± 5.9^{D}	182.5 ± 15.5^{CD}	510.4 ± 55.8^{B}	1235.8 ± 136.5^{A}
17.34	**2,5-dimethyl-pyrazine**	1315	1290-1358	25.9 ± 7.4^{F}	307.6 ± 42.4^{F}	1360.8 ± 144.6^{DE}	2486.2 ± 234.9^{B}	58.4 ± 12.0^{F}	450.5 ± 42.0^{F}	1175.9 ± 68.7^{E}	2048.4 ± 145.3^{BC}	46.8 ± 9.9^{F}	444.9 ± 73.1^{F}	1719.1 ± 220.4^{CD}	3681.4 ± 343.0^{A}
17.56	**2,6-dimethyl-pyrazine**	1321	1296-1358	39.7 ± 6.2^{G}	192.7 ± 23.4^{EFG}	593.6 ± 66.7^{D}	1024.9 ± 107.9^{B}	67.6 ± 4.7^{FG}	259.5 ± 22.8^{E}	546.3 ± 48.5^{D}	862.3 ± 67.7^{BC}	63.7 ± 3.5^{FG}	251.4 ± 44.5^{EF}	687.0 ± 83.6^{CD}	1392.2 ± 129.6^{A}
17.71	**ethyl-pyrazine**	1325	1323-1343	n.d.	58.1 ± 2.5^{CD}	183.6 ± 36.6^{B}	385.7 ± 33.8^{A}	n.d.	69.0 ± 7.9^{C}	205.9 ± 31.1^{B}	330.8 ± 44.7^{A}	n.d.	55.6 ± 8.3^{CD}	156.3 ± 13.8^{B}	376.4 ± 5.4^{A}
18.17	**2,3-dimethyl-pyrazine**	1337	1315-1344	94.1 ± 12.7^{F}	272.9 ± 16.2^{EF}	727.4 ± 84.6^{D}	1194.3 ± 161.0^{C}	123.8 ± 13.2^{F}	226.4 ± 22.9^{EF}	379.9 ± 35.5^{EF}	539.6 ± 71.3^{DE}	237.0 ± 10.7^{EF}	546.2 ± 78.0^{DE}	1655.9 ± 243.1^{B}	2541.4 ± 231.4^{A}
19.56	**2-ethyl-6-methyl-pyrazine**	1376	1381-1415	n.d.	204.6 ± 30.5^{D}	678.9 ± 59.8^{B}	1295.1 ± 119.5^{A}	n.d.	125.7 ± 11.2^{DE}	427.3 ± 31.4^{C}	735.0 ± 75.7^{B}	n.d.	141.2 ± 41.0^{DE}	629.2 ± 76.7^{B}	1320.8 ± 110.2^{A}
19.76	**2-ethyl-5-methyl-pyrazine**	1381	1386-1453	n.d.	115.4 ± 22.3^{E}	1198.1 ± 95.0^{C}	1741.0 ± 141.4^{B}	n.d.	239.7 ± 86.1^{E}	874.1 ± 41.5^{D}	1212.6 ± 129.5^{C}	n.d.	177.7 ± 28.3^{E}	1517.0 ± 219.8^{BC}	2337.1 ± 191.8^{A}
20.34	**trimethyl-pyrazine**	1397	1381-1413	264.7 ± 28.3^{G}	1168.9 ± 90.7^{FG}	3658.5 ± 329.8^{CD}	5417.4 ± 389.7^{C}	282.2 ± 50.6^{G}	778.1 ± 58.8^{FG}	1782.1 ± 72.9^{EFG}	2393.8 ± 207.6^{DEF}	884.5 ± 28.1^{FG}	3039.1 ± 329.4^{DE}	9230.8 ± 1166.8^{B}	15353.8 ± 1606.7^{A}
21.59	**2,5-dimethyl-3-ethyl-pyrazine**	1431	1435	n.d.	703.2 ± 240.1^{DE}	1513.7 ± 152.9^{C}	2478.3 ± 164.7^{B}	n.d.	n.d.	783.4 ± 58.3^{DE}	1062.4 ± 67.2^{D}	n.d.	470.5 ± 84.2^{E}	1503.6 ± 232.3^{C}	3055.7 ± 281.8^{A}
22.08	**2,3-dimethyl-5-ethyl-pyrazine**	1445	1493	87.3 ± 16.7^{E}	385.5 ± 42.1^{DE}	1001.1 ± 117.3^{C}	1920.4 ± 162.8^{B}	83.8 ± 13.1^{E}	170.2 ± 3.5^{E}	429.7 ± 45.2^{DE}	766.6 ± 29.2^{CD}	138.9 ± 16.5^{E}	572.8 ± 45.4^{CDE}	2095.8 ± 259.2^{B}	4090.6 ± 574.0^{A}
22.53	**tetramethyl-pyrazine**	1457	1438-1474	1116.8 ± 94.5^{E}	1971.7 ± 164.2^{E}	2467.5 ± 269.7^{E}	2956.2 ± 293.3^{E}	730.6 ± 74.9^{E}	674.9 ± 63.0^{E}	777.2 ± 27.8^{E}	758.5 ± 57.8^{E}	7215.8 ± 131.0^{D}	10141.9 ± 1097.3^{C}	14325.2 ± 1970,2^{B}	18542.3 ± 2260.2^{A}
22.81	**2-methyl-6-vinyl-pyrazine**	1465	1521	n.d.	80.5 ± 14.9^{C}	128.0 ± 19.1^{ABC}	119.8 ± 13.1^{ABC}	n.d.	96.6 ± 13.4^{BC}	101.5 ± 9.2^{BC}	81.5 ± 8.6^{C}	n.d.	101.5 ± 6.7^{BC}	143.4 ± 41.6^{AB}	152.8 ± 16.9^{A}
23.16	**3,5-diethyl-2-methyl-pyrazine**	1474	1524	n.d.	123.3 ± 14.7^{DEF}	407.6 ± 49.1^{B}	653.5 ± 67.9^{A}	n.d.	55.9 ± 7.7^{EF}	144.9 ± 11.8^{DE}	221.0 ± 21.2^{CD}	n.d.	n.d.	273.9 ± 61.1^{C}	687.9 ± 99.5^{A}
23.78	**2,3,5-trimethyl-6-ethylpyrazine**	1491	-	85.4 ± 11.4^{E}	476.9 ± 22.1DE	876.9 ± 106.5^{D}	1325.8 ± 105.4C	76.1 ± 10.5^{E}	138.4 ± 22.3^{E}	271.8 ± 9.8^{E}	328.7 ± 12.5^{E}	193.0 ± 10.6^{E}	826.0 ± 107.6^{D}	1950.7 ± 271.1^{B}	3250.0 ± 395.4^{A}
27.03	**2-isoamyl-6-methyl-pyrazine**	1581	-	n.d.	n.d.	39.0 ± 5.9^{D}	74.0 ± 15.1^{B}	n.d.	n.d.	40.6 ± 3.0^{D}	43.9 ± 2.2^{CD}	n.d.	n.d.	64.0 ± 12.8^{BC}	144.6 ± 15.2^{A}
29.49	**2-methyl-6,7-dihydro-5H-cyclopentapyrazine**	1648	-	n.d.	n.d.	92.2 ± 9.0^{C}	225.9 ± 14.6^{B}	n.d.	n.d.	83.0 ± 17.4^{C}	176.1 ± 35.9^{B}	n.d.	n.d.	196.2 ± 43.6^{B}	518.5 ± 55.5^{A}
29.99	**2,3,5-trimethyl-6-propylpyrazine**	1662	-	n.d.	n.d.	33.5 ± 3.5^{CD}	60.4 ± 10.9^{C}	n.d.	n.d.	24.2 ± 6.3^{CD}	32.4 ± 6.8^{CD}	n.d.	56.2 ± 5.9^{C}	116.2 ± 19.9^{B}	218.9 ± 39.5^{A}
31.89	**2,3-dimethyl-6,7-dihydro 5H-cyclopentapyrazine**	1714	-	n.d.	n.d.	n.d.	113.3 ± 29.6^{B}	n.d.	n.d.	n.d.	56.3 ± 13.7^{BC}	n.d.	n.d.	130.1 ± 26.0^{B}	545.7 ± 101.6^{A}
	Total			1732.5 ± 174.8^{H}	6242.2 ± 689.6^{FG}	15533.5 ± 1549.2^{D}	24732.1 ± 2023.3^{C}	1473.5 ± 174.3^{H}	3573.0 ± 371.0^{GH}	8676.6 ± 514.0^{EF}	12892.9 ± 966.2^{DE}	8814.6 ± 150.0^{EF}	17007.3 ± 1895.8^{D}	36904.7 ± 4841.5^{B}	59446.0 ± 6453.8^{A}

alkylpyrazines are presented. The most abundant compound was tetramethylpyrazine (> 18 µg/g), followed by trimethylpyrazine (> 15 µg/g) after 7PS at 160°C. Higher roasting temperatures resulted in the formation of more alkylpyrazines compared to lower roasting temperatures. In addition, at roasting temperatures of at least 140°C, four extra pyrazines were formed: 2-isoamyl-6-methylpyrazine, 2-methyl-6,7-dihydro-5H-cyclopentapyrazine, 2,3,5-trimethyl-6-propylpyrazine and 2,3-dimethyl-6,7-dihydro-5H-cyclopentapyrazine; the latter was only formed at a temperature of 160°C.

At 100°C only 8 pyrazines were detected. At 120°C, already 14 pyrazines were formed and the total amount of pyrazines increased by a factor 3.6 for 0PS; 2.4 for 3PS and 1.9 for 7PS compared to a roasting temperature of 100°C. Starting from 140 °C, for all PS, an increase in the level of pyrazines was observed by a factor greater than 2, compared to a roasting temperature of 120°C. At a temperature of 160 °C, only a rise by a factor 1.5-1.6 for all PS was reached, compared to a roasting temperature of 140°C. In summary, increasing the roasting temperature gives rise to more complex alkylpyrazine profiles in cocoa, and quantitatively higher levels in alkylpyrazines.

Influence of pod storage on formation of pyrazines

Figure 1 represents the biplot from PCA on all samples and all detected alkylpyrazines. The amount of variance explained by the two factors in the biplot was 94% (PC1:84%, PC2:10%). From the PCA, it became clear that there is an influence of both roasting temperature and PS. Samples of 7PS were clearly distinct from all other samples. This was mainly due to an exponential increase in tetramethylpyrazine and trimethylpyrazine (Table 1). Samples of 0PS and 3PS migrated in another, but similar way as a function of roasting temperature. At 3 days PS, the total amount of formed pyrazines was always lower than after 0 days PS. Both the data shown in Table 1 and the PCA biplot in Figure 1, point to a pronounced impact of extended PS (7 days) on the formation of alkylpyrazines, independent of the roasting temperature. Even at the lowest roasting temperature (100°C), PS for 7 days gives rise to highly increased alkylpyrazine formation, compared to 0 days PS (difference by a factor 5.1).

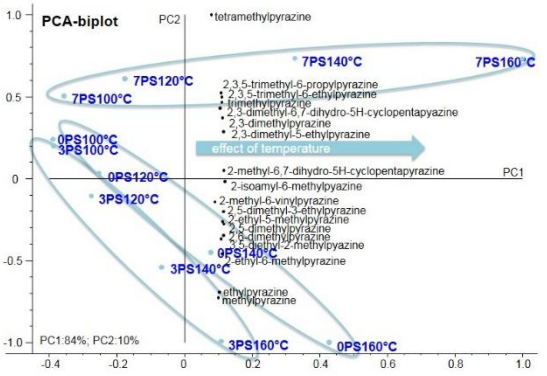

Figure 1: PCA-biplot of different alkylpyazines in Ghanaian liquor samples as a function of pod storage and roasting temperatures

References

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