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Effect of polyphenol concentration on pyrazine formation during cocoa liquor roasting

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Abstract

A study on effect of polyphenol on pyrazine formation during cocoa liquor roasting has been carried out. Cocoa liquors, having polyphenol concentrations of 41, 58, 116, 143 and 170 g kg^{-1}, were roasted at 120 °C for 15, 25, 35 and 45 min. Results of the study showed that, as polyphenol concentration increases, pyrazine formation decreases; this was due to the binding of polyphenol against pyrazine precursors, i.e. free amino acids and reducing sugars, and pyrazines formed during roasting. Reduction of the formations of 2,3,5-TrMP and 2,3,5,6-TMP occurred throughout roasting times, starting at 116 g polyphenol kg^{-1} and reaching maximum reduction at 116–170 g polyphenol kg^{-1}. The reduction against 2,5-DMP was not significant over roasting times, except at 35 min; however, formation of 2,3-DMP was reduced at roasting times of 25, 35 and 45 min. These results implied that the presence of polyphenol in cocoa has to be considered in many aspects, including its reduction of flavour formation during roasting, besides productions of astringency and bitterness of the product and its beneficial effect as an antioxidant.

Keywords: Cocoa; Aroma; Pyrazine; Polyphenol; Roasting, Amino acid; Reducing sugar

1. Introduction

Flavour is one of the most important properties of cocoa products; 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 (Barel, Leon, & Vincent, 1985; Mohr, Landschreiber, & Severin, 1976).

Through the Maillard reactions, all of the cocoa aroma precursors interact to produce cocoa flavour components, such as alcohols, ethers, furans, thiazoles, pyrones, acids, esters, aldehydes, imines, amines, oxazoles, pyrazines and pyrroles (Hoskin & Dimick, 1994; Jinap, Wan Rosli, Russly, & Nurdin, 1998; Puziah, Jinap, Sharifah, & Asbi, 1998b). In a series of intensive studies, Biehl and co-workers (1982–1994) have successfully generated cocoa aroma in vitro from its precursors in the presence of cocoa butter, although the real chocolate flavour remained obscured.

Furthermore, researches have indicated that unfermented cocoa beans do not develop any chocolate flavour when roasted and are excessively astringent and bitter in taste (Biehl & Voigt, 1996; Puziah, Jinap, Sharifah, & Asbi, 1998a). Polyphenols are compounds in cocoa, which are responsible for the astringency and contribute to bitter and green flavours (Bonvehi & Coll, 1997, 2000; Luna, Crouzillat, Cirou, & Bucheli, 2002). Hagerman and Butler (1981) stated that the most important characteristic of polyphenol is its propensity to form complexes with proteins, polysaccharides and alkaloids.

The above evidence leads to a hypothesis that the presence of polyphenol in cocoa not only gives astringent, bitter and green flavours and interaction with protein, but also causes interaction with aroma precursors as well as aroma compounds which are produced during roasting, such as pyrazines. This paper is addressed to effects of polyphenol on pyrazine formation during cocoa roasting.

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2. Materials and methods

2.1. Crude polyphenol extract

Cocoa fruits were obtained from the Cocoa Production and Processing Station, Malaysian Cocoa Board, Jengka, Pahang. The ungerminated beans were collected from healthy pods and their testa were removed before being shock-frozen in liquid nitrogen. The frozen cocoa cotyledons were then lyophilized and freeze-dried at a pressure of <13.3 Pascal (Labconco Freezone 6, USA), followed by grinding, refining and defatting in a Soxhlet apparatus for 16 h with petroleum ether (b.p. 40–60 °C). Polyphenol was collected by extraction of the powder, twice, using chilled 80% aqueous acetone, followed by twice using chilled 100% acetone. The extract was centrifuged at 4000 rpm and 4 °C for 15 min (Kubota 788, Japan); the supernatant was then evaporated under vacuum at 45 °C using a rotary evaporator (Heidolph WB/VV 2000, Germany), followed by freeze-drying at a pressure of <13.3 Pascal.

2.2. Design of study

The study was carried out in two factors, using complete randomized design (CRD) with three replications. The first factor was polyphenol concentration in cocoa liquor, 41, 58, 71, 116, 143 and 170 g polyphenol kg^{-1}; whereas the second factor was time of roasting, 15, 25, 35 and 45 min. Cocoa liquor with polyphenol concentrations of 41 and 71 g kg^{-1} was prepared from Ghanaian fermented cocoa powder which was previously dephenolized by thrice extracting with chilled 100% acetone. These treatments were designed to study effects of low polyphenol concentrations on cocoa flavour. Cocoa liquor with polyphenol concentrations of 58, 116, 143 and 170 g kg^{-1} was prepared from Ghanaian fermented cocoa powder. All of the cocoa liquors were adjusted to contain 55% fat using deodorized cocoa butter and the respective polyphenol concentration using crude polyphenol extract.

2.3. Roasting condition

Roasting of cocoa liquor was carried out in an oven (Memmert UL 40, Germany), which was set at 120 °C and maintained for 1 h to reach equilibrium, before being used. Fifty grammes of cocoa liquor were placed in a 10 cm Petri dish at 5 mm thickness before being inserted into the oven. The door was opened and closed as quickly as possible after inserting the Petri dish. After cooling to ambient temperature (26 °C), the liquor was then defatted in a Soxhlet apparatus with petroleum ether (b.p. 40–60 °C) for 16 h. After drying at room temperature (27 °C) for 3 h, the petroleum ether residue was discarded under vacuum at a pressure <13.3 Pascal for 3 h. The sample was then kept in a sealed container for further analyses.

2.4. Pyrazines

Pyrazines were determined by simultaneous steam-distillation and extraction (SDE), followed by detection using gas chromatography (GC). Cocoa powder was extracted by distillation (1 h) in a Lickens and Nickerson apparatus (Schultz, Flath, Mon, Eggling, & Terranishi, 1977) using 3 g of cocoa powder and 200 ml of distilled water. Internal standard of 4-Picoline (Aldrich, USA) was applied to the mixture before the distillation. The extracted pyrazines was trapped with 20 ml of pentane. Anhydrous sodium sulphate was later added to the distillate and set aside for 2 h to absorb moisture. The distillate was dried to less than 1 ml using a nitrogen stream before analysis.

Measurement of different types of pyrazine was accomplished using an HP 6890 GC (Hewlett-Packard, USA). The capillary column used was Fused Silica BP 20 (50 m × 0.33 mm i.d. with 0.25 μm film). The column temperature was programmed at 60 °C for 3 min, increased to 180 °C at 5 °C min^{-1} and held at the final temperature for 5 min (Puziah et al., 1998a). Injector and detector (Flame Ionization Detector) were set at 200 °C and carrier gas employed was helium at 30 ml min^{-1}.

2.5. Free amino acids and reducing sugars

Free amino acids and reducing sugars were determined using a high performance liquid chromatography method, as described by Misnawi, Jinap, Jamilah, and Nazamid (2002a).

3. Results and discussion

3.1. Pyrazine formations

This study showed that 2,5-dimethylpyrazine (DMP), 2,3-dimethylpyrazine (DMP), 2,3,5-trimethylpyrazine (TrMP) and 2,3,5,6-tetramethylpyrazines (TMP) were the pyrazines obtained in sufficient quantities after roasting, i.e. in the range of trace–2.25 mg kg^{-1}, 0.15–4.43 mg kg^{-1}, 0.21–3.69 mg kg^{-1} and 0.44–12.82 mg kg^{-1}, respectively (Table 1). This is in agreement with the results found by Bonvehi and Coll (2002), Puziah et al. (1998a, 1998b) and Jinap et al. (1998) where dimethyl-, trimethyl- and tetramethylpyrazines were found to be the major pyrazines produced during cocoa bean roasting. About 95 pyrazines have been identified in cocoa aroma, with concentration depending on the time and temperature of the thermal treatments (Hashim & Chaveron, 1994; Jinap et al., 1998).

Table 1
Effect of polyphenol concentration during roasting on pyrazine concentrations of cocoa liquor[a]

Roasting time (min)	Polyphenol (g kg^{-1})	2,5-DMP	2,3-DMP	2,3,5-TrMP	2,3,5,6-TMP	Total
15	41[b]	trace	0.20c	0.31c	0.52e	1.03e
	71[b]	trace	0.15d	0.26c	0.45e	0.87e
	58[c]	0.78a	0.43a	3.69a	12.8a	17.7a
	116[c]	0.68a	0.29b	1.25b	10.4b	13.9b
	143[c]	0.79a	0.28b	1.44b	8.02c	10.5c
	170[c]	0.67a	0.27b	1.25b	6.48d	9.12d
25	41[b]	0.16c	0.48d	0.28c	0.69c	1.61d
	71[b]	0.13d	0.16e	0.28c	0.46d	1.03e
	58[c]	0.35a	2.27a	1.81a	7.91a	12.3a
	116[c]	0.35a	1.75b	0.66b	3.61b	7.64b
	143[c]	0.34a	0.68c	0.65b	3.68b	6.57c
	170[c]	0.30b	0.71c	0.67b	3.74b	6.86c
35	41[b]	0.20c	0.20d	0.33d	0.72d	1.45d
	71[b]	0.15c	0.15d	0.33d	0.63d	1.25d
	58[c]	2.25a	2.53a	2.31a	9.91a	17.0a
	116[c]	1.56b	1.57b	1.96b	9.70a	17.1a
	143[c]	1.47b	1.10c	2.16ab	6.77b	14.9b
	170[c]	1.37b	0.91c	0.65c	3.84c	7.84c
45	41[b]	0.15d	0.18d	0.22d	0.52d	1.07c
	71[b]	0.13d	0.15d	0.21d	0.44d	0.94c
	58[c]	1.09a	4.43a	1.84a	8.06a	15.4a
	116[c]	0.92bc	2.94b	1.43b	6.00b	13.7a
	143[c]	0.81c	1.13c	0.77c	3.12c	7.15b
	170[c]	0.99ab	1.24c	0.67c	2.98c	6.93b
Average		0.64	1.00	1.08	4.72	8.13
Range		trace–2.25	0.15–4.43	0.21–3.69	0.44–12.8	0.87–17.7

Means with same letter in the same column at every roasting time are not significantly different according to Duncan's Multiple Range Test ($P > 0.05$).

[a] DMP, dimethylpyrazine; TrMP, trimethylpyrazine; TMP, tetramethyl-pyrazine.

[b] Prepared from dephenolized cocoa powder.

[c] Prepared from undephenolized cocoa powder.

Formation of various groups of pyrazine has been reported as a result of thermal treatment via the Maillard reaction, involving amine of amino acids and carbonyl of reducing sugars (Davies, Wedzicha, & Gillard, 1997; Hofmann, 1998; Hofmann, Munch, & Schieberle, 2000; Hofmann & Schieberle, 2000). It has been demonstrated that time, temperature, pH, reactant concentration and water activity are important variables in determining the nature and quantity of the products (Shibamoto & Bernhard, 1977).

Polyphenol concentration in cocoa liquor significantly affected pyrazine formations during roasting. The first two treatments, at 41 and 71 g kg^{-1} polyphenol, which were prepared from dephenolized cocoa powder, indicated a significant decrease in pyrazines formed with an increase in polyphenol concentration. Reduction was shown mainly on 2,3-DMP at the first 15 min of roasting and on 2,5-DMP, 2,3-DMP and 2,3,5,6-TMP at 25 min of roasting; however, there was no more significant reduction after 35 min of roasting. In the 58, 116, 143 and 170 g polyphenol kg^{-1} samples, which were prepared from fermented cocoa bean, as the polyphenol concentration increased, the formations of 2,3-DMP, 2,3,5-TrMP and 2,3,5,6-TMP at all roasting periods, and 2,5-DMP at 25 min of roasting were found to be significantly reduced ($P < 0.05$).

Low formations of pyrazine, occurring in the first to second treatments as compared to the third to fifth treatments, may be due to losses in pyrazine precursors during removal of polyphenol from the former. The total free amino acids (Table 2) and reducing sugars (Table 3) in unroasted dephenolized liquor (treatment I) were found to be 10.6 g kg^{-1} and 19.7 g kg^{-1}, respectively, which were significantly lower than that in undephenolized liquer (treatment III) (28.8 g kg^{-1} and 52.6 g kg^{-1}, respectively).

The above results show that it is indeed possible that polyphenol in cocoa liquor decreases pyrazine formation during roasting. There is no previous research which has reported this finding. This study also reveals that the reduction level was different for different pyrazines (Figs. 1 and 2). Reduction against formation of

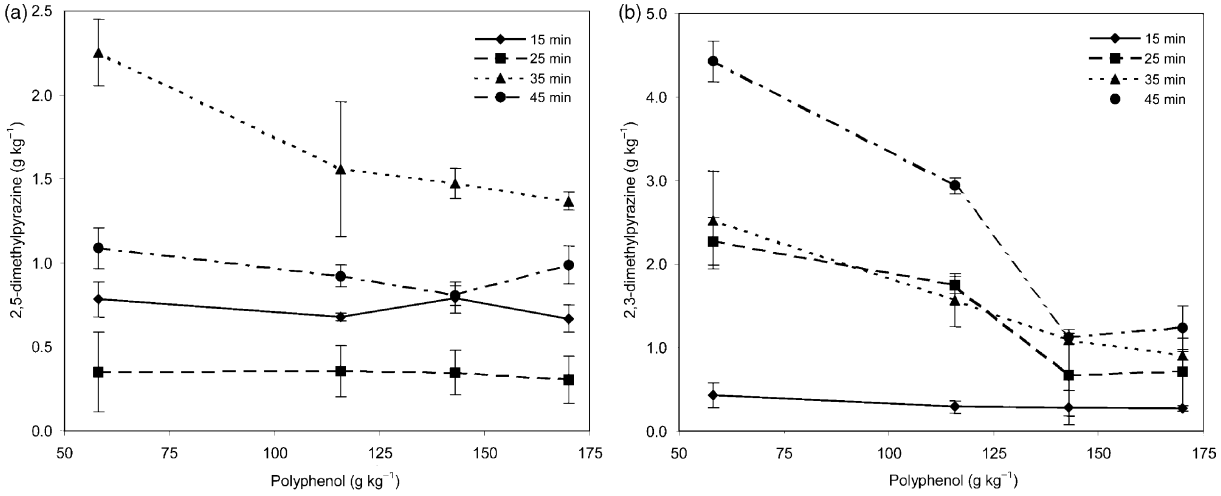

Fig. 1. Effects of polyphenol concentration on 2,5-dimethylpyrazine (a) and 2,3-dimethylpyrazine (b) concentrations during roasting.

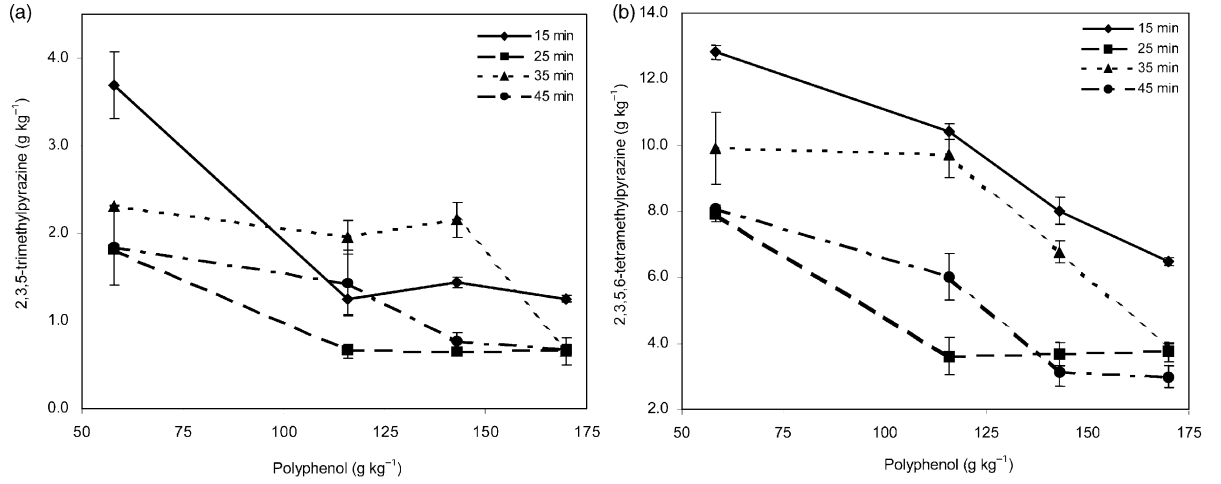

Fig. 2. Effects of polyphenol concentration on 2,3,5-trimethylpyrazine (a) and 2,3,5,6-tetramethylpyrazine (b) concentrations during roasting.

2,5-DMP was not significant over roasting times, except at 35 min at a polyphenol concentration of 116 g kg^{-1}. Formation of 2,3-DMP was reduced at roasting times of 25, 35 and 45 min, starting at a polyphenol concentration of 116 g kg^{-1} and reaching a maximum level at 143 g kg^{-1}. Significant reductions of formations of 2,3,5-TrMP and 2,3,5,6-TMP were observed to occur throughout the roasting times; the reduction started at 116 g kg^{-1} and reached maximum level at 116–170 g polyphenol kg^{-1}.

These results also showed that a maximum pyrazine production formation occurred at different roasting times; high concentrations of both 2,5-DMP and 2,3-DMP were formed at longer roasting time, i.e. for 35 and 45 min, respectively. However, both 2,3,5-TrMP and 2,3,5,6-TMP were formed at high concentrations in a shorter roasting time, i.e. at 15 min (Figs. 1 and 2).

Bindings of polyphenol on pyrazine and its precursors may be responsible for the reduction of pyrazine formations during roasting; however, further research is still needed to verify this suggestion.

3.2. Binding effect of polyphenol on pyrazine precursors

The suggestion is supported by the fact that, prior to roasting, concentrations of free amino acids and reducing sugars, which were the precursors for pyrazine formation, were decreased with the increase of polyphenol concentration (Fig. 3). According to Hagerman (1992), the phenolic hydroxyl group is an excellent hydrogen bond donor and forms strong hydrogen bonds with the amide carbonyls of the peptide backbone. Kattenberg and Kemmink (1993) also found that enzymatic oxidation of polyphenols produces quinones, which are very reactive agents. They can react further with amino acids and proteins, or polymerize with each other to form a higher molecular weight the so-called "condensed tannin", whereas at molecular weights

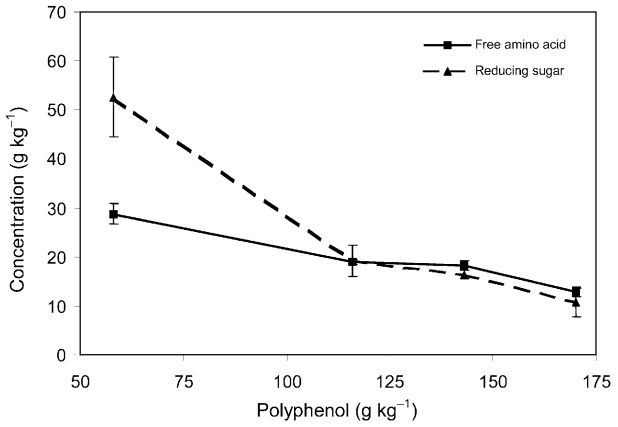

Fig. 3. Effects of polyphenol concentration on free amino acid and reducing sugar concentrations prior to roasting.

above 3000 they form complexes with protein through hydrogen bonding.

3.3. Binding effect of polyphenol on pyrazines formed

There is a possibility that pyrazines formed during roasting were bound to polyphenols. As has been mentioned previously, polyphenols have a strong inclination to combine with other compounds, both in unoxidized and oxidized forms. Our previous study found that total polyphenol decreases by only 3% during 45 min of roasting at 120 °C of Ghanaian cocoa liquor (Misnawi, Jinap, Jamilah, & Nazamid, 2002b), suggesting a great possibility that pyrazine-polyphenol binding might occur.

3.4. Free amino acid degradation

Cocoa aroma compounds, including furans, thiazoles, oxazoles, pyrroles, pyridines and alkyl pyrazines, are produced during roasting through Maillard (non-enzymatic) browning (Bonvehi & Coll, 2002; Hoskin & Dimick, 1994; Jinap et al., 1998). The Maillard reaction mainly involves the reaction between α-amino acids and reducing sugars. This study detected 17 amino acids present in unroasted cocoa liquor and the amino acids were degraded by as much as 76% during 45 min of roasting at 120 °C (Table 2). According to Bonvehi and Coll (2002) the degradation of amino acids during roasting could be the result of Maillard reaction and oxidative deamination. They found degradation of amino acids during 3 min of roasting of cocoa powder at 125 and 135 °C at 76 and 84%, respectively.

Hydrophobic amino acids, namely alanine, tyrosine, valine, iso-leucine, leucine and phenylalanine, along with hydrophilic peptides and reducing sugars, are specific precursors for cocoa aroma formation (Barel et al., 1985; Biehl & Voigt, 1994; Mohr et al., 1976; Voigt, Biehl, Heinrichs, Kamaruddin, Gaim Marsoner, & Hugi, 1994a; Voigt, Voigt, Heinrichs, Wrann, & Biehl, 1994b; Voigt, Wrann, Heinrichs, & Biehl, 1994c; Ziegleder & Biehl, 1988). Degradation rates of these hydrophobic amino acids in cocoa liquor containing 58 g polyphenol kg^{-1} were similar to that of total amino acid, i.e. at 76%. However, increase in polyphenol concentration significantly decreased the degradation rates of hydrophobic amino acids. The degradation rates of 116, 143 and 170 g polyphenol kg^{-1} were 61, 56 and 45%, respectively. These results coincide with the reduction of pyrazine formations by polyphenol.

Higher degradation rates of the hydrophobic amino acids than of total amino acids were observed at 143 and 170 g polyphenol kg^{-1}; the degradation of hydrophobic amino acids were 56 and 45%, respectively, which were significantly higher than that of total amino acids at 36 and 26%. There are two possible causes of this phenomenon: either the hydrophobic amino acid was consumed to a greater extent during pyrazine synthesis and deamination, or the polyphenol binding was preferred on hydrophobic amino acids. According to Hagerman and Butler (1981) polyphenol has a propensity to form complexes with proteins, polysaccharides and alkaloids. There are five potential types of interaction between polyphenol and protein: hydrogen bonding, π-bonding, hydrophobic, ionic and covalent linkage. Charlton et al. (2002) reported that intermolecular binding (polyphenol-peptide) is dominated by stacking of polyphenols onto planar hydrophobic surfaces and strengthened by multiple cooperative binding of polyphenolic rings. From this point of view, the second possibility is favourable.

3.5. Sugar degradation

Fructose was found to be the major reducing sugar in cocoa beans, comprising 92% of total reducing sugar or 64% of total sugar (Table 3). This is in agreement with reports by Redgwell, Trovato, and Curti (2003), Puziah et al. (1998a) and Bonbehi and Coll (2002) that fructose and glucose were the main reducing sugars present in cocoa beans. The percentage of fructose obtained in this study was higher than those found by Bonbehi and Coll (2002) and Puziah et al. (1998a) at 61 and 72% of total reducing sugar, respectively. The difference may be due to the sources of cocoa bean and the methods of analysis used. However, Reineccius, Anderson, Kavanagh, and Keeney (1972), using Ghanaian bean, which is similar to the bean used in this study, found that the fructose was 87% of total reducing sugars.

Both fructose and glucose were degraded in great quantities (as much as 83 and 79%, respectively); this is slightly lower than the reducing sugar degradation found by Bonvehi and Coll (2002) at 84%. However,

Table 2
Effects of polyphenol concentration during roasting on free amino acid concentrations of cocoa liquor

Polyphenol (g kg^{-1})	Roasting time (min)	Asparagine			Glutamine			Serine			Glycine			Histidine			Arginine			Threonine		
		g kg^{-1}	Degradation[a]		g kg^{-1}	Degradation[a]		g kg^{-1}	Degradation[a]		g kg^{-1}	Degradation[a]		g kg^{-1}	Degradation[a]		g kg^{-1}	Degradation[a]		g kg^{-1}	Degradation[a]	
			g kg^{-1}	%		g kg^{-1}	%		g kg^{-1}	%		g kg^{-1}	%		g kg^{-1}	%		g kg^{-1}	%		g kg^{-1}	%
41[b]	0	0.443$^{\pm 0.229}$			0.364$^{\pm 0.0335}$			0.604$^{\pm 0.0502}$			0.245$^{\pm 0.345}$			0.092$^{\pm 0.0071}$			0.510$^{\pm 0.114}$			0.201$^{\pm 0.0376}$		
58[c]	0	0.547$^{\pm 0.054}$			0.786$^{\pm 0.0268}$			0.906$^{\pm 0.0742}$			0.188$^{\pm 0.0150}$			13.01$^{\pm 0.989}$			0.923$^{\pm 0.118}$			0.813$^{\pm 0.0197}$		
	45	0.110$^{\pm 0.008}$	0.438	80	0.068$^{\pm 0.0049}$	0.717	91	0.215$^{\pm 0.0438}$	0.690	76	0.065$^{\pm 0.0039}$	0.124	66	3.33$^{\pm 0.4276}$	9.72	74	0.235$^{\pm 0.0654}$	0.688	74	0.064$^{\pm 0.0095}$	0.749	92
116[c]	0	0.313$^{\pm 0.0557}$			0.294$^{\pm 0.0213}$			0.549$^{0.0410}$			0.114$^{\pm 0.0084}$			12.23$^{\pm 0.7692}$			0.178$^{\pm 0.0167}$			0.734$^{\pm 0.0607}$		
	45	0.107$^{\pm 0.0212}$	0.206	66	0.088$^{\pm 0.0118}$	0.206	70	0.247$^{\pm 0.0068}$	0.302	55	0.041$^{\pm 0.0033}$	0.074	65	4.11$^{\pm 0.6269}$	8.14	66	0.078$^{\pm 0.0045}$	0.100	56	0.034$^{\pm 0.0098}$	0.700	95
143[c]	0	0.185$^{\pm 0.0074}$			0.238$^{\pm 0.0218}$			0.496$^{\pm 0.0194}$			0.100$^{\pm 0.0085}$			12.3$^{\pm 1.10}$			0.158$^{\pm 0.0070}$			0.727$^{\pm 0.0364}$		
	45	0.075$^{\pm 0.0102}$	0.109	59	0.083$^{\pm 0.0079}$	0.155	65	0.221$^{\pm 0.0054}$	0.276	56	0.039$^{\pm 0.0012}$	0.061	61	9.4$^{\pm 0.924}$	2.92	25	0.064$^{\pm 0.0059}$	0.094	60	0.024$^{\pm 0.0050}$	0.703	97
170[c]	0	0.176$^{\pm 0.0022}$			0.186$^{\pm 0.0023}$			0.443$^{\pm 0.0023}$			0.036$^{\pm 0.0046}$			8.38$^{\pm 0.781}$			0.132$^{\pm 0.0019}$			0.044$^{\pm 0.0012}$		
	45	0.073$^{\pm 0.0056}$	0.103	58	0.076$^{\pm 0.0009}$	0.110	59	0.204$^{\pm 0.0166}$	0.239	54	0.013$^{\pm 0.0002}$	0.022	61	7.16$^{\pm 0.501}$	1.22	15	0.061$^{\pm 0.0076}$	0.071	54	0.014$^{\pm 0.0002}$	0.030	68

Polyphenol (g kg^{-1})	Roasting time (min)	Alanine			Proline			Tyrosine			Valine			Methionine			Cystine			Iso-Leucine		
		g kg^{-1}	Degradation[a]		g kg^{-1}	Degradation[a]		g kg^{-1}	Degradation[a]		g kg^{-1}	Degradation[a]		g kg^{-1}	Degradation[a]		g kg^{-1}	Degradation[a]		g kg^{-1}	Degradation[a]	
			g kg^{-1}	%		g kg^{-1}	%		g kg^{-1}	%		g kg^{-1}	%		g kg^{-1}	%		g kg^{-1}	%		g kg^{-1}	%
41[b]	0	0.755$^{\pm 0.239}$			0.654$^{\pm 0.132}$			0.511$^{\pm 0.0485}$			0.643$^{\pm 0.0400}$			0.234$^{\pm 0.0389}$			0.318$^{\pm 0.451}$			0.282$^{\pm 0.0398}$		
58[c]	0	1.19$^{\pm 0.0906}$			0.952$^{\pm 0.133}$			0.810$^{\pm 0.0326}$			1.01$^{\pm 0.0842}$			0.370$^{\pm 0.0494}$			0.088$^{\pm 0.0093}$			0.558$^{\pm 0.0428}$		
	45	0.216$^{\pm 0.086}$	0.971	82	0.317$^{\pm 0.0574}$	0.635	67	0.213$^{\pm 0.0063}$	0.597	74	0.234$^{\pm 0.0132}$	0.771	77	0.141$^{\pm 0.0146}$	0.228	62	0.025$^{\pm 0.0007}$	0.063	72	0.294$^{\pm 0.0360}$	0.263	47
116[c]	0	0.149$^{\pm 0.0174}$			0.323$^{\pm 0.121}$			0.561$^{\pm 0.0086}$			0.658$^{\pm 0.0413}$			0.249$^{\pm 0.0270}$			0.096$^{\pm 0.0107}$			0.126$^{\pm 0.0090}$		
	45	0.036$^{\pm 0.0056}$	0.112	75	0.130$^{\pm 0.0478}$	0.192	59	0.139$^{\pm 0.0014}$	0.423	75	0.198$^{\pm 0.0120}$	0.460	70	0.105$^{\pm 0.0054}$	0.145	58	0.027$^{\pm 0.0025}$	0.069	72	0.051$^{\pm 0.0039}$	0.075	60
143[c]	0	0.145$^{\pm 0.0162}$			0.215$^{\pm 0.0427}$			0.437$^{\pm 0.0141}$			0.484$^{\pm 0.0199}$			0.189$^{\pm 0.0159}$			0.096$^{\pm 0.0102}$			0.126$^{\pm 0.0082}$		
	45	0.036$^{\pm 0.0001}$	0.109	75	0.101$^{\pm 0.0065}$	0.114	53	0.121$^{\pm 0.0114}$	0.316	72	0.169$^{\pm 0.0059}$	0.316	65	0.083$^{\pm 0.0047}$	0.106	56	0.032$^{\pm 0.0026}$	0.064	67	0.064$^{\pm 0.0008}$	0.062	49
170[c]	0	0.144$^{\pm 0.0011}$			0.294$^{\pm 0.0748}$			0.312$^{\pm 0.0271}$			0.311$^{\pm 0.0039}$			0.129$^{\pm 0.0049}$			0.031$^{\pm 0.0004}$			0.122$^{\pm 0.0015}$		
	45	0.081$^{\pm 0.0074}$	0.063	44	0.235$^{\pm 0.0366}$	0.059	20	0.116$^{\pm 0.0014}$	0.196	63	0.140$^{\pm 0.0017}$	0.171	54	0.062$^{\pm 0.0040}$	0.068	53	0.017$^{\pm 0.0007}$	0.014	45	0.073$^{\pm 0.0018}$	0.049	40

Polyphenol (g kg^{-1})	Roasting time (min)	Leucine			Phenylalanine			Lysine			Total hydrophobic amino acid			Total amino acid		
		g kg^{-1}	Degradation[a]		g kg^{-1}	Degradation[a]		g kg^{-1}	Degradation[a]		g kg^{-1}	Degradation[a]		g kg^{-1}	Degradation[a]	
			g kg^{-1}	%		g kg^{-1}	%		g kg^{-1}	%		g kg^{-1}	%		g kg^{-1}	%
41[b]	0	3.51$^{\pm 0.308}$			1.14$^{\pm 0.484}$			0.131$^{\pm 0.0157}$			6.84$^{0.35}$			10.6$^{\pm 1.25}$		
58[c]	0	5.00$^{\pm 0.317}$			1.31$^{\pm 0.223}$			0.301$^{\pm 0.0343}$			9.87$^{0.665}$			28.8$^{\pm 2.06}$		
	45	1.24$^{\pm 0.196}$	3.758	75	0.181$^{\pm 0.0301}$	1.13	86	0.017$^{\pm 0.0042}$	0.284	94	2.38$^{0.136}$	7.49	76	6.97$^{\pm 0.515}$	21.8	76
116[c]	0	1.68$^{\pm 0.134}$			0.491$^{\pm 0.0439}$			0.137$^{\pm 0.0347}$			3.67$^{0.098}$			18.9$^{\pm 0.530}$		
	45	0.793$^{\pm 0.0299}$	0.890	53	0.227$^{\pm 0.0122}$	0.264	54	0.023$^{\pm 0.0052}$	0.114	83	1.44$^{0.0098}$	2.22	61	6.44$^{\pm 0.619}$	12.5	66
143[c]	0	1.69$^{\pm 0.145}$			0.489$^{\pm 0.0286}$			0.138$^{\pm 0.0347}$			3.37$^{0.118}$			18.2$^{\pm 1.042}$		
	45	0.81$^{\pm 0.0142}$	0.881	52	0.273$^{\pm 0.0148}$	0.216	44	0.022$^{\pm 0.0061}$	0.115	84	1.47$^{0.0240}$	1.90	56	11.6$^{\pm 0.915}$	6.62	36
170[c]	0	1.480$^{\pm 0.0186}$			0.451$^{\pm 0.0293}$			0.178$^{\pm 0.0360}$			2.82$^{0.0533}$			12.9$^{\pm 0.847}$		
	45	0.826$^{\pm 0.0100}$	0.653	44	0.302$^{\pm 0.0227}$	0.149	33	0.023$^{\pm 0.0019}$	0.156	88	1.54$^{0.0269}$	1.281	45	9.47$^{\pm 0.511}$	3.38	26

[a] Degradation (g kg^{-1}) = conc. before roasting — conc. after roasting. Degradation (%) $= \frac{\text{degradation in conc.}}{\text{conc. before roasting}} \times 100$.
[b] Prepared from dephenolized cocoa powder.
[c] Prepared from undephenolized cocoa powder.

Table 3
Effects of polyphenol concentration during roasting on sugar concentrations of cocoa liquor

Polyphenol (g kg^{-1})	Roasting time (min)	Fructose			Glucose			Sucrose		
		g kg^{-1}	Degradation[a]		g kg^{-1}	Degradation[a]		g kg^{-1}	Degradation[a]	
			g kg^{-1}	%		g kg^{-1}	%		g kg^{-1}	%
41[b]	0	$16.3^{\pm 0.92}$			$3.41^{\pm 0.77}$			$2.12^{\pm 0.18}$		
58[c]	0	$48.6^{\pm 7.81}$			$3.95^{\pm 0.62}$			$23.1^{\pm 0.83}$		
	45	$8.25^{\pm 0.44}$	40.4	83	$0.84^{\pm 0.59}$	3.11	79	$0.46^{\pm 0.20}$	22.6	98
116[c]	0	$15.2^{\pm 2.82}$			$3.97^{\pm 0.44}$			$21.4^{\pm 2.14}$		
	45	$4.91^{\pm 0.81}$	10.3	68	$1.20^{\pm 0.54}$	2.77	70	$5.82^{\pm 0.66}$	15.6	73
143[c]	0	$12.7^{\pm 1.18}$			$3.66^{\pm 0.34}$			$18.3^{\pm 3.53}$		
	45	$9.80^{\pm 0.32}$	2.92	23	$1.09^{\pm 0.57}$	2.57	70	$6.28^{\pm 0.81}$	12.0	66
170[c]	0	$8.30^{\pm 3.06}$			$2.51^{\pm 0.22}$			$6.10^{\pm 0.42}$		
	45	$6.18^{\pm 0.54}$	2.12	26	$0.99^{\pm 0.22}$	1.52	61	$5.97^{\pm 0.65}$	0.13	2

[a] Degradation (g kg^{-1}) = conc. before roasting — conc. after roasting. Degradation (%) $= \frac{\text{degradation in conc.}}{\text{conc. before roasting}} \times 100$.
[b] Prepared from dephenolized cocoa powder.
[c] Prepared from undephenolized cocoa powder.

computation of sugar losses during roasting in the same table showed that polyphenol concentration in cocoa liquor significantly decreased the degradation of the three sugars.

The degradations of fructose at 58, 116, 143 and 170 g polyphenol kg^{-1} were 83, 68, 23 and 26% respectively. However those of glucose were 79, 70, 70 and 61%. These results indicate that the reduction effect of polyphenol on reducing sugar degradation during roasting was higher for fructose than for glucose. This is because fructose was more bound to polyphenol than was glucose; however, BeMiller and Whistler (1996) reported that glucose undergoes browning reaction faster that does fructose. The former suggestion indicated that 70% of fructose was bound to polyphenol as the polyphenol concentration in the liquor increased to 116 g kg^{-1} (Fig. 4), whereas glucose was not bound at all. At 170 g polyphenol kg^{-1}, the bound fructose increased to more than 80%; however, that of glucose was ±35%.

Reduction of the degradation rate of fructose and glucose could explain the reduction of pyrazine formation, as previously discussed. However, degradation of sucrose during roasting is frequently ignored by researchers since it is not directly involved in aroma synthesis through Maillard reactions. Bonvehi and Coll (2002) found that degradation of sucrose during roasting was negligible; thus, they concluded that sucrose concentration in unroasted cocoa beans was not an important variable in the generation of alkylpyrazines. However, this study showed that 98% of the sucrose in cocoa liquor were degraded during 45 min of roasting. The degradation of sucrose was possibly associated with formation of insoluble complexes, induced by the high temperature during roasting. According to Redgwell et al. (2003), cocoa bean roasting did promote an interaction between polysaccharides, proteins, polyphenols and Maillard products.

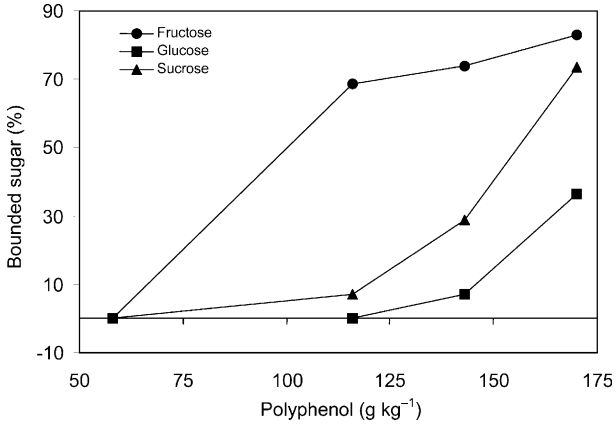

Fig. 4. Effects of polyphenol concentration on bounded sugar prior to roasting Note: Data were calculated from Table 3.

$$\text{Bounded sugar (\%)} = \frac{\text{concentration at 58g kg}^{-1} - \text{concentration at X}_i}{\text{concentration at 58g kg}^{-1}} \times 100$$

X_i = 58, 116, 143 and 170 g polyphenol kg^{-1}.

4. Conclusion

Increase in polyphenol concentration of cocoa liquor reduced pyrazine formation during roasting. The reduction in the formations of 2,3,5-TrMP and 2,3,5,6-TMP occurred throughout the roasting period, starting at 116 g polyphenol kg^{-1} and reaching maximum reduction at 116–170 g polyphenol kg^{-1}. The reduction against 2,5-DMP was not significant over roasting times, except at 35 min; however, formation of 2,3-DMP was reduced at 25, 35 and 45 min. The increase in polyphenol caused free amino acids and reducing sugars to be less available for the pyrazine formation due to the polyphenol binding with these compounds. Further-

more, the presence of polyphenols in cocoa liquor may bind part of the pyrazine formed during roasting, thus reducing pyrazine concentration.

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