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Extended Abstract

The Evaluation of Desirable Aromatic Components and Polyphenolic Compounds in the Process of Cocoa Powder Production

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Abstract

Cocoa powder and chocolate are one of the most popular ingredients used in food products. The flavor of these products plays an important role in the consumer's popularity and acceptance. Pyrazines are one of the main constituents of the heterocyclic group of volatiles and key components of odor in cocoa flavor. In order to evaluate the effect of the process of cocoa powder production on the composition of desirable fragrances, the stages of cocoa powder production line were sampled from Cameroon seeds and the samples were analyzed and analyzed by gas chromatography-mass spectrometry. The results showed that the alkalization stage is one of the important and effective steps on the flavor of cocoa powder. Therefore, the effect of three types of alkali (NaOH, K_2CO_3 and NH_4HCO_3) was investigated at different concentrations. The data indicated that the polyphenolac and alkylpyrazines amounts significantly changed. Non-alkaline powder had higher polyphenol and tetramethylpyrazine to trimethylpyrazine (TMP/TrMP) ratios than alkaline cocoa powder. In addition, in alkaline cocoa samples, the amounts of polyphenol and alkylpyrazine decreased with increasing alkali concentration. At the same concentration, alkalization with a NaOH solution produced a higher polyphenol content and TMP/TrMP ratio, but lower alkylpyrazine value than that with a K_2CO_3 solution. Light alkalization cocoa powder with K_2CO_3 solution (pH 6.89) produced the highest amount of alkylpyrazine

Keywords: Alkalization, Alkyl pyrazine, Cacao powder, Polyphenol, Roasting

Introduction

Cocoa (*Theobroma cacao* L.) beans are among the crops of top economic interest in several countries. They are widely used to supply a wide range of products. The cocoa flavors are made from over 400 compounds, among which there are aldehydes and pyrazines (Li *et al.*, 2014 ; Serra Bonvehí & Ventura Coll, 2002). The alkylpyrazines are the main class of

heterocyclic compounds and the key odor components in cocoa aroma (Aprotosoaie, Luca, & Miron, 2016; Serra Bonvehí & Ventura Coll, 2002). Among these alkylpyrazines compounds, trimethylpyrazine and tetramethylpyrazine are the most important ones (Aprotosoaie, Luca, & Miron, 2016). The alkaline treatment was devised by Van Houten, and this process, known as ‘‘Dutching’’, is mostly offered partially omitting acetic acid created during fermentation and raise the pH as well as to develop a darker color, lessening the bitterness, and enhance the suspension of the cocoa particles in the aqueous media. (Giacometti, Jolić, & Josić, 2015; Kothe, Zimmermann, & Galensa, 2013; Mazor Jolić, Radojčić Redovniković, Marković, Ivanec Šipušić, & Delonga, 2011). Alkalization, occurring during roasting, can raise the reaction between free amino groups of amino acids and reducing sugars, leading to the production of the aroma compounds of cocoa (Bonvehí & Coll, 2000; Li *et al*., 2014; Serra Bonvehí & Ventura Coll, 2002). One of the main factors affecting the quantity and quality of polyphenols in cocoa powder production is alkalization process. Miller’s studies show that alkalization reduces polyphenols and their antioxidant activity (Miller *et al*., 2008). The most significant characteristic of polyphenol is its tendency to form complexes with polysaccharides, proteins, and alkaloids. According to the evidence, not only does the presence of polyphenol in cocoa powder make bitter, astringent, and green flavors and interaction with protein, but it also causes interaction with flavor precursors, such as pyrazines (Misnawi *et al*., 2004). The previous literature showed that a number of researches have been conducted on cocoa alkalization and roasting process, but the changes of the flavor volatile compounds of cocoa occurring at different stages of the cocoa powder production process have not been investigated yet. Since alkalization treatments are commonly used in the processing of cocoa powder, the attributes of cocoa powder (polyphenol and alkylpyrazine amount) can be controlled via various parameters of alkalization in order to meet the demands of customers. The main aim of the present study was to study the changes of the flavor volatile compounds of cocoa in the process of producing cocoa powder at each stage (i.e., cacao beans, alkalization stage, roasting stage, milling stage, pressing stage, cocoa powder) at an industrial factory in Iran and to determine the alkalization effects. The cocoa nibs were alkalized under three types of alkali solution (sodium hydroxide, potassium carbonate, and ammonium bicarbonate) at their different concentrations and combinations. Then, the influences of performed alkalizing conditions on the alkylpyrazine and polyphenol contents and pH of cocoa powder were investigated.

Materials and methods

Stage 1: Cameroon cacao beans (*Forastero* cultivar, Cameroon) were used in the current study. Cocoa powder production was carried out by a factory located in Tabriz, Iran. After the alkaline treatment with potassium carbonate, cacao beans were roasted. The milling was done under a two-stage process. The cocoa liquor was exited from containers and pressed (by hydraulic press) and converted to the cocoa powder and cocoa butter. The samples obtained from the raw cacao beans, alkalized cacao beans, roasted cacao beans, milled cacao beans, pressed liquor, and cocoa powder in order to find out the effects of the production process on the cocoa powder flavor.

Stage 2: Cameroon cacao beans underwent cleaning, drying at 100 ºC, shelling and crushing to convert to cocoa nibs. The resultant nibs, then, were alkalized with the solutions of NaOH, K_2CO_3 and NH_4HCO_3 at their different concentrations and combinations. The alkalized cocoa nibs were roasted at 130 ºC for 20 min. Finally, the samples were pulverized in a laboratory mill to obtain the cocoa powders. Total

polyphenols content was evaluated with the Folin-Ciocalteu procedure matching to (Li *et al*., 2014). Absorption was evaluated at 760 nm in a 722 spectrophotometer (HACH, DR/ 4000U, USA). Finally, gallic acid was exerted as a standard and the total polyphenols amount was reported as gallic acid equivalent in mg/g. The aromatic compounds were identified and confirmed by GC-MS. For the purpose of GC-MS analysis, an Agilent 7890 A gas chromatograph coupled to a 5975A mass spectrometer using a HP-5 MS capillary column (5% phenyl methylpolysiloxane, 30 m length, 0.25 mm i.d., 0.25 μm film thickness) was used.

Statistical analysis

Statistics on a completely randomized design (CRD) were performed by the analysis of variance (ANOVA) procedure, using Mstatc software version 1.1.0 (Michigan State University). The Duncan test was applied to compare the difference among mean values at a significant level of 0.01 ($P\leq0.01$). All experiments were carried out in three replications.

Results and discussion

Many volatile aromatic materials included alcohols, acids, aldehydes, esters, ketones, pyrazines, and pyrrole compounds (Table 1), which, according to previous studies, are the most effective compounds in creating the cocoa products' flavor. Therefore, the investigation on the production stages was focused on these compounds. In the case of alkalized cocoa powder, the most significant processes were the degree of roasting temperature to which the cacao beans are exposed and the varying degrees of alkalization. Pyrazine compounds are the key type of heterocyclic volatiles and the main components forming the cocoa aroma. They exhibit nutty, roasty, green, and earthy aromas (Hashim & Chaveron, 1994; Huang & Barringer, 2010; Serra Bonvehí & Ventura Coll, 2000). Most of the pyrazine compounds in the current study were formed during the roasting stage and maintained in the subsequent processes, thus found in cocoa powder compounds. 2, 3, 5, 6-tetramethylpyrazine was detected in the cacao beans (0.5%) and increased to 2.69% in the cocoa powder. The analysis of variance (ANOVA) showed that the total polyphenol content of the cocoa powders changed significantly ($P\leq0.01$) with changing the type and concentration of alkali solution. As shown in Table (2), the lowest total value of polyphenol of the alkalized samples was observed in the A9 samples with 3% K_2CO_3 solution. The analysis of variance (ANOVA) showed that the alkylpyrazine content (2-methylpyrazine, 2, 3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,3,5-trimethylpyrazine, and 2,3,5,6-tetramethylpyrazine) of the cocoa powders changed significantly ($P\leq0.01$) with changing the type and concentration of alkali solution. The cocoa powder alkalized via 1% (w/w) of K_2CO_3 solution had the highest value of total alkylpyrazine and tetramethylpyrazine content (44047.40 and 16767.10 ppb, respectively, Table 2). While, the cocoa powder alkalized via 3% (w/w) of NaOH solution had the lowest value of total alkylpyrazine and tetramethylpyrazine content.

Table 1. Main cocoa on-odor volatile compounds of samples in different stages of production*

Flavour Compounds	R.T*	Content of volatile compounds in total volatile flavour components (w/w %)					
		Cacao bean	Alkalization stage	Roasting stage	Milling stage	Pressing stage	Cocoa powder
Alcohols							
Linalool	9.49	0.05±0.01^{c}	0.50±0.15^{b}	1.05±0.20^{a}	1.09±0.10^{a}	1.13±0.13^{a}	1.38±0.18^{a}
2- Hexanol	4.61	0.03±0.02^{a}	0.03±0.02^{a}	0.05±0.04^{a}	0.05±0.04^{a}	0.06±0.04^{a}	0.05±0.01^{a}
1-Hexanol	4.55	0.02±0.01^{a}	0.03±0.02^{a}	0.05±0.04^{a}	0.06±0.04^{a}	0.06±0.04^{a}	0.04±0.02^{a}
Aldehydes							
Benzaldehyde	6.04	0.38±0.16^{d}	0.56±0.20^{d}	0.80±0.32^{cd}	1.40±0.20^{bc}	1.70±0.20^{b}	4.67±0.33^{a}
Phenylacetaldehyde	8.05	0.10±0.02^{d}	1.10±0.10^{c}	2.02±0.20^{b}	2.17±0.17^{b}	3.97±0.40^{a}	4.21±0.30^{a}
5-methyl -2-Phenyl -2-hexenal	19.81	-	-	0.42±0.30^{b}	0.96±0.30^{b}	2.68±0.10^{a}	2.16±0.30^{a}
2-hexenal	36.06	0.16± 0.05^{c}	-	0.40±0.25^{bc}	1.30± 0.20^{a}	0.70±0.30^{bc}	0.92±0.35^{ab}
Esters							
Isoamyl acetate	4.29	0.23± 0.10^{b}	-	1.66± 0.10^{a}	1.60± 0.20^{a}	1.23±0.23^{ab}	1.53± 0.23^{a}
2-Phenethyl acetate	13.78	0.17± 0.10^{c}	0.18± 0.10^{c}	0.55± 0.67^{c}	1.70± 0.02^{b}	2.50± 0.15^{a}	2.68± 0.20^{a}
Isobutyl benzoate	17.40	0.38± 0.07^{b}	-	3.30± 0.30^{a}	3.50± 0.25^{a}	3.60± 0.20^{a}	3.22± 0.22^{a}
Ethyl laurate	22.21	0.07± 0.02^{c}	-	1.10± 0.10^{b}	1.28±0.28^{ab}	1.06± 0.06^{b}	1.62± 0.20^{a}
ketones							
2-Heptanone	4.59	-	-	0.38± 0.10^{a}	0.28± 0.15^{a}	0.27± 0.10^{a}	0.26± 0.20^{a}
Acetophenon	8.64	-	-	0.53± 0.21^{a}	0.50± 0.15^{a}	0.78± 0.20^{a}	0.77± 0.20^{a}
Methyl heptyl ketone	9.28	0.14± 0.07^{b}	-	0.97± 0.40^{a}	0.95± 0.05^{a}	1.00± 0.10^{a}	0.90± 0.30^{a}
2-Pentadecanone	29.30	-	-	1.13± 0.15^{b}	0.98± 0.20^{b}	1.04± 0.20^{b}	1.52± 0.20^{a}
Pyrazines							
2-Ethyl-3-methylpyrazine	6.90	-	0.21± 0.20^{b}	0.23± 0.10^{b}	0.25± 0.20^{a}	0.31± 0.10^{a}	0.26± 0.20^{a}
2,3,5,6 Tetramethylpyrazine	9.15	0.50± 0.10^{c}	0.30± 0.20^{c}	1.96± 0.20^{b}	2.90± 0.20^{a}	3.00± 0.10^{a}	2.69± 0.20^{a}
2,5-Dimethylpyrazine	13.46	-	-	0.30± 0.20^{a}	0.31± 0.10^{a}	0.37± 0.17^{a}	0.23± 0.13^{a}
2,3,5-trimethyl pyrazine	14.32	0.05± 0.04^{b}	-	0.09± 0.04^{b}	0.73± 0.20^{a}	0.74± 0.20^{a}	0.66± 0.33^{a}
pyrrole							
1H-Pyrrole, 1-pentyl	8.31	-	0.62±0.20	-	-	-	-
Indole (1H-Indole)	14.77	-	0.52± 0.20^{a}	-	-	0.29± 0.20^{a}	-

Values are mean ±standard deviation of three separate determinations.

* Retention Time (min)

Mean values with different superscript letters are significantly different ($P \leq 0.01$).

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Table 2. Alkylpyrazines* (ppb) with different pH values and Total polyphenol content (mg/g), and pH of cocoa powders

Sample	Alkylpyrazine*							pH	Total polyphenol (mg/g)
	2-MP	2,3-DMP	2,5-DMP	2,3,5-TrMP	2,3,5,6-TMP	TMP/TrMP	Total pyrazine		
A_0	1967.80±40.00^{d}	1353.20±25.20^{c}	1967.80±40.00^{c}	10475.00±20.00^{f}	12871.90±20.00^{c}	1.20±0.02^{a}	30611.80^{c}	5.35±0.10^{d}	16.75±0.25^{a}
A_1	1755.00±50.00^{efg}	1272.50±20.00^{d}	1755.00±20.00^{e}	9526.00±20.00^{g}	11671.90±30.00^{f}	1.10±0.06^{ab}	27801.90^{h}	7.02±0.02^{bc}	15.85±0.10^{b}
A_2	2797.50±60.00^{a}	2237.70±20.00^{a}	2797.50±10.00^{a}	16731.40±20.00^{a}	16767.10±30.00^{a}	1.00±0.00d^{a}	44047.40^{a}	6.89±0.01^{c}	14.34±0.10^{c}
A_3	1849.10±72.00^{def}	1292.70±20.00^{d}	1849.10 ±20.00^{e}	10743.40±20.00^{e}	11767.80±30.00^{e}	1.00±0.05^{cd}	29240.00^{f}	6.83±0.06^{c}	15.44±0.19^{b}
A_4	1876.30±15.10^{de}	1387.40±30.00^{c}	1876.30 ±40.00^{d}	12603.70±26.20^{c}	13279.20±20.00^{b}	1.00±0.02^{d}	32914.90^{d}	7.04±0.12^{bc}	15.74±0.30^{b}
A_5	2115.50±83.270^{c}	1668.00±20.00^{b}	2115.50 ±29.70^{cd}	16497.50±19.90^{b}	13327.80±20.00^{b}	0.80±0.01^{g}	37442.50^{e}	7.00±0.10^{bc}	13.86±0.13^{c}
A_6	1681.00±20.00^{g}	1123.30±20.00^{f}	1681.10 ±20.00^{f}	9021.70±20.00^{h}	10133.80±30.00^{h}	1.10±0.00^{bc}	24491.80^{i}	7.23±0.19^{b}	13.27±0.07^{d}
A_7	2274.60±20.00^{b}	1699.40±20.00^{b}	2274.60 ±30.00^{b}	12488.10±20.00^{d}	12345.60±30.00^{d}	0.93±0.06^{ef}	34141.70^{c}	6.94±0.03^{bc}	13.07±0.19^{d}
A_8	1303.40±45.80^{h}	1155.10±20.00^{ef}	1303.40 ±20.00^{g}	7339.00±30.00^{i}	7637.90±20.00^{i}	1.00±0.00^{de}	19664.60^{j}	8.28±0.10^{a}	10.95±0.20^{e}
A_9	1722.00±10.00^{fg}	1188.70±20.00^{e}	1747.00 ±30.00^{cd}	10721.20±20.00^{e}	10665.50±30.00^{g}	0.90±0.00^{f}	28134.30^{g}	8.38±0.29^{a}	10.49±0.09^{e}

Values are mean ± standard deviation of three separate determinations.
* MP, methylpyrazine; DMP, dimethylpyrazine; TrMP, trimethylpyrazine; TMP, tetramethylpyrazine.
Mean values with different superscript letters in the same column are significantly different ($P \leq 0.01$).

Conclusions

The roasting and alkalization stages are important variables which affect the development of the cocoa flavor. Cacao beans alkalization is one of the important factors of Maillard reaction with the alteration of pH and also advances an interaction between polysaccharides, proteins, polyphenols, and Maillard products. Many volatile compounds were detected in samples. Among them, pyrazines and esters were two major groups which existed in the cocoa volatiles. One of the main factors affecting the formation of flavor compounds during the Maillard reaction is the parameters of alkalization stage (pH, type and concentration of alkali); therefore, the flavor properties of cocoa powder could be modified by improving alkalization parameters.

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