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Optimization of cocoa beans roasting process using Response Surface Methodology based on concentration of pyrazine and acrylamide

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Abstract

Roasting is an important process that contribute to formation of flavour compounds in cocoa beans. Pyrazines, a by-product of Maillard reaction is one of the character impact compounds that contribute to unique cocoa flavour. Unfortunately during roasting, carcinogenic acrylamide are also produced through Maillard reaction. Therefore, this study was focussed on optimising the roasting conditions using Central Composite Design (CCD) to produce superior quality cocoa beans with high concentration of pyrazines and low concentration of acrylamide. The roasting conditions used were temperatures in the range of 110°C to 160°C and time ranging from 15 min to 40 min. Roasting conditions significantly ($p<0.005$) affect the concentration of pyrazines in cocoa beans. However, the RSM analysis shows that the concentration of acrylamide in the beans was not influenced by the roasting conditions. Statistical optimisation based on maximum pyrazines and minimum acrylamide at temperature of 116°C and a time of 23 min produced the desirable value of 0.73. Hence, the optimized roasting conditions were able to produce high quality cocoa beans.

Keywords
Cocoa bean
pyrizines
acrylamide
roasting
Maillard reaction

Introduction

Roasting is an important operation for the development of aroma and flavour of cocoa beans at temperatures of 110°C to 140°C and time range of 20 to 50 min (Jinap *et al*., 1998). Pyrizines has generally been agreed as the main compounds to indicate the quality and quantity of cocoa flavour (Misnawi and Teguh, 2010). Approximately 95 pyrizines have been identified in cocoa aroma and their concentration varied depending on the time and temperature of thermal treatment (Jinap *et al*., 1998).

The precursors of flavour are developed during fermentation and drying of cocoa beans, which include the free amino acid, peptides and reducing sugar, contributing to the development of cocoa specific aroma through Maillard reaction during roasting (Misnawi and Teguh, 2010). During the reaction, aroma precursors interact with each other to produce cocoa flavour such as alcohol, pyrizines, alcohols, ethers, furans, esters, aldehydes and pyroles (Jinap *et al*., 1998; Puziah *et al*., 1998). Unfortunately, during roasting, carcinogenic acrylamide are also produced through Maillard reaction.

The presence of acrylamide in roasted cocoa beans is due to interactions between asparagines as one of the reactants and dicarbonyl compounds as co-reactant in Strecker Degradation (Maillard reaction) during roasting (Sander *et al*., 2002; Mottram *et al*., 2002; Becalski *et al*., 2003; Yasuhara *et al*., 2003; Zyzak *et al*., 2003). Acrylamide is categorised as human carcinogen (IARC, 2004) and the exposure to high level of acrylamide may cause damage to the nervous system. Acrylamide has been detected in cocoa product at level up to 909 µg/kg (FAO/WHO, 2005). The safe level of acrylamide in cocoa product has yet to be determined. According to the Food and Drug Administration, the safe limit of acrylamide in fries for consumption is 0.077 ppm. Although the presence of acrylamide in food has not been shown to have effect on human health, it has been shown to be carcinogenic in laboratory animal, thus making acrylamide as a potential carcinogen to human.

Several researchers had reported on the effect of cocoa beans roasting conditions on the formation of cocoa flavour compounds (Finken, 1996;

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Swiechowski, 1996; Nebesny and Rutkowski, 1998; Misnawi *et al*., 2003; Ramli *et al*., 2006). However, optimisation of roasting condition in term of flavour compounds (methyl pyrizine, 2,3 di-methyl pyrizine, 2,3,4 tri-methyl pyrizine and tetra pyrizine) and formation of acrylamide have not been done. The objective of this study is to optimise roasting conditions (time and temperature) for pyrizines and the presence of acrylamide by using rResponse Surface Methodology (RSM).

Materials and Methods

Cocoa beans

Indonesian cocoa beans were obtained from Banyuwangi Plantation, Indonesia - Post Harvest Laboratory of Indonesian Coffee and Cocoa Research Institute (ICCRI). Cocoa beans were de-shelled and cut to particle size of 10 - 5 mm. The desired cocoa beans were collected using a sieve. The samples were kept sealed in plastic and store at room temperature for further analysis.

Roasting Conditions

Roasting was carried out as suggested by Central Composite Design (CCD) using Design-Expert software version 6.0 (Stat Ease Software). Two independent variable were used, temperature (110-160oC) and time (15 - 40 min). Six dependent variable (responses) were determined; 2-methylpyrizine, 2,3-dimethylpyrizine, 2,5-dimethyl pyrizine, 2,3,5-trimethylpyrizine, 2,3,5,6-tetra-pyrizine and acrylamide. The models with statistically significant parameters ($P \leq 0.05$) were considered and the non-significant parameters ($P \geq 0.05$) were withdrawn from the model (Jinap et al., 1995). Cocoa beans were roasted in a roaster (Mitsubishi Probat – Magnetic Contractor model S-N20, the Pascall Engineering Co. Ltd., England).

Volatile components

To produce cocoa liquor, 5 g of grounded roasted cocoa beans were transferred into capped glass vial. Internal standard of 4-Picoline was applied to the cocoa liquor. SPME fiber–Polydimethylsyloxane-Divinylbenzene (PDMS-DVB) polymer was used for extraction. The extraction was held for 30 min at a temperature of 60^{o}C (Misnawi and Ariza, 2011). The flavour compounds in the extract were analysed using GC-FID equipped with Rtx-5(*dimethylpolisiloxane crossbone*) capillary column, helium with 30 ml/min constant flow as carrier gas and injector SPL-1 operating in splitless mode. The injector temperature was maintained at 260^{o}C and the GC temperature were programmed from 60^{o}C (3 min) to 180^{o}C at 5^{o}C/min for 3 min, following the method described by Puziah *et al*. (1998). Identification of the component of the standard was carried out by comparing the retention time for the component.

Analysis of acrylamide

Ground roasted cocoa beans were defatted using hydraulic press. The residue was extracted using solid phase extraction (SPE). Five gram of the defatted cocoa powder was added with 10 mL 0.1% formic acid solution and was shaken for 20 min. The supernatant was filtered through 0.45 µm nylon syringe filter. SPE C18 was conditioned with 2 mL acetone and 2 mL 0.1% formic acid. Extracted cocoa powder solution was allowed to pass through the tube by gravity flow and the SPE tube was washed with 1.0 mL water. Excess water was vacuum dried from the tube and 2 mL acetone was used for elution. Sample (1.0 µL) was injected and held for 0.5 min in the GC-FID equipped with Rtx-5 (*dimethylpolisiloxane crossbone*) capillary column using helium as carrier gas with constant flow of 1.2 ml/min. The injector temperature was maintained at 260^{o}C and the GC temperature programme was from 100^{o}C (0.5 min) to 200^{o}C at 15^{o}C/min.

Result and Discussion

Preliminary studies were conducted at different temperature and time in order to get suitable roasting conditions. The effect of two independent variables (A: temperature of 110 to 160^{o}C and B: time of 15 to 40 min) at five levels were investigated. Response variables were pyrazines (2-methylpyrizine, 2,3-Dimethylpyrizine, 2,5-Dimethylpyrizine, 2,3,5-trimethylpyrizine, 2,3,5,6-tetra-pyrizine) and acrylamide. Pyrizines are heterocyclic nitrogen-containing compound and are extremely important in cocoa flavours. According to Misnawi and Teguh (2010), pyrizines formation and their concentration varied depending on time and temperature of roasting conditions. Prior to roasting, the formation of pyrizines via Maillard reaction, involved amino acids and reducing sugar. Fourteen treatments were assigned based on the CCD for the Response Surface Methodology (RSM) analysis. Central Composite Design (CCD) for pyrizines and acrylamide in the roasted cocoa beans are shown in Table 1.

The summary of the results obtain from the effect of independent variables; temperature and time on each independent from CCD are shown in Table 2. By using lack-of-fit and coefficient of determination (R^2), adequacy of the model can be determined. The significance of equation parameter for all response

Table 1. Central Composite Design (CCD) for pyrizines and acrylamide in the roasted cocoa beans

Exp. No	Temp. (°C)	Time (min)	2-MP (g/100g)	2,3-DMP (g/100g)	2,5-DMP (g/100g)	2,3,5-TMP (g/100g)	2,3,5,6-TP (g/100g)	Acrylamide (g/100g)
1	135	28	211.42	512.88	1218.84	1319.10	3883.06	1.07
2	135	28	227.98	476.08	929.34	1175.60	3864.60	1.05
3	135	28	213.42	512.88	1218.84	1319.10	3883.06	1.06
4	135	28	380.16	475.50	1945.12	1224.98	3893.66	1.08
5	135	28	271.96	492.68	1566.96	2070.90	3894.92	1.09
6	135	28	271.96	492.68	1566.96	2070.90	3894.92	1.09
7	110	28	271.96	492.68	1566.96	2070.90	2794.92	0.25
8	160	28	271.96	492.68	1566.96	2070.90	894.92	0.43
9	135	40	173.24	333.36	681.52	784.64	1290.58	0.65
10	135	15	166.58	171.46	1432.42	863.46	2920.92	0.53
11	118	36	129.30	521.98	683.10	661.44	1859.88	0.86
12	153	19	53.74	425.18	562.38	643.16	1505.50	1.18
13	153	36	59.26	71.68	139.54	389.12	1065.06	1.56
14	118	19	129.24	69.24	639.84	482.32	1737.28	0.15

Table 2. ANOVA for response surface for major compound and yield employing Central Composite Design (CCD)

Parameters	Model	Lack of Fit	R	Equation	Significant model terms
2-methyl Pyrizine	Quadratic Significant	Not Significant	0.813	2 MP = 262.82 -18.19 A + 1.87 B -27.44 A2 -78.47 B2 +1.37 AB	A, B^2
2,3 di-methyl pyrizine	Quadratic Significant	Significant	0.956	2,3 DMP = 493.78 -11.80 A +41.03B - 25.68 A2 -145.82 B2 -201.56 AB	B, A^2, B^2, AB
2,5 di-methyl pyrizine	Quadratic Significant	Not Significant	0.829	2,5 DMP = 1407.68 -77.63 A -180.19 B - 121.80 A2 -376.79 B2 -116.52	B, B^2, AB
2,3,4 tri-methyl pyrizine	Quadratic Significant	Not Significant	0.807	2,3,5 TMP = 1530.10 -13.94 A -23.30 B + 44.54 A2 -578.89 B2 -108.29	B^2, AB
2,3,4,5 tetra pyrizine	Quadratic Significant	Not Significant	0.949	2,3,5,6 TP = 3885.70 -464.20 A -327.94 B - 1128.74 A2 -998.33 B2 -140.76	A, B, A^2, B^2, AB
Acrylamide	Quadratic Significant	Significant	0.924	Acrylamide = 1.07 +0.25 A +0.16 B - 0.25 A2 -0.12 B2 -0.082 AB	A, B, B^2

variables was assessed by F-ratio at probability (p) of 0.05. Zaibunnisa *et al*. (2009) suggested that R^2 should be at least 0.80 to have good fit of the model. The closer the value of R^2 is to unity, the better the empirical model fits the actual data. Polynomial regression equations relating the responses to the independent variable were generated to obtain the optimal level of two factors (A and B).

Numerical optimisation was also carried out to determine the exact optimum level of independent variable leading to desirable roasting condition. Optimizations of the roasting process was based on major flavor compounds (2-methylpyrizine, 2,5-dimethyipyrizine, 2,3,5-trimethylpyirizine and 2,3,5,6-tetra-pyrizine) that have significant model and not significant lack of fit with R^2 more than 0.80 were set as maximun, meanwhile 2,3-dimethylpyrizine was not use in optimisation and acrylamide set as minimum.

Figure 1 shows the chromatrogram for pyrizines with the addition of internal standard, 4-picoline. The major compounds obtained in this study were 2,3,5-trimethylpyrizine and 2,3,4,6-tetra-pyrizine (Table 1). This result correlates well with the finding obtained by Misnawi and Teguh (2010). The results obtained from this study indicate that the concentration of pyrizines increases with temperature, from 110°C to 160°C but dramatically reduced when roasting time was increased (Table 1). This is most likely due to cocoa flavour that will develop better at high temperature, but the cocoa beans will be burnt if the roasting temperature is too high. This burnt flavour will mask the cocoa flavour. During roasting, diketopiperazines or known as cyclic dipeptides, compound that induce bitter taste will be generated and mix with theobromine (Beckwett and Ziegleder, 2009). As temperature increase, more formation of diketopiperazines will be generated contributing to the burnt taste in cocoa.

Consumption of cocoa products with high concentration of acrylamide is hazardous to human health. 3D plot of acrylamide is as shown in Figure 2. In this study, the range of concentration of acrylamide obtained was 0.15-1.56 g/100g. The formation of acrylamide was increased with temperature and time but up to a certain level and reduced gradually thereafter.

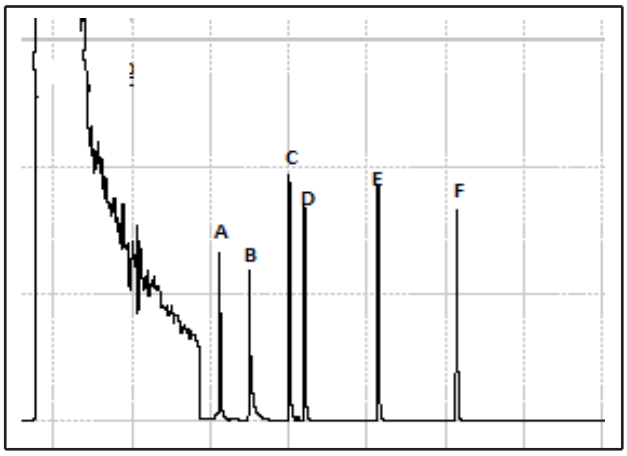

Figure 1. GC-FID chromatogram of external standards pyrizines and internal standard 4-picoline
A = 2,3-Dimethylpyrizine, B= 4-Picoline, C=2,5-Dimethylpyrizine, D=2-Methylpyrizine, E=2,3,5-Trimethylpyrizine, F=2,3,5,6-Tetra-pyrizine

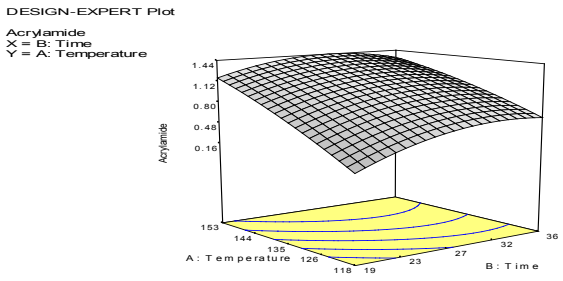

Figure 2. Design-Expert 3D plot of acrylamide (Time x Temperature)

These results were agreeable with acrylamide level for cocoa products that ranged between 17-23 g/100g from countries such as Norway, Sweden, United Kingdom and United State of America (FAO/WHO, 2005) and with an average of 6.7 g/100g in cocoa powder (Adriana *et al*. 2008). Average intake for the general population was estimated to be in the range of 0.3-0.8 mg of acryalamide intake/kilogram of body weight/day (FAO/WHO, 2005). There is no limit of acrylamide in food, but Centre for Science in the Public Interest (CSPI, 2003), said that the limit of acrylamide in fries should be set as 0.077 ppm as an interim acceptable level, which is much less than the amount of acrylamide detected in the roasted cocoa bean analyzed in this study. Acrylamide formation in cocoa based product in found minimum level at 2 ppm and higher level at 826 ppm state by FAO/WHO, 2006.

Conclusion

Based on the statistical optimization to obtain maximum concentration of the major compounds (2-methylpyrizine, 2,5-dimethylpyrizine, 2,3,5-trimethylpyrizine and 2,3,5,6-tetra-pyrizine) and low concentration of acrylamide, temperature of 116°C and time of 23 min gave a desirable value of 0.73.

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