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Changes in Procyanidins and Tannin Concentration as Affected by Cocoa Liquor Roasting

Perubahan Konsentrasi Prosianidin dan Tanin Akibat Pemanasan Pasta Cokelat

Misnawi[1*)]

Summary

Changes in cocoa procyanidins and tannin concentration as affected by cocoa liquor roasting were studied by heating up cocoa liquor of fermented beans containing 58 g kg^{-1} of polyphenols and the same liquor which was enriched with crude polyphenols, extracted from freeze-dried unfermented cocoa beans to a final concentration of 170 g kg^{-1}. The liquors were roasted at 120 ^{o}C for 15, 25, 35 and 45 min with three replications. Result of the study showed that cocoa bean polyphenol was resistant against high temperature during heating of cocoa liquor 120 ^{o}C for up to 45 min. The resistance was stronger with the unfermented cocoa bean polyphenol than with fermented cocoa. High temperature promoted a small quantity of monomers up to pentamers of the fermented cocoa bean polyphenol to polymerize into higher oligomers, but did not with unfermented cocoa bean polyphenol. These results imply that the problem of lack in cocoa flavor in terms high astringency and bitterness due to high polyphenol content cannot be overcome by the application of high temperature during chocolate processing, meanwhile cocoa bean polyphenol could still give beneficial as an antioxidant even after high temperature application.

Ringkasan

Perubahan konsentrasi prosianidin dan tanin sebagai akibat pemanasan pasta dikaji dengan cara memanaskan pasta biji kakao fermentasi dengan kandungan polifenol 58 g kg^{-1} dan pasta yang sama yang diperkaya dengan ekstrak polifenol dari biji kakao non-fermentasi sehingga mencapai konsentrasi akhir 170 g kg^{-1}. Penelitian dilakukan menggunakan rancangan acak lengkap dengan tiga ulangan. Pasta kakao dipanaskan pada suhu oven 120^{o}C selama 15, 25, 35 dan 45 menit. Hasil penelitian menunjukkan bahwa polifenol biji kakao memiliki ketahanan yang tinggi terhadap kerusakan akibat pemanasan pasta pada suhu 120^{o}C selama 45 menit. Ketahanan polifenol dari biji kakao non-fermentasi lebih tinggi dibanding dengan polifenol dari biji kakao hasil fermentasi. Adanya panas yang tinggi secara terbatas memicu polimerisasi prosianidin rantai pendek, yaitu monomer sampai pentamer menjadi oligomer-oligomer yang lebih tinggi, walaupun gejala ini tidak

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terjadi pada polifenol dari kakao non-fermentasi. Hasil penelitian ini memberikan gambaran yang jelas bahwa masalah citarasa berupa tingginya rasa sepat dan pahit karena kandungan polifenol yang terlampau tinggi, tidak dapat diatasi dengan penggunaan suhu tinggi selama pembuatan cokelat. Di sisi lain, tampak juga bahwa kandungan polifenol dalam produk kakao kemungkinan besar masih memberikan nilai positif sebagai zat antioksidan, kendati produk tersebut telah melewati pemanasan selama pengolahan.

Key words: Cocoa bean, polyphenol, procyanidin, tannin, roasting, antioxidant, flavor, astringency.

INTRODUCTION

Beneficial effect of cocoa bean polyphenol on human health has been studied intensively, as recently described by Ding *et al.* (2006), Andres-Lacuena *et al.* (2008) & Ruzaidi *et al.* (2008). Cocoa bean polyphenols have gained much attention owing to their antioxidant activity and possible beneficial implications on human health, such as treatments and preventions of cancers, cardiovascular diseases and other pathologies (Kattenberg, 2000; Wollgast & Anklam, 2000b). Experiments using in vitro and animal models demonstrated that most of the cocoa bean polyphenol fractions were found to have antioxidant activities and give health beneficial, such as in inhibition of hydrogen peroxide and superoxide anion, protection against lipid peroxidation and deterioration, antiulceric, inhibition of oxidative stress and reduction of low density lipoprotein (LDL) oxidative cardiovascular disease, antimutagenic, inhibition of tumor development and carcinogenic and antimicrobial (Kattenberg, 2000; Osakabe *et al.*, 2000, 1998a, 1998b; Sanbongi *et al.*, 1998).

Nevertheless, Misnawi *et al.* (2004a, 2004b) found that the increase in polyphenol caused free amino acids and reducing sugars to be less available for the pyrazine formation during cocoa flavour formation, and the presence of polyphenols in cocoa liquor may bind part of the pyrazine formed during roasting, thus reducing pyrazine concentration. Bonvehi & Coll (2000, 1997) & Lindsay (1996) also found that polyphenols are compounds which responsible for astringency and contributing to bitterness. Luna *et al.* (2002) showed that polyphenols in cocoa beans positively correlated with astringency, bitterness and green note and negatively correlated with fruity flavor. Bonvehi & Coll (2000) found that the contribution of polyphenol to the bitterness is in combination with the presence of alkaloids, some amino acids, peptides and pyrazines.

Biehl & Voigt (1996) specified cocoa flavor to consists of both taste, mainly bitter and astringent, and aroma in term of smelling from aromatic volatile compounds. Accoding to Lindsay (1996), flavor is defined as an overall integrated perception of all the contributing senses, includes smell, taste, sight,

feeling and sound. Taste is frequently attributed by the responsible substances, which are water soluble and relatively non-volatile. Sweet, bitter (and astringent), sour and salty are commonly basic taste occurred in food; however bitterness and astringency are the basic main taste associated in cocoa products.

Polyphenols also affect the stability and digestibility of foods (Bonvehi & Coll, 2000; Haslam *et al.*, 1992); meanwhile, polyphenol concentration in cocoa beans and their product is in abundant quantity (Wollgast & Anklam, 2000a, 2000b; Kim & Keeney, 1983). Unfermented cocoa beans contain 120–180 g kg^{-1} of polyphenolic compounds (Kim & Keeney, 1984). Three groups of the polyphenols present are catechins or flavan-3-ols (ca. 37%), anthocyanins (ca. 4%) and proanthocyanidins (ca. 58%) (Wollgast & Anklam, 2000a).

During cocoa fermentation, polyphenols are subjected to biochemical modification through polymerization and binding with protein, and hence decreasing their solubility and astringency effect (Bonvehi & Coll, 1997; Kim & Keeney, 1984). Polyphenol concentrations and compositions would also be expected to alter during chocolate manufacturing processes such as roasting, grinding, refining and conching, where high temperatures (120–180 ^{O}C) are used and air (oxygen) is present. However, information related to polyphenol changes during cocoa manufacturing is still limited (Wollgast & Anklam, 2000a). Kealey *et al.* (1998) found that total polyphenol decreased from 24.618 to 12.786 mg g^{-1} as the nib roasting temperature was increased from 127 to 181 ^{O}C.

Kim & Keeney (1983) identified and estimated the concentration of major polyphenols in Forastero cocoa bean. Eight compounds in the three main fractions viz. catechin, leucocyanin and anthocyanin were identified, however, another fraction which moved very slow on the paper chromatography suggested as complex tannins. Wollgast & Anklam (2000a) stated that cocoa polyphenols are mainly monomers and oligomers of flavan-3-ol basic compound and they also classified cocoa polyphenol into three groups i.e. catechins or flavan-3-ols ca. 37%, anthocyanins ca. 4% and proanthocyanidins ca. 58%. Changes in procyanidins and tannin as affected by heating, mainly during roasting of cocoa liquor and its possible impact on flavor has never been studied.

MATERIAL AND METHOD

Cocoa Bean Liquors

Cocoa liquor containing two different polyphenol contents was used in this study. The first was prepared from Ghanaian cocoa beans which were peeled, ground using a Limprimita cocoa breaker (John Gordon & Co., England) and refined to the size of < 15 μ in a triple roll refiner (Pascal Eng., England).

The second was the above fermented cocoa liquor enriched with crude cocoa polyphenols extract to obtain a final con-

centration of 170 g kg^{-1}. Crude polyphenol extract was obtained from extraction of healthy cocoa beans. Beans of healthy bulk cocoa pods after being removed from their testa was shock frozen in liquid nitrogen. The frozen cocoa cotyledons were then lyophilized and freeze-dried at a pressure of < 13.3 Pascals using a Labconco Freezone 6 (Kansas, USA), followed by grinding, refining and defatting in a Soxhlet apparatus for 16 h using petroleum ether (bp. 40–60°C). Polyphenols were then extracted twice using chilled 80% acetone followed by twice chilled 100% acetone. The extract was then centrifuged using a Kubota 7800 Centrifuge (Tokyo, Japan) at 4,000 rpm and 4°C for 15 min. The supernatant was then evaporated under vacuum at 45°C using a Heidolph WB/VV 2000 rotary evaporator (Heidolph, Germany) followed by freeze-drying at a pressure of < 13.3 Pascals.

Roasting - heating Conditions

Roasting of cocoa liquor was carried out in a Memmert UL 40 oven (Schwabach FRG, Germany). The oven was set at 120°C and maintained for 1 h to reach equilibrium. Fifty grams of cocoa liquor from each treatment and replication were placed in a 10 cm petri dish at 5 mm thickness and inserted into the oven. The door was opened and closed as quick as possible after inserting the petri dish. The time of roasting started immediately when the door was closed.

The liquor was roasted for 15, 25, 35 or 45 min. After cooling to ambient temperature (26 °C), the liquor was then defatted in a Soxhlet apparatus using petroleum ether (bp 40—60 °C) for 16 h. After drying at room temperature (27 °C) for 3 h, the rest of the petroleum ether was discharged under vacuum using a Labconco Frezone 6 (Kansas, USA) at pressure < 13.3 Pascals for 3 h then the sample kept in a sealed container for further analyses. The roasting procedure was repeated three times for each treatment.

Procyanidin Concentration

Cocoa procyanidins were determined using normal-phase HPLC as described by Rigaud *et al*. (1993) with slight modification in terms of column used and elution condition. One gram of defatted cocoa powder was diluted in 10 ml of 80% aqueous acetone and homogenized for 5 min. The suspension was then centrifuged in an Eppendorf Centrifuge 5415C (Eppendorf, Germany) at 14,000 rpm for 20 min. The supernatant was collected and salted out using NaCl followed by re-centrifugation at the same condition.

Ten microliters of the supernatant was then injected into the HPLC equipped with a m Bondapak-NH2 (300 x 3.9 mm i.d.) stainless steel tube column protected with a guard column (20 x 4 mm i.d.) packed with the same material. The solvents were dichlo-romethane-methanol-formic acid-water at 5:43:1:1 for solvent A and 41:7:1:1 for solvent B. The elution condition was as follows: flow rate, 1 ml min^{-1}; oven temperature, 30 °C; elution, linear gradients from 0 to 20% A for 30 min,

from 20 to 50% A for 20 min, from 50 to 100% A for 5 min and standing at 100% A for 10 min.

The eluted individual polyphenol was monitored by measuring the absorbance of the effluent at 280 nm. Cocoa polyphenol monomers was quantified using authentic standards from SIGMA Chemical Co. in term of (–)-epicatechin, however procyaidins from dimers to pentamers were quantified using partial purified of cocoa procyanidins.

Total Polyphenol and Tannin

The amounts of total polyphenol and tannin were determined using the method of Shamsuddin & Dimmick (1986) and Bonvehi & Coll (2000). Results obtained were stated as g kg^{-1} equivalent to epicatechin. (-)-Epicatechin standard was purchased from Sigma Chemical Co. Decreases in total polyphenol and tannin were calculated as follows:

$$\% \text{ Decrease} = \frac{\text{Concentration prior to} - \text{after roasting}}{\text{Concentration prior to roasting}} \times 100$$

Total polyphenol was determined spectrophotometrically. Five hundred milligram of defatted cocoa powder and 80 ml of 80% aqueous acetone were placed in a 125 ml conical flask and the mixture was sonicated in a Sonicor C-125 (Sonicor Inst., New York, USA) for 30 min. During sonication, the extraction mixture was kept cold by filling the sonicator vessel with ice water. Sonication was preferred over shearing as an aid in solubilizing polyphenol, since shearing promotes browning of the polyphenol extract by oxidation (Shamsuddin & Dimmick, 1986).

Clear extract was obtained by vacuum filtration of the mixture through Whatman no. 1 filter paper. The residue and all glasswares were washed with the 80% aqueous acetone and total volume of the extract was made up to 100 ml in a volumetric flask. One milliliter of the extract was pipetted into a 100 ml volumetric flask and diluted with 70 ml of distilled water. The extracted polyphenol was then reacted with 5 ml of 2N Folin-Ciocalteau's reagent for 2 min. Then 15 ml of saturated Na_2CO_3 solution was added to stabilize the color formed. The blue color was allowed to develop for at least 2 hr and its absorbance was measured at 765 nm. (–)-Epicatechin standard of nine known concentrations of 1 to 9 mg L^{-1} was used for calculation.

Tannin (and non-tannin) was determined using combination method of Bonvehi & Coll (1997) and Singleton & Rossi (1965). One gram of cocoa powder was extracted by agitating with 50 ml of 70% aqueous methanol. Phenols in the extract were then determined using Folic-Ciocateu method as described before. Tannin concentration was calculated based on absorbance differences by precipitating an aliquot of the extract at pH 4 using 1% NaCl in 10% gelatin solution.

RESULTS AND DISCUSSION

Changes in Procyanidin

Major phenolic compounds in cocoa beans are (–)-epicatechin and its C4–C8 linked oligomers (Rigaud *et al.*, 1993). Adamson *et al.* (1999) and Hammerstone *et al.* (1999) detected cocoa polyphenols from monomers up to decamers, with monomers to hexamers were dominant. Result of this study (Figure 1) showed that roasting of fermented cocoa liquor without polyphenol extract enrichment significantly decreased monomers up to pentamers concentrations; however, hexamers and higher oligomers concentration were increased. The decrease in the concentration of the first five monooligomerics was directly followed by the increase in the hexamers and higher oligomers concentration. This evidence shows the possibility of polymerization from the lower into a higher degree oligomerics; it is also assumed that a few parts of the procyanidins was reduced through heat damages. Explanation of the latest is referring to the changes of procyanidin concentration in the roasting of fermented cocoa liquor enriched with polyphenol extract below, however, the mechanism of procyanidin polymerization hypothetically is described in Figure 2.

According to Wollgast *et al.* (2001) and Porter *et al.* (1991) leucocyanidins (procyanidins) found in cocoa, most are based on epicatechin structure. This condition could be explained since most of the procyanidin monomeric is (–)-epicatechin. Analysis carried by Misnawi *et al.* (2002b) found the concentration of (–)-epicatechin in fermented and unfermented cocoa bean were 40.2 and 17.2 g kg^{-1}, respectively; meanwhile that of (+)-catechin were 7.7 and 6.9 g kg^{-1}. In the studies of competition between (+)-catechin and (–)-epicatechin in acetaldehyde-induced polymerization of flavanols, Es-Safi *et al.* (2000, 1999) found that the reaction of the latter was faster. Porter *et al.* (1991) also found that (+)-catechin was not associated with polymer biosynthesis, thus it was not significantly decreased during fermentation. Furthermore, (+)-catechin was more resistant to enzymatic oxidation, compared to (–)-epicatechin (Misnawi *et al.*, 2002a). The binding site in the polymerization of procya-nidin among the (–)-epicatechins is suggested to be at between atom number 4β and 8 (Figure 2). However, Weisburger (2001) assumed that the binding also could occur between atom number 4β and 6.

Changes in procyanidin concentration during roasting of cocoa liquor enriched with crude polyphenols extracted from unfermented bean showed a higher resistance against high temperature of roasting as indicated by the no significant changes in monomers and procyanidins concentration was observed during the roasting, except on tetramers (Figure 3). This evidence may be due to the fact that polyphenols in fermented bean had derived from their original structure, mainly through the enzymatic oxidation by polyphenol oxidase activity. However, crude polyphenol in freeze-dried unfermented bean did not have any extensive structure modifications. According to Bonvehi & Coll (1997), cocoa polyphe-

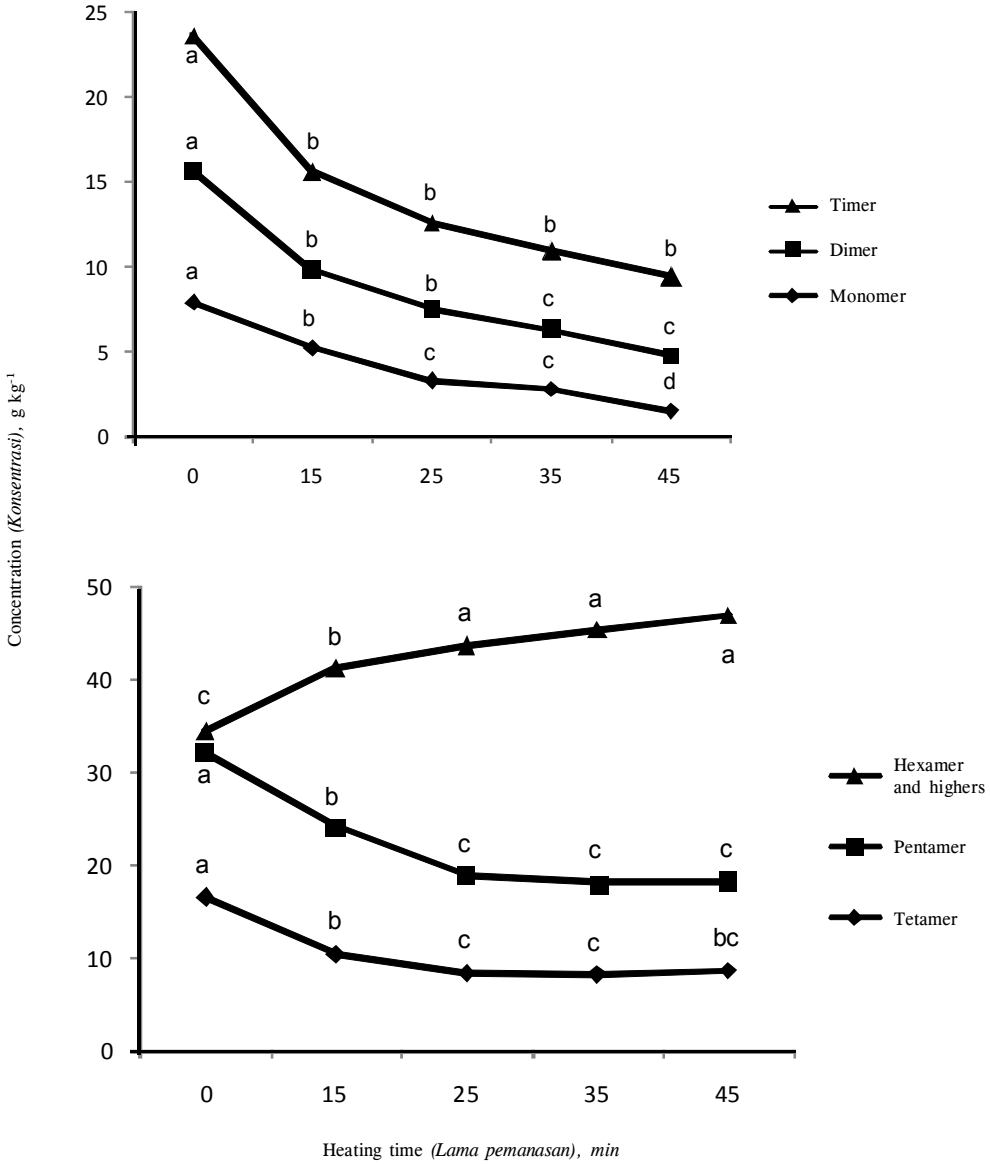


Figure 1. Effects of heating time on monomers and procyanidins concentrations in cocoa liquor of fermented bean.

Gambar 1. Pengaruh lama pemanasan terhadap konsentrasi monomer dan prosianidin dalam pasta kakao fermentasi.

Note (*Catatan*) : Values with the same letter in respective figure are not significantly different according to Duncan's Multiple Range Test at p of 5% (*Nilai dengan huruf sama di dalam gambar yang sama menunjukkan tidak ada perbedaan nyata menurut uji jarak Duncan pada taraf 5%*).

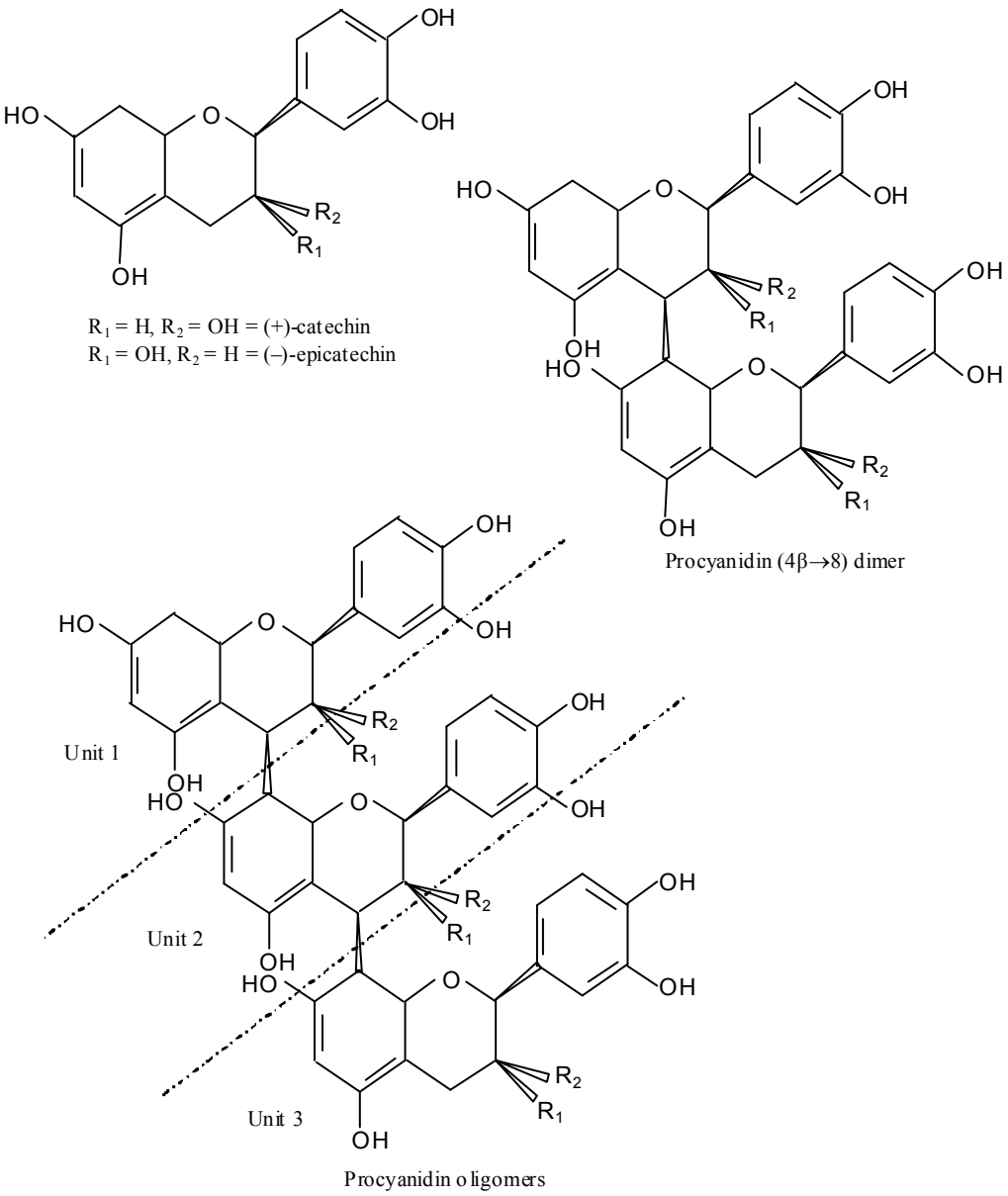


Figure 2. Hypothetical polymerization of cocoa bean procyanidin during heating.
Gambar 2. Hipotesis polimerisasi prosianidin kakao selama pemanasan.

Heating time, min
Lama pemanasan, min

Heating time, min
Lama pemanasan, min

Heating time, min
Lama pemanasan, min

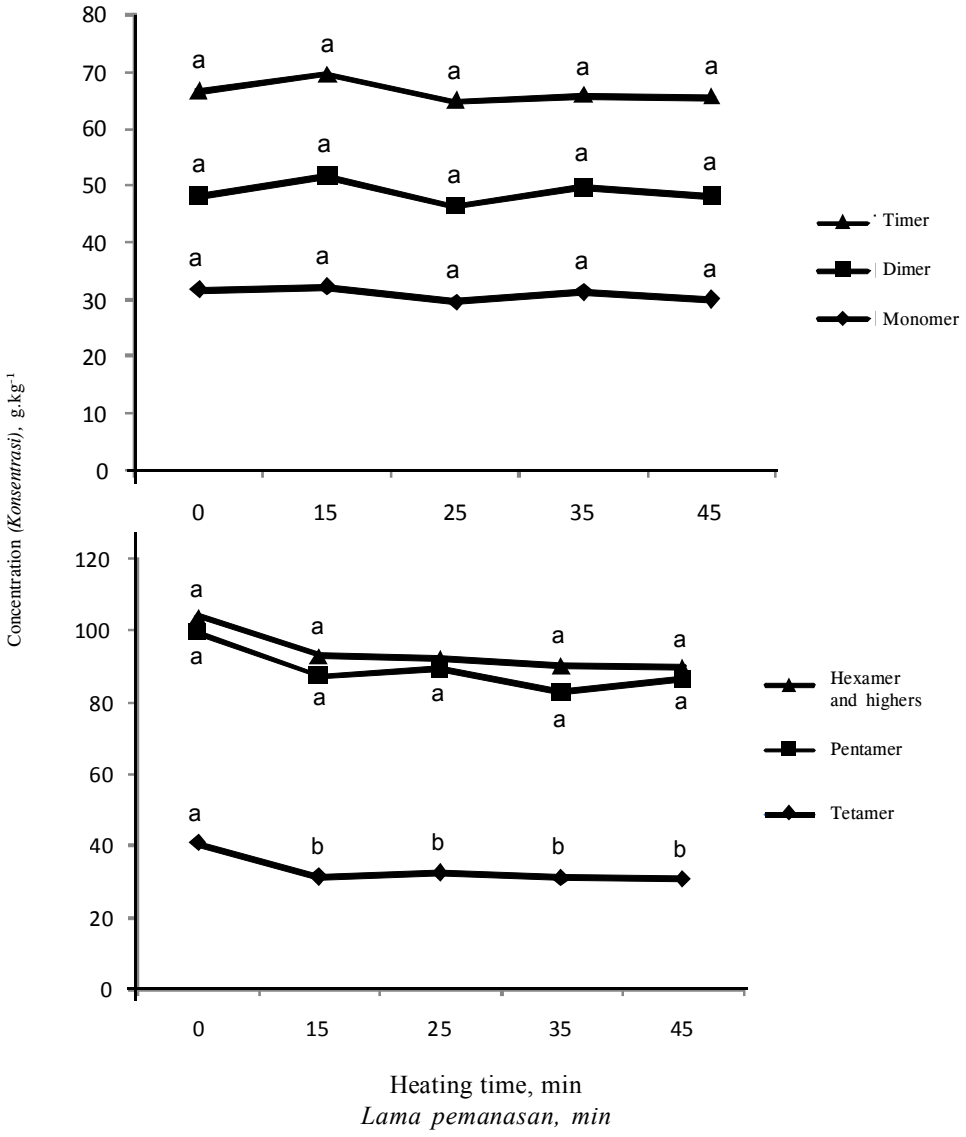


Figure 3. Effects of heating time on monomers and procyanidins concentrations in cocoa liquor of fermented bean enriched with cocoa polyphenol extract.

Gambar 3. Pengaruh lama pemanasan terhadap konsentrasi monomer dan prosianidin dalam pasta kakao fermentasi yang diperkaya dengan ekstrak polifenol kakao.

Note (*Catatan*) : Values with the same letter in respective figure are not significantly different according to Duncan's Multiple Range Test at p of 5% (*Nilai dengan huruf sama di dalam gambar yang sama menunjukkan tidak ada perbedaan nyata menurut uji jarak Duncan pada taraf 5%*).

nols are subjected to biochemical modification during cocoa fermentation through oxidation and polymerization and their binding with protein, hence decreasing the solubility. Kim & Keeney (1984) and Forsyth & Quesnel (1963) found that during the fermentation, anthocyanins are hydrolyzed to produce anthocyanidins, galactose and arabinose; beside dimerisation of leucocyanidins and exudation of the flavonoids from the bean.

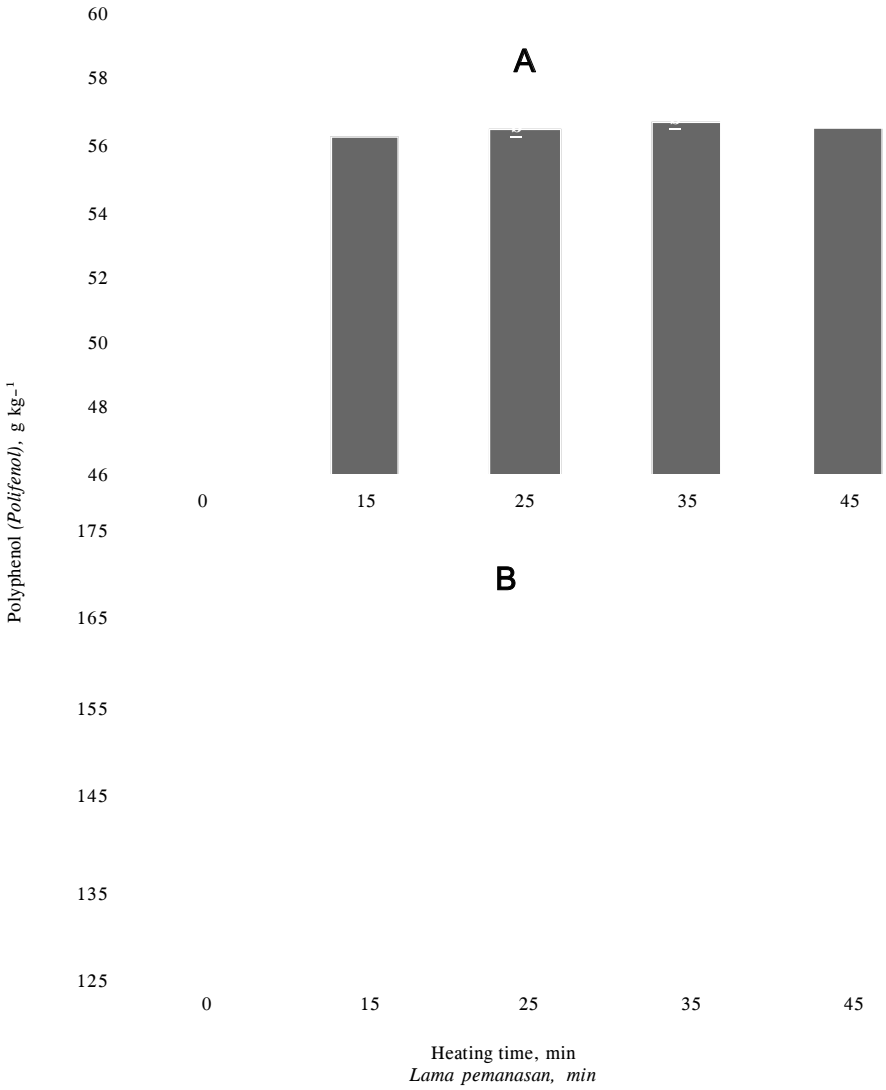
Total Polyphenol and Tannin Concentration

Results of total polyphenol and tannin analyses (Figure 4 and 5) showed that roasting of cocoa liquor significantly decreased both the polyphenol and tannin concentrations, mainly during the first 15 min. In fermented cocoa liquor without polyphenol enrichment, total polyphenol decreased to 56.2–56.4 g kg^{-1} after roasting which are 2.6–3.3% lower than its original concentration at 58.1 g kg^{-1}; however a higher decrease was obtained with tannin. Tannin concentration decreased from 48.6 g kg^{-1} to 32.5–39.1 g kg^{-1}, equal to 19.5–33.1% decreases.

These results indicated that roasting of cocoa liquor reduces tanning ability of cocoa polyphenol, thus astringency of the liquor would hypothetically be reduced. The results supported the fact that the astringency of cocoa liquor would decrease during roasting and conching processes. The reduction of tannin may be due to oxidation and interaction of polyphenols with proteins, amides or other related compounds, which are induced by heat during roasting (Katternberg & Kemmink, 1993; Hagerman, 1992).

Roasting of cocoa liquor enriched with crude polyphenol extract showed a different mode of changes compared with the cocoa liquor without enrichment. Tannin concentration decreased by only 2.3–7.5% from 112.3 g kg^{-1} in the liquor before roasting. This phenomenon may be due to the fact that polyphenols in fermented bean have derived from their original structure, mainly through the enzymatic oxidation by polyphenol oxidase activity. However, the crude polyphenols, from freeze-dried fresh unfermented bean, had not had any extensive structure modification.

Bonvehi & Coll (2000) and Wollgast & Anklam (2000a) stated that during fermentation of cocoa beans, polyphenol diffuses with cell liquids from their cell storage and undergoes oxidation to form condensed high molecular mostly insoluble tannin. Katternberg & Kemmink (1993) found that enzymatic oxidation of polyphenols produces quinones, which can react further with amino acids and proteins, or polymerize with each other to form high molecular weight, the condensed tannin. At molecular weight above 3,000 Da, they form complexes with protein through hydrogen bonding. Peleg *et al.* (1999) also stated that polymerization of polyphenols increases the intensity formation of phenol-protein complex. These are the hypotheses why tannin concentration in fermented cocoa during roasting decreased faster than in unfermented cocoa.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Polyphenol (Polifenol), g kg⁻¹
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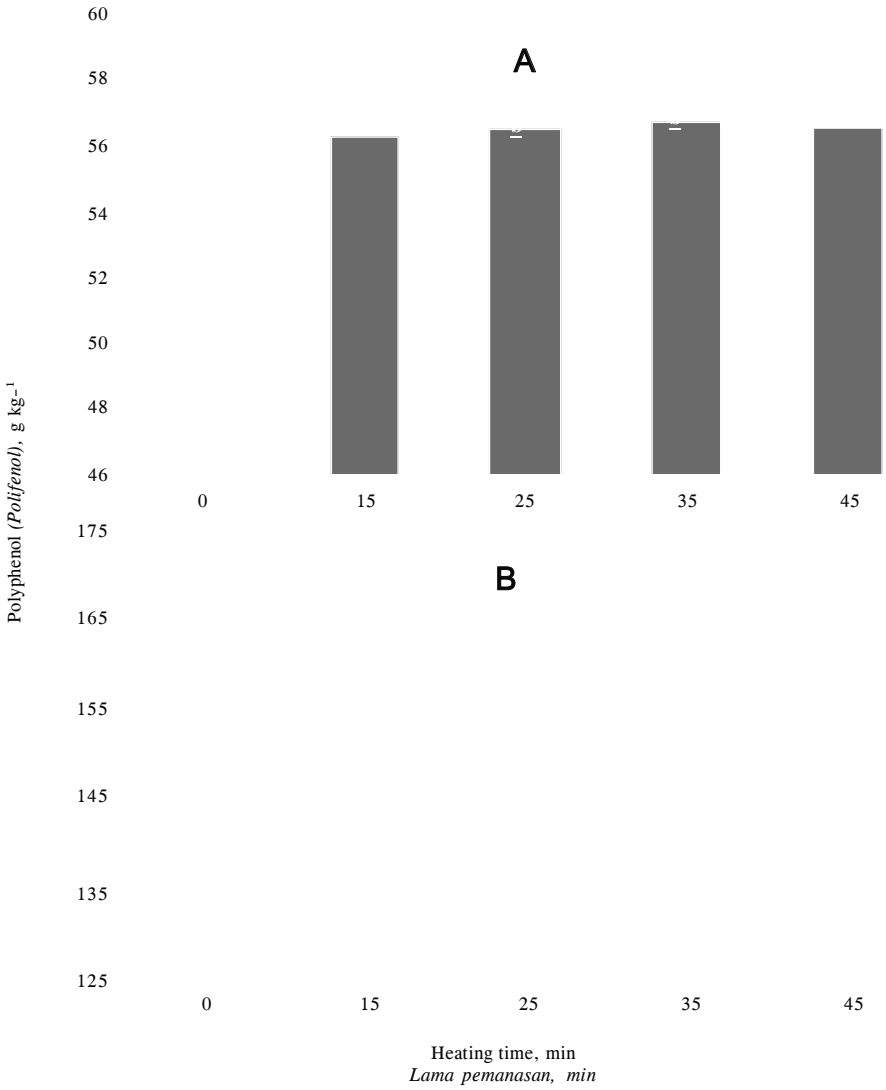
Lama pemanasan, min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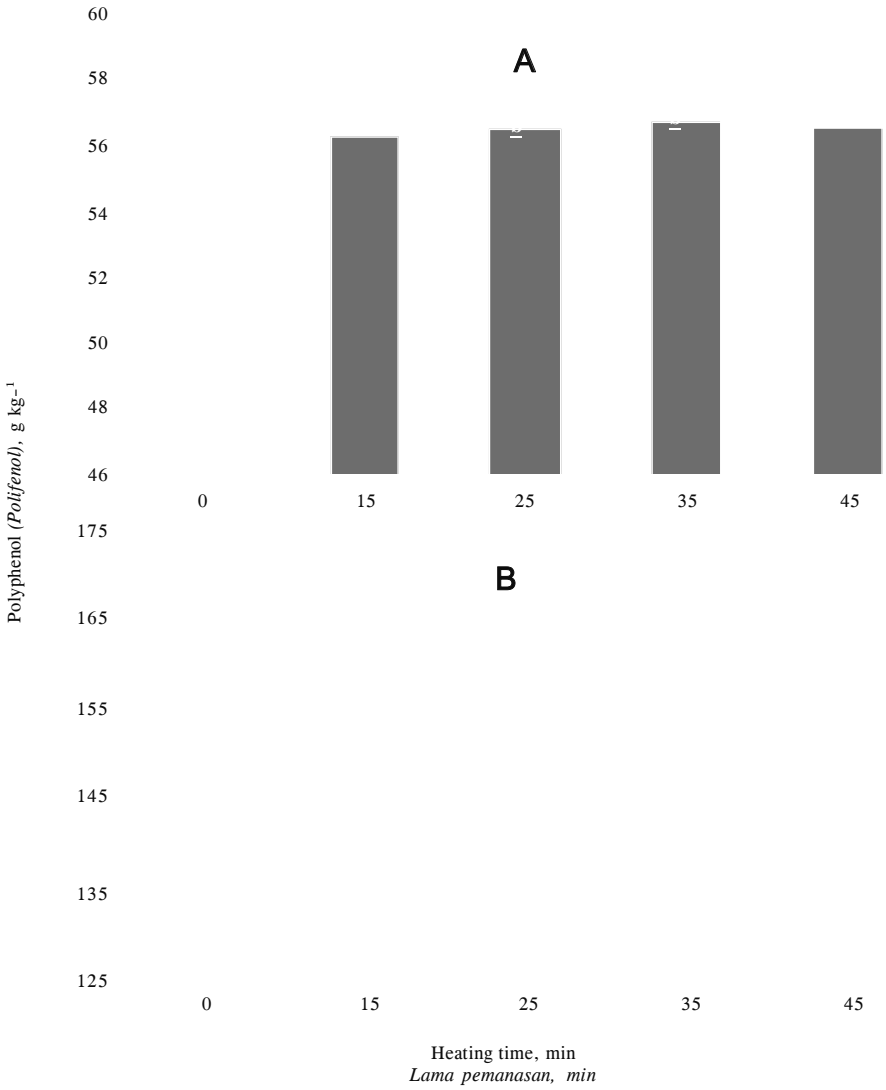
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Polyphenol (Polifenol), g kg⁻¹
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Lama pemanasan, min

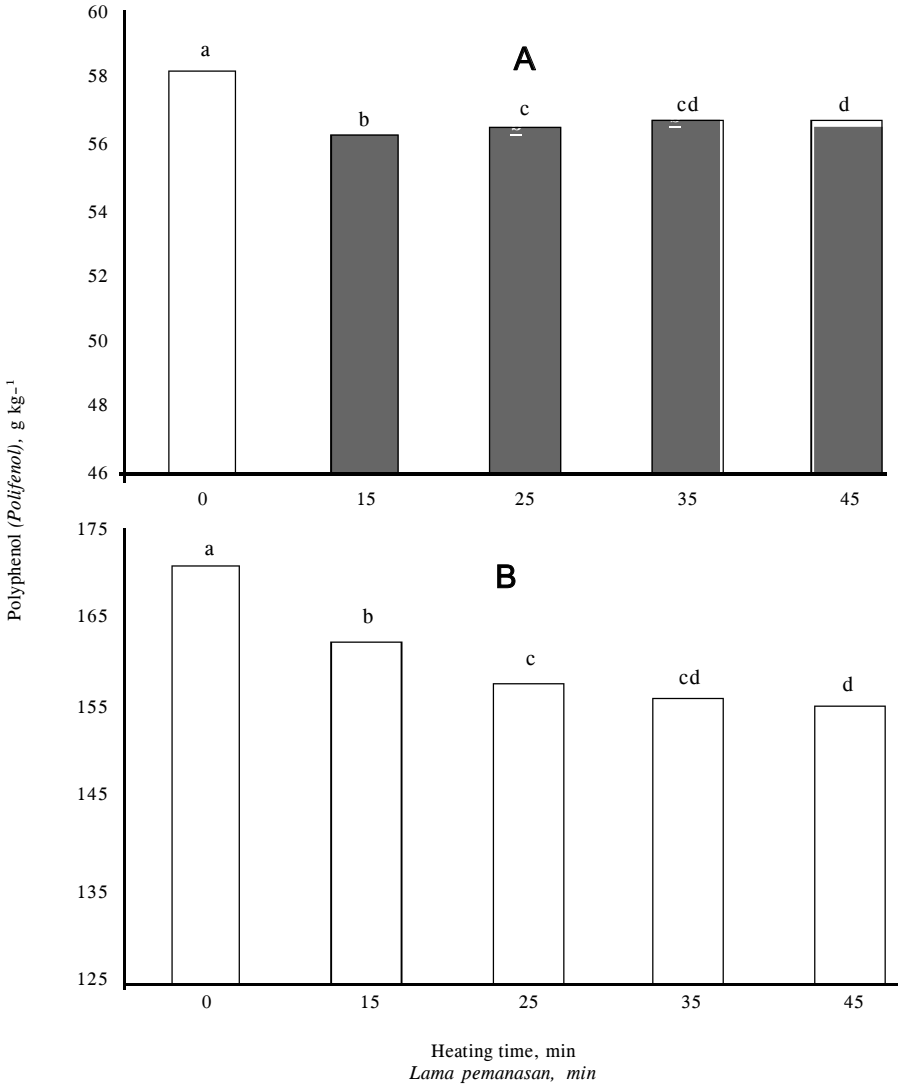
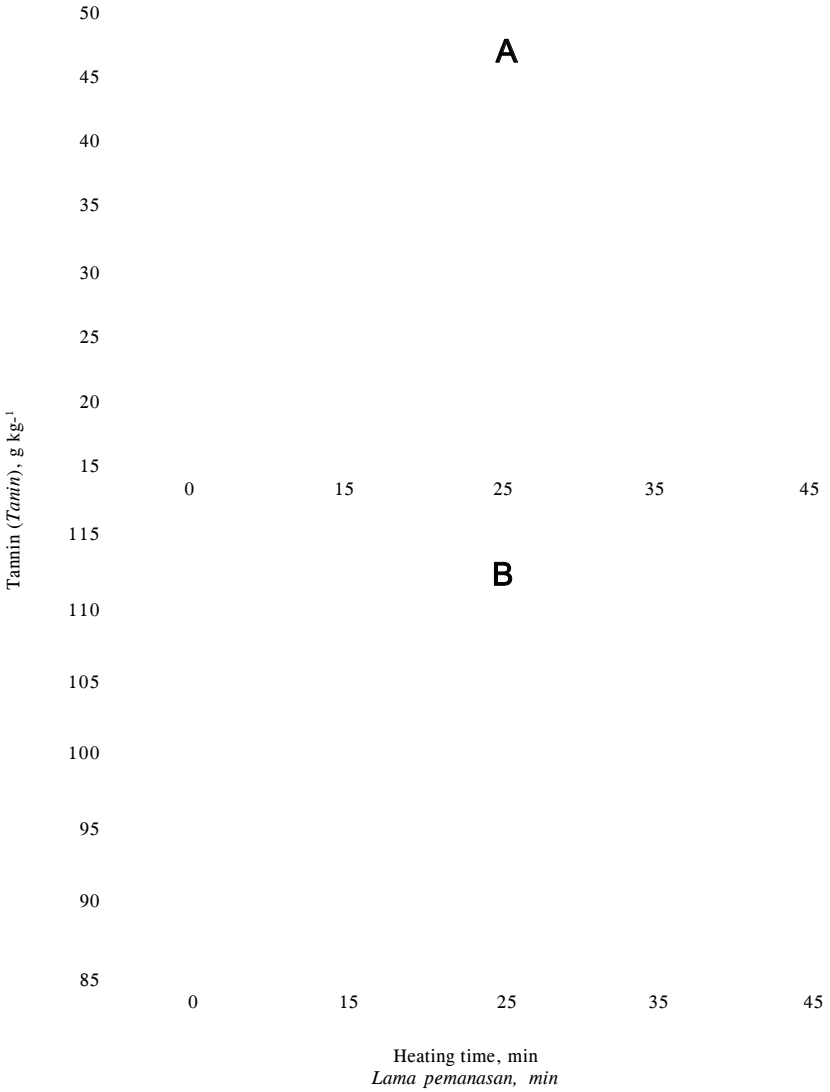


Figure 4. Effects of cocoa liquor heating time on polyphenol concentration (A. Cocoa liquor, B. Polyphenol enriched cocoa liquor).

Gambar 4. Pengaruh lama pemanasan pasta cokelat terhadap konsentrasi polifenol (A. Pasta kakao, B. Pasta kakao dengan penambahan ekstrak polifenol).

Note (*Catatan*) : Bars with the same letter in respective figure are not significantly different according to Duncan's Multiple Range Test at p of 5% (*Bar dengan huruf sama di dalam gambar yang sama menunjukkan tidak ada perbedaan nyata menurut uji jarak Duncan pada taraf 5%*).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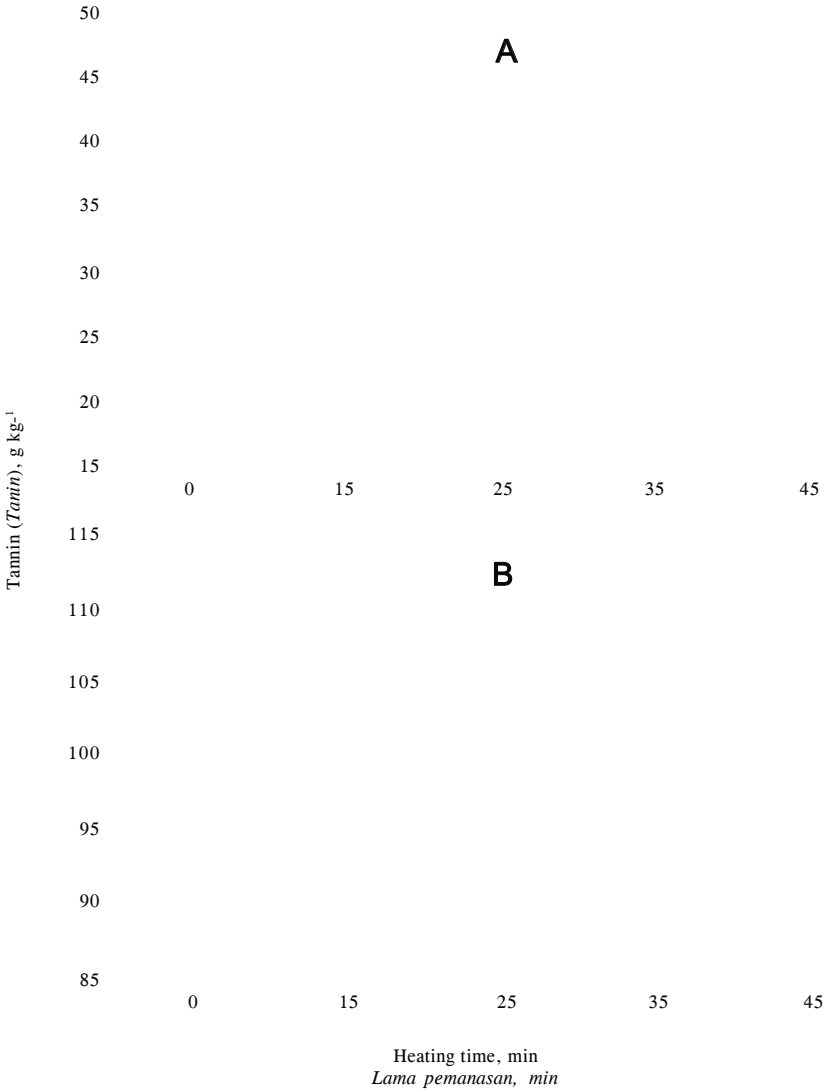
Tannin (Tanin), g kg-1
Heating time, min
Lama pemanasan, min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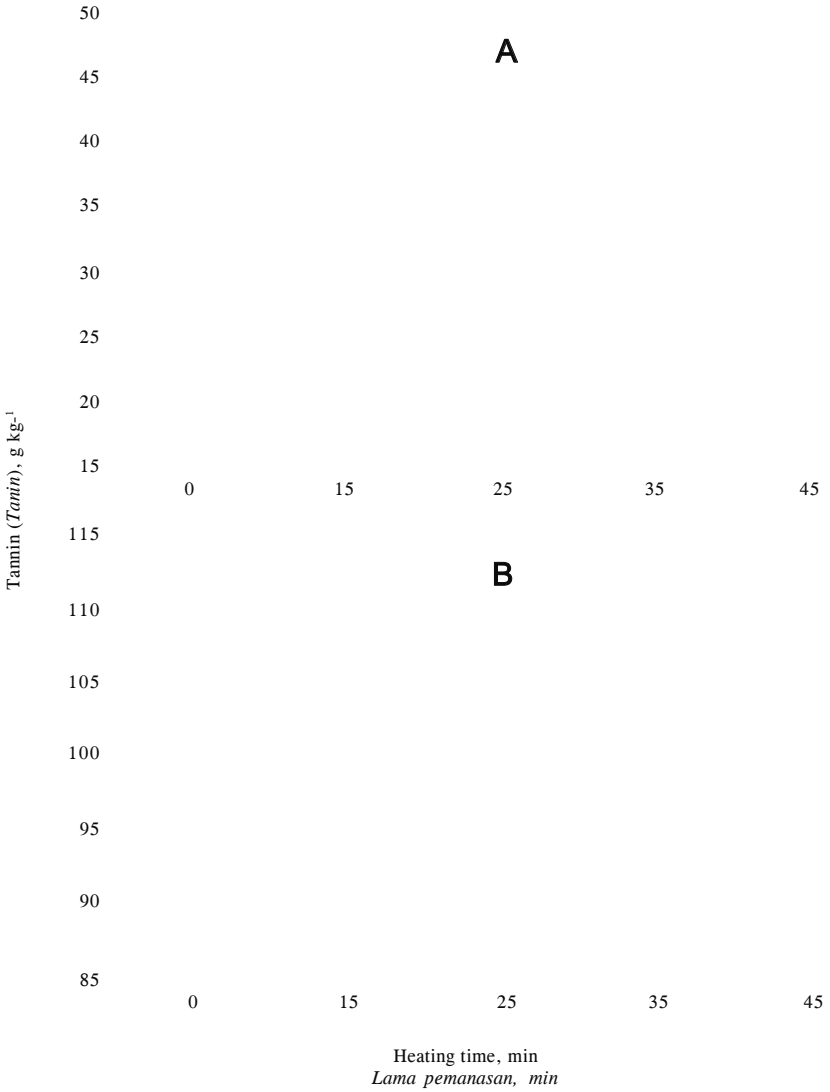
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Lama pemanasan, min

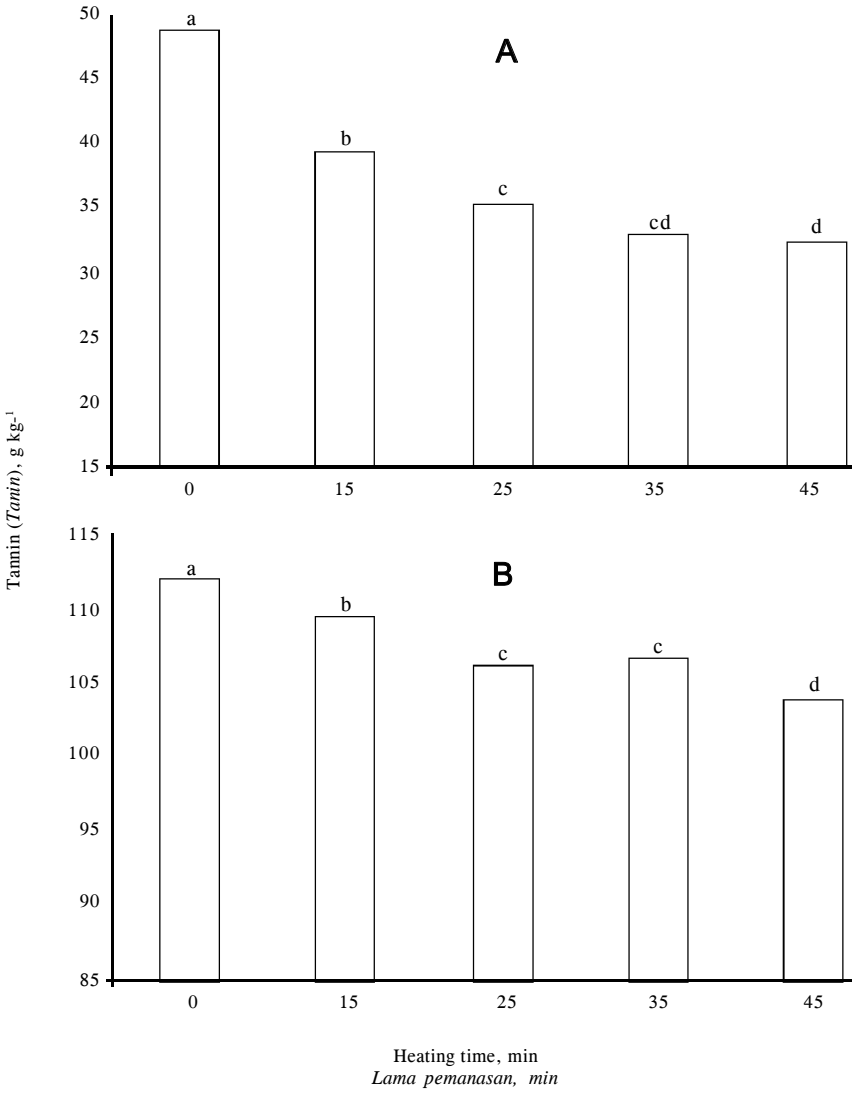

Figure 5. Effects of cocoa liquor heating time on tannin concentration (A. Cocoa liquor, B. Polyphenol enriched cocoa liquor).

Gambar 5. Pengaruh lama pemanasan pasta cokelat terhadap konsentrasi tanin (A. Pasta kakao, B. Pasta kakao dengan penambahan ekstrak polifenol).

Note (*Catatan*) : Bars with the same letter in respective figure are not significantly different according to Duncan's Multiple Range Test at p of 5% *(Bar dengan huruf sama di dalam gambar yang sama menunjukkan tidak ada perbedaan nyata menurut uji jarak Duncan pada taraf 5%*).

CONCLUSSION

Cocoa bean polyphenol showed a resistance against high temperature during heating of cocoa liquor at 120°C up to 45 min. The resistance was stronger with the unfermented cocoa bean polyphenol than with of fermented cocoa. High temperature promoted a small quantity of monomers up to pentamers of the fermented cocoa bean procyanidin to polymerize into higher oligomerics, but did not with unfermented bean polyphenol. These results imply that the problem of lack in cocoa flavor in terms high astringency and bitterness due to high polyphenol content cannot be beat by the application of high temperature during chocolate processing, meanwhile cocoa bean polyphenol could still give beneficial as an antioxidant even after high temperature application.

REFERENCES

Andres-Lacueva, C.; M. Monagas; N. Khan; M. Izquierdo-Pulido; M. Urpi-Sarda; J. Permanyer & R. M. S. Lamuela-Raventos (2008). Flavanol and flavonol contents of cocoa powder products: Influence of the manufacturing process. *Journal of Agriculture and Food Chemistry*, 56, 3111–3117.

Biehl, B. & J. Voigt (1996). Biochemistry of chocolate flavour precursors. *International Cocoa Conference,* Salvador, Bahia, November 1996.

Bonvehi, J.S. & F.V. Coll (1997). Evaluation of purine alkaloids and diketopirazines contents in processed cocoa powder. *Food Chemistry,* 60, 365–370.

Bonvehi, J.S. & F.V. Coll (2000). Evaluation of purine alkaloids and diketopiperazines contents in processed cocoa powder. *European Food Research and Technology,* 210, 189–195.

Ding, E. L., S.M. Hutfless, X. Ding & S. Girotta (2006). Chocolate and prevention of cardiovascular disease: A systematic review. *Nutrition & Metabolism*, 3, 1–12.

Es-Safi, N.E.; H. Fulcrand; V. Cheynier & M. Moutounet (1999). Competition between (+)-catechin and (–)-epica-techin in acetaldehyde-induced polymerization of flavanols. *Journal of Agricultural and Food Chemistry,* 47, 2088–2095.

Es-Safi, N.E.; H. Fulcrand; V. Cheynier & M. Moutounet (2000). Study on the reaction between (+)-catechin and furfural derivates in the presence or absence of anthocyanins and their implication in food color change. *Journal of Agricultural and Food Chemistry,* 48, 5946–5954.

Forsyth, W.G.C. & V.C. Quesnel (1963). Mechanism of cocoa curing. *Advance Enzymology,* 25, 457–492.

Hagerman, A.E. (1992). Tannin-protein interaction. p. 237–247. ***In:*** Ho, C.T.; C.Y. Lee & M.T. Huang (Eds.), *Phenolic Compounds in Food and Their Effects on Health I: Analysis, Occurrence and Chemistry*. ACS Symposium Series 506.

Haslam, E.; T.H. Lilley; E. Warminsky; H. Liao; Y. Cai; R. Martin; S.H. Gaffney; P.N. Goulding & G. Luck (1992). Polyphenol complexation: a study in molecular recognition. p. 8–50. ***In:*** Ho, C.T.; C.Y. Lee & M.T. Huang (Eds.), *Phenolic Com-*

pounds in Food and Their Effects on Health I: Analysis, Occurrence and Chemistry. ACS Symposium Series 506.

Kattenberg, H.R. (2000). Nutritional functions of cocoa and chocolate. *The Manufacturing Confectioner*, 33–37.

Kattenberg, H.R. & A. Kemmink (1993). The flavor of cocoa in relation to the origin and processing of the cocoa beans. p. 1–22. ***In:*** Charalambous, G. (Ed.), *Food Flavor, Ingredients and Composition*. New York: Elsivier Sci. Publ.

Kealey, K.S.; R.M. Snyder; L.J. Ramanczyk; H.M. Geyer; M.E. Myers; E.J. Withcare; J.F. Hammerstone & H.H. Schmitz (1998). Cocoa components, edible products having enhached polyphenol content, methods of making same and medical uses. *Patent Cooperation Treaty (PCT) WO 98/09533,* Mars Incorporated, USA.

Kim, H. & P.G. Keeney (1983). Method of analysis for (–)-epicatechin in cocoa beans by high performance liquid chromatography. *Journal of Food Science*, 48, 548–551.

Kim, H. & P.G. Keeney (1984). (–)-Epicatechin content in fermented and unfermented cocoa beans. *Journal of Food Science,* 49, 1090–1092.

Lindsay, R.C. (1996). Flavor. p. 723–765. ***In:*** Fennema, O.R. (Ed.), *Food Chemistry. 3rd edition*. New York: Marcel Dekker Inc.

Luna, F.; D. Crouzillat; L. Cirou & P. Bucheli (2002). Chemical composition and flavor of Ecuadorian cocoa liquor. *Journal of Agricultural and Food Chemistry,* 50, 3527–3532.

Misnawi; S. Jinap; B. Jamilah & S. Nazamid (2002a). Oxidation of polyphenols in unfermented and partly fermented cocoa beans by cocoa polyphenol oxidase and tyrosinase. *Journal of the Science of Food and Agriculture,* 82, 559–566.

Misnawi; S. Jinap; B. Jamilah & S. Nazamid (2002b). Activation of remaining key enzymes in dried under-fermented cocoa beans and its effect on aroma precursors formation. *Food Chemistry,* 78, 407–417.

Misnawi; S. Jinap; B. Jamilah & S. Nazamid (2004a). Effects of polyphenol on pyrazines formation during cocoa liquor roasting. *Food Chemistry*, 85, 73—80.

Misnawi; S. Jinap; B. Jamilah & S. Nazamid (2004b). Sensory properties of cocoa liquor as affected by polyphenol concentration and roasting duration. *Food quality and Preference*, 15, 403—409.

Osakabe N.; M. Yamagishi; M. Natsume; T. Takizawa; T. Nakamura & T. Osawa (2000). Antioxidative polyphenolic substances in cacao liquor. p. 88–101. ***In:*** Parliament, T.H.; Chitang Ho & P. Schieberle (Eds.), *Caffeinated Be-verages: Health Benefits, Physiological Effects, and Chemistry*. ACS Symposium Series 754.

Osakabe N.; C. Sanbongi; M. Yamagishi & T. Takizawa (1998a). Effects of polyphenol substances derived from *Theo-broma cacao* on gastric mucosal lesion induced by methanol. *Bioscience Biotechnology and Biochemistry,* 62, 1535–1538.

Osakabe N.; M. Yamagishi; C. Sanbongi; M. Natsume; T. Takizawa & T. Osawa (1998b). The antioxidative substances in cacao liquor. *Jounal of Nutrition Science and Vitaminology,* 44, 313–321.

Peleg, H.; K. Gacon; P. Schlich & A.C. Noble (1999). Bitterness and astringency of flavan-3-ol monomer, dimer and trimer. *Journal of the Science of Food and Agriculture*, 79, 1123–1128.

Porter, L.J.; Z. Ma & G. Chan (1991). Cacao procyanidins: major flavonoids and identification of some minor metabolites. *Phytochemistry,* 30, 1657–1663.

Rigaud, J.; M.T. Escribano-Mailon; C. Prieur; J.M. Souquet & V. Cheynier (1993). Normal-phase high-performance liquid chromatographic separation of procyanidins from cacao and grape seeds. *Journal of Chromatography A,* 654, 255–260.

Ruzaidi, A.; A. Maleyki; A. Ismail; A. G. Nawalyah; H. Muhajir; M. B. S. M. J. Pauliena & M. S. Muskinah (2008). Hypoglycaemic properties of Malaysian cocoa (*Theobroma cacao*) polyphenols-rich extract. *International Food Research Journal,* 15, 305—312.

Sanbongi C.; N. Osakabe; M. Natsume; T. Takizawa; S. Gomi & T. Osawa (1998). Antioxidative polyphenols isolated from *Theobroma cacao*. *Journal of Agricultural Food Chemis-try,* 46, 452–457.

Shamsuddin, S.B. & P.S. Dimick (1986). Qualitative and quantitative measurement of cacao bean fermentation. p. 55–78. ***In:*** Dimmick, P.S. (Ed.), *Proceeding of the symposium Cacao Biotechnology*. The Pennsylvania State University.

Singleton, V.L. & J.A. Rossi (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16, 144–158.

Weisburger, J. H. (2001). Chemopreventive effects of cocoa polyphenols on chronic diseases. *Experimental Biology and Medicine*, 226, 891–897.

Wollgast, J. & E. Anklam (2000a). Review on polyphenols in *Theobroma cacao:* changes in composition during the manufacture of chocolate and methodology for identification and quantification. *Food Research International,* 33, 423–447.

Wollgast, J. & E. Anklam (2000b). Polyphenols in chocolate: is there a contribution to human health?. *Food Research International,* 33, 449–459.

Wollgast, J.; L. Pallaroni; M.E. Agazzi & E. Anklam (2001). Analysis of procyanidins in chocolate by reversed-phase high performance liquid chromatography with electrospray ionization mass spectrometric and tandem mass spectrophotometric detection. *Journal of Chromatography A*, 926, 211–220.

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