UNIVERSITI PUTRA MALAYSIA

INFLUENCES OF COCOA POLYPHENOLS AND ENZYME REACTIVATION ON THE FLAVOR DEVELOPMENT OF UNFERMENTED AND UNDER-FERMENTED COCOA BEANS

MISNAWI

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By

MISNAWI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

June 2003

This Thesis is specially dedicated to my beloved:

Wife
F a t i m a h

Daughter
Miftalia Aisah

Son
Fahmiandi Kabul Ahsan

For the unconditional patient, love and support.

Abstract of dissertation submitted to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

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Faculty : Food Science and Biotechnology

Polyphenols are mainly responsible for the formation of astringency and bitterness in cocoa beans. Due to the propensity of polyphenols to interact with other compounds, studies have been carried out to evaluate influences of polyphenols in the development of cocoa flavor. The studies started with the evaluation of polyphenol changes during cocoa fermentation and roasting, followed by the study on the development of flavor during roasting of cocoa liquor at different polyphenol concentrations. In order to overcome the low aroma and excessive astringency problems in unfermented and under-fermented cocoa beans, studies on the reactivation of the remaining key enzymes in these beans were also carried out.

The study found that polyphenol concentration was reduced as high as 53% during cocoa fermentation. Oxidation and derivatization of the cocoa polyphenols decreased polyphenol ability to interact with protein and

produce astringency as supported by the decrease in tannin concentration as high as 39% from the concentration prior to fermentation and decrease in more hydrophobic polyphenols proportion during cocoa fermentation. Monomers, trimers and tetramers at 36, 20 and 16%, respectively were the predominant polyphenols present in unfermented cocoa bean. However, in fermented cocoa bean they were monomers, dimers and trimers which were 29, 16 and 16% of the total polyphenol, respectively. Pentamers, tetramers and dimers were highly correlated ($p < 0.01$) with astringency and bitterness.

Roasting of cocoa liquor at 120°C for 15 to 45 min significantly ($p < 0.05$) reduced polyphenol hydrophobicity and concentrations of polyphenol and tannin. During the roasting process, concentrations of polyphenol and tannin in fermented cocoa liquor decreased 2.6–3.3% and 20–33% from the concentration prior to roasting, respectively. However, in the fermented cocoa liquor enriched with unfermented cocoa polyphenol at 170 g kg^{-1}, the decreases were 4.7–8.9% and 2.3–7.5%, respectively.

Increases in polyphenol concentration in cocoa liquor from 58 to 170 g kg^{-1} did not only produce excessive astringency and bitterness, but also caused reduction on pyrazine formation during roasting. The reduction on the formations of 2,3,5-trimethyl- and 2,3,5,6-tetramethy- occurred through out roasting period. However, the reduction against 2,5-dimethyl- only occurred

at 35 min roasting time. Reduction on the formation of 2,3-dimethylpyrazine occurred at 25, 35 and 45 min roasting time.

Sensory evaluation indicated that the increases in polyphenol concentration significantly ($p < 0.05$) lowered the cocoa flavor and viscosity, and increased astringency and bitterness; however, it did not influence acidity, fruity/floral/bouquet, raw/green, smoky and mouldy/earthy properties of the liquor. Cocoa flavor scored at 6.4 out of maximum score of 10 was decreased to 5.2 due to the polyphenol increases from 58 to 170 g kg^{-1}. However, viscosity score was decreased from 4.2 to 3.0. In contrary, astringency score was increased from 3.6 to 5.3, and bitterness score was increased from 3.2 to 4.9.

Key enzymes remained in dried unfermented cocoa bean, namely polyphenol oxidase, aspartic endoprotease, carboxypetidase and invertase bean were found at 1, 33, 20 and 19% from the original activities, respectively; however, those in under-fermented cocoa bean were 0.08, 31, 16 and 7%, respectively. Reactivation of these enzymes through incubation at 45°C, pH 3.5–6.5 reduced the excessive polyphenol concentration and produced aroma precursors in the dried cocoa beans. Crude polyphenol oxidase extracted from fresh freeze-dried unfermented cocoa bean and tyrosinase from mushroom at concentration of 88–8,800 U g^{-1} could also be used to enhance oxidation of cocoa bean polyphenols.

Abstract disertasi yang dikemukakan kepada Senat Universiti Putra Malasysia sebagai memenuhi syarat untuk ijazah Doktor Falsafah

KESAN POLIFENOL DAN PENGAKTIFAN KEMBALI ENSIM KE ATAS PERKEMBANGAN PERISA KOKO YANG TIDAK DAN KURANG DITAPAI

Oleh

MISNAWI

Jun 2003

Pengerusi : Profesor Jinap Selamat, Ph.D.

Fakulti : Sains Makanan dan Bioteknologi

Polifenol merupakan unsur yang menghasilkan rasa kelat dan pahit di dalam biji koko. Berasaskan kepada kecenderungan polifenol membentuk ikatan dengan unsur-unsur lain, beberapa kajian telah dijalankan untuk mengetahui pengaruh polifenol ke atas pembentukan perisa koko. Kajian dimulakan dengan penyelidikan ke atas perubahan yang berlaku kepada polifenol semasa penapaian dan pemanggangan koko, dan selanjutnya kesan polifenol ke atas pembentukan perisa semasa pemanggangan. Untuk mengatasi masalah perisa yang lemah dan rasa kelat yang berlebihan di dalam biji koko yang tidak dan kurang ditapai, kajian selanjutnya telah dijalankan dengan pengaktifan kembali enzim-enzim utama yang tertinggal di dalam biji koko tersebut.

Hasil penyelidikan menunjukkan bahawa kandungan polifenol berkurang sebanyak 53% semasa penapaian. Oksidasi dan perubahan yang berlaku ke

atas polifenol boleh menurunkan kecenderungannya untuk berikatan dengan protein dan menghasilkan rasa kelat, sepertimana ditunjukkan oleh penurunan kandungan tannin sebanyak 39% dan penurunan proporsi polifenol yang hidrofobik semasa penapaian. Monomer, trimer dan tetramer adalah polifenol yang paling banyak terdapat di dalam biji koko segar, iaitu sebanyak 36, 20 dan 16%; manakala di dalam biji koko yang telah ditapai pula, ianya terdiri daripada monomer, dimmer dan trimer sebanyak 29, 16 dan 16%. Pentamer, tetramer dan dimer berhubungan erat ($p < 0.01$) dengan rasa kelat dan pahit pada likur koko.

Pemanggangan likur pada suhu 120°C selama 15–45 minit boleh mengurangkan sifat kehidrofobikan polyphenol dan kandungan polifenol serta tanin di dalam koko. Semasa pemanggangan likur koko yang ditapai kandungan polifenol dan tanin berkurang sebanyak 2.6–3.3% dan 20–33%, manakala pada likur koko yang ditapai di mana kandungan polifenolnya ditingkatkan kepada 170 g kg^{-1}, pengurangannya adalah 4.7–8.9% dan 2.3–7.5%.

Peningkatan kandungan polifenol di dalam koko dari 58 kepada 170 g kg^{-1} bukan sahaja menyebabkan rasa kelat dan pahit, tetapi juga mengurangkan penghasilan pirazin semasa pemanggangan. Pengurangan ke atas penghasilan 2,3,5-trimetil- dan 2,3,5,6-tetrametil- berlaku di sepanjang masa pemanggangan, manakala ke atas penghasilan 2,5-dimetil- hanya berlaku

pada masa pemanggangan 35 min. Pengurangan ke atas penghasilan 2,3-dimetilpirazin berlaku pada masa pemanggangan 25, 35 dan 45 min.

Ujian deria membuktikan bahawa peningkatan kandungan polifenol tersebut secara nyata ($p < 0.05$) menurunkan intensiti perisa koko dan sifat kelikatan, meningkatkan intensiti rasa kelat dan pahit, tetapi tidak memberikan kesan ke atas rasa asid, bau buah/bunga, bau rumput/mentah, bau asap dan bau kulat/tanah. Skor perisa koko menurun daripada 6.4 menggunakan skor maksimum 10 menjadi 5.2, sedangkan sifat kelikatan menurun daripada 4.2 menjadi 3.0. Sebaliknya, skor rasat kelat meningkat daripada 3.6 menjadi 5.3, dan rasa pahit meningkat daripada 3.2 kepada 4.9.

Enzim-enzim utama yang tersisa di dalam biji koko kering tidak ditapai, iaitu *polyphenol oxidase*, *aspartic endoprotease*, *carboxypetidase* dan *invertase* didapati 1, 33, 20 dan 19% daripada aktiviti semula; apabila di dalam biji yang kurang ditapai ianya adalah 0.08, 31, 16 dan 7%. Pengaktifan kembali enzim-enzim tersebut melalui inkubasi pada 45°C, pH 3.5-6.5 boleh mengurangkan kandungan polifenol yang berlebihan dan menghasilkan pelopor aroma dalam biji-biji koko tersebut. Enzim daripada luar koko seperti *polyphenol oxidase* kasar daripada biji koko segar yang dikeringkan secara sejuk beku dan *tyrosinase* daripada kulat juga boleh ditambahkan pada konsentrasi 88-8,800 U g^{-1} untuk mempercepat pengoksidaan polifenol.

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