# Development Of Technology To Improve Quality Of Under-Fermented Cocoa Through Enzymatic Modification

*Jinap Selamat, Jamilah Bakar and Nazamid Saari*

Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
43400 UPM, Serdang, Selangor
Malaysia

*Telephone Number of Corresponding Author: 03-89486314*
*E-mail of Corresponding Authorjinap@putra.upm.edu.my*

*Key words*: cocoa, flavour, fermentation, polyphenol, indigenous enzyme

## Introduction

Malaysia is well known as one of the main world producers of cocoa-based products and is the biggest in Asian region. Due to the cocoa bean's production shortage, this country is currently importing about 44,000 ton annually from Indonesia, which constitutes about 57% of the total import. However, most of these cocoa beans are under-fermented, which are characterized by low cocoa aroma intensity and excessive astringency and bitterness. Aroma precursors in cocoa beans which include the free amino acids, peptides and reducing sugars develop into cocoa specific aroma through maillard reaction during roasting. During cocoa fermentation, proteolysis catalyzed by aspartic endoprotease and carboxypeptidase gives rise to amino acids and oligopeptides The aspartic endoprotease from cocoa beans cleaves protein substrate preferentially at hydrophobic amino acids residues to produce oligopeptides having hydrophobic amino acids residues at their carboxyl terminal ends. Carboxypeptidase plays an important role in converting hydrophobic oligopeptides to cocoa specific aroma precursors namely hydrophilic oligopeptides and hydrophobic free amino acids, which are required for the formation of the typical cocoa aroma components in the presence of reducing sugar upon roasting. However, the predominant sugars in cocoa beans are sucrose, fructose and glucose. In unfermented cocoa beans, sucrose was found to be present in significant concentration (18.78 g $kg^{-1}$), comprising about 95% of the total sugars. During cocoa fermentation, sucrose was almost completely hydrolysed to fructose and glucose by invertase present in the beans. This project aimed to find a technology to improve flavour quality of under-fermented cocoa beans so that could be used for the production of quality cocoa products.]

## Materials and Methods

The project was carried out in three studies. First study is the study of remaining key enzymes in unfermented and under-fermented cocoa beans and their potential to be activated. Cocoa beans of PBC 140 obtained from Jengka, Pahang was used for the study. Aspartic endoprotease, carboxypeptidase, invertase and polyphenol oxidase activities were the parameters observed. Second study was reactivation of the indigenous enzymes and enzyme enrichment (polyphenol oxidase and carboxypeptidase) through incubation in phosphate buffer pH 3.5-6.5 for 1-32 hr at 45°C. Free amino acids, reducing sugars, peptide pattern, polyphenols, pyrazine and sensory properties of the resultant powder were evaluated. Third study was focused on the reduction of high polyphenol concentration in unfermented and under-fermented cocoa beans. Changes in polyphenol during fermentation and roasting (120° for 15-45 min), effects of polyphenol concentration (58-170 g $kg^{-1}$) on the production of pyrazine during roasting and effects of polyphenol on sensory properties of cocoa liquor were the main treatment in this study. The second and the third studies was using Ghanaian cocoa beans, however sensory evaluation was conducted by trained panelist in collaboration with Malaysian Cocoa Board.

## Results and Discussion

This study found that 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, tyrosinase from mushroom and carboxypeptidase from porcine could be used to enhance the production of flavour precursors and reduction of astringency and bitterness. The study also found that unpleasant excessive astringency and bitterness in unfermented and under-fermented cocoa beans' flavour is due to high polyphenol concentration. Furthermore, the polyphenol is reduced as high as 53% during cocoa fermentation. Oxidation and derivatization of cocoa polyphenols during the fermentation also 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.

## Conclusions

Reactivation of remaining key enzymes in dried unfermented and under-fermented cocoa beans, namely polyphenol oxidase, aspartic endoprotease, carboxypetidase and invertase can be employed to overcome low aroma and excessive astringency and bitterness in the beans. Exogenous polyphenol oxidase from freeze-dried fresh cocoa bean or mushroom and carboxypeptidase can also be used to enhance production of flavour precursors and reduction of polyphenol. Chemical characteristics and sensory properties of fermented bean can be obtained in unfermented and under-fermented cocoa beans treated with the enzyme reactivation.

## Benefits from the study

These findings would be beneficial for Malaysian cocoa and chocolate manufactures in which they can import cheap raw under-fermented cocoa beans to control the production cost; at the same time the beans can be treated to utilize for the production of quality chocolate products.

## Patent(s), if applicable:

Nil

## Stage of Commercialization, if applicable:

Nil

## Project Publications in Refereed Journals

Misnawi, Jinap, S., Jamilah, B. and Nazamid, S. 2002. Activation of remaining key enzymes in dried under-fermented cocoa beans and Its Effect on Aroma Precursors Formation. *Food Chemistry*, 78, 407-417.

Misnawi, **Jinap, S.**, Jamilah, B. and Nazamid, S. 2002. Changes in polyphenol ability to produce astringency during roasting of cocoa liquor. *Journal of the Science of Food and Agriculture* (accepted).

Misnawi, Jinap, S., Jamilah, B. and Nazamid, S. 2002. 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, **Jinap, S.**, Jamilah, B. and Nazamid, S. 2002. Polyphenol changes during fermentation and their impacts on astringency and bitterness of cocoa bean. *ASEAN Food Journal* (submitted)

Misnawi, Jinap, S., Jamilah, B. and Nazamid, S. 2003. Effects of incubation and polyphenol oxidase enrichment of unfermented and partly fermented dried cocoa beans on color, fermentation index and (−)-epicatechin content. *International Journal of Food Science and Technology*, 38, 1–11.

Misnawi, **Jinap, S.**, Jamilah, B. and Nazamid, S. 2003. Effects of cocoa liquor roasting on polyphenols content, their hydrophobicity and relation to astringency. *ASEAN Food Journal*, 12(2), 103–113.

Misnawi, **Jinap, S.**, Jamilah, B. and Nazamid, S. 2003. Inhibition effects of polyphenol on pyrazines formation during cocoa liquor roasting. *Food Chemistry* (in press)

Misnawi, **Jinap, S.**, Jamilah, B. and Nazamid, S. 2003. Sensory properties of cocoa liquor as affected by polyphenol concentration and duration of roasting. *Food Quality and Preference* (in press).

Yusep, I., Jinap, S., Jamilah, B. and Nazamid, S. 2003. Influence of carboxypeptidases on free amino acids, peptides pattern and methylpyrazines of under-fermented cocoa beans. J. Sci. Food Agric. (in press)

## Project Publications in Conference Proceedings

Misnawi, Jinap, S., Jamilah, B. and Nazamid, S. 2000. Polyphenol oxidase regeneration of under-fermented dried cocoa beans and its possibility to improve cocoa flavor quality. *Proc. Of the 13th International Cocoa Research Conference* (pp. 1025-1032. Kota Kinabalu, Sabah, Malaysia: 9-14 October 2000.

Misnawi, Jinap, S., Jamilah, B. and Nazamid, S. 2000. New approach in handling of under-fermented cocoa beans: reduction of polyphenols through enzymatic oxidation. *Proc. Of the 7th ASEAN Food Conference 2000,* (pp. 342-348). Manila, Philippine.

Jinap, S., Misnawi, Jamilah, B. and Nazamid, S. 2001. Malaysian high-polyphenol chocolate from partly fermented cocoa beans. *Pameran Penyelidikan Institusi Pengajian Tinggi Awam (IPTA).* Kuala Lumpur, 25-28 Oktober, 2001.

Misnawi, **Jinap, S.**, Jamilah, B. and Nazamid, S. 2002. Effects of cocoa liquor roasting on polyphenols content, hydrophobicity and tanning capacity. *Scientific Conference on Food Antioxidant 2002.* Bangi, Selangor DE, Malaysia: 23-24 April 2002.

**Jinap, S.** and Misnawi 2002. Effects of cocoa liquor roasting on polyphenol, hydrophobicity and antioxidant activity. *2nd International Meeting on Free Radicals in Health and Disease: The role of oxidants and antioxidants in the regulation of chronic diseases.* Istambul, Turkey, May 8-12, 2002.

Misnawi, Jinap, S., Jamilah, B., Nazamid, S. 2002. Polyphenol changes during cocoa bean fermentation and their impacts on astringency and bitterness. Maysian Science and Technology Congress 2002. Kuching, Serawak, Malaysia: 12-14 December, 2002.

**Jinap, S.**, Amin, I. and Jamilah, B. 1996. Cocoa aspartic endoprotease and carboxypeptidase activity during fermentation". In Proceedings of the 8th International Biotechnology Seminar, 25-27 November, 1996, Johor Baharu, Hasanah, M.C., Khatijah, M.Y and Marziah, M. (eds.), UPM. pp 359-362

Yusep, I., **Jinap, S.**, Jamilah, B. and Nazamid 2000. Influence of carboxypeptidase on specific aroma formation in underfermented dried cocoa beans". Proceedings of 13th International Cocoa Research Conference, 9th – 14th October, 2000, Kota Kinabalu, Sabah, Malaysia.

***Graduate Research***

| Name of Graduate | Research Topic | Field of Expertise | Degree Awarded | Graduation Year |
|---|---|---|---|---|
| Yusep Ikrawan | Influence of Carboxypeptidases on Cocoa Specific Aroma Precursors and Methylpyrazines in Under-Fermented Cocoa Beans | Enzymology, especially on proteases | Ph.D. | 2003 |
| Misnawi | Influences of Cocoa Polyphenols and Enzyme Reactivation on The Flavour Development of Unfermented and Under-Fermented Cocoa Beans | Flavour | Ph.D. | 2003 |
| Noor Soffalina S.S. | Study on Flavour Precursor – Polyphenol Interaction during Roasting of Under-fermented and Fermented Cocoa Beans | Flavour | Ph.D. | On going |

**IRPA Project number**01-02-04-0466
**UPM Research Cluster**AFF