



UNIVERSITI PUTRA MALAYSIA

**ALPHA-TOCOTRIENOL AS A POSSIBLE INDICATOR FOR
MONITORING THE PRESENCE OF PALM MID-FRACTION IN DARK
CHOCOLATE**

ELHAM MOAZAMI FARAHANY

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**MASTER OF SCIENCE
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By

ELHAM MOAZAMI FARAHANY

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfillment of the Requirements for the Degree of Master of Science**

October 2006



DEDICATIONS

**THIS PIECE OF WORK IS SPECIALLY DEDICATED TO Dr. AZIZ
NAGHDIVAND**



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

**TOCOTRIENOL AS A POSSIBLE INDICES FOR THE MONITORING OF
PALM MID-FRACTION IN DARK CHOCOLATE**

By

ELHAM MOAZAMI FARAHANY

October 2006

Chairman : Professor Jinap Selamat, PhD

Faculty : Food Science and Technology

The compositional variations of tocopherols and tocotrienols composition of the genuine CB and PMF were investigated to introduce a more reliable indicator in detecting as well as quantifying the PMF in CB. The results suggested that the α -tocotrienol data presented could be utilized for the detection of the PMF admixture to CB. Detection and of PMF admixture to CB in a chocolate model system was conducted using α -tocotrienol as an indicator. This study focused on mono-addition of PMF to CB. The PMF was added to CB at 1, 2, 3, 4, 5, 10, 15, 20 and 25% levels. HPLC was used to detect the presence of PMF admixture to CB. The results derived from the model system indicated that by increasing the PMF amount at 0 to 15% to CB resulted in an increase in the concentration of the α -tocotrienol significantly ($P < 0.05$). However, the addition of PMF amount more than 15% did not have any effect on the α -tocotrienol concentration. A linear plot with a high correlation of 0.9967 was obtained with standard error (SE) of 1.527. The PMF



amount in chocolate was at 1.4, 2.8, 4.2, 5.6, 5.9, 6.2, 6.4, 6.7 and 7% levels. There was a significant difference ($P < 0.05$) between the detected concentrations of the α -tocotrienol when the amount of the PMF was increased from 1.4% to 5.6%. However, the addition of PMF amount at 5.6 to 6.4% level did not have any effect on α -tocotrienol concentration. The interesting results were obtained when the amount of the PMF was increased from 6.4 to 7% where a significant difference was observed. A linear plot ($R^2 = 0.9837$) was obtained with SE of 1.986. A validation test was conducted to verify the equation obtained from the regression analysis. The high correlation obtained indicated a good accuracy, reflecting a close relationship between the predicted and actual values obtained by theoretical and experimental, respectively. From these studies the use of α -tocotrienol as a promising indicator for detection and quantification of PMF in chocolate was demonstrated. The results suggested that the amount of the added PMF in the CB in chocolate was predictable in the condition when smaller and more than 5% PMF was added. Therefore, it can be concluded that the identification of the foreign fat added was made possible because only one type of the foreign fat was added in this experiment scheme.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana

**PENGGUNAAN ALPHA-TOKOTRIENOL SEBAGAI BAHAN PETUNJUK
DALAM PENGESANAN PECAHAN PERTENGAHAN MINYAK KELAPA
SAWIT DI DALAM COKLAT GELAP**

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Kepelbagaian komposisi tokoferol dan tokotrienol di dalam CB asli dan PMF telah dikaji untuk mencari bahan petunjuk yang lebih sesuai dan berkesan dalam mengesan dan mengira kuantiti PMF di dalam CB. Hasil kajian mencadangkan bahawa data-data α -tokotrienol yang diperolehi boleh digunakan untuk mengesan PMF yang telah dicampur ke dalam CB. Pengesanan dan pengiraan kuantiti PMF di dalam CB menggunakan sistem model coklat telah dijalankan. Kajian ini tertumpu kepada penambahan PMF ditambah ke dalam CB secara tunggal. PMF ditambah ke dalam CB pada tahap 1, 2, 3, 4, 5, 10, 15, 20 dan 25%. HPLC telah digunakan untuk mengesan kehadiran PMF di dalam CB dengan menjadikan α -tokotrienol sebagai bahan petunjuk. Hasil kajian daripada sistem model menunjukkan bahawa penambahan jumlah PMF pada tahap 0% hingga 15% ke dalam CB



menyebabkan peningkatan yang bererti ($P < 0.05$) kepada tahap kepekatan α -tokotrienol. Walaubagaimanapun, penambahan jumlah PMF melebihi 15% tidak memberikan sebarang kesan ke atas kepekatan α -tocotrienol. Plot linear dengan korelasi yang tinggi sebanyak 0.9967 bersama sisihan piawai (SE) sebanyak 1.527 telah diperolehi.

Jumlah PMF di dalam coklat ialah pada aras 1.4, 2.8, 4.2, 5.6, 5.9, 6.2, 6.4, 6.7 and 7%. Perbezaan yang bererti ($P < 0.05$) di antara kepekatan α -tokotrienol telah diperolehi apabila jumlah PMF ditingkatkan daripada aras 1.4% kepada 5.6%. Walau bagaimanapun, penambahan jumlah PMF daripada aras 5.6% kepada aras 6.4% tidak mempunyai sebarang kesan ke atas kepekatan α -tokotrienol. Keputusan yang memberansangkan di mana perbezaan yang bererti telah diperolehi apabila jumlah PMF ditingkatkan daripada aras 6.4% kepada 7% plot linear ($R^2 = 0.9837$) dengan SE sebanyak 1.986. Ujian pengesahan juga telah dijalankan untuk menentusahkan persamaan yang telah didapati daripada analisis regresi. Korelasi tinggi yang diperolehi menunjukkan ketepatan yang tinggi dan ini juga menunjukkan hubungan rapat di antara nilai-nilai yang diramal dan nilai-nilai sebenar yang didapati masing-masingnya secara teori dan praktikal.

Hasil kajian ini menunjukkan bahawa α -tokotrienol boleh digunakan sebagai bahan petunjuk yang berpotensi untuk pengesanan dan pengiraan kuantiti PMF di dalam coklat. Hasil kajian juga mencadangkan bahawa jumlah penambahan PMF ke dalam CB di dalam coklat boleh diramal apabila PMF telah ditambah melebihi 5%. Oleh itu, boleh disimpulkan bahawa pengenpastian lemak-lemak asing yang ditambah boleh dilakukan

kerana hanya terdapat satu jenis lemak asing digunakan atau ditambah di dalam eksperimen yang dijalankan di dalam skema kajian ini.



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I certify that an Examination Committee met on 19th Oct 2006 to conduct the final examination of Elham Moazami Farahany on her Master of Science thesis entitled “The Use of α -Tocotrienol for Detection of Palm Mid-Fraction in Dark Chocolate” in accordance with Universiti Pertanian Malaysia (higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

ELHAM MOAZAMI FARAHANY

Date:



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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
AOAC	Association of official Analytical Chemists
AOCS	American Oil Chemists' Society
CB	Cocoa butter
CBA	cocoa butter alternative
CBE	cocoa butter equivalent
CBS	cocoa butter substitute
C12:0	lauric acid
C14:0	myristic acid
C16:0	palmitic acid
C16:1	palmitoleic acid
C18:0	stearic acid
C18:1	oleic acid
C18:2	linoleic acid
C18:3	linolenic acid
C20:0	arachidic acid
C22:0	behenic acid
DSC	differential scanning calorimetry
EC	European Commission
FAME	Fatty acid methyl ester
FAO	Food and Agricultural Organization
FDA	Food and drug administration



GC	gas chromatography
g	gram
HPLC	high performance liquid chromatography
IUPAC	International Union of Pure and Applied Chemistry
IV	iodine value
J	journal
MCB	Malaysian Cocoa Board
mg	milligram
min	minute
mL	Milliliter
MP	melting point
MD	mean of differences
NMR	nuclear magnetic resonance
PKO	palm kernel olein
PMF	palm mid-fraction
POO	1-palmitoyl-2,3-dioleoyl -sn-glycerol
POP	1,3-dipalmitoyl-2- oleoyl-sn-glycerol
PORIM	Palm Oil Research Institute of Malaysia
POS	palmitoyl-oleoyl- stearoyl-sn-glycerol
SAS	statistical analysis system
SDD	Standard deviation of difference
SE	Standard Error
SOO	1-stearoyl-2,3-dioleoyl -sn-glycerol



T	Tocopherol
T3	Tocotrienol
TAG	triacylglycerol
TLC	thin layer chromatography
UM	unsaponifiable matter
USDA	United State Drug Administration



CHAPTER I

GENERAL INTRODUCTION

Fats and oils from various sources are important ingredients in the manufacture of confectionery products. They provide unique characteristics to food products, mainly in chocolate and sugar confectionery. In order to develop new products, it is essential to understand the important roles of both fats and oils in influencing consumers' perceptions of the food items. Fat is typically a major component of most recipes and in general, it imposes the highest cost as compared to other ingredients. Weyland (1999) and Herzing (1989) suggested that fats also served as the primary carriers of many flavors.

In dark chocolate, fat is cocoa butter (CB) whereas in milk chocolate, it is with cow's milk fat. As both fats and oils serve a variety of applications in the productions of chocolate and confectionery items, no application has received more attention than the development of vegetable fats for cocoa butter alternatives. The high proportion of the vegetable fats to other fats and oils as well as the volatile CB prices caused by the uncertain supplies of cocoa beans each year have brought potential for these fats to replace CB in chocolate and confectionary applications (Simoneau *et al.*, 1999; Pease, 1985).

The vegetable fats for use in chocolate must comply with the outlined analytical criteria in order to allow qualitative and quantitative control. These include i) the



level of triacylglycerol type SOS which must be greater than 65%, 2) the fractions of unsaturated fatty acids occupying 2-positions in triacylglycerol must be greater than 85%, 3) the total content of unsaturated fatty acids must be less than 45%, and 4) if they have less than 5% of two or more double bonds, the level of lauric acid has to be lesser than 2% (Padley and Timms, 1980).

The substitution of CB with CBEs by manufacturers of chocolate products is mainly caused by economic reasons; CBEs possess technical advantages such as the structure of the chocolate, better milk fat tolerance as well as lesser bloom on the surface of the chocolate (Buchgraber *et al.*, 2004). Non-cocoa fats used in confectionery are of mixtures known as cocoa butter alternatives (CBA), of which the most important is the cocoa butter equivalents (CBEs). They are formulated from the non-hydrogenated fat fractions with a triacylglycerol composition which is almost identical with the CB. By definition, they must be fats which are low in lauric acid (non-lauric) but rich in 1,3-dipalmitoyl-2-oleoyl-sn-glycerol (POP), 1-palmitoyl-2-oleoyl-3-stearoyl -sn-glycerol (POS) and 1,3-distearoyl -2-oleoyl-sn-glycerol (SOS) triacylglycerols, miscible with CB and obtained only by refining and fractionation.

Six non-cocoa vegetable fats are specified, illipe which is derived from *Shorea* species, palm oil and its related products from *Elaeis guineensis*, sal from *Shorea Robusta*, Shea from *Butyrospermum parki*, kokum from *Garcinia indica* and mango kernel from *Mangifera indica*. Palm mid fraction (PMF) is one of the components that can be used as CBE other than shea, illipe and sal fat in chocolate. Due to the fact that certain fractions of these fats are similar to CB in all respects, there are potentials

for them to be added to it fraudulently without proper detection. Other alternative fats such as cocoa butter replacers (CBRs) and cocoa butter substitutes (CBSs) are particularly used in manufacturing special forms of chocolate application such as coating (Simoneau *et al.*, 1999; Codex Alimentarius, 1981; Aljowder *et al.*, 1997).

With regards to cocoa and chocolate products, the new directive of the Codex Alimentarius (1981) allows an addition of vegetable fats other than cocoa butter at levels up to only 5% based on the finished products. The main reasons of this directive was to monitor the distributions of goods by providing a definition of chocolate suitable for sale, more defined packaging and labeling requirements as well as informing the consumers of the product's contents in term of the vegetable origins of the fats used. The substitution of CB is crucial in several respects such as the melting behavior of CBE has to be very similar to CB in order to achieve the same mouth feeling. Therefore, if CB is to be partly substituted, the addition of other fats must not alter the melting behavior of CB.

Various components of cocoa butter have been suggested as indicators for the detection of added vegetable fats other than cocoa butter in chocolate. The fatty acid and the triacylglycerol data can be utilized for the detection and quantification of the CBEs in plain chocolate. In principle, the triacylglycerol composition of the CBE is similar but mostly not identical to the genuine CB. These differences can be exploited to determine the presence of the CBE in confectionery products (Simoneau *et al.*, 1999; Lipp and Anklam, 1998a).