UNIVERSITI PUTRA MALAYSIA

PRODUCTION OF PROCESSED CHEESE USING LIPASE-CATALYZED TRANSESTERIFIED PALM KERNEL OLEIN AND ANHYDROUS MILK FAT MIXTURES

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MARIAM MOHAMED ISMAIL

Thesis submitted in Fulfillment of the Requirements for the Degree of Master of Science in the Faculty of Food Science and Biotechnology
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To those who are proud of my success

my life partner,

Fareg

and my beloved daughter,

Eman

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All praise due to Allah, the Most Gracious and Merciful, for giving me the strength, health and determination to complete my study.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

Symbol	Meaning
AMF	Anhydrous Milk Fat
AOCS	American Oil Chemist Society
BS	British Standard
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
C22:1	Erucic Acid
Ca^{2+}	Calcium
CBS	Cocoa Butter Substitute
DG	Diglycerides
DSC	Differential Scanning Calometry
FA	Fatty Acids
FFA	Free Fatty Acids

HMG	High Melting Glycerides
HPLC	High Performance Liquid Chromatography
IDF	International Dairy Federation
IV	Iodine Value
LMG	Low Melting Glycerides
LMP	Last Melting Peak
LOO	1-linoleoyl-dioleoyl glycerol
LOP	1-linoleoyl-2-oleoyl-palmitoyl glycerol
MG	Monoglycerides
MMG	Medium Melting Glycerides
Na^+	Sodium
$Na_3C_6H_5O_7$	Sodium Citrate
NBD	Neutralized Bleached Deodorized
NMR	Nuclear Magnetic Resonance
NTF	Non Transesterified Fat
OOO	Triolein
pH	Hydrogen Ion
PKO	Palm Kernel Olein
POP	1,3-dipalmitoyl -2-oleoyl glycerol
PORIM	Palm Oil Research Institute of Malaysia
POS	1-palmitoyl-2-oleoyl-stearoyl glycerol
PPP	Tripalmitin

PPS	1,2-dipalmitoyl-stearoyl glycerol
r	Correlation Coefficient
RBD	Refined Bleached Deodorized
rpm	Revelation Per Minute
SFC	Solid Fat Content
SMP	Slip Melting Point
SMS	Stable Micro System
Sp	Species
TA	Titrable Acidity
TF	Transesterified PKO and AMF Fat
TG	Triglyceride
TLC	Thin Layer Chromatography
v/v	Volume Per Volume
w/w	Weight Per Weight
XRD	X-Ray Diffraction

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

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By

MARIAM MOHAMED ISMAIL

July 1999

Chairman: Professor Hasanah Mohd. Ghazali, Ph.D.

Faculty: Food Science and Biotechnology

In recent years, lipase-catalyzed transesterification has received considerable attention from the fats and oils industry due to its potential to modify the physical and chemical properties of edible fats and oils. In this study, enzymatic transesterification of three different ratios of palm kernel olein (PKO) and anhydrous milk fat (AMF) was carried out using immobilized lipase from *Rhizomucor miehei* (Lipozyme IM 60) to modify their chemical and physical properties, such that they can be used in the production of processed cheese.

After transesterification for 8 hours, there were either increases or decreases in the concentrations of several existing triglycerides (TG) of the reaction mixtures and, in some cases, the formation of new peaks. The results also showed that increasing the reaction time, increased the % free fatty acid (FFA) between 1 to 2%.

The slip melting points (SMP) of the fat mixtures, which were determined after removal of free fatty acids, declined after the transesterification process. The solid fat content (SFC) decreased with respect to the control samples. At 40°C, the PKO:AMF (7:3) transesterified fat mixture was completely melted, making it a potential candidate in the formulation of processed cheese. Thermal profiles showed that the original peaks of the transesterified mixtures have either shifted or completely changed and some new peaks were also formed. It was also found that the transesterified fat mixtures were in the β' form, compared to a predominantly β form before transesterification.

After the preparation of experimental processed cheese, several chemical and physical analyses of this final product were carried out. The results showed that the experimental processed cheese had a satisfactory aroma and texture compared to the commercial sample. It also recorded a high total solid content and a low amount of moisture, with a good workability and overall general behaviour at cool, ambient, and elevated temperatures.

Sensory evaluation and texture analysis studies were also conducted on the final product. The experimental processed cheese samples achieved an acceptable response from the panelists, where there was no significant difference between the samples in terms of colour and texture. The texture profiles indicated that the experimental processed cheese samples exhibited close texture profiles compared to their counterpart commercial sample.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

PENGHASILAN KEJU TERPROSES MENGGUNAKAN CAMPURAN OLEIN ISIRONG KELAPA SAWIT DAN LEMAK SUSU TANPA AIR YANG DITRANSESTERIFKASI MELALUI PEMANGKINAN BERLIPASE

Oleh

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Pada masa kini, transesterifikasi yang dimangkin oleh lipase telah menerima perhatian dari industri minyak dan lemak disebabkan potensinya untuk mengubah ciri-ciri fizikal dan kimia minyak dan lemak. Dalam kajian ini, transesterifikasi secara ber enzim di antara campuran minyak sawit isirong olein (PKO) dan lemak susu anhidrus (AMF) pada tiga nisbah yang berbeza telah dijalankan menggunakan lipase tersekat-gerak dari *Rhizomucor miehei* (Lipozyme IM 60) untuk mengubah ciri-ciri kimia dan fizikal dalam campuran untuk membolehkan campuran minyak untuk menghasilkan keju terproses.

Selepas transesterifikasi selama 8 hours, terdapat pengurangan atau penambahan kepekatan beberapa trigliserida yang menang wujud dalam campuran yang bertindakbalas, dan pembentukan puncak-puncak baru dalam kes-kes tertentu. Keputusan juga menunjukkan bahawa peningkatan masa tindakbalas telah menyebabkan penambahan % asid lemak bebas di antara 1 hingga 2%. Titik lebur (SMP) campuran-campuran lemak yang ditentukan setelah dikeluarkan asid-asid lemak bebas didapati menurun selepas proses transesterifikasi. Kandungan pepejal lemak (SFC) telah berkurangan berbanding dengan sampel-sampel kawalan. Pada suhu 40°C, campuran lemak PKO:AMF (7:3) tertransesterifikasi telah lebur sepenuhnya. Ini menjadikan campuran tersebut berpotensi dalam formulasi keju terproses. Gambarajah terma menunjukkan bahawa puncak-puncak asal dalam campuran-campuran tertransesterifikasi telah samada beralih tempat atau berubah sepenuhnya dan beberapa puncak baru telah terbentuk. Selain itu, keputusan juga menunjukkan bahawa campuran-campuran lemak tertransesterifikasi menghasilkan bentuk kristal jenis β', dimana bentuk β adalah dominan sebelum transesterifikasi.

Selepas penyediaan keju terproses, beberapa analisis kimia dan fizikal produk akhir telah dilakukan. Keputusan yang diperolehi menunjukkan behawa keju terproses yang disediakan mempunyai aroma yang memuaskan dan tekstur yang setanding dengan sampel komersial. Produk juga merekodkan kandungan pepejal yang tinggi dan kuantiti lembapan yang rendah dengan kebolehan kerja yang baik dan sifat umum keseluruhan pada suhu-suhu sejuk, ambien dan tinggi.

Kajian penilaian deria dan analisis tekstur juga dijalankan pada produk akhir. Sampel-sampel keju terproses yang diediakan telah mencapai respon yang boleh diterima daripada para ahli panel dari segi warna dan tekstur sifat sampel-sampel ini tidak menunjukkan perbezaan yang bererti. Sampel-sampel keju terproses menunjukkan sifat tekstur yang hampir sama dengan sampel komersial.

CHAPTER I

INTRODUCTION

Fats and oils are complex mixtures of simple and mixed triglycerides that function as energy reserves. Triglycerides differ according to the types and composition of fatty acids and their distribution along the glycerol backbone. These characteristics play an important role in the physical, rheological, and nutritional properties of fats and oils.

In recent years, the use of lipases as catalysts has received considerable attention because of its potential ability to modify the physical and chemical properties of edible fats and oils (Kyotani et al. 1988; Yamane et al. 1989; Miller et al., 1991; Choo and Rhee, 1993; Foglia et al., 1993; Ghazali et al., 1995b; Lai et al., 1998). The main technical application of lipases is to modify the fatty acid compositions of triglycerides by interesterification.

Lipases are a group of enzymes that preferentially catalyze the hydrolysis and synthesis of triglycerides. Immobilized lipases show many advantages over traditional inorganic catalysts (Eigtved et al., 1986; Hansen et al., 1986; Miller et al., 1988). They have large catalytic activity under mild operating conditions. They show large selectivity to the desired product with no significant side reactions, leading to products of high purity. They are easily recovered from the reaction mixture and can be reused. There is no contamination of the final product, saving time and cost in the purification stage (Coteron et al., 1998).

Transesterification with lipase provides a useful way to improve the properties of triglyceride. Through enzymatic transesterification, it is possible to incorporate a desired acyl group onto specific positions of the glycerol, whereas chemical transesterification does not possess this regiospecificity due to the random nature of the reaction (Sreenivasa, 1978; Norris, 1982; Gunstone and Norris, 1983). Thus, lipase-catalyzed transesterification can provide regio- or sterospecific structured lipids for nutritional, medical, and food applications (Akoh, 1995; Lee et al., 1997; 1998).

Transesterification reaction is most often confirmed by closely monitoring changes in the product's physico-chemical properties (Goh et al., 1993; Kerry, et al., 1993). Physical properties such as melting characteristics, crystallization, and thermal behaviour are commonly used as specifications for fats and oils. The melting characteristics including melting point and solid fat content of fat are among the most important predictors of its functionality used in industry today, and many fat ingredients are selected for applications based on these characteristics (Kaylegian et al., 1993). The SFC profile over a given temperature range is an important characteristic in fat functionality that can be used to predict how the fat will behave at different stages of manufacturing (rolling and baking of doughs). Another important indicator of fat functionality is its crystallization behaviour, which dictates the crystal form present and the respective stabilities of crystals. Fats, in particular their component triglyceride molecules, can crystallize in more than one polymorphic forms which differ from each other in terms of melting point, heat of fusion and crystallization, and density. The α form is a form that crystallises from the melt, has a strong single short spacing at 0.415nm and exhibits an enantiotropic transition on further cooling Hagemann (1988). According to Hagemann (1988), D'Souza et al. (1990) and Timms (1991), the β' form is a form exhibiting two strong short spacing bands at 0.42 and 0.38nm, and the β form exhibits three strong short spacings bands at 0.46, 0.39 and 0.37nm. Thermal analyses of fats and oils are also indicators of their physical properties. Differential scanning calorimetry (DSC) is the most convenient method for determining the thermal behaviour of fats and oils (Hagemann, 1988; Che Man et al., 1995; Haryati et al., 1996). It can provide much information on the behaviour of fats and oils.

Cheese is a commercial product, which is widely consumed and almost the one food of good keeping ability, high in protein with fat, calcium phosphorus, riboflavin, and other vitamins, in a concentrated form (Sukumar, 1980). Cheeses of various kinds always have been important sources of nutrients wherever milk-producing animals could be raised. It has been an art handed down from generation to generation and was known as a staple food with bread since 6000BC (Scott, 1986). Natural cheese is made from the curd of the milk of cows and other animals, the curd being obtained by the coagulation of milk casein with an enzyme (usually rennin), an acid (usually lactic acid), and with or without further treatment of the curd by heat, pressure, salt, and ripening with selected microorganisms (Sukumar, 1980).

Progress in the development of cheese production, combined with a considerable increase in the quality of the finished product, made its appearance at the end of the 19th century (Kosikowski, 1982). However, there are some constraints (from a nutritional viewpoint) on the use of cheese depending on the interpretation of the word 'cheese' (Meyer, 1973). Therefore, some fresh curds still hold lactose in the free whey and this may cause allergic reactions in susceptible persons, while riper cheese with no free lactose is safe. Extremely ripe cheese, where the protein is well broken down to peptones and amino acids, can be absorbed very quickly and so create difficulty in some persons, who should, therefore, avoid overripe cheese. Although these difficulties when consuming cheese do exist for a minority of the population, the individual has usually a wide assortment of varieties from which to choose so that some of the difficulties can be avoided (Potter, 1978). However, the cheese may reach a condition that makes it unfit for consumption. This is due to uncontrollable protein decomposition, where all types of cheese, including the hard cheeses, are unsuitable for long storage, in the sense of forming an "iron ration". In addition, the harder cheeses are also dependent on temperature, for instance, it is a well-known fact that hard and semi-hard cheese in tropical countries become unfavorably altered in flavour and texture (Meyer, 1973).

To overcome the shortcoming of the natural cheese, the processed cheese becomes a solution. The invention of processed cheese in 1911 has been a very important step forward in the dairy field (Kosikowski, 1982). Processed cheese is the