PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 11 (4): 360-366, 2012
ISSN 1680-5194

Potential of a Soft Spreadable Margarine Produced from Waste Chicken Fat and Corn Oil Catalyzed by the *Thermomyces lanuginosus* (TI lm) Lipozyme

Afida Tasirin[1] and Mamot Said[2]
[1]Department of Chemistry, Jalan Sultan 46661, Petaling Jaya, Selangor, Malaysia
[2]Food Science Programme, School of Chemical Sciences and Food Technology,
Faculty of Science and Technology, University Kebangsaan Malaysia, Bangi, Selangor, Malaysia

Abstract: Low-fat bread spreads have become very popular since conventional spreads have been shown to be unhealthy due to high amounts of saturated fat and *trans* fatty acids. Two interesterified products, sample 16 (4% *Thermomyces lanuginosus* lipozyme, 4:1 molar ratio of chicken fat to corn oil and 42 h of interesterification at 50ºC) and sample 17 (4% lipozyme, 2:1 molar ratio of chicken fat to corn oil and 42 h of interesterification at 30ºC), were selected that had the highest Solid Fat Content (SFC) at 30ºC. Both the samples contained high proportions of low melting triglycerides, which explains the lower melting temperatures (sample 16, -37.45ºC to 31.40ºC; sample 17, -39.78ºC to 35.4ºC) and crystallization temperatures (sample 16, 0.58°C to -38.90ºC; sample 17, -2.45ºC to -34.27ºC for sample 17) and solid fat content (sample 16, 3.2% at 20ºC; sample 17, 3.5% at 20ºC). The enzymatic process caused the Free Fatty Acid (FFA) values to increase from 0.13-0.48% (sample 16) and 0.16-0.66% (sample 17). The final product (sample 16) had a smaller and less dense fat particle and is a low-cost alternative for soft spreads. The crystallization and melting properties of blends of chicken fat and corn oil result in a product that has a wide plastic range but still contains an unsaturated fatty acid content nearly equal to those based on plant sources.

Key words: Solid fat content, soft spread, triglycerides, melting and crystallization

INTRODUCTION

Margarine was first introduced by Michel Eugène Chevreul in 1813. Soon after the hydrogenation process was discovered, it became a popular process for producing margarine. Hydrogenation is capable of producing a more solid fat as a result of the elimination of double bonds in lipids. However, the process results in the formation of *trans* fatty acids, which are associated with Coronary Heart Disease (CHD). Enzymatic interesterification is the best alternative for producing the spreadable product since it can reduce the *trans* fatty acid. In their research, Zhang *et al.* (2001) used palm stearin and coconut oil to produce margarine on a large scale using *Thermomyces lanuginose* and Yap *et al.* (1989) blended palm oil and canola oil, both of which resulted in less *trans* fatty acid formation. According to Mat Shahri *et al.* (2008), the recommended saturated fat content in food products is less than 33% and *trans* fatty acids is less than 1%.

Margarine can now be obtained from various sources of animal fat, vegetable oil, or blends thereof with a skim milk mixture, salt and emulsifiers. However, to produce a good quality spreadable product, the most important factors are the solid fat range, texture, crystal size of less than 5 μm and diversity of glyceride chains (Sato and Ueno, 2005). The solid fat content influences the properties of plasticity at room temperature (25ºC) and results in a desirable mouth feel at temperatures between 33 and 38ºC (Brekke, 1980). Enzymatic interesterification of chicken fat and liquid oil high in polyunsaturated fatty acids, such as corn oil, is predicted able to produce a soft spreadable comparable to the product from pure vegetable sources.

The goal of this study was to evaluate the potential of soft margarine produced from the byproduct of chicken fat and corn oil by enzymatic interesterification using selected physicochemical properties.

MATERIALS AND METHODS

Immobilized lipase from *Thermomyces lanuginose* (TL IM) was provided by Novo Nordisk. Chicken fat was prepared by wet rendering process in laboratory, while corn oil was purchased from local stores.

Preparation of chicken fat: Fresh chicken fat was collected from wet market and washed to remove the dirt. The chicken fat was placed into a 500 ml beaker and boiled at 100°C for 30 min. The mixture containing molten fat (oil) and water was transferred into a beaker and kept in a refrigerator overnight. The solidified fat (top layer) was collected, warmed, filtered through Whatman 4 filter paper. The filtrate was transferred into an airtight bottle and kept in a fridge until further use.

Corresponding Author: Afida Tasirin, Department of Chemistry, Jalan Sultan 46661, Petaling Jaya, Selangor, Malaysia

Table 1: Actual factor levels corresponding to coded factor level

		Degree of factor				
Factors	Symbol	-2	-1	0	1	2
Percentage of enzyme (%)	A	1	2.5	4	5.5	7
Ratio of mole subtract (mole of chicken fat: 1 mole of corn oil)	B	0:1	1:1	2:1	3:1	4:1
Temperature (°C)	C	30	40	50	60	70
Period of reaction (h)	D	6	24	42	60	78

Experimental design for RSM study: Factors of interesterification reaction were determined by the Experts Design software using Central Composite Design (CCD) in the program of Response Surface Methodology (RSM). The Factors selected in this design were percentage of enzyme (A), the mole ratio of substrate (B), temperature (C) and reaction time (D). Independent variables and the experimental design were shown in Table 1. Experiments were conducted at random. Total of 30 analyses were performed with five levels (-2, -1, 0, 1, 2) and six central points (0, 0, 0) were designed.

Enzymatic Interesterification: Enzymatic interesterification of chicken fat and corn oil blends (30 g) were performed in a 250 mL beaker, catalyzed by immobilized lipase (TL IM). The optimization of the reaction parameters (substrate mole ratio, enzyme concentration, temperature, duration) were based on Response Surface Methodology (RSM) using Design Expert® version 6. The mixtures were incubated in an orbital shaking water bath agitated at 300 rpm. The molecular masses of fat and oil were estimated based on their saponification values (Kuntom *et al*., 2005) determined earlier. The reaction was stopped by removing the immobilized enzyme through filtration by using Whatman no. 4 filter paper. The products of interesterification were transferred into a separating funnel and neutralized to remove free fatty acids as described by (Lee and Akoh, 1998). The dried samples were transferred into airtight containers and stored in a freezer (4°C) until used for further analysis.

Triacylglycerol (TAG) profile analysis: The reaction mixtures were analyzes according (Shekarchizadeh *et al*., 2009) with slight modification. HPLC equipped with an auto sampler and UV/vis scanning detector was used in the study. A non-aqueous spherical RP-C18 column (15 cm x 4.6 mm, 5 µm) with solvent mixture (acetonitrile/tetrahydrofuran (75:25, v/v (solvent A)) and water (solvent B)) were used for the separation. The following solvent gradient profile: initial condition 80:20 (Volume percent A/B) for 10 min, then to 100% A held for 25 min and then returned to original conditions over 5 min. The UV spectra of TAG were determined at 220 nm and the oven set at 40°C.

Free fatty acid analysis: The Free Fatty Acid (FFA) content was determined in accordance with MPOB official method MPOB p2.5: 2004 (Kuntom *et al*., 2005).

Peroxide value analysis: The Peroxide Value (PV) content was determined in accordance with MPOB official method MPOB p2.3: 2004 (Kuntom *et al*., 2005).

Fatty acid profile analysis: Samples were analyzed by gas chromatography-flame ionization detector. Silica column (SP 2560, 100 m x 0.25 mm ID, 0.25 µm)) was used for the separation. Elution was carried out with temperature programming from 140-240°C at 4°C/min (Kuntom *et al*., 2005).

Solid fat content analysis: The percentage of Solid Fat Content (SFC) in the samples was analyzed according to (Zainal and Yusoff, 1999) by using Pulsed Nuclear Magnetic Resonance Equipment (PNMR), Bruker Minispec 20 MHz PC (Karlsruhe, Germany). Prior to analysis, the samples were placed in an oven at 60°C for 30 min then were cooled at 0°C for 90 min. Solid fat content was then measured after equilibrating the samples at 0°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 37°C for 30 min each.

Morphology analysis: The morphology of the interesterified products was analyzed by using polarized light microscope (model Leica) equipped with PixelLink uScope software as reported by (Rousseau and Marangoni, 1998). A small amount of samples were first melted by heating it in an oven at 60°C and then transferred onto a glass slide, covered and cooled at 5°C in a chiller for 24 h. Eyepiece and objective lenses with 10x magnification was used to give photographic magnification of 100x. The images were recorded at room temperature as quickly as possible.

Melting and crystallization analysis: Melting and crystallization profiles of the interesterified and commercial products were determined by using Mettler-Toledo Differential Scanning Calorimeter (DSC) model 822e (Schwerzenbach, Switzerland) according to the method reported by (Lee and Foglia, 2000). Calibration was done by using indium as a reference standard (melting point 176°C). A total of 3-5 mg samples were placed in aluminum plates with an empty plate used as a reference. Plates were heated to 80°C from room temperature for 10 min to destroy crystal memory. Then the temperature was decreased to -60°C at 10°C/min for 10 min to record the crystallization behavior. The temperature was raised back to 80°C at 5°C/min to determine the melting profile of the samples.

Table 2: ANOVA analyses for the response for the reaction catalyzed by Lipozyme TL IM

Source	Sum of squares	DF	Mean squares	F-value	Prob>F-value
Model	994.00	14	71.00	16.56	<0.0001
A	22.00	1	22.00	5.13	0.0387
B	694.02	1	694.02	161.91	<0.0001
C	6.667E-005	1	6.667E-005	1.555E-005	0.9969
D	42.88	1	42.88	10.00	0.0064
A^2	102.17	1	102.17	23.83	0.0002
B^2	94.76	1	94.76	22.11	0.0003
C^2	2.02	1	2.02	0.47	0.5031
D^2	7.31	1	7.31	1.71	0.2113
AB	0.41	1	0.41	0.096	0.7615
AC	53.36	1	53.36	12.45	0.0030
AD	2.74	1	2.74	0.64	0.4366
BC	3.28	1	3.28	0.76	0.3958
BD	1.44	1	1.44	0.34	0.5708
CD	0.25	1	0.25	0.057	0.8143
Lack of fit	64.30	15	4.29		
Pure error	50.78	10	5.08	1.88	0.2521
	13.51	5	2.70		
Cor total	1058.30	29			
R^2	0.9392				
Adjusted R^2	0.8825				
Predicted R^2	0.7052				
AdeqPrecision	15.979				
CV	15.97				

Table 3: Free fatty acid (%) and peroxide value (meq/kg) of sample 16 and sample 17 before and after interesterification process

Analysis	Sample 16	Sample 17	Sample 16 (EIE)	Sample 17 (EIE)
FFA (%)	0.13	0.16	0.56	0.48
PV (meq/kg)	11.92	12.66	12.76	8.21

RESULTS AND DISCUSSION

Response Surface Methodology (RSM) study: The aim of this study was to modify the physical and chemical characteristics of a chicken fat and corn oil blend by enzymatic interesterification to produce a soft margarine with features similar to oil produced from pure vegetable oil but using animal by-products as the fat source. A melting range close to soft margarine (30-37°C) was achieved through Response Surface Methodology (RSM) and the respective design points are shown in Table 1. Results are shown in Table 2 of the interesterification reactions of chicken fat and corn oil blends catalyzed by Lipozyme TL IM (oleic acid incorporation, w/w). We obtained a quadratic model for the incorporation of oleic acid using multiple regressions. An ANOVA analysis was used to determine which factors in the model were significant ("Prob > F" less than 0.05). A signal greater than 15.979 and a signal to noise ratio, referred to as "Adeq Precision", of greater than 4 and are adequate. This model can be used to navigate the design space. However, R^2 and CV values were not significant, at 0.9392 and 15.97, respectively. We found that the only four factors that significantly affected the incorporation of oleic acid in the sample blends were B, A^2, B^2 and AC (for the meaning of the symbols please refer to Table 1). The optimum value for the incorporation of oleic acid into the Structure Lipid (SL) as shown by the experimental design was 4.7%. The reaction parameters for obtaining the optimum incorporation of oleic acid were an enzyme concentration of 5.47%, a molar ratio of chicken fat to corn oil of 3:1, a temperature of 43.47°C and reaction time of 59.97 h. From a total of 30 blends, sample 16 (4% lipozyme, 4:1 molar ratio of chicken fat to corn oil and 42 h of interesterification at 50°C) and sample 17 (4% lipozyme, 2:1 molar ratio of chicken fat to corn oil and 42 h of interesterification at 30°C) were selected since they produced the highest SFC at 30°C.

Free fatty acid and peroxide value: Free Fatty Acid (FFA) is one of the by-products of the interesterification process and can be measured in order to determine the rate of hydrolysis of oil. Unlike other by-products such as diacylglycerol and monoacylglycerol, free fatty acids reduce the yields of and degrade the final product due to oxidative rancidity. The amount of FFA increased in both samples 16 and 17 after interesterification from 0.13-0.48% and 0.16-0.66%, respectively (Table 3). Generation of FFA during the initial phase of enzymatic interesterification can be attributed to water activity in the system, medium composition and the biocatalyst (Reshma *et al*., 2008).

The extent of oxidation and the level of freshness of oil can be determined by measuring peroxide. Peroxide Values (PV) obtained for the interesterified of chicken fat was relatively high at 11.25 meq/kg, compared to 12.76 meq/kg for sample 16 and 8.21 meq/kg for sample 17

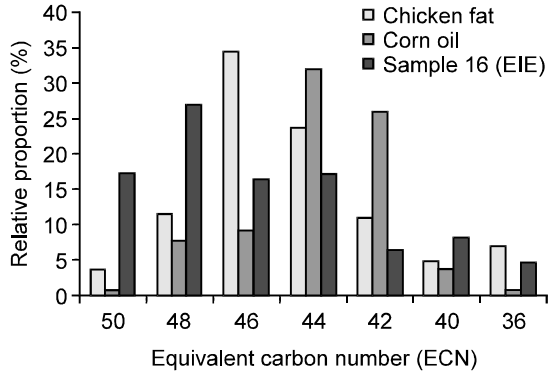

Fig. 1: Equivalent carbon number (ECN) for raw material and final product (sample 16)

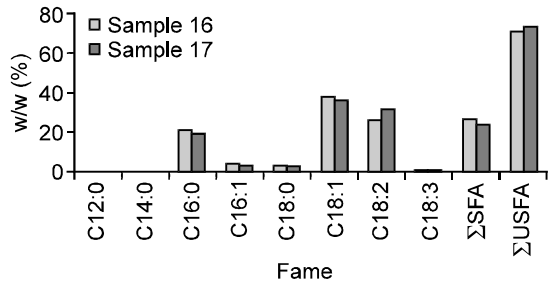

Fig. 2: Fatty acid composition (w/w%) of final products (16 and 17) for the synthesis of soft spreadable fat

(Table 3). The wet rendering technique for preparing chicken fat, which used a high temperature, may influence the PV of the sample blends. High temperatures give the atoms sufficient energy to break the covalent C-C and C-H bonds in the TAG backbone to form a variety of fatty acid radicals that initiate the process of oxidation.

Triacylglycerol (TAG) and fatty acid profiles: Changes in the composition of TAG in chicken fat, corn oil and the interesterified blend (sample 16) were monitored (Fig. 1). Since TAG is a key component in fat and oil resources, its physical characteristics influence the look, texture, morphology and rheology of the product (Sato and Ueno, 2005). The most abundant TAGs in chicken fat were ECN 48 at 12.1% (PPO, POO and OOO), ECN 46 at 34.38% (OOL and POL) and ECN 46 at 23.7% (OLL and POLn). Corn oil was composed of two major TAGs, namely ECN 44 (OLLn and LLL) and ECN 42 at 26.99% (OLL). The final product (sample 16) contained seven types of TAGs, with ECN 44, ECN 46 and ECN 48 at 17.10%, 16.40% and 26.95%, respectively. ECN 50 and 48 were clearly increased after interesterification as well as low the melting TAGs ECN 36 and ECN 40.

The fatty acid content of the final products is shown in Fig. 2. Large amounts of oleic acid (C18:1) and linoleic acid (C18:2) were present in the final products. Lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0), atherogenic and thrombogenic fatty acids that are considered as the main promoting factors of Coronary Heart Disease (CHD) (Shin *et al*., 2010) were detected at low amounts in both sample 16 and sample 17. The high amount of total unsaturated fatty acid ($\sum$USFA) in samples 16 and 17 is consistent with the high proportion of low melting TAGs in the final products. The amount of palmitoleic acid (C16:1) in both samples 16 and 17 (4.9% w/w and 4% w/w, respectively) was high due to the high proportion of palmitoleic acid (C16:1) in the raw chicken fat.

Solid fat content and morphological properties: Margarine is a fat with the features and functionality of nutrients that can be manipulated according to user requirements. Solid fat content is one of the features that can be adjusted. However, the percentage of solid fat required varies depending on the ingredients. The amount of solid fat content in the interesterified products is shown in Table 4. The high proportion of low melting TAGs in the both samples affected the solid fat content (Lee and Foglia, 2000). The spreadability of margarine at refrigerator temperature is related to Solid Fat Content (SFC) at 2-10°C (Seriburi and Akoh, 1998), SFC at 33-38°C is used as an indicator of a good mouth feel. In the case of bread spread, the required solid fat content at room temperature is 10-15% (deMan *et al*., 1995). Therefore, samples 16 and 17 have potential for being developed into a cold-spreadable spread since the SFC of sample 16 and sample 17 at refrigerator temperature was only 9-10% and 11-15%, respectively. Based on their SFC profiles, both interesterified products melted at body temperature, which is essential for ensuring the absence of a waxy after-taste.

The interesterification of chicken fat and corn oil produced a product (sample 16) with a large crystal size

Table 4: Solid fat contents of interesterifed samples (sample 16 and 17)

Sample/Temp (°C)	0	10	15	20	25	30	35	37
Sample 16	17.925	10.130	4.765	3.225	2.350	0.760	0.250	0.165
Std Error	0.046	0.007	0.004	0.004	0.014	0.028	0.021	0.025
Std Dev	0.092	0.014	0.007	0.007	0.028	0.057	0.042	0.049
Sample 17	14.090	8.775	4.750	3.560	2.700	1.290	0.460	0.305
Std Error	0.014	0.004	0.028	0.028	0.014	0.014	0.028	0.004
Std Dev	0.028	0.007	0.057	0.057	0.028	0.028	0.057	0.007

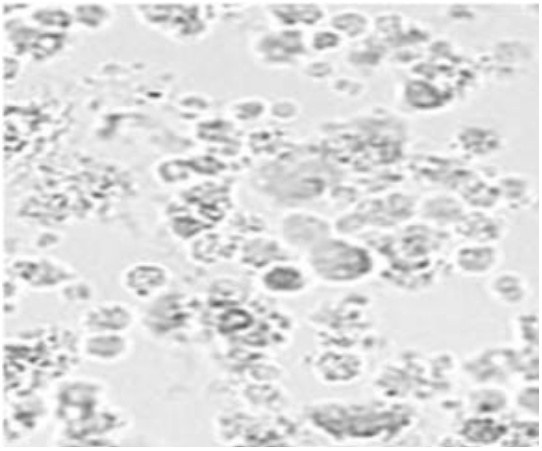

Fig. 3: Microscopic image for sample 16

and its structure changed from consisting of symmetrical to asymmetrical shapes (Fig. 3). The larger crystal size increases the interaction between the particles, decreasing the value of solid fat content and reducing the crystals formed in the space (Rye *et al*., 2005). Characteristics of crystal size are very important in the consistency and acceptability of the final product. Smaller crystals will produce a smoother texture while larger crystals may result in a grainy texture (Rodriguez *et al*., 2001). In addition, the interesterification of chicken fat with corn oil was found to reduce the number of sperulites, which cause aggregation of fine crystals to form clusters. The morphology of fat crystals can affect the rheological behavior and melting profile (Mulder and Walstra, 1974). Hence, the formation of the fat crystal network is strongly influence by the structure of individual crystals or crystal aggregates (Rodriguez *et al*., 2001).

Melting and crystallization properties: As shown in Fig. 4(a, b), the interesterified samples had endothermic peaks in the temperature range of -40°C to 35°C. Samples 16 and 17 melted at 31.40°C and 35.41°C, respectively. The recommended melting point of margarine is 35-36°C, which prevents a waxy taste (Tan and Che Man, 2009). The melting enthalpies (ΔH) for the interesterified products (samples 16 and 17) were 58.45

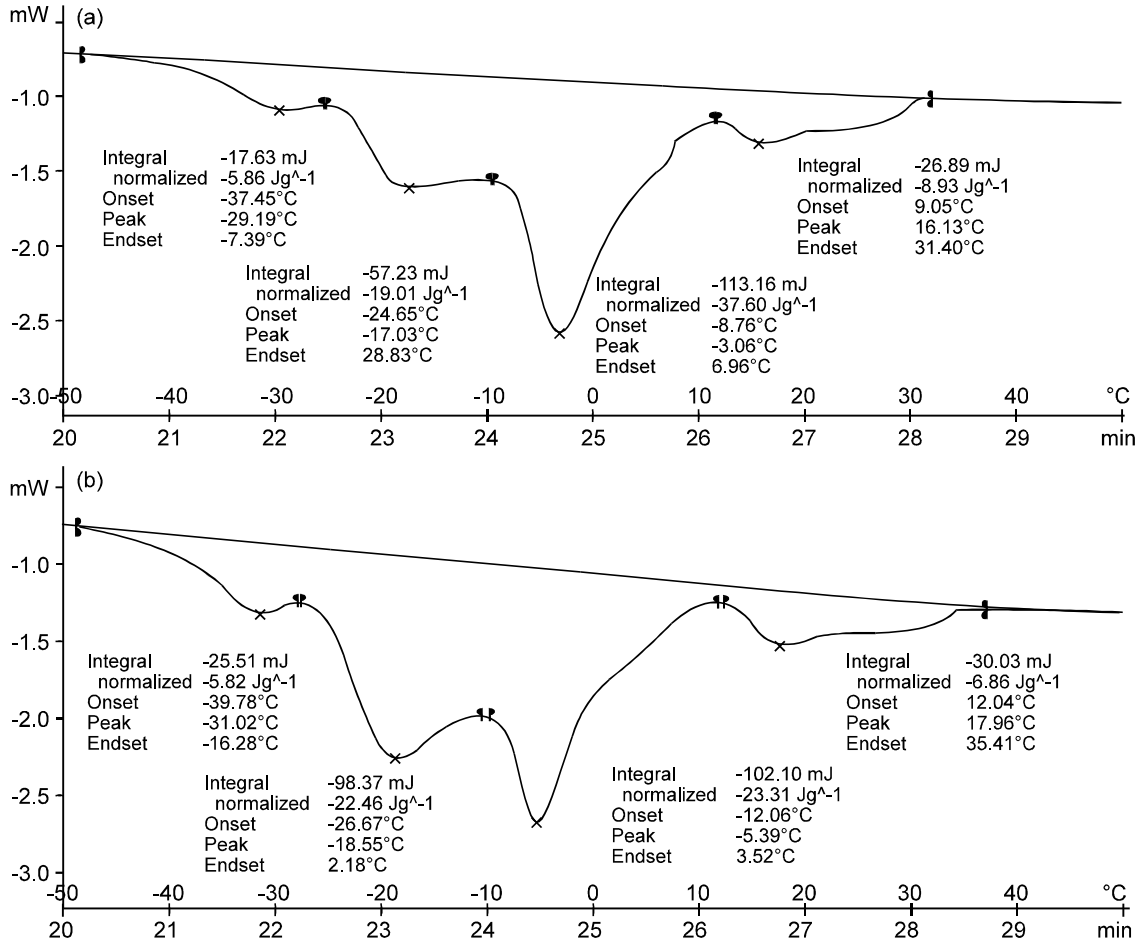

Fig. 4: Cont.

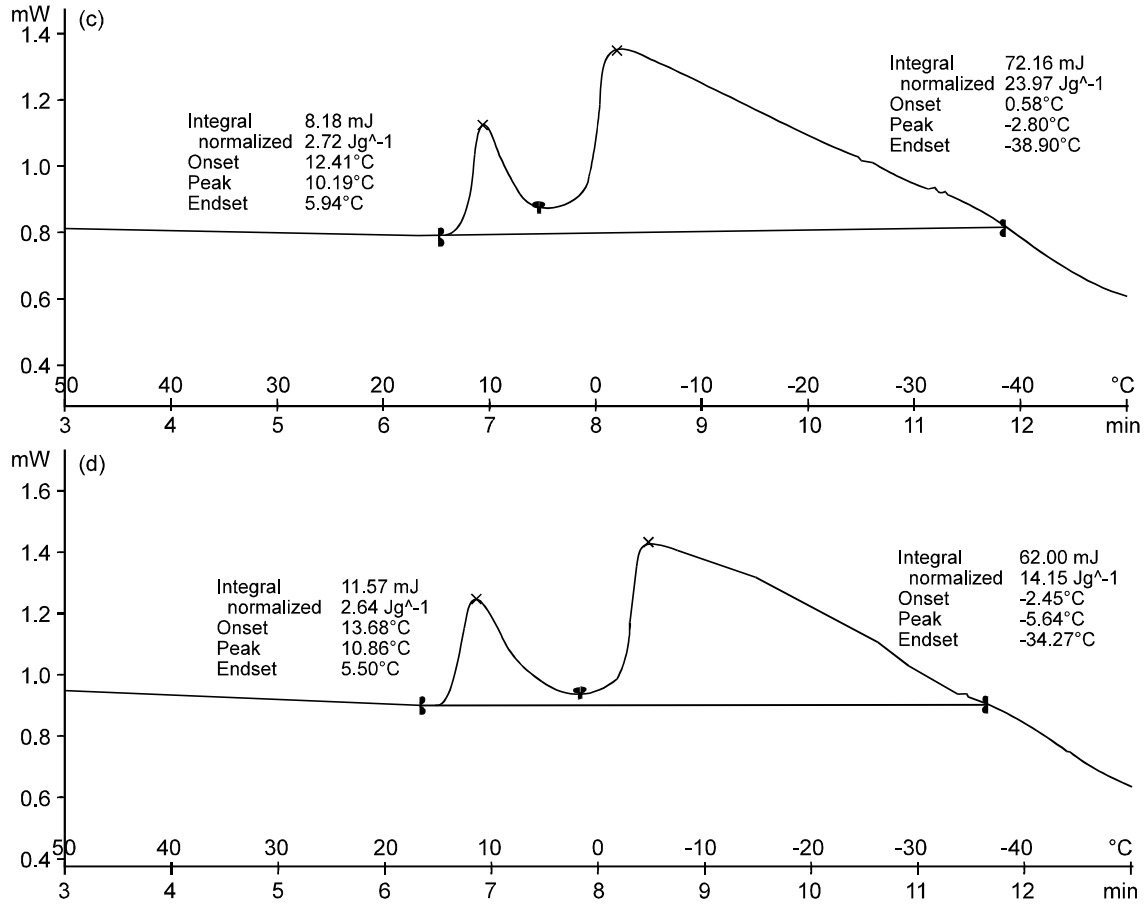

Fig. 4: (a) Melting thermogram of sample 16, (b) Melting thermogram of sample 17, (c) Crystallization thermogram of sample 16 (d) Crystallization thermogram of sample 17

J/g and 71.40 J/g, respectively, indicating that the final products contained low amounts of high melting triacylglycerols since more energy is needed to cleave the glyceride bonds.

As shown in Fig. 4(c, d), the first peak of sample 16 was at 10.19°C and sample 17 was at 10.86°C, which represents the crystallization temperature of the triacylglycerols rich in saturated fatty acids. The second peak at 0.58°C for sample 16 and -2.45°C for sample 17 represents the crystallization temperature of the triacylglycerols rich in unsaturated fatty acids. Recrystallization involves the transformation of unstable polymorphic forms to more stable polymorphic forms (Yap *et al*., 1989). Sample 16 started to crystallize at 12.41°C and finished crystallizing at -38.90°C, while sample 17 started at 13.68°C and finished at -34.27°C.

Conclusion: Two final products (samples 16 and 17) had a good composition of total saturated fat and melted completely at body temperature. Although the solid fat content of the interesterified samples was low, this was expected due to the smaller amount of high-melting triacylglycerols. The interesterified product showed a small crystal size, which is favorable for margarine production. Based on the crystallization and melting behaviors, the interesterified chicken fat-corn oil has potential to be developed into a product that has a wide range of plasticity and a high unsaturated fatty acid content similar to pure vegetable spreadable product.

REFERENCES

Brekke, L.O., 1980. Soyabean oil food products-Their preparation and uses. In: Erickson, E.H., Pryde, O.L., Brekke, T.L., Mounts and Falb, R.A. (Eds.), Handbook of SoyOil Processing and Utilization, pp: 389-438.

deMan, L., J.M. deMan and B. Blackman, 1995. Effect of tempering on the texture and polymorphic behavior of margarine fats. Fat Sci. Technol., 97: 55-60.

Kuntom, A., S.W. Lin, T.Y. Aini, N.A. Idris, M. Yusof, T.T. Sue and N.A. Ibrahim, 2005. Malaysian Palm Oil MPOB Test Method, Bangi.

Lee, K.T. and C.C. Akoh, 1998. Solvent free enzymatic synthesis of structured lipids from peanut oil and caprylic acid in a stirred tank batch reactor. J. Am. Oil Chem. Soc., 75: 1533-1537.

Lee, K.T. and T.A. Foglia, 2000. Synthesis, purification and characterization of structured lipids produced from chicken fat. J. Am. Oil Chem. Soc., 77: 1027-1034.

Mat Shahri, M., N.A. Idris and Z. Omar, 2008. Trans-free soft spread. MPOB Information Series, 387: 1511-7871.

Mulder, H. and P. Walstra, 1974. The milk fat globule, Centre for Agricultural Publishing and Documentation, Wageningen. The Netherland, pp: 33-52.

Reshma, M.V., S.S. Saritha, C. Balachandran and C. Arumughan, 2008. Lipase catalyzed interesterification of palm stearin and rice bran oil blends for preparation of zero trans shortening with bioactive phytochemicals. Bioresour. Technol., 99: 5011-5019.

Rodriguez, A., E. Castro, M.C. Salinas, R. Lopez and M. Miranda, 2001. Interesterification of tallow and sunflower oil. J. Am. Oil Chem. Soc., 78: 431-436.

Rousseau, D. and A.G. Marangoni, 1998. Tailoring the textural attributes butterfat/canola oil blends via Rhizopus arrhizus lipase-catalysed interesterification. 2. Modification of physical properties. J. Agric. Food Chem., 46: 2375-2381.

Rye, G.G., J.W. Litwinenko and A.G. Marangoni, 2005. Fat Crystal Networks. In Bailey's Industrial Oil and Fats Products, Shahidi, F. Eds; 6th Edn., New Jersey: John Wiley and Sons Inc, 1st vol, pp: 121-135.

Sato, K. and S. Ueno, 2005. Polymorphism in fats and oils. In: Bailey's Industrial Oil and Fats Products, 6th Edn., Shahidi, F. Eds; New Jersey: John Wiley and Sons Inc 3th vol., pp: 77-85.

Seriburi, V. and C.C. Akoh, 1998. Enzymatic interesterification of lard and high oleic sunflower oil with candida antartica lipase to produce plastic fats. J. Am. Oil Chem. Soc., 75: 1339-1345.

Shekarchizadeh, H., M. Kadivar, H.S. Ghaziaskar and M. Rezayat, 2009. Optimizing of enzymatic synthesis of cocoa butter analog from camel hump fat in supercritical carbon dioxide by response surface method (RSM). J. Supercritical Fluids, 49: 209-215.

Shin Jung-Ah, C.C. Akoh and Ki-Tek Lee, 2010. Enzymatic interesterification of anhydrous butterfat with flaxseed oil and palm stearin to produce low-trans spreadable fat. Food Chemistry, 120: 1-9.

Tan, C.P. and Y.B. Che Man, 2009. Differential scanning calorimetric analysis of palm oil based products and coconut oil: Effects of scanning rate variations. Food Chem., 76: 89-102.

Yap, P.H., J.M. deMan and L. deMan, 1989. Polymorphic stability of hydrogenated canola oil as affected by addition of palm oil. J. Am. Oil Chem. Soc., 66: 1784-1791.

Zainal, Z. and M.S.A. Yusoff, 1999. Enzymatic interesterification of palm stearin and palm kernel olein. J. Am. Oil Chem. Soc., 76: 1003-1007.

Zhang Hong, Xu Xuebing, J. Nilsson, Mu Huiling, J.A. Nissen and C.E. Hoy, 2001. Production of margarine fats by enzymatic interesterification with silica granulated Thermomyces lanuginosa lipase in a large scale study. J. Am. Oil Chem. Soc., 78: 57-64.