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Evaluation of soft spreadable margarine properties produced by lipase-catalysed interesterification of chicken fat and corn oil

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Abstract: Chicken fat is a potential bioresource that can be developed into a commercial product. In this study, chicken fat, which is rich in unsaturated fatty acids, including oleic acid (C18:1) and linoleic acid (C18:2), was enzymatically interesterified with corn oil to produce a soft spread. Two interesterified products, sample 16 (4% enzyme, 4:1 mole ratio of chicken fat to corn oil, 50°C and 42 h of the interesterification process) and sample 17 (4% enzyme, 2:1 mole ratio of chicken fat to corn oil, 30°C and 42 h of the interesterification process), were selected based on the highest SFC at 30oC which were close to SFC values of commercial product. A morphological study showed that the final products had smaller and less dense fat particles, which explained the lower melting temperatures and solid fat content (3.2 and 3.5% for samples 16 and 17, respectively, at 20°C) compared to the commercial products (9.7, 6.8 and 7.7% for products A, B and C, respectively, at 20°C). However, both sample 16 and 17 had similar thermal properties to a vegetable-oil-based commercial product, with melting enthalpies (ΔH) of 58.45 J/g and 71.40 J/g, and were fully melted at 31.40°C and 35.41°C, respectively.

Keywords: Chicken fat, morphology, solid fat content, soft spread

Introduction

Bioresources from animal and vegetable waste are produced in large volumes every year, and serious attention should be directed towards these bioresources to avoid environment pollution. For example, chicken fat is produced in increasing quantities every year because the consumption of chicken meat has increased around the world due to cheaper prices compared to other meats. Chicken meat consumption is estimated to be approximately 69% of the total meat consumption in Malaysia. In 2000 alone, the consumption of chicken meat was estimated to be 635,110 t in Malaysia (Serin and Haji Lias, 2007). However, the use of unmodified or modified chicken fat as ingredients in food products is still limited. Lee and Foglia (2000) successfully synthesised a new structured lipid from chicken fat by acidolysis with caprylic acid while Guru *et al.* (2010) attempted to produce biodiesel from chicken fat. Despite having low cholesterol content compared to other animals, chicken fat is high in unsaturated fatty acids, especially oleic acid, which is desirable because it ameliorates the risk of coronary artery disease (Lee and Foglia, 2000). Enzymatic interesterification of chicken fat and liquid oil high in polyunsaturated fatty acids, such as corn oil, is predicted to produce a soft spreadable margarine comparable to products from pure vegetable sources. One of the unique properties of a soft margarine is low-temperature spreadability. The spreadability of margarine at refrigerator temperatures (2 to 10°C) is related to its solid fat content (SFC) at this temperature.

The aim of this study was to evaluate the potential of soft margarine produced from the byproduct of chicken fat and corn oil by enzymatic interesterification with regard to selected physicochemical properties compared to commercial products.

Materials and Methods

Materials

Immobilised lipase from *Thermomyces lanuginose* (TL IM) was provided by Novo Nordisk. Chicken fat was prepared by a wet rendering process in the laboratory. The corn oil and selected commercial soft margarines were purchased from local stores.

Experimental design for the RSM study

Factors of the interesterification reaction were determined by Design Expert software using a central composite design (CCD) with response surface methodology (RSM). The factors selected in this design were as follows: percentage of enzyme

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(A), mole ratio of substrate (B), temperature (C) and reaction time (D). The independent variables and experimental design are shown in Table (1). Experiments were conducted at random. A total of 30 analyses were performed with five levels (-2, -1, 0, 1, and 2) and six central points (0, 0, and 0).

Enzymatic interesterification

Enzymatic interesterifications of the chicken fat and corn oil blends (30 g) were performed in a 250-mL beaker and catalysed by the immobilised lipase, TL IM. The optimisation of the reaction parameters (substrate mole ratio, enzyme concentration, temperature, and duration) was 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 the fat and oil were estimated based on their saponification values as previously determined (Kuntom *et al.*, 2005). The reaction was stopped by removing the immobilised enzyme through filtration using Whatman No. 4 filter paper. The interesterification products were transferred into a separating funnel and neutralised to remove free fatty acids as described by Lee and Akoh (1998). The dried samples were transferred into airtight containers and stored in a refrigerator (4°C) until further analysis.

Fatty acid profile analysis

Samples were analysed by a gas chromatography flame ionisation detector. A 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 to 240°C at 4°C/min.

Determination of solid fat content

The percentage of solid fat content (SFC) in the samples was analysed according to Zainal and Yusoff (1999).

Morphology analysis

The morphology of the interesterified and commercial products was analysed using a polarised light microscope (model Leica) equipped with PixelLink uScope software as reported by Rousseau et al. (1998).

Differential scanning calorimetry (DSC)

Melting profile of the interesterified and commercial products were determined by a Mettler-Toledo differential scanning calorimeter (DSC) according to the method reported by Lee and Foglia (2000).

Results and Discussion

Response surface methodology (RSM) study

The optimization of reaction parameters to produce SL having 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. A total of 30 blends according to the central composite design (CCD) were interesterified by enzymatic interesterification. The response (% linoleic acid incorporation; w/w) was observed to ensure the high unsaturated fatty acids content was maintained in the final product to produce SL having melting range close to soft margarine (30- 37°C). The optimum value for the incorporation of linoleic acid into the SL as given by the experimental design was 104.86% respectively. The reaction parameters suggested to obtain the optimum incorporation of linoleic acid were 5.47% enzyme concentration, the mole ratio of chicken fat to corn oil of 3 moles:1 mole, temperature of 43.47°C and reaction time of 59.97 hours.

Table 1. Actual factor levels corresponding to coded factor level.

Factors	Symbol	Degree of Factor				
		-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 (hrs)	D	6	24	42	60	78

From the ANOVA analysis (Table 2), the Model F-value of 60.99 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. The Prob>F was less than 0.0551, which indicates that the model factors were significant with the R^2 0.9587 was very good. "Adeq Precision" measures the signal-to-noise ratio, and a ratio greater than 4 is desirable. Moreover, a ratio of 31.494 indicates an adequate signal. This model can be used to navigate the design space. Through backward elimination regression method, several factors were removed and added to the model. Only six factors were found to be significant factors (B, C, A2, B2, C2, D2) for incorporation of linoleic acid (Please refer Table (1) for the meaning of the symbols). The other factors did not significantly affect the inclusion of linoleic acid. From the 3D surface graph in Figure 1, the low moles subtract and enzyme, the lower linoleic acid was incorporated into the structure lipid. However, from a total of 30 blends, sample 16 and sample 17 were selected based on the highest SFC at 30ºC which were close to SFC values of commercial product. The following equation was the equation model of the factors coded using the quadratic for a response:

Linoleic Acid Incorporation:
= +126.70+0.095* A-18.98 * B+2.84* C-1.56* D-2.74*A2+5.24* B2+2.80* C2-3.37* D2 (Eq. 1)

Table 2. ANOVA analyses for the response to the reaction catalysed by the TL IM lipozyme.

Source	Sum of Squares	DF	Mean squares	F Value	Prob>F
Model	10615.05	8	1326.88	60.99	<0.0001
A	0.22	1	0.22	0.010	0.9211
B	8642.35	1	8642.35	397.22	<0.0001
C	194.20	1	194.20	8.93	0.0070
D	58.38	1	58.38	2.68	0.1163
A^2	205.94	1	205.94	9.47	0.0057
B^2	753.45	1	753.45	34.63	<0.0001
C^2	214.45	1	214.45	9.86	0.0050
D^2	310.60	1	310.60	14.28	0.0011
Residual	456.90	21	21.76		
Lack of Fit	426.54	16	26.66	4.39	0.0551
Pure error	30.36	5	6.07		
Cor total	11071.94	29			
R^2	0.9587				
Adjusted R^2	0.9430				
Predicted R^2	0.8675				
Adeq Precision	31.494				
C.V.	3.64				

Fatty acid profile

The fatty acids of the commercial spreads and selected final products are shown in Table 2. Oleic acid (C18:1) and linoleic acid (C18:2) were the main fatty acids present in the commercial products and final products. The total saturated fatty acid (∑SFA) of commercial products A, B and C were 23.6, 35.7 and 15.5% (w/w), respectively. Interestingly, sample 16 and sample 17 had similar total saturated fatty acid contents to product A with values of 27 and 24.2% (w/w), respectively. According to Mat Shahri *et al.* (2008), the recommended amount of saturated fatty acids for the production of low saturated margarine is 33% and 1% of trans fatty acids. A lower amount of saturated fatty acids is required to avoid oil separation, graininess and grittiness in the final product. Lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0) are atherogenic and thrombogenic fatty acids, which are considered as main promoting factors of coronary heart disease (CHD) (Shin *et al.,* 2010), and low amounts of these acids were detected in both samples. However, the levels of palmitoic acid (C16:1) in samples 16 and 17 (4.9 and 4%, respectively; w/w) were higher than the levels in the commercial spreads (0.2%; w/w) due to the high proportion of palmitoleic acid (C16:1) found in the chicken fat.

Solid fat content

Margarine is a fat with features and nutrients that can be manipulated according to user requirements. Solid fat content is one of the features that can be adjusted according to the needs of users. DeMan (1992) proposed a 15 to 35% range of ideal solid fat content in the spreadable products. However, the percentage of required solid fat varies depending on the ingredients. The solid fat contents of the commercial spreads and interesterified products are shown in Figure 2. The interesterified products had a lower solid fat content than the three commercial spreads. Products A and C were made from a mixture of a small portion of butterfat and other vegetable oils, and product B was based on pure palm oil. At a temperature range of 0 - 25°C, the solid fat content of the interesterified products were different when compared to the commercial spreads, but the SFCs were more similar among the products at a temperature range of 30 - 37°C, which may have been due to the presence of a high melting glyceride content in the commercial spreads at the early stage (0 - 25°C). However, the SFCs of the interesterified products were almost equal to the SFC of soft margarine made from a mixture of 70% soy oil and 30% partial hydrogenation of oils (Seriburi and Akoh, 1998).

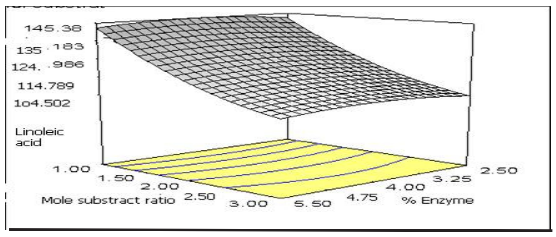

Figure 1. Three Dimension (3D) surface for the optimum value of linoleic acid (Mole subtract ratio vs % of enzyme)

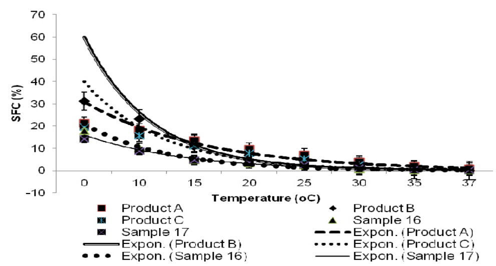

Figure 2. Comparison of solid fat contents between the interesterifed and commercial products

In addition, Mat Shahri *et al.*, (2008) successfully produced a trans-free soft spread that can be used directly from a refrigerator (5 - 10°C) with low SFC. Samples 16 and 17 in this study have the

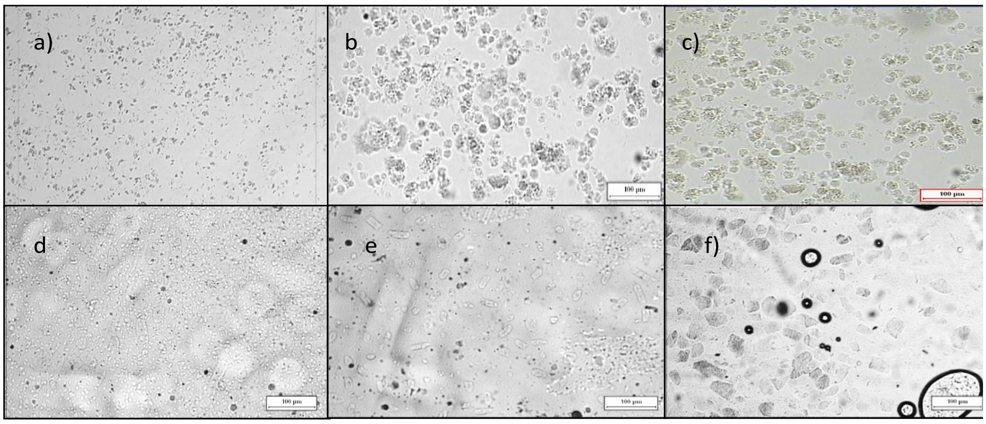

Figure 3. Microscopic images of (a) Interesterified chicken fat (b) Sample 16 (c) Sample 17 (d) Product A (e) Product B (f) Product C

potential to be developed into a cold-spreadable margarine because the SFC of these samples at 0 and 10°C were only 10 and 9% and 15 and 11%, respectively. The high content of total unsaturated fatty acid in both of the final products lowered the SFCs of these products. Based on the SFC profile, both the interesterified products (samples 16 and 17) and commercial products were melted at body temperature. This melting characteristic is essential to ensure the absence of a waxy aftertaste.

Morphological properties

The interesterification of chicken fat and corn oil produced a product (sample 16 and sample 17) (Figure 3 (b) and 3 (c)) with a larger crystal size than the interesterified chicken fat (Figure 3 (a)), and the structure changed from symmetrical to unsymmetrical. Larger crystal sizes increase the interaction between particles, resulting in larger void spaces. The larger void spaces cause a decrease in the value of solid fat content and reduce the number crystals in the space (Rye *et al*., 2005). Previous studies have shown that the addition of vegetable oil to the mixture changes the morphology of fat crystals in interesterified lard/canola oil blends (Rousseau *et al*., 1998) and beef tallow/sunflower oil blends (Rodriguez *et al*., 2001). Characteristics of the crystal size are important in the final product for the purpose of consistency and acceptability. Smaller crystals produce a smoother texture, and larger crystals may result in a grainy texture (Rodriguez *et al.*, 2001).

.In addition, the interesterification of chicken fat with corn oil reduced the number of spherulites, which caused an aggregation of fine crystals, thereby, forming clusters. Mulder and Walstra (1974) found that the morphology of fat crystals can affect the rheological behaviour and melting profile. The required solid fat content of bread spread at room temperature is 10 to 15% (deMan *et al.*, 1995). Hence, the formation of the fat crystal network is strongly influenced by the structures of individual crystals or crystal aggregates (Rodriguez *et al*., 2001). The morphology of the interesterified product showed no similarities with any of the commercial products (Figure 3 (d) - 3 (f)). However, the morphology of the interesterified sample demonstrated the significant result.

Melting properties

The interesterified samples and commercial products had endothermic peaks in the temperature range of -40 to 38°C. The melting enthalpy (ΔH) values for the interesterified products (sample 16 and 17) were much lower (58.45 and 71.40 J/g, respectively) (Table 4) compared to products A, B and C with melting enthalpy values of 81.35, 78.17 and 77.57 J/g, respectively, indicating that the commercial products contained more high melting triacylglycerols because more energy was needed to

Table 4. Enthalpy (ΔH), onset temperature (°C), endset temperature (°C) and peak temperature (°C) obtained from the melting thermogram

Sample	ΔH (J/g)	Onset °C	End °C	Onset °C	End °C	Onset °C	End °C	Onset °C	End °C	Peak 1	Peak 2	Peak 3	Peak 4
Product A	-81.35	-27.38	-11.96	-36.20	37.88	-	-	-	-	-19.74	32.93	-	-
Product B	-78.17	-29.35	26.76	-33.90	5.32	-2.07	9.69	7.20	26.79	-17.70	-3.21	2.94	15.11
Product C	-77.57	-26.53	-19.1	10.56	37.12	-	-	-	-	-19.07	18.11	-	-
Sample 16	-58.45	-37.45	-7.39	-24.65	28.83	-8.76	6.96	9.05	31.40	-29.19	-17.03	-3.06	16.13
Sample 17	-71.40	-39.78	-16.28	-26.67	2.18	-12.06	3.52	12.04	35.41	-31.02	-18.55	-5.39	17.96

cleave the glyceride bonds. For products A and C, a broad endothermic band existed starting from -27.38 to 37.88°C for product A and from -26.53 to 37.12°C for product C. A low melting (Tp) and high melting (Tp) peak were found for products A and C at -19.74 and 32.93°C, respectively, for product A and -19.07 and 18.11°C, respectively, for product C.

Although the interesterified products (samples 16 and 17) had a lower onset temperature (-37.45 and -39.78°C, respectively) than the commercial products, the endset temperatures of the interesterified products (31.40 and 35.41°C, respectively) were similar to the endset temperatures of the commercial products (37.88°C for product A and 37.12°C for product C). Product B started to melt at -29.35°C and was fully melted at 26.79°C, and it had two main peaks (2.94 and 15.11°C) and shoulder peaks (-17.70 and -3.21°C). The low melting glyceride component (Tp = -17.70°C) may have been composed of glycerides rich in oleic acid (C18:1) in addition to other unsaturated acids. Moreover, high melting glyceride components (Tp = 15.11°C) can be represented by palmitic and other saturated acids. The recommended melting point of margarine is between 35 and 36°C to avoid a waxy taste (Tan and Che Man, 2002).

Conclusions

Both samples 16 and 17 had a good composition of total saturated fat. Therefore, they are ideal for use as a soft spreadable margarine product. In addition, the interesterified products in this study completely melted at body temperature, indicating that they lacked the unwanted waxy aftertaste. The morphology study of the interesterified products demonstrated that these products had a small crystal size, which would be good for margarine production.

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