

The Production of an Experimental Table Margarine Enriched with Conjugated Linoleic Acid (CLA): Physical Properties

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Abstract Even though conjugated linoleic acid (CLA) is known to have some beneficial effects on the human body, its consumption has decreased over the past 20 years due to the replacement of animal fats by vegetable oils. In this study, using the structured lipid (SL) containing CLA, an experimental table margarine enriched with CLA was produced and stored for 3 months at two temperatures prior to performing the relevant analyses. The GC results showed that the margarine fat had 10.6% CLA. The solid fat content was the highest in week 0 in all samples, which then decreased during storage but the hardness increased. An increment in dropping point was also observed in the samples. In week 0, all the samples had the β' crystal as the predominant crystal form but a crystal transformation from β' to β was observed during storage.

Keywords Conjugated linoleic acid (CLA) ·
Table margarine · Physical properties

Introduction

Conjugated linoleic acid (CLA) is a mixture of various isomers of octadecadienoic acid with double bonds at conjugated positions. The two important forms of CLA, the

cis9, *trans11* and *trans10*, *cis12* isomers, are naturally found in milk fat and meat from ruminants. CLA is known to exert beneficial effects such as inhibiting tumor growth, reducing body fat, and reducing atherosclerotic risks [1, 2].

Commercial vegetable oils contain only small amounts of CLA (0.1–0.5 mg/g fat), which is due to the oil refining and hydrogenation processes [3]. Even though the beneficial effects of CLA have been revealed, the consumption of dietary CLA has decreased during the past 20 years due to the replacement of animal fats by vegetable oils. It is suggested that relatively low amounts of CLA (3.5 g/d for a 70-kg person) in human diet would have an anticarcinogenic effect [4]; many studies have been conducted on the production of modified oil and fat (structured lipid, SL) containing CLA, especially by an enzymatic reaction [4, 5]. However, to the best of our knowledge, there are still no reports on the production of food products enriched with CLA.

Margarine, namely table, bakery, and puff pastry, is a water-in-oil (W/O) emulsion food product that must contain at least 80% fat [6, 7]. Table margarine is of two main types: soft and stick. Soft margarines should be spreadable at refrigerator temperature but not oil off when left at room temperature for a short period of time, whereas stick margarine should be spreadable at 15 °C [8, 9]. The production of margarine fats is usually carried out by partial hydrogenation which causes the formation of a high content of *trans*-fatty acids (TFAs) as high as 50% during the process; therefore, the trend nowadays is to produce *trans*-free products by using hard stocks such as palm stearin or fully hydrogenated fats [10–12]. Moreover, there is a great interest in incorporating as much liquid oil as possible into the margarines to claim the highest possible amount of polyunsaturated or monounsaturated fatty acids following nutritional recommendations [8].

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The quality of a margarine product is usually defined by terms such as texture, consistency, hardness, plasticity, and spreadability [7]. For margarine, storage stability is critical in practical usage. During storage, the changes of the crystal size and network influence changes in the physical properties of margarine. Thus, it is very important to focus on those changes during storage whenever a new product is developed [13].

In this study, an experimental table margarine enriched with CLA was produced using SL containing CLA, palm stearin (as hard stock) and super palm olein (as a liquid oil) and then stored along with a commercial margarine (as a control sample) at 5 °C (refrigerated temperature) and 15 °C (non-refrigerated temperature) for 3 months and evaluated for physical and textural properties at intervals.

Experimental Procedure

Materials

Refined, bleached, and deodorized (RBD) canola oil was obtained from Naz Vegetable Oils Co. (Isfahan, Iran). RBD palm stearin (Iodine value, 27.6) and super palm olein (Iodine value, 61.3) were provided by Mewah Oleo Food Industries, a local refinery (Selangor, Malaysia). Other ingredients including emulsifier (distilled monoacylglycerols, 90% monoester), antioxidant (vitamin E), color (beta-carotene), flavor, skim milk powder and vacuum-dried salt were from Danisco Ingredients (M) Sdn. Bhd; Prai Industrial Estate, Penang, Malaysia.

Commercial margarine formulated (as labeled on the product) by canola and sunflower oils, palm fractions, water, salt, and skim milk powder was purchased from a local supermarket (Kuala Lumpur, Malaysia). Immobilized lipase (*Candida antarctica*) was a gift from Novozymes (Tehran, Iran). Chemical and solvents were all of analytical grade and purchased from Merck.

Synthesis of Structured Lipid Containing CLA

Conjugated linoleic acid (CLA) was produced by alkali isomerization of safflower oil and purified using urea, then interesterified with canola oil by lipase to synthesize SL containing CLA according to Goli et al. [14].

Enzymatic Interesterification (EIE)

Structured lipid containing CLA and melted palm stearin (PS) were mixed in ratio of 60:40 (w/w), then the lipase from *Candida antarctica* (1% of total substrate weight) was added and EIE was carried out at 60 °C and 200 rpm under nitrogen gas in an orbital shaker for 7 h.

Fat Phase Preparation

The oil mixture obtained from EIE was analyzed for its physical properties such as solid fat content profile at different temperatures, melting curve, slip melting point, and crystal form [15]. The results showed that for preparation of a suitable margarine fat phase, it is necessary to add another source of liquid oil to the mixture; therefore, super palm olein was simply blended into the mixture at a ratio of 50:50 (w/w).

Determination of Fatty Acids Composition

Fatty acids composition of the blend was determined using GC. Fatty acid methyl esters (FAMES) were made by methylation with sodium methoxide (0.5 N) and separated on DB-23, 60 m × 0.25 mm × 0.25 μm capillary column, equipped with a flame ionization detector (FID). Injection (1 μL) was performed in the split mode at a split ratio of 1:100. Helium was the carrier gas with a flow rate of 0.8 mL/min. The temperature program was isothermal and the column temperature was set at 185 °C. The injector and detector temperature was 240 °C. The analyses of FAMES were carried out in duplicate for each sample.

Margarine Formulation and Production

Experimental margarine (EM) samples were prepared using the following formulation: fat, 81.7%; water, 16%; salt, 0.8%; skim milk powder, 0.8%; emulsifier, 0.3%; flavor, 0.2%; antioxidant, 0.01%; and color, 0.003%. The melted oil was weighed followed by dissolving emulsifier, antioxidant, flavor, and color in the heated oil phase. The salt and skim milk powder were dissolved in the water phase. The water phase was then added slowly to the oil phase with agitation by a mixer to form a good emulsion. The emulsion was stirred for 10 min and then poured into a double insulated bowl of an ice cream maker equipped with a liquid refrigerant located between the walls. The emulsion was mixed and crystallized in the machine at a temperature range of 10–12 °C. A commercial margarine (CM) was melted down and recrystallized using the same procedure. This sample was then used as the control to be compared with the experimental margarine. Both margarine samples (EM and CM) were immediately stored at two test temperatures: 5 °C (as refrigerated temperature) and 15 °C (as non-refrigerated temperature) for 3 months and analyzed every 2 weeks. The initial week (days 0–7) was termed week 0 and the following weeks designated as weeks 1–12.

Solid Fat Content (SFC) Determination

A Bruker wideline NMR (Minispec20, Germany) was employed for the isothermal SFC measurements. Margarine samples were loaded into the NMR tubes of 0.8 cm in diameter and 2 cm in height using a stainless steel plunger. After that, the tubes were kept at the test temperatures and measurements were carried out in the specified weeks [16].

Hardness Evaluation

Hardness, or consistency, of the samples was determined in terms of penetration yield value (g/cm^2) [8, 17] using a cone penetrometer (Stanhope-Seta, Surrey, England) with a 40° angle, where the weight of the cone assembly was 79.03 g. The cone penetrometer is a rapid and empirical method used in the evaluation of texture [7]. The penetrating cone was placed just above the surface of the sample before it was released. The penetration time was 5 s and penetration depth was read directly from the instrument in 0.1 mm unit. Yield values were calculated using Eq. 1:

$$\text{Yield value } (\text{g}/\text{cm}^2) = KW/P^{1.6} \quad (1)$$

where, K = constant (5840 for 40° cone angle), W = weight of the cone assembly (79.03), P = mean of penetration depth from two replicates (mm).

Dropping Point Measurement

Dropping points of the products were measured using a Mettler FP90 thermosystem. The product was placed directly into the cup with a small opening, while the excess of the product was carefully removed. The cup containing the sample was held at the test temperature (5 or 15°C) for 5 min, and then heated at a rate of $2^\circ\text{C}/\text{min}$. The temperature at which a drop of the sample falls through under its own weight is the dropping point.

Polymorphic Forms

Polymorphic forms of the samples were determined using Rigaku's X-ray diffractometer Rint Series (Rigaku, Japan). The short spacings of β' form is at 4.2 and 3.8 Å and that of β form is at 4.6 Å [18].

Statistical Analyses

The statistical analyses were carried out using the ANOVA program in the Statistical Analyses System (SAS) software. The treatments were margarine type and temperature at two levels with factorial experiment according to the complete randomized design (CRD) with two replications for each analysis. The means evaluation was done by the Least Significant Difference (LSD) test at a confidence level of 95%.

Results and Discussion

Fatty Acids Composition

Fatty acids profile of margarine fat as well as the inter-esterified blend of PS/SL and super palm olein determined by GC is shown in Table 1. The CLA content in the interesterified blend was about 17.7% and the major fatty acid in super palm olein was oleic acid (44.0%) followed by palmitic acid (35.3%). After a simple blending, the margarine fat now contained oleic and palmitic acids as the main fatty acids due to the presence of palm stearin and super palm olein. The amount of CLA was about 10.6% of total fatty acids which was considerably high. This means that in one serving (100 g) of the margarine containing 80% fat, closely 8.5 g CLA would be available which is about 7.5 times higher than the natural content of CLA in milk [3]. However, the margarine fat has high nutritional value, not only due to the presence of CLA but also for the palm stearin as hard stock which is devoid of TFAs in the margarine formulation.

Storage Effect on SFC

Solid fat content is an important property of the oil or fat. The ratio of the solid fats composing of fat crystals which incorporate liquid oil in a crystal network, to the total phase at a particular temperature is one of the determining factors in the texture of plastic fats [8]. Excessive changes in SFC of the product during storage are not desirable; ideally, the SFC should not change from the time of filling the product into containers throughout the storage period [18].

Table 1 Fatty acids composition of PS/SL, super palm olein and margarine fat

Sample/fatty acids	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	CLA
PS/SL	0.5 ± 0.0	26.3 ± 0.3	2.7 ± 0.1	35.8 ± 0.1	12.1 ± 0.0	3.0 ± 0.0	17.7 ± 0.1
Super palm olein	1.2 ± 0.0	35.2 ± 0.2	3.2 ± 0.1	44.0 ± 0.0	12.2 ± 0.2	–	–
Margarine fat	0.8 ± 0.0	30.2 ± 0.0	2.9 ± 0.0	39.0 ± 0.1	12.4 ± 0.3	1.9 ± 0.4	10.6 ± 0.4

Table 2 SFC percentage of experimental and commercial table margarines during the storage

Samples	Weeks							
	0	1	2	4	6	8	10	12
EM5	23.3 ± 0.3 ^{Aab}	22.1 ± 0.3 ^{Ab}	22.7 ± 0.2 ^{Aab}	24.1 ± 0.0 ^{Aa}	23.9 ± 0.6 ^{Aa}	23.0 ± 0.4 ^{Aab}	22.8 ± 0.7 ^{Aab}	22.0 ± 0.8 ^{Ab}
EM15	17.3 ± 0.2 ^{Bab}	17.8 ± 0.4 ^{Bab}	17.4 ± 0.2 ^{Bab}	18.4 ± 0.4 ^{Ba}	18.0 ± 0.5 ^{Bab}	17.7 ± 0.1 ^{Bab}	17.3 ± 0.0 ^{Bab}	16.9 ± 0.5 ^{Bb}
CM5	7.8 ± 0.2 ^{Ca}	7.7 ± 0.1 ^{Ca}	7.5 ± 0.1 ^{Ca}	7.9 ± 0.2 ^{Ca}	7.9 ± 0.0 ^{Ca}	7.7 ± 0.1 ^{Ca}	7.7 ± 0.2 ^{Ca}	7.5 ± 0.0 ^{Ca}
CM15	6.5 ± 0.0 ^{Da}	6.1 ± 0.1 ^{Db}	5.7 ± 0.1 ^{Dc}	5.9 ± 0.1 ^{Dbc}	6.0 ± 0.0 ^{Cb}	5.9 ± 0.0 ^{Cbc}	6.1 ± 0.1 ^{Cb}	5.4 ± 0.1 ^{Dd}

Capital letters in the same column and lowercase letters in the same row represent significant different ($p < 0.05$)

In all samples, the SFC was the highest in week 0 and then decreased during storage (Table 2). For commercial margarine stored at 5 °C (CM5), this reduction was not significantly different ($p > 0.05$). The experimental margarine sample stored at 5 °C (EM5) had the highest SFC followed by the experimental margarine stored at 15 °C (EM15). During storage, the SFC in EM5 and EM15 reduced by 1.3 and 0.4%, respectively. However, this reduction was statistically significant ($p < 0.05$). The commercial margarines had the lowest values, which was probably due to their formulation using more liquid oil compared to the experimental ones. In EM samples, the content of saturated fatty acids (34%) was much more than that of commercial margarines (19%). For CM5, only 0.3% reduction was observed after 3 months of storage; however, the commercial margarine stored at 15 °C (CM15) showed a reduction of 1.1% in SFC. Statistical analysis indicated that in the two types of margarines, there was a significant difference between the temperatures, meaning that not only the storage time, but also storage temperature was effective on SFC content of the products.

Storage Effect on Hardness

The hardness of the fats, in general, is a contributory factor of margarine characteristics such as appearance, ease of packaging, workability, spreadability, and oil exudation. The hardness of margarine usually increases due to the aggregation of fat crystal network during storage [19].

The yield values of the margarine samples as shown in Table 3, indicated that as storage proceeded, product

hardness also increased significantly ($p < 0.05$). The highest yield value belonged to the EM5 sample which also had the highest SFC content, followed by EM15, CM5, and CM15. According to Haighton's classification [16] for margarine hardness, commercial margarines (with yield values between 50 and 100 g/cm²) were categorized as very soft and not spreadable whereas the experimental margarines were classified as satisfactorily plastic and spreadable products (with yield values between 200 and 800 g/cm²). Commercial margarines had a lower consistency compared to experimental margarines, probably due to their lower SFC contents. The microstructure of plastic fats consists of a three-dimensional network containing solid fat crystals which are held together by primary (non-reversible) and secondary (reversible) bonds. The strength of this crystal network depends on the SFC percentage, polymorphic form, and crystal size [7]. The yield values increased during the storage time probably due to crystal growths and the bridges made between them [19]. The relationship between yield value and SFC in table margarines was determined by the equation below:

$$\text{Yield value} = 29.747(\text{SFC}) - 138.68 \quad (2)$$

This equation is graphically displayed in Fig. 1. The linear correlation coefficient was 0.96 ($p < 0.01$), which represented a strong relationship between the SFC and the yield value. Using this equation, we concluded that for a yield value of zero, a minimum of 4.66% SFC is required in the products to form a crystal structure. The correlation of yield value with SFC appeared to indicate a small increase in SFC can result in a large variation in hardness, showing

Table 3 The yield value (g/cm²) of experimental and commercial margarines during storage

Samples	Weeks							
	0	1	2	4	6	8	10	12
EM5	585.3 ± 3.0 ^{Ad}	585.3 ± 4.8 ^{Ad}	602.7 ± 4.2 ^{Ac}	600.2 ± 0.0 ^{Ac}	615.4 ± 4.5 ^{Aab}	616.2 ± 0.0 ^{Aa}	605.2 ± 3.3 ^{Abc}	625.7 ± 1.2 ^{Aa}
EM15	295.9 ± 5.3 ^{Bcd}	291.2 ± 3.5 ^{Bd}	305.7 ± 3.3 ^{Bbc}	291.2 ± 3.0 ^{Bd}	316.1 ± 3.7 ^{Bb}	316.1 ± 0.0 ^{Bb}	305.7 ± 0.0 ^{Bbc}	327.0 ± 3.5 ^{Ba}
CM5	76.2 ± 0.0 ^{Cc}	77.3 ± 2.4 ^{Cc}	77.3 ± 0.5 ^{Cc}	76.8 ± 0.0 ^{Cc}	81.8 ± 1.6 ^{Cabc}	81.2 ± 3.2 ^{Cbc}	86.8 ± 0.3 ^{Cab}	87.5 ± 3.1 ^{Ca}
CM15	71.2 ± 1.3 ^{Cc}	71.7 ± 1.9 ^{Dc}	72.7 ± 0.0 ^{Cbc}	73.7 ± 0.6 ^{Cbc}	74.2 ± 0.2 ^{Cbc}	77.8 ± 1.0 ^{Cab}	80.1 ± 4.0 ^{Ca}	80.7 ± 0.0 ^{Ca}

Capital letters in the same column and lowercase letters in the same row represent significant different ($p < 0.05$)

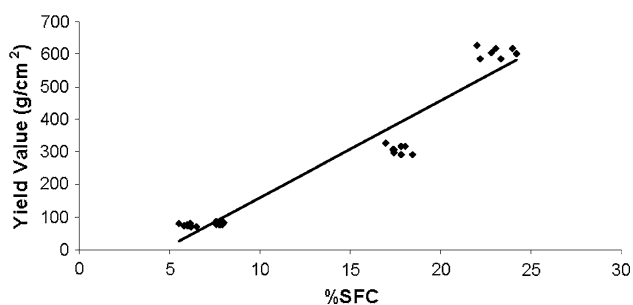


Fig. 1 Regression correlation of yield value and SFC of table margarines during storage

this relationship might be explained much better by Power Law equation.

Storage Effect on Crystal Forms

The fat crystals in margarine might exist in two polymorphic forms, β' and β . β' crystals are the most desirable, as they are small and able to incorporate a large amount of liquid oil in the crystal network, resulting in smooth texture of the product. β crystals are initially small but they grow together into large needle-like agglomerates, which leads to sandy texture [8].

The crystal forms of margarine samples during storage as shown in Table 4 reveal that the crystals were a mixture of β and β' crystals. In week 0, the β' crystal form was predominant in all margarines but after 3 month of storage, due to the crystal transformation of β' to β , the β crystal became more dominant. The β' crystal was predominant in CM15 only for days 0–7, when the crystal transformation from β' to β occurred. This phenomenon was observed a week later for CM5. However, EM samples were more stable than CM samples. The transformation of β' to β crystals in EM15 occurred only after the 8th week of storage. Another experimental sample, EM5, also experienced the crystal transformation from β' to β after the 10th week of storage. Soft margarines are more prone to polymorphic transition as compared to hard ones. In low-SFC samples, the formation of a crystal network which provides consistency is more difficult. The formation of only a few nuclei causes crystal formation to concentrate on these nuclei, forming bigger

agglomerates and giving rise to transformation to the β crystal form [16]. For this reason, in CM samples, the transformation of crystals toward a more stable form (β) could be observed as early as the first and second weeks. According to Czerniak, *trans* fatty acids in fat and oil may prevent transformation of crystals to the β form [20]; therefore, CLA in EMs might be effective in keeping the β' crystal form for a longer time. Moreover, due to the presence of high palmitic acid (>20%) in both oils used in EM samples (PS/SL blend and super palm olein), the β' crystal form was more predominant whereas CM margarines contained only 15% palmitic acid (<20%) [21].

The results showed that samples stored at the lower temperatures (5 °C) had more stability in β' crystal in comparison to those stored at 15 °C. These results are in agreement with those reported by Zhang [22] who reported that storage temperature has a significant effect on crystal stability and crystal transformation. According to Zhang, higher storage temperatures can accelerate crystal transformation [22].

Storage Effect on Dropping Point

Changes in the dropping point (DP) of the samples during storage are shown in Table 5. An increment in dropping points were observed in all samples during storage, which were (except for EM5) significantly different ($p < 0.05$). After production (week 0), CM15 and CM5 had the highest DPs (35.0 °C), but during storage, the DP of CM15 increased more than that of CM5. Consequently, at the end of the storage time, CM15 had the highest DP followed by CM5, EM15, and EM5. There was also a significant difference between the samples at the end of the storage time ($p < 0.05$). Since the changes in DPs during storage of margarines reflect changes in crystal size and crystal network [20], it might be concluded that the higher DP in commercial margarines was probably due to the occurrence of more crystal transformation compared to experimental samples. As mentioned previously, the low SFC in CMs caused earlier transformation of β' to β , leading to higher DPs in the samples compared to EMs. At higher temperatures, the low melting TAGs of the sample were melted

Table 4 Polymorphic forms of experimental and commercial margarines during storage

Samples	Weeks								
	0	1	2	4	6	8	10	12	
EM5	$\beta' \gg \beta$	$\beta' \gg \beta$	$\beta' > \beta$	$\beta' > \beta$	$\beta' > \beta$	$\beta' = \beta$	$\beta > \beta'$	$\beta > \beta'$	
EM15	$\beta' \gg \beta$	$\beta' > \beta$	$\beta' = \beta$	$\beta' = \beta$	$\beta' = \beta$	$\beta > \beta'$	$\beta > \beta'$	$\beta > \beta'$	
CM5	$\beta' > \beta$	$\beta' = \beta$	$\beta > \beta'$	$\beta > \beta'$	$\beta > \beta'$	$\beta > \beta'$	$\beta > \beta'$	$\beta \gg \beta'$	
CM15	$\beta' > \beta$	$\beta > \beta'$	$\beta \gg \beta'$	$\beta \gg \beta'$	$\beta \gg \beta'$	$\beta \gg \beta'$	$\beta \gg \beta'$	$\beta \gg \beta'$	

Table 5 Dropping point (°C) of experimental and commercial table margarines during storage

Samples	Weeks								
	0	1	2	4	6	8	10	12	
EM5	34.6 ± 0.6 ^{Aa}	34.9 ± 0.3 ^{Ba}	35.1 ± 0.5 ^{Ba}	35.3 ± 0.3 ^{Aa}	34.9 ± 0.5 ^{Ba}	34.9 ± 0.5 ^{Ca}	35.0 ± 0.0 ^{Aa}	35.1 ± 0.3 ^{Ca}	
EM15	34.6 ± 0.2 ^{Ad}	34.9 ± 0.3 ^{Bcd}	35.4 ± 0.3 ^{Bbc}	35.8 ± 0.1 ^{Abcd}	35.7 ± 0.1 ^{ABbc}	35.3 ± 0.4 ^{Bab}	35.4 ± 0.2 ^{Aab}	36.4 ± 0.0 ^{Ba}	
CM5	35.0 ± 0.2 ^{Ab}	35.0 ± 0.1 ^{Bb}	36.8 ± 0.4 ^{Aa}	36.4 ± 0.4 ^{Aa}	36.1 ± 0.4 ^{ABab}	36.2 ± 0.0 ^{Ba}	37.1 ± 0.6 ^{Aa}	36.6 ± 0.3 ^{Ba}	
CM15	35.0 ± 0.0 ^{Ad}	36.0 ± 0.1 ^{Ac}	36.4 ± 0.4 ^{Abc}	36.6 ± 0.3 ^{Abc}	36.9 ± 0.2 ^{Ab}	36.9 ± 0.2 ^{Ab}	36.8 ± 0.3 ^{Ab}	37.9 ± 0.1 ^{Aa}	

Capital letters in the same column and lowercase letters in the same row represent significant different ($p < 0.05$)

and eventually recrystallized at the sites of the existing crystals forming bigger crystals. Hence, transforming from the low melting crystal types into higher melting ones is indicated by higher DPs.

Even though the experimental margarine in this study was produced by an ice cream maker, the data were promising. The results showed that the EM samples were classified as satisfactorily plastic and spreadable margarines. During storage, their SFC decreased, and the crystal form was transformed from β' to β which might be due to the production conditions in the ice cream maker. Dropping point of the samples increased consistently with the crystal transformation of β' to β crystal form.

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