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Pilot-Scale Interesterification of Special Fat Blends to Prepare Zero-Trans Margarine: Monitoring the Content and Physicochemical Properties of Fatty Acids

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ABSTRACT

The chemical interesterification (CIE) process is a promising approach to modifying and improving oils and the fat structure. In this study, the CIE of the fully hydrogenated soybean oil (FHSO) and sunflower oil (SFO) was performed. Different initial blends with various mass ratios of 20-45 % of FHSO (coded as S1, S2, S3, and S4) were converted to interesterified samples (Si-1, Si-2, Si-3, and Si-4, peer-to-peer). The interesterified samples (60 % content) were used in different margarine formulas with 40 % of palmolein PO (M1, M2, M3, M4), and the margarine enriched with beta-carotene, to compensate for the reduction of carotene during the oil decolorization process during refining. Esterification caused a significant decrease in the solid fat content (SFC) in the initial fat blends and the fatty acid profile analysis confirmed that the content of trans fatty acid was just less than 0.17-0.3 % (According to the definition of zero trans being less than 0.5 g/12 g per serving). The differential scanning calorimetry (DSC) measurement indicated that the interesterified samples possess lower melting points while showing binary or ternary crystallization peaks. The Polarized light microscopy (PLM) confirmed the presence of fine, desirable β′ spherulite crystals, which are effective in creating the proper texture in margarine. Different types of formulated margarine were evaluated and compared with one type of the commercial margarine (as a control sample). According to the texture profile analysis (TPA) and organoleptic results, the M3 formula was chosen as the best formulation for margarine preparation (using Si-3 blending with the 35: 65 ratios of FHSO to SFO).

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1. Introduction

In the new era, oils and fats in their natural state are modified to answer health concerns and improve the physical properties of foods [1]. Developing and applying new formulations of fats and oils for preparing margarine and other fat-based products have been considered to achieve new physical and functional properties [2].

The CIE process comprises the breakage and formation of the new ester bonds in the glyceride molecules simultaneously. Chemical randomization causes the redistribution and rearrangement of fatty acids in the structure of triacylglycerol molecules (TAGs), and these phenomena lead to form new fats with different melting points. The CIE reaction is an alternative process for the design of safe fat-based functional food products to develop a wide variety of formulations from pilot plants to industrial scale. These new fats are used as raw materials to prepare lipid-based products [3]. The new functional properties of oils and fats, depending on the application, have to include better palatability, plasticity, melting points, spreadability, and compatibility. The modification of the composition of acylglycerols affects the size, morphology, and stabilization of the network of fats [4].

Firstly, margarine was introduced as a butter substitute for the market to meet nutritional and economic needs [5]. Margarine is a water-in-oil emulsion, and the oil phase is a blend of crystalline fat and liquid oil at ambient temperature. Quoted from the *Codex Alimentarius Commission,* margarine has a semi-solid texture and consists of 80 % fat (equal to or greater than>>) and 16 % water (equal to or less than <<). The 3-D continuous sheet-like network of fat crystals entraps suspended liquid crystals in the oils and tiny water droplets [6].

Changes in physical properties to diversify the application were the main reasons for the growth of margarine manufacturing. More than ten kinds of margarine, such as regular, whipped, spreads, soft tub, no fat, restaurant, bakers, liquid, diet, and specialty types, have been produced and offered in different packages [5]. Each unique/particular sample has its serving temperature. Soft margarine is consumed at 4-5 °C, while hard margarine is used at either 4–5 °C or 22–24 °C [6]. One of the unique functional properties of margarine is its spreadability. Of course, there are ambiguities about the similarity of margarine flavor with butter. As the formulation of margarine consists of two or more fat blending, different degrees of hardness are achievable. This feature provides margarine with spreadability immediately after leaving the refrigerator (4 ℃) whereas it keeps its consistency at room temperature [5].

The hydrogenated vegetable oil is still the most suitable and preferred one for table-grade margarine with steep SFC slopes to create an appropriate melting point. The Unfavorable aspect of the final product is that it contains excessive amounts of trans fatty acids. From a nutritional point of view, it is better to use a larger ratio of polyunsaturated to saturated acylglycerols by using a high level of liquid oils [5]. Recently, different studies have been done on the oil crystallization process, originating many patents for new formulations [2, 7].

These days considering there has been more concern about the side-effects of trans fatty acids on health and it has been shown that CIE is an excellent method to prepare fats without trans isomers. Partial hydrogenation promotes the isomerization of the double bonds, while CIE does not [8]. The CIE of oils and fats is a suitable alternative to replace the common

method of partial hydrogenation to prepare margarine. The knowledge of controlling the ratio of oil blends is necessary for setting up the desired processing parameters to prepare margarine with improved physicochemical properties.

Margarine with smooth and uniform texture has a predominant number of β′ crystals, which have intermediate stability [9]. Palmolein (PO) is an initiator to form β′ crystals, and the presence of a significant amount of it is essential for creating the desirable plasticity and consistency of margarine [4, 10, 11]. Their physical and functional properties are mainly governed by the amount of solid fat present, the shape, size, and interrelationship of (interaction between) the fat crystals [12].

The problem is that nowadays, semi-hydrogenated fats are used to prepare commercial margarine in Iran and, these kinds of products are not considered healthy food. To replace the commercial margarine with a healthier type of margarine, we have investigated the optimal formulation of margarine using the CIE method. The art of the correct use of processes and blending of ingredients can improve the quality of the final product on a commercial scale. In this study, margarine was prepared using interesterified SFO and FHSO (60 % substitution) as a new formulation to prepare zero trans margarine (monitoring the content of elaidic acid) and enriched with β-carotene. Subsequently, the effect of CIE on the thermal behavior, microstructure, crystallization properties, and SFC of ternary blends (interesterified fat blends and PO) for the intended margarine was on the agenda. This ternary combination has not been studied so far. The prepared samples were compared, in terms of physicochemical properties, with the commercial margarine as control (made of partially hydrogenated SFO).

Finally, organoleptic properties were evaluated to choose the best formulation of margarine for the scale-up of the process in the next step.

2. Materials and methods
2.1. Materials
Materials used in this research include refined sunflower oil (MP: -17, IV:128.5), fully hydrogenated soy oil (MP: 63, IV:6), and palmolein (MP: 19, IV:62), which were provided by a regional industry of Nahangol Co. Sodium methoxide powder (Sigma–Aldrich), emulsifier (Palsgaard 6151, Denmark), butter essence (IFF, Turkey), β-carotene, sodium chloride, citric acid, and all the other chemicals were of the particular type of analysis grade made by the German Merck company.

The general equipment used in this research include a laboratory scale (DENVER model APX-203 with an accuracy of 0.001 g, USA and model GR-200 with an accuracy of 0.0001 g, Japan), magnet hot plate (FALC, Turkey), laboratory oven (BINDER, Germany), rancimet (Metrohm model 743, Switzerland), lovibond (UK), digital colorimeter (model PFX 995, England), refractometer (ATAGO model RX-9000a, Japan), carl Fischer (Metrohm, model KF 787, Switzerland), and two-stage distilled water maker (Fater Electric, Iran).

2.2. Methods
2.2.1. Preparation of fat blends
First, the properties of Sunflower oil, soy oil, FHSO, PO, and the commercial margarine were determined (acidity (%), refract (%), peroxide, moisture, color, the saponification value, the apparent melting point). The analysis performed on the mixtures based on internal and international standards, were: the

fatty acid profile analysis (ISIRI 4091), % free fatty acids (ISIRI 4178), peroxide value (ISIRI 4179), stability (Rancimet) (ISIRI 3734), residual soap (ISIRI 6082), iodine value (AOCS method Cd 1c-85), moisture (carl Fischer) (AOCS method Tb 2-64), slipping point (open capillary tube) (AOCS method Cs 3-25) and refractive index (AOCS method Cs 6-25).

The binary mixtures of refined oils, including SFO and FHSO (with saturated fatty acids equal to 5 % and 95 % respectively), were used for the CIE reaction. Four treatments (coded as S1, S2, S3, S4) at different ratios (SFO: FHSO) and with the following specification were prepared (Table 1). Prior to each CIE reaction, oils (1600 g) were heated to 100 °C (30 min), under a vacuum condition while stirring (at 400 rpm) to dry the oil and decompose the excess amounts of peroxides. Afterward, the samples were cooled to 65 °C to minimize oxidation reactions. The excess acid was neutralized by ethanol to reach the acidity of less than 0.1 % and finally was dried under vacuum in the converter. In the following, the vacuum was cut off.

Table 1
Formulation of primary fat blends (4 treatments).

Sample	Total saturated fat (%)	Sunflower oil (w/w %) SFO	Soy oil (w/w %) FHSO
S_1	26.2	80	20
S_2	30.5	75	25
S_3	38.8	65	35
S_4	47.7	55	45

2.2.2. CIE

For the CIE reactions, a stainless steel 16-jacketed converter (2500 ml, Di = 15.24 cm) was designed with a bottom outlet, equipped with coupled to two turbine stirrers (D = 10 cm), with 45° inclined blades and six blades (for producing FHSO and maintaining the solubility of the hydrogen gas in the oil by the vortex created by the top stirrer), vacuum pump (Emerson, model C55JXHRL-4205, USA), and digital skewer type thermometer (Incoterm) and recirculating thermo-statized bath.

According to the optimization process performed before, sodium methoxide (0.5 % w/w) was added to each treatment (Table 1) in the converter (at 800 rpm), and esterification reactions were performed at 80 °C (under vacuum, with 500 rpm agitation, 20 min) [2, 13, 14]. The catalyst began to disperse in the oil, and a milky white color appeared, indicating that the catalyst was adequately mixed with the oil. The heating was used again and, after five minutes, the brown color began to appear. As the reaction progressed, the intensity of the brown color increased and reached its maximum intensity after about 15 minutes, while the temperature during this process reached 80 °C. the reaction was finalized through the addition of 2 % w/w of an aqueous citric acid solution (20 % w/w) to the mixture in the converter while stirring for 10 min. The brown color of the oil gradually changed to the initial milky white color as an indicator of the inactivation of the catalyst.

The sample was transferred to a decanter (2000 ml) for settling (1 hr), and the soap paste was removed while preventing the oil

temperature from rising or falling considerably. The sample was washed several times (400 ml of distilled warm and soft water at 90 °C), which took half an hour to complete phase separation. After five washes, the residual soap test was performed (the remaining soap of less than five ppm is required). Subsequently, the sample was dried under vacuum (at 95 °C; stirred at 400 rpm). Due to the water in the oil, the temperature did not rise above 95 °C until the most of the moisture evaporated. The gradual increase of the temperature indicated the complete drying of the sample. At 105 °C, the heating was stopped to prevent further increase in temperature while stirring was continued for about 30 min until the sample was dehydrated [2, 13, 14].

2.2.3. Preparation of margarine

The production of margarine in the pilot plant was carried out on the interesterified samples (coded as Si-1, Si-2, Si-3, Si-4), and to improve crystallinity, palmolein was used as part of the formulation. The constant ratio of interesterified samples to palmolein was 60:40.

According to ISIRI #143, solidification, working, and resting are the main steps in the preparation of margarine.

The oil phase (the interesterified sample and PO) of margarine was heated to 80 °C to melt the remaining crystals. The emulsifier (0.5 %) and butter essential oil (0.02 %) were added and stirred at 160 rpm until the emulsifier completely dissolved. The aqueous phase contained a solution of deionized distilled water (18 %) heated to 75 °C with NaCl (0.2 %) and citric acid (0.5 %) (% w/w according to the final formula). The emulsion was formed by gradually adding an aqueous phase to the oil phase over about 2-3 min of stirring (700 rpm) at room temperature. The emulsion of β-carotene (0.2 %) was used as a colorant and source of pro-vitamin A. It was protected by encapsulation in the o/w emulsion of the 90 % (v/v) aqueous solution, 10 % (v/v) palm oil, and 2 % E472c (citric acid esters of mono- and diglycerides based on fatty acids) [15].

Afterward, the temperature was reduced from 80 °C to 40 °C to induce crystallization. The emulsion was cooled to -10 °C, and stirring increased to 1000 rpm (Due to the gradual increase in the viscosityof the emulsion) to ensure that the emulsion was appropriately and thoroughly mixed. After the temperature gradually passes through the melting points of various fats, the formation of crystals starts (exothermic process). The full crystallization occurs when a noticeable increase in temperature (1-3 ºC) is observed. At this point, stirring was stopped, and the resulting emulsion was immediately poured into special containers and kept in the refrigerator for at least 4 hr to create the sufficient consistency. Finally, the samples were compared, in terms of physical and chemical properties, with the commercial margarine as a blank (Mandegar brand, Iran).

2.3. Physicochemical analysis of samples
2.3.1. GC analysis
Gas chromatography (young liner, model Acme6000), with a 50-meter cap column CPSIL88 and an FID detector, was used to detect the profile of the composition of fatty acids in primary and interesterified samples and also in the commercial and prepared margarine. The injection rate was 1 μl with the injector temperature of 250 °C and 1.100 of isothermal split conditions. The oven temperature was 175 °C, and the detector temperature was 260 °C. The flow rate of helium as the carrier gas was 0.8 ml/min. In

this study, elaidic acid was monitored as an indicator of the content of trans fatty acid in samples to check the validation of CIE for the preparation of the zero trans margarine.

2.3.2. Determination of SFC

The determination of the SFC of the interesterified samples was carried out by the p-NMR method, which can measure the difference in solid and liquid protons and determine the content of fat in the samples. The specimens were heated to 70 °C in a utensil in the Nuclear Magnetic Resonance (NMR) device (Bruker, Minispec model, Germany). The content of the solid fat in samples (before and after esterification) was measured and recorded at 5 ° C and afterward at higher temperatures (10, 15, 20, 30, and 35 °C), and each analysis was repeated three times.

2.3.3. Thermal analysis (crystallization and melting)

The melting profile is one of the most critical tests in examining the treatment differences. The differential scanning calorimetry (DSC), Diamond model (Perkin Elmer, USA) was used to study the profile of the melting point of the samples [16, 17]. 4-5 g of the pre/post-esterified sample was placed in an aluminum container. The heating rate was 30 °C/min until the temperature reached 80 °C (the holding time of 10 minutes) until the complete melting of the fat crystals. Then, the sample cooled down at the rate of 10 °C/min until the temperature reached -20 °C (the holding time of 20 min) and the sample was completely crystallized. Finally, the sample was heated again at a rate of 5 °C/min to reach the temperature of 80 °C. The thermometric scanner has recorded the thermogram curve [18].

2.3.4. Texture profile analysis (TPA)

The analysis of the force-time curve led to finding texture parameters. Three types of the prepared margarine and commercial margarine, were analyzed. A minor mold of each sample was put in a particular place of the TPA apparatus (CT3™ Texture Analyzer, AMETEK Brookfield, USA). Measurements were performed at ambient temperature under these conditions of: the TA-BT-KI Fixture, probe TA4 / 1000, 4500 g cell Capacity, Spool mold with the length and width of 20 mm, data speed of 10 point/s [18]. In this study, hardness, adhesion, elasticity, and chewing indexes were investigated.

2.3.5. PLM analysis

Images were recorded using the binocular polarized light microscope (LEITZ LABORLUX 12 POL, Plain/ Cross polarized Light, PPL/ and XPL type, Germany) at ambient temperature. A capillary tube was used to transfer a drop of the melted sample (at 70 °C) onto a glass slide and was covered with a glass cover and spread evenly to create a uniform thickness of the sample (duplicates of glass slides). Samples were stored at the experimental temperature (25 ºC ± 1.0) overnight. The microscopic images were recorded under PLM connected to a camera at a magnification of 60 [16].

2.4. Hedonic sensory evaluation

For the sensory evaluation of the prepared margarine and its comparison with the commercial margarine, a five-point hedonic scale was used to evaluate the parameters of the taste, smell and aroma, spreadability, and general acceptance of the product of which the results are as follows: Very bad, Bad, Medium, Good, and Very good (with the score of 1, 2, 3, 4, and 5, respectively). Ten trained test-

panelists were used to evaluate the kinds of the margarine.

2.5. Statistical analysis

The obtained data were statistically analyzed by the one-way ANOVA at least three times (n = 3) using the SPSS® 21.0 software (IBM, USA). The comparison of means was done based on Tukey's test at the confidence level of 95 %. Experiments were performed in three replicates.

3. Results and discussion

The properties of the Sunflower oil, soy oil, FHSO, PO, and commercial margarine were determined and recorded in Table 2.a. All specifications are within the permitted range for use.

In refined oils, the maximum amount of free fatty acids, according to all national and international standards, is 0.1 % w. The values of the acidity of the primary sunflower and fully hydrogenated soybean oils were 0.04 % and 1.6 % respectively. The acidity of the esterified product has increased significantly compared to that of the initial mixture (Table 2.b), which indicates the good activity of the catalyst.

The peroxide index, shows the degree of the fat oxidation and the formation of hydroperoxides in the initial stages of the reaction. The maximum allowed value for the peroxide is 10 meq/kg. The peroxide index of the fat blends has slightly increased compared to that of the raw materials, due to heating the primary oils and mixing under a vacuum condition. The peroxide index decreased after the esterification reaction (Table 2.b), because hydroperoxides are easily broken at temperatures above 100 °C, and the use of a suitable vacuum can quickly reduce the amount of peroxide to zero.

Refractive index is one of the physical characteristics of oils and fats, which has a close relationship with the chemical characteristics such as the average molecular weight and degree of the unsaturation of fatty acids, and the spatial arrangement of triglycerides. The refractive index of symmetric triglycerides is greater than that of the asymmetric type, the decrease in the refractive index after the reaction is probably due to the decrease in the amount of symmetric tristearin triglycerides and the formation of new asymmetric triglycerides.

Table 2.a

Specifications of raw materials before any mixing or CIE.

Specification	Sunflower oil (SFO)	Soy oil	FHSO	PO	Commercial margarine
M.P* (°C)	-17	-	63.5	19	36.5
Acidity (%)	0.045	0.07	1.6	0.05	0.24
Refract (%)	1.4672	1.4669	1.4536	1.4585	1.4594
Peroxide (meq/Kg)	0.16	0.4	1.5	0.6	1.5
Moisture (%)	0.015	0.02	0	0.023	
Color	20 Y, 2 R	11 Y, 1.1 R	11 Y, 1 R	2.5 Y, 25 R	
Saponification Value (mg KOH/g oil)	0	0	0	0	0
Saturated fat content %	9	13	95	46	

*Melting Point
fully hydrogenated soybean oil (FHSO)

Table 2.b
Specifications of fat blends before and after CIE.

Oil/Fat	S1	Si-1	S2	Si-2	S3	Si-3	S4	Si-4
M.P* (ºC)	57	30	58	31.5	59	41	61	48
Acidity (%)	0.9	0.34	0.1	0.35	0.13	0.48	0.16	0.51
Refract (%)	1.4645	1.4623	1.4639	1.4617	1.4626	1.4609	1.4613	1.4603
Peroxide (meq/Kg)	0.58	0.36	0.54	0.35	0.44	0.3	0.42	0.32

3.1. Monitoring the content of the fatty acid

The content of elaidic acid as an indicator of trans fatty acid (C18:1 t), has been shown in Table 3 according to the results of GC. There was a significant difference between the content of elaidic acid in the prepared margarine (using an interesterified fat blend) and commercial margarine (using partially hydrogenated fats).

Trans-free or no trans claims mean that the product contains less than 0.5 g of trans-fat per serving. One serving is defined as one tablespoon or about 12 g of trans fat. In this case, food manufacturers are allowed to insert "trans-free" or "no trans" claims on the package. Rarely has an article addressed this issue with such precision as our research has. In the research of Danthine et al. (2022), in the zero-trans, interesterified rapeseed oil, the amount of trans fatty acids has been reported in the range of 0.03-0.3 [19].

Beef tallow/rice bran oil (BT/RBO) has been interesterified (at different proportions: 10, 20, 30, 40, and 50 wt % of RBO), for the preparation of trans-free fats. In the 50:50 BT/RBO blend the percentage of SUU and SSS type of TAG were 19 and 4 % respectively. These variations in composition greatly improved the plasticity range of BT [20].

Table 3
Profile of the fatty acids in the fully hydrogenated soybean oil (FHSO), sunflower oil (SFO), palmolein (PO), fat blend in different ratio (S1-S4), interserified margarin (M1-M4) and commercial margarine (CM).

	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1cis	**C18:1 t**	C18:2	C18:3	C20:0	C20:1	C22:0
FHSO	0.07[b]	0.03[a]	9.27[f]	0.20[a]	84.55[l]	2.10[a]	**0.91[g]**	1.56[a]	0.18[a]	0.65[h]	-	0.42[b]
SFO	0.02[a]	0.026[a]	4.28[a]	0.11[ab]	3.98[a]	32.06[g]	**0.91[g]**	54.63[l]	0.29[b]	0.31[a]	0.20[d]	0.79[h]
PO	0.50[f]	0.03[a]	25.94[j]	0.28[ab]	5.67[b]	46.57[l]	**0.48[f]**	18.38[c]	0.85[g]	0.52[g]	0.07[a]	0.17[a]
S1	0.03[a]	0.22[g]	5.28[b]	0.09[b]	20.09[f]	26.07[e]	**0.18[b]**	46.42[k]	0.27[b]	0.38[b]	0.16[c]	0.72[h]
S2	0.03[a]	0.20[f]	5.53[c]	0.09[a]	24.12[h]	24.57[d]	**0.22[b]**	43.61[j]	0.26[b]	0.40[bc]	0.15[c]	0.70[g]
S3	0.04[a]	0.18[e]	6.02[d]	0.08[a]	32.18[j]	21.58[c]	**0.32[c]**	38.01[i]	0.25[b]	0.43[c]	0.13[c]	0.66[f]
S4	0.04[a]	0.16[d]	6.52[e]	0.06[a]	40.24[k]	18.58[b]	**0.41[d]**	32.40[a]	0.24[b]	0.46[de]	0.11[b]	0.62[e]
M1	0.25[d]	0.11[b]	14.36[h]	0.15[ab]	14.02[d]	34.27[j]	**0.17[a]**	35.06[h]	0.48[c]	0.53[f]	0.09[a]	0.51[d]
M2	0.21[c]	0.14[c]	13.98[g]	0.17[ab]	16.34[e]	34.05[i]	**0.31[c]**	33.18[g]	0.62[f]	0.43[c]	0.11[b]	0.46[c]
M3	0.22[c]	0.11[b]	14.23[h]	0.19[ab]	21.08[g]	32.70[h]	**0.37[d]**	29.46[e]	0.58[e]	0.49[ef]	0.12[b]	0.45[c]
M4	0.23[c]	0.13[c]	14.91[i]	0.15[ab]	25.03[i]	31.09[f]	**0.36[e]**	26.48[d]	0.52[d]	0.45[d]	0.15[c]	0.40[b]
CM	0.47[e]	0.03[a]	29.28[k]	0.26[ab]	7.85[c]	40.17[k]	**_2.6[h]_**	17.50[b]	1.22[h]	0.47[e]	-	0.15[a]

3.2. SFC analysis

Interesterification reduces SFC and consequently improves the physical properties without affecting the chemical characteristics. The SFC results showed the effect of CIE and the degree of SFC at different temperatures (Figure 1). The further addition of FHSO to SFO promoted the increase in SFC, proportionally to the added content. SFC increases from the first to the fourth samples. So, the trend of reduction in SFC increased from S1 to S4 after the interesterification. As a general rule, CIE has reduced the SFC. For example, in sample S1, at 10 °C, the esterification reaction reduced SFC from 20.6 to 8 % (in Si-1), and in sample S4, at the same temperature, SFC reduced from 45.9 % (S4) to 35.7 % (Si-4).

The amount of the SFC reduction after esterification is dependent on the initial content of the saturated fat in the samples and also on the temperature. Original blends showed the maximum SFC increased from 20.6 % to 45.9 % at 10 °C and from 16.6 % to 40.5 % at 35 °C. Interesterified samples showed the SFC values in the range of 8 % to 35.7 % at 10 °C and 0.8 % to 12 % at 35 °C. CIE caused the decreased values of SFC in all blends. It is attributed to the decrement of S3 TAGs and simultaneously increase in the percentage of S2U and SU2 TAGs [21]. In this study, the reaction of CIE significantly decreased SFC. However, the percentage of the SFC reduction in all samples is not uniform. The percentage of change in SFC after CIE was reduced from S1 to S4. The reduction of SFC (%), after CIE for the 80:20, 75:25, 65:35, and 55:45 blends at 35 °C, caused by randomization corresponded to 95.19 %, 94.29 %, 80.95 %, and 70.37 % respectively. The less content of the saturated fat in fat blends, the more changes in SFC after CIE [22-24].
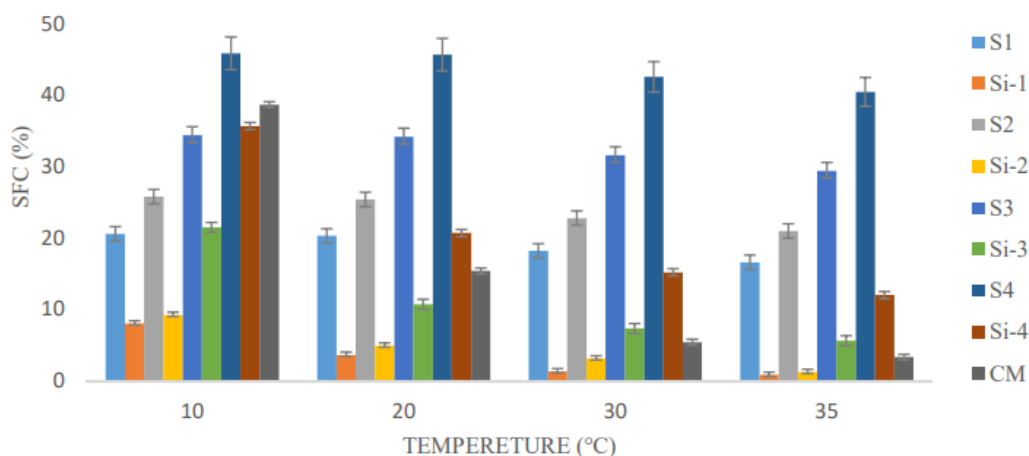

Figure 1. SFC % of the original blend (S1-S4), interesterified samples (Si1-Si4) at 10, 20, 25, and 35 ℃.

3.3. Thermal behavior

The DSC thermograms are valuable tools to monitor alteration in the enthalpy and phase transitions of the TAG mixtures after applying the CIE reactions in fats [25, 26]. The content of the TAGs with middle-melting-points (S2U and SU2) shows a significant increase because of the randomization in the CIE process. CIE led to the reduction of tri-saturated and an increase in the content of mono- and di-unsaturated acylglycerols by making significant changes in the temperature and enthalpy associated with the melting and crystallization profiles.

In the DSC profile analysis, interesterification has led to a decrease in both the melting point (Figure 2) and crystallization temperature (Table 4). The changes were more pronounced for samples with lesser SFC. Table 3 shows the prominent endothermic and exothermic peaks of pre and post-esterified samples. The endothermic and exothermic regions are related to the melting and crystallization of sample components respectively. Figure 3 shows the DSC thermogram of sample S3 in detail.

Exothermic thermograms are affected by the chemical composition and content of acylglycerols. The higher temperature is related to the crystallization of more saturated TAGs (the components with higher melting points are prone to the faster crystallization). Before esterification, in all samples, there was one exothermic peak. After esterification samples exhibited more multiple exothermic peaks. The onset (Toc) and the endset crystallization temperatures (Tec) of interesterified blends were lower than those of the initial blends, allowing them to have a softer texture. Hence, the CIE of SFO: FHSO blends eventuates a decrease in the growth of crystals (due to the less thermodynamic or kinetic desirability [21].

Different amounts of solids and liquids cause differences in the points of the beginning, the end, the height, and the width of the peaks. A broad curve with more overlap is related to the cooling rate or wider distribution of TAGs. The higher content of DAGs also results in the delayed crystallization. The minor exotherms are associated with less saturated components.

The complex and irregular curve show different amounts/types of fat crystals. The unsharp and broad melting areas indicate the diversity in melting points because of the varied content of the saturated fat in TAGs, and the variety in the type, percentage, and size of crystalline spherulites. Thermograms with sharp peaks confirm the uniform fatty acid distribution in crystalline TAGs.

In all cases, the width of curves in the crystallization period has been increased after CIE due to the rearrangement of new acylglycerols with more variety relatively to the original mixture. In a comparison between the S1 and S4 treatments (with the minimum and maximum content of the saturated fat in TAGs in the formulation respectively), the S4 curve has narrower width in the melting regime and a sharper peak due to the lower TAGs diversity (Table 4).

The ingredients with higher melting points show rapid crystallization. Albeit, any change in the heating/cooling rate, generally, was associated with a change in the peak size. The crystallization peak size shows an increase (broadened) and shifts to lower temperatures as soon as the cooling rate increases [16]. As the cooling rate increases, so does the rate of crystallization.
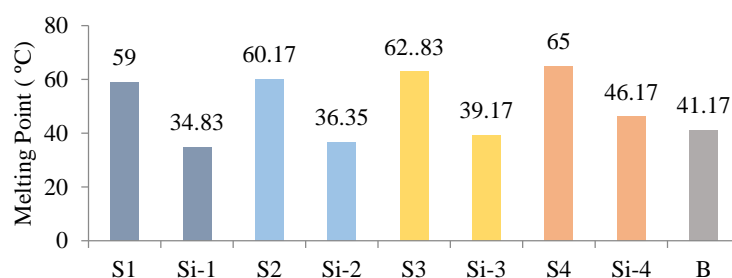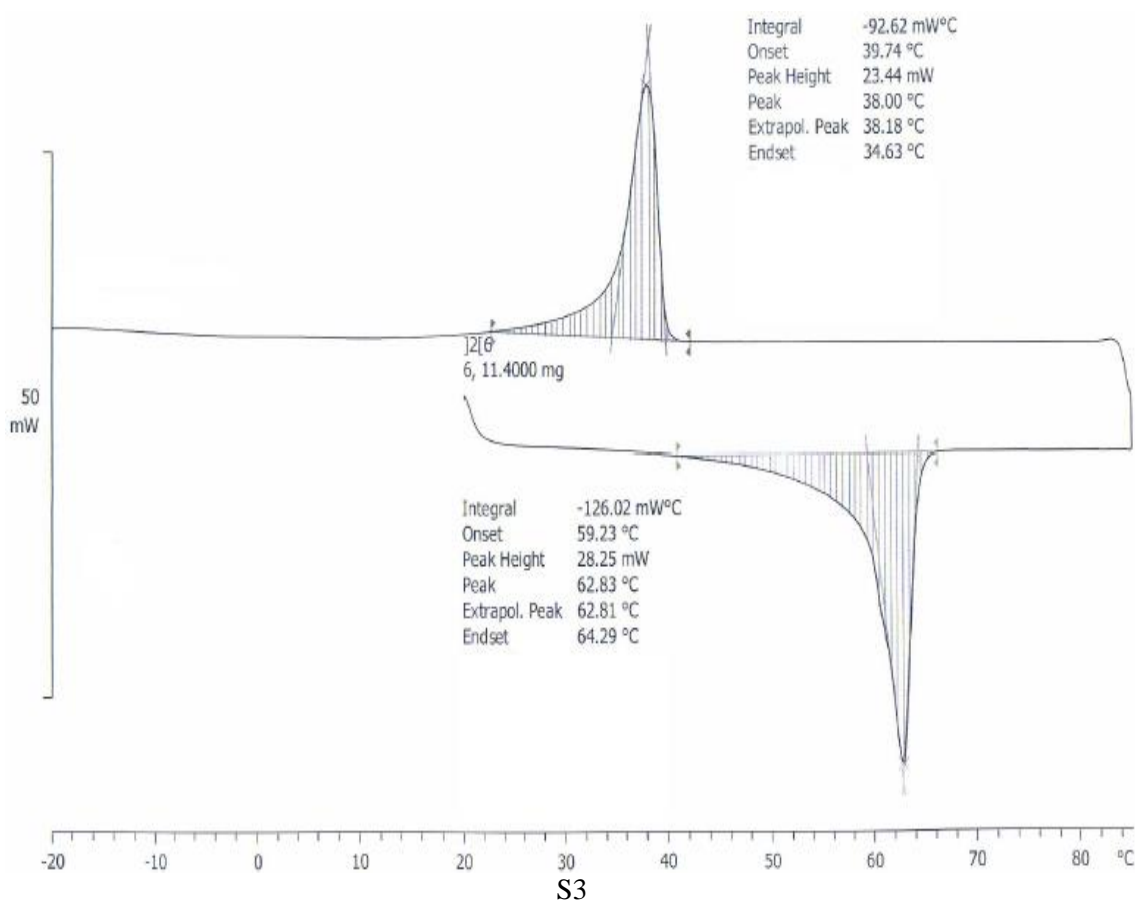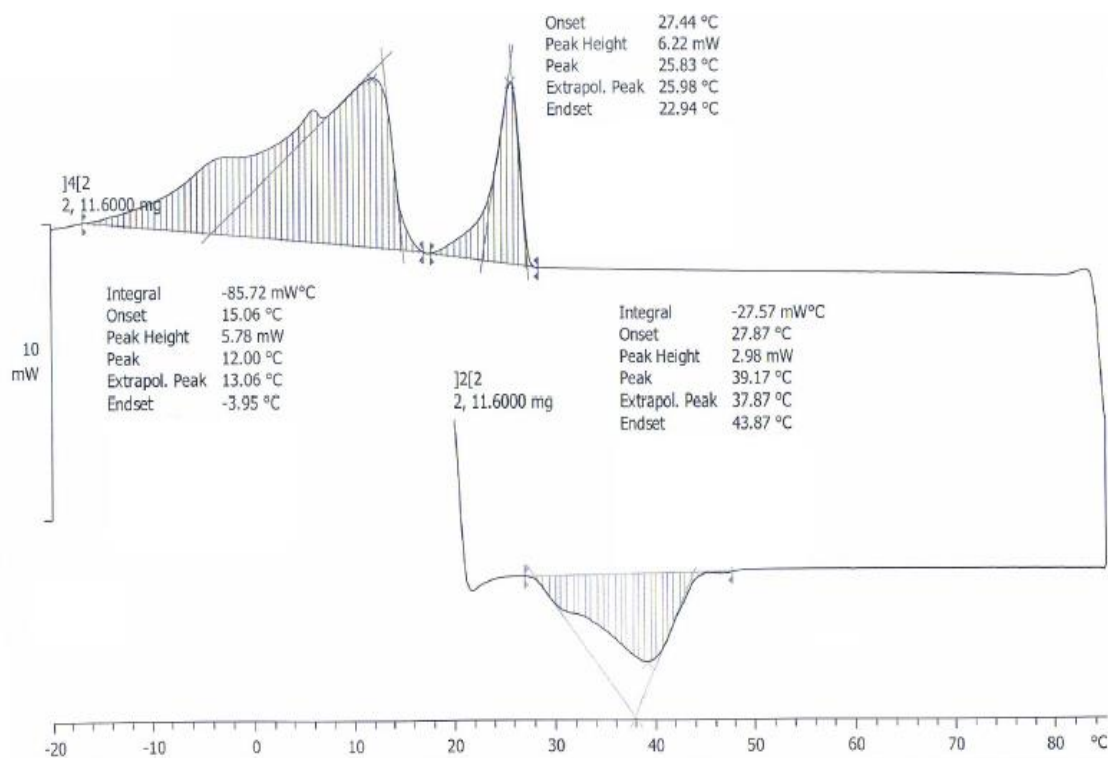

Figure 2. Melting point (°C) of samples (peak of the thermogram), before (S1-S4) and after (Si-1 to Si-4) CIE, extracted from DSC themograms.

Table 4
Details of melting and crystallization temperatures in the DSC thermograms of the samples
(the main endothermic and exothermic peaks of pre and post-esterified samples).

Samples	Melting temperature (endothermic region)			Crystallization temperature (exothermic region)		
	Onset (ºC)	Peak (ºC)	Endset (ºC)	Onset (ºC)	Peak (ºC)	Endset (ºC)
S1	48.60	59.00	61.35	35.72	34.17	30.5
				23.4	20.67	16.13
Si-1	28.95	34.83	44.08	13.82	11.67	10.39
				9.58	1.67	-7.48
S2	53.12	60.17	62.24	37.37	35.83	32.69
Si-2	28.65	36.5	40.34	22.73	20	15.96
				11.9	7.17	-12.22
S3	59.23	62.83	64.29	39.74	38	34.63
Si-3	27.87	39.17	43.87	27.44	25.83	22.94
				15.06	12.00	-3.95
S4	62.29	65.00	66.61	42.8	40.83	37.2
				32.06	30.63	27.78
Si-4	45.15	46.17	48.12	17.63	14.5	7.15
				6.33	5.17	3.64

Integral	-92.62 mWºC
Onset	39.74 ºC
Peak Height	23.44 mW
Peak	38.00 ºC
Extrapol. Peak	38.18 ºC
Endset	34.63 ºC

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Integral	-126.02 mWºC
Onset	59.23 ºC
Peak Height	28.25 mW
Peak	62.83 ºC
Extrapol. Peak	62.81 ºC
Endset	64.29 ºC

S3

Si-3

Figure 3. Overview of the DSC thermogram before and after CIE in samples 3 (the main endothermic and exothermic peaks of pre and post-esterified samples, S3 and Si-3 respectively).

3.4. Texture analysis

The crystal network is responsible for creating the unique structure in the fat-containing compounds. The polymorphism and size of crystals influence this structure and consequently affect the texture and functionality of fats [21, 27]. Based on the texture analysis results, by increasing SFC and the fat saturation in margarine samples, the amount of hardness, adhesion force, and elasticity showed an increasing trend. The amount of hardness in the samples (from M1 to M2 and M3) showed an increase by increasing the percentage of SFC. The amount of deformation due to hardness has an upward trend. Considering the market sample, the quantity of hardness of the market sample (CM) was between the samples M1 and M2 (Table 5 and Figure 4). The greater hardness in formulated margarine with a similar level of SFC ascribed to the existence of a more robust fat crystal network [9]. The chewing rate also had an upward trend (from M1 to M2 and M3) due to the increase in the saturation percentage of the fat.

The adhesion force of the market sample (CM) was not significantly different from that of the M1 sample, which had the lowest SFC among the samples formulated by CIE. These peaks are gentle, have less sharpness and slope, and appear similar to those of M1 (Figure 4). In this diagram, the elasticity of M2 and M3 with the market control sample (B) is almost in the same range. Regarding the chewing evaluation of treatments M1 and M2, there was a significant difference with the market sample, which can be due to the long storage time of the control sample.–In the control sample (CM), the adhesion force was similar to that of sample M1, and the elasticity of the sample was similar to that of sample M3.

Table 5

TPA analysis of margarine products (M1, M2, M3), the control sample of the commercial margarine (CM) and the spread sample of the margarine esterified by [18] (C).

Sample	M1	M2	M3	CM	C
Hardness (g)	687 ± 1.1^a	1535 ± 3^c	3010 ± 5^d	744.5 ± 2.7^b	1611
Adhesive force	149 ± 2.1^c	43 ± 1.56^a	65 ± 2.2^b	160.5 ± 0.5^d	34
Elacticity	0.15 ± 3^a	0.23 ± 0.03^b	0.3 ± 0.02^d	0.28 ± 0.03^c	0.33
Chewingness (g)	22.4 ± 0.4^a	31.1 ± 0.8^b	89 ± 1.2^c	166.9 ± 0.45^d	170
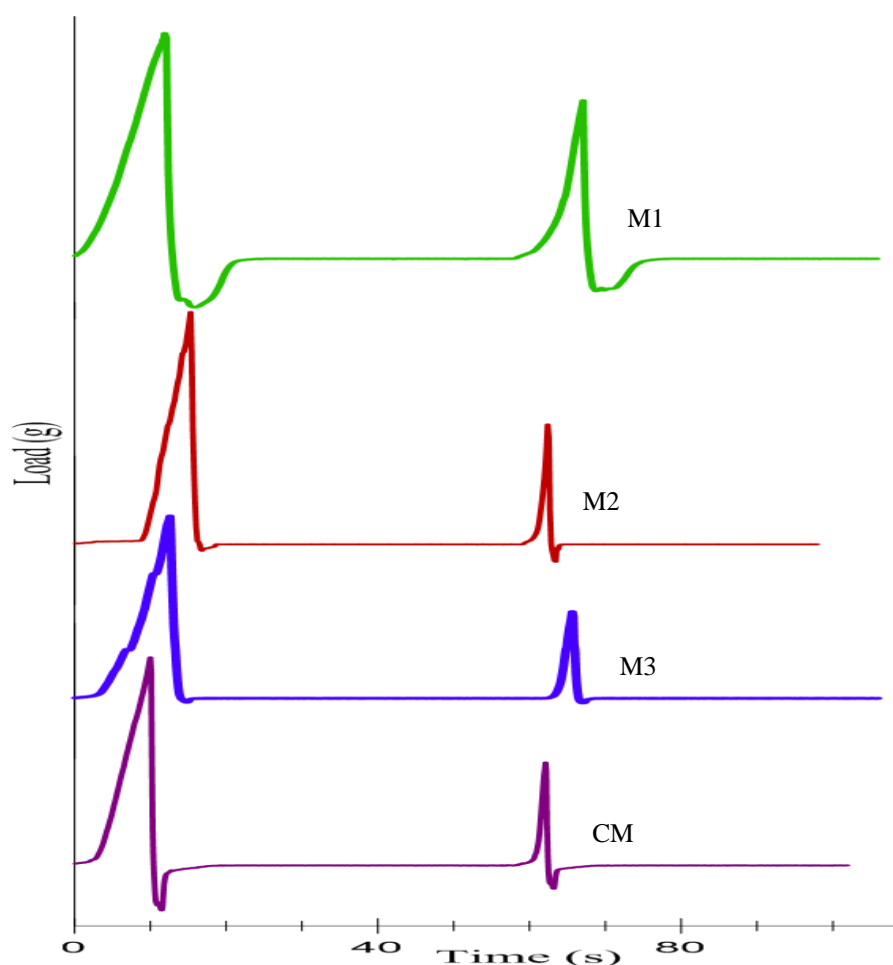
Figure 4. TPA analysis of three margarine samples (M1, M2, M3), and the commercial margarine (CM).

3.5. Morphological observation of crystals by PLM

The macroscopic physical properties of fats are related to their microstructure. The change in SFC after the CIE process specifies and determines the crystal network. In this study, the PLM examination of native blends (pre-esterified sample: S2) showed the presence of asymmetrical, large, and dense spherulite aggregates. The presence of large spherulites attributed to the significant content of long-chain TAGs in the blends, giving an undesirable sandy texture to the fat [21, 28].

The β′ crystals are tiny delicate crystals (1 μm long), while the β form crystals are large and coarse (25-50 μm in length) and have the

highest order and melting point. The β modification does not occur very often, while the β' modification is the predominant one in margarine crystals. The β modification often happens in the products made from canola or sunflower oils (used in the formulation of margarine) [12, 29, 30]. In this study, CIE causes the formation of the β'crystal. In the M2 and M3 samples, the halo around the core zone had blurred borders, and more desirable β'crystals, entirely dependent on the SFC and fat saturation, were observed. Many studies have shown that CIE reduces the spherulitic nature of fat crystals and increases the halo-to-core ratio within the spherulites [21]. CIE is associated with the alteration in the microstructure of the fat blends, creation of the more disordered packing of terminal methyl groups, and the formation of lower-density crystal lattices. These modifications in fats create smaller spherulites and changes in the crystal's halo-nucleus ratio, which eventually affects the texture and functional properties of margarine [2, 21, 31, 32]. The CIE process forms more symmetrical spherulitic particles and smaller spherulites, and increases the halo-to-core ratio. After CIE, the number of spherulites decreased, and low density fine crystals were observed (Figure 5). The alteration of the TAGs composition, generating the new structures, caused by CIE can modify the strength of bonds among crystals either in a spherulite (intraparticle) or among the spherulite (interparticle) [33]. Crystals were similar to one another, having spherulitic needle-like crystal structures radiating and branching out from the central nucleus. However, they were different in terms of crystal packing [34]. Other researchers have shown that the incomplete removal of the liquid oil may cover the crystals and prevent observing the details of the fat crystals by a microscope [29].

In general, the incorporation of SFO and PO into FHSO caused a decrease in the size of the spherulite, and the spherulitic particles became more symmetrical. According to the reports, the addition of soya oil to PO resulted in a decrease in the size of the spherulite with the same morphology after the CIE process. We observed that the structure of margarine crystals varied from that of pre-esterified samples. The tiny needle-shaped crystals were observed in all samples (Figure 5). The margarine obtained from the CIE of (FHSO: SFO: PO) the blend exhibited the highest capability to be used as the desirable margarine as it showed a similar SFC curve to that of the commercial margarine fat and crystallized into the β' polymorph. The disparity might be due to different compositions of TAG and distinct textural properties. The structure was similar to the same in previous findings [33, 35, 36].

Palm oil (PO) is categorized as a β'-tending fat because of asymmetric mixed-TAGs such as POO and PPO [37]. Using 20–25 % of PO (as the promoter of the formation of the β' crystal) is a good suggestion for the attainment of a more desirable texture (i.e., consistency, plasticity, and aeration properties) in margarine. It was seen that in the ratio of 75POP: 25OPO, large (150 μm) and many small spherulites (less than 20 μm) are grown from neat liquid, and small spherulites are between the large spherulites [38]. PO spherulites are dense and large with a low-density halo. In the macroscopic scale, PO shows an increase in the halo: core ratio after CIE, so a wider surface is provided for the interaction between the spherulites, resulting in a more robust network. At the microscopic level, the spherulite boundary in PO is diffused, and a higher hardness index confirmed this hypothesis [21, 39]. Silva et al.

showed that the induction time of crystallization for tripalmitin was 2.9 times higher than the same for tristearin (C16 vs. C18) [35], so the presence of PO in the formulations of margarine produces fine crystals. The physical structure of fats may change through time passing at a constant temperature because of polymorphic transformations [40]. However, the experimental time difference in this study was too short for the polymorphic transformation.

As confirmed in this study, when SFC decreases, the formation of crystals is more remarkable because of the driving force induced by the higher molecular mobility [22]. CIE causes a significant increase in the number of crystals of fats dispersed in the blends. The existence of more small crystals can provide the favorable properties, such as good spreadability [31, 35, 41].
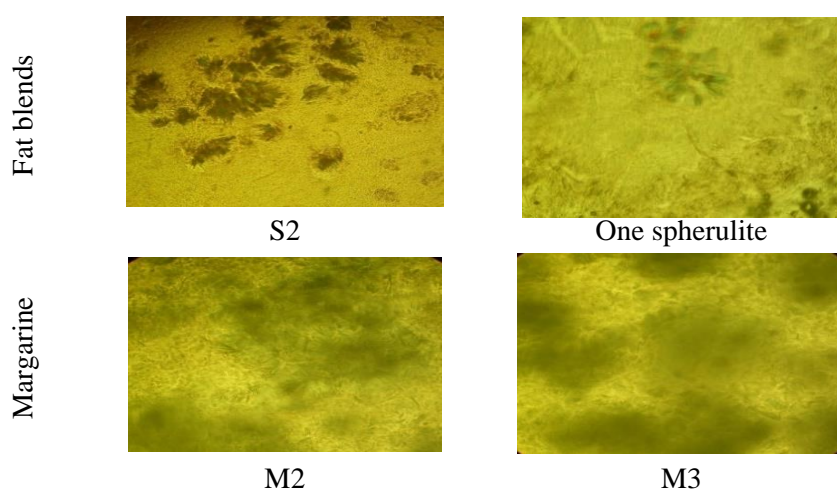

Figure 5. Polarized light micrographs (x60) of M2, M3, the blank margarine and the distinct shpherulite, before esterification (increased halo around the core after esterification).

For the diversification of utilizing rice bran oil (RBO), the interesterification of the blend of RBO and the fully hydrogenated RBO has been done. The interesterification process reduced the concentration of triglycerides (either tri-saturated or tri-unsaturated) causing the reduction of the melting point temperature. The final product has a high tendency to crystallize under the β' polymorph. β' crystals are preferred for structuring different types of foods. The quantitative determination of TAGs was performed using HPLC [42].

3.6. Organoleptic evaluation
As shown in Table 5, the organoleptic evaluation of the formulated margarine showed that the probability values (Sig) for the overall acceptance, taste, and spreadability are significant among the samples (< 0.05). In other words, changing the samples causes significant changes (which are not due to random changes) in the sensory scores (the total acceptance, spreadability, and taste). The only characteristic of margarine which indicates insignificant changes in all samples was aroma. There are demands for the fat crystals smaller than 30 μm to prevent a sandy sensation in the mouth (Herrera et al., 1999). For all margarine evaluated, CIE was effective at modifying the feeling in mouth.

The results of Table 6 show the effect of the formulation on the mean sensory properties of margarine and the control sample. As it is clear, M3 is the selected choice of organoleptic evaluators, according to taste, aroma, spreadability, and total acceptance.

Table 5

Mean squares of the sensory scores of the different types of the formulated margarine.

S.V	df	MS			
		Taste	Aroma	Spreadability	Total acceptance
T	3	2.2*	0.358 ns	3.233*	2.7*
e	36	0.383	0.297	0.228	0.25
Total	39	-	-	-	-

*: At 5% level, the difference is significant; ns: The difference is not significant.

Table 6

Effect of the formulation on the mean sensory characteristics of margarine and the control sample.

Sample code	Taste	Aroma	Spreadability	Total acceptance
M1	4.6[a]	4.7[a]	4.8[a]	4.7[a]
M2	4.5[a]	4.7[a]	4.7[a]	4.7[a]
M3	3.6[b]	3.3[a]	3.6[b]	3.6[b]
CM	4.5[a]	4.6[a]	4.7[a]	4.4[a]

Means that have at least one common letter are not statistically significant. M1, M2, M3 are the formulated margarine samples and CM is the commercial margarine.

4. Conclusions

CIE is an excellent candidate to formulate healthier foods containing fats without trans fatty acids. The interesterified blend of FHSO and SFO were used for the preparation of margarine, caused the properties like commercial margarine (ie. the improved texture, microstructure, and thermal properties). Thus, the art of the correct use of various vegetable fats and oils have a significant influence on the properties of margarine and shortenings. In this study, interesterified FHSO and SFO were blended with PO to obtain a new formulation of margarine with zero trans fatty acids. The formulated margarine samples have been evaluated instrumentally by NMR, TPA, PLM, DSC, and organoleptic tests. The spherulite needle-like crystal structure was dominant in the commercial margarine (not shown), while in two formulations (M2 and M3), greater halo to core ratios were dominant which is comparable with β' crystals in terms of polymorphism. The best margarine formulation had a 35: 65 proportion (FHSO: SFO), and 60 % of the oil formula was used in the emulsion, plus 40 % of PO with the best texture and total acceptance.

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