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Diffraction Scanning Calorimetric Analysis of Fully Hydrogenated Soybean Oil and Soybean Oil Blends

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Abstract

In this study, a binary combination consisting of native soybean oil (SO) and fully hydrogenated soybean oil (FH) in varying ratios of 40:60 (F4S6) and 45:55 (F45S55) was compared with partly hydrogenated shortening (PH) as the control. Thermophysical characteristics of F4S6, F45S55, and control were investigated by the application of differential scanning calorimetry (DSC). With a 60m 8×0.32mm×0.25um DB-23 capillary column and a flame ionisation detector (FID), fatty acid and methyl esters of fat were measured using gas-liquid chromatography (HP 6890 GC capillary) The shelf life of all samples was determined. The results observed that melting occurs at approximately 27, 39, and 48 for soybean oil as a control sample, F4S6 and F45S55, respectively. So, the addition of fully hydrogenated soybeans increases the melting point of oil. For the melting enthalpy ΔHm, the lowest was for PH, and the highest ΔHm was for F4S6. The GC data represented the highest value of C18: I found in FH followed by PH, F45S55, and then F4S6 85.07, 23.64, 14.13 and 13.35 %w, respectively. The data illustrated that F4S6 and F45S55 had no trans fatty acid (TFA) of oleic acid (C18:1T) compared with the control (PH), which had around 16.976. However, F4S6 and F45S55 had very small amounts of trans fatty acid of linoleic acid (C18:2T) compared with control (PH), which recorded 0.09, 0.09, 0.01 and 0.49 %w, respectively. In general, the blended fatty from soybean oil and fully hydrogenated soybean oil (F4S6 and F45S55) improved sample sensory properties compared with the control.

Keywords: DSC, Soybean oil, Fully hydrogenated oil, Thermal properties

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Introduction

Glycine max L. Merrial, or soybean, is the most produced crop in the world and accounts for around 56% of all oilseed goods. Antioxidants, vitamins, lecithin, and nutraceuticals are among the high-value and secondary co-products that are thought to originate mostly from soybeans. Low in saturated fat and free of trans fat, soybean oil is a suitable source. On the other hand, we must combine completely hydrogenated (FHSBO) with soybean oil (SO) in order to raise its melting point [1]. It is also believed that soybean oil is trans-free [2]. Therefore, instead of using partial hydrogenation to make shortening, margarine, and confections, a binary combination of FHSBO and SO is employed [3, 4]. One of the few non-fish forms of omega-3 polyunsaturated fatty acids, which offer a number of physiological advantages, including cardioprotective properties, is soybean oil. The American Journal of Clinical Nutrition [5] states that, in contrast, fish oil is the main source of omega-3s in the American diet. A new piece of research released by the American Heart Association suggests that omega-6 fatty

acids, which are naturally present in soybean oil, may also lower the risk of heart disease itself. Numerous phytosterols found in soybean oil, such as stigmasterol, campesterol, and ß-sitosterol, have been demonstrated to lower LDL cholesterol and blood cholesterol by at least 5 to 10%. A common practice is partial hydrogenation. However, some of the cis double bonds isomerize during the partial hydrogenation of vegetable oils, which results in around 50% of TFA [6]. Compared to cis-fatty acids, trans-fatty acids are more stable and have higher melting points. It is challenging to pack the molecules of cis-fatty acids together due to their kink form [5]. In contrast, TFA has a linear form that facilitates the packing of its molecules. Numerous research investigations on nutrition have proposed a connection between TFA levels and coronary heart disease [7]. TFA has been linked to increased levels of high-density cholesterol (HDL) and low-density lipoprotein (LDL) [1-8].

According to studies, cutting back on our daily calorie intake by 5% of saturated fat will lower our risk of cardiovascular disease by 22–37%. In [9].

Differential Scanning Calorimetry (DSC) technique is used in this study to obtain the melting points associated with the polymorphic transformations formed at different cooling rates and the thermal characteristics of enthalpy of fats and oils [10]. Additionally, the way in which materials exhibiting thermal transitions like melting and crystallisation are affected by differential heat flow with temperature [11, 12].

Through the use of differential scanning calorimetry (DSC), the thermal characteristics of many samples (PH as a control, F4S6, and F45S55) were investigated. The movement of heat modifications to the sample is linked to the development or absorption of heat, which is then noted as a peak [13]. Thermal enthalpy must be taken in or released during the two basic physical processes—melting and crystallisation—that are used to describe the thermal behaviour of samples combined with fat. DSC may be used to ascertain the physical characteristics of various samples that have been combined with fat. DSC was used to establish the thermal curves for partly hydrogenated soybean oil (PH) and fat blends including both fully hydrogenated and partially hydrogenated soybean oil (F45S55, F4S6).

Finding the thermal properties of a low-trans and low-saturated-fatty acid healthy shortening made from a blended blend of fully hydrogenated soybean oil and soybean oil is the goal of this study in order to assist manufacturers in finding a substitute for partially hydrogenated oils, which are a major source of harmful trans fatty acids.

Materials and Methods

Safola Oil Company, Suez Governorate, Egypt, provided the soybean oil and partly hydrogenated soybean oil, while General Mills Company, Canada, provided the completely hydrogenated soybean oil.

Thermal characteristics determination by DSC

Differential Scanning Calorimetry (DSC) (TA instrument DSC Q 100, Newcastle, DE, USA) conducted at Dalhousie University, Canada, 1913. The DSC technique was used in this study to determine the thermal characteristics of enthalpy and the melting points associated with the polymorphic transformations formed at different cooling rates. Three cycles were conducted for all samples compared with the control one. **Figures 1 and 2** revealed all experiments (3 cycles) for F4S6, F45S55, and control, respectively. Using three temperature cycles, the DSC was utilised to compare the sample's rate of heat transfer to that of the reference, an empty pan. In order to eliminate any crystallised history, samples were heated to 70°C in the first cycle at a rate of 25°C/min. They were then cooled to 20°C at a rate of 10°C/min, and they were maintained at this temperature for one hour. The samples were heated once again in the second cycle at a rate of 25°C/min to achieve 70°C/min. They were then cooled to 10°C at a rate of 10°C/min and stored for about an hour. The samples were eventually heated to 70°C by the rete 25°C/min in the third cycle. After that, they were cooled by 10°C/min to 1°C and were maintained for almost an hour.

Analysis of fatty acids using Gas Liquid chromatography

Preparation of fatty acids methyl ester

The fast process described in [14] would be used to create fatty acid methyl esters from total lipids. Comparatively, before saponification occurs, fatty acid methyl esters undergo esterification using methanolic potassium hydroxide as a transitional step. A 5-ml screwtop test tube containing around 0.1 g of the oil was filled with 2 ml of heptan, and the tube was then shaken. A 0.2 ml (2N) methanolic potassium hydroxide solution was added to the cap and allowed to stratify until the top solution turned clear. The upper layer containing the methyl ester was then decanted. It is OK to inject the heptane solution into the gas chromatography.

Separation and identification of fatty acid methyl esters by Gas Liquid Chromatography (GLC)

A GLC (HP 6890 GC capillary) fitted with a flame ionisation detector (FID) and a 60-m 8×0.32mm×0.25um DB-23 capillary column was used to measure the methyl esters of fat and fatty acids. At 2300C and 2500C, respectively, the injector and detector temperatures were set. A carrier gas of 40 millilitres per minute (ml/min) of hydrogen was used, and the temperature programming ranged from 150 to 170 0C at 100 c/min, followed by 1700 to 1920 °C at 50 c/min for five minutes, and finally 1920

to 2200 °C for ten minutes. Through comparison with established standards, individual methyl esters were identified [14]. In 2000, IUPAC.

Shelf-life determination

The Rancimat (679 Metroham) apparatus was used to measure the stability of each sample (5 g of each sample at 120 °C and an air flow rate of 20 L/h) and calculate the shelf life of the novel shortening mix with varied concentrations of FHSO: SO (40:60 and 45:55 by weight).

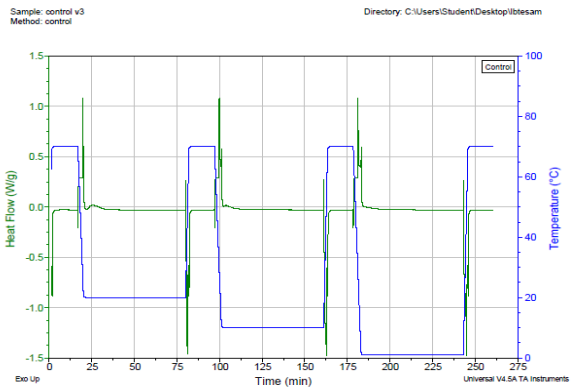
Statistical analysis

Software [15] (CoStat, 2005) would be used to statistically analyse the results obtained. First, an analysis of variance (ANOVA) was performed on each multiple comparison. The least significant difference (LSD), as selected by Steel and Torrie [16] and Gomez [17], would be used to compare means in order to identify any significant differences between different treatments at $P \leq 0.01$ and 0.01.

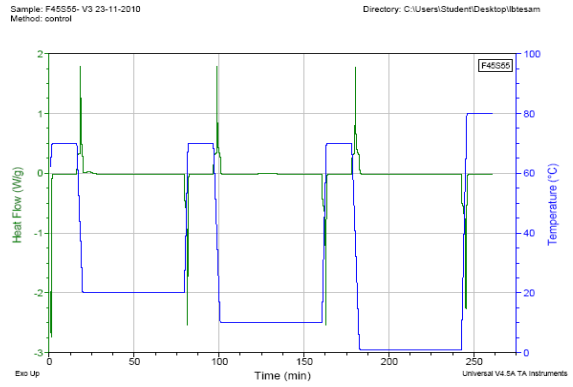
Results and Discussion

Thermal analysis of samples by DSC

Thermal properties of different samples (PH as control, F4S6, and F45S55) were examined using differential scanning calorimetry (DSC). A peak is created by variations in the sample that are linked to the development or absorption of heat and result in a change in the distinct heat flow [13]. As shown in **Figures 1a-1c** and **Table 1**, the thermal curves were established using DSC as the control for partly hydrogenated soybean oil (PH) and fat blends of fully hydrogenated soybean oil (FH) and soybean oil (SO) (F45S55, F4S6). The thermal behaviour of oil samples has been characterised by the two main physical phenomena, melting and crystallisation, which both require the intake or release of thermal enthalpy. As shown in **Figures 2a-2c** and **Table 1**, thermal curves were calculated using DSC for samples of F45S55, F4S6, and soybean oil (SO). When a sample is heated or cooled below its phase transition temperature, it stays isothermal for a brief period of time before being heated or cooled again to its starting temperature. The results observed that melting occurs at approximately 27, 39, and 48 for soybean oil as control samples, F4S6 and F45S55, respectively. The addition of fully hydrogenated soybean increases the melting point of oil due to the fact that crystallization was influenced only by the chemical composition of the sample. Also, exothermic peaks are observed in all samples, indicating recrystallization.



a)



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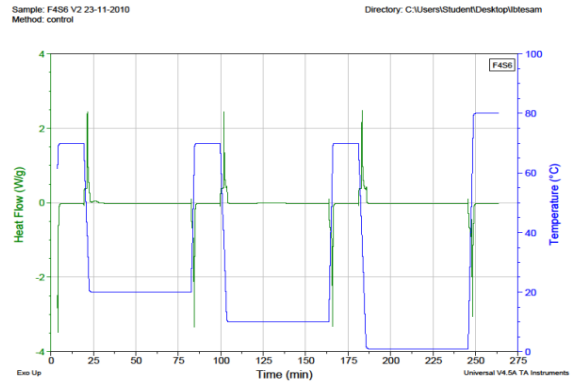
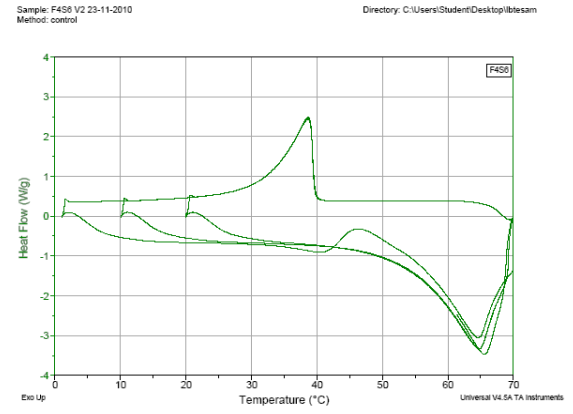


c)

Figure 1. Represented the three temperature cycles conducted on DSC a) for control, b) for F45S55 and c) for F4S6



a)

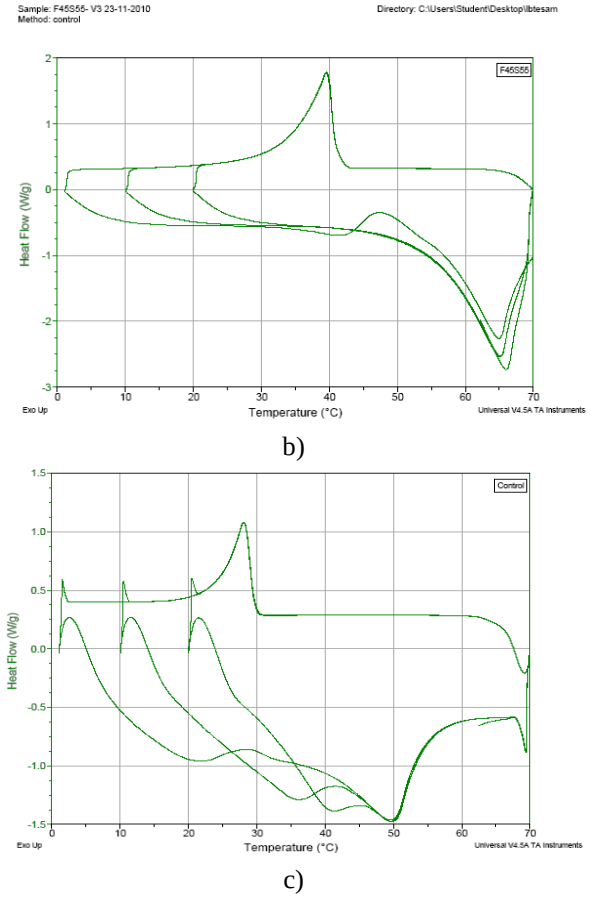


Figure 2. Differential scanning Calorimetric crystallization curves: a) for F4S6, b) for F45S55 and c) for control

The following five DSC parameters were determined for the primary thermal curve in all fat and oil samples: onset temperature (To), offset temperature (Tf), temperature range (Tr) (the difference between to and Tf), peak temperature, and melting enthalpy (ΔHm) and melting temperature (Tm): This is the first peak temperature; the peak is reached at this point when the measured data's curve starts to diverge from the baseline. The temperature at its highest point is TM. This temperature is the greatest value of the deviation between the interpolated baseline and the observed value curve. The temperature at which the measured value curve returns to the baseline is the final peak, or Tf. At this point, the peak is finished.

A summary of the DSC parameters is provided in **Table 1**. According to the data, sample F4S6 had the greatest peak melting temperature (39 °C), and sample SO had the lowest peak melting temperature (27°C). The peak melting temperatures varied from 27°C to 39°C. Variations were observed in the melting enthalpies (ΔHm). According to the statistics, F45S55 had the lowest ΔHm (35.73 J/g), followed by F4S6 (45.75 J/g). The control group had a much higher ΔHm (113.9 J/g), which might be related to the medium chain's high content of saturated fatty acids.

At a certain temperature, the fraction of solid fat that remains is typically equal to the partial area beneath the melting peak, or endothermic event. According to [18-20], the amount of fat that crystallises in liquid oil is determined using the DSC method. The findings were in line with Veerle's [21] findings, according to which DSC trans-free samples exhibited a two-step exothermic crystallisation that was produced by DSC at 10°C and was thought to be an α-mediated and β' polymorphism.

Table 1. Thermal profile (To, Tp, Tf, Tr, ΔHm) obtained from DSC

Samples	To, °C	Tm, °C	Tf, °C	Tr, °C	ΔHm (J/g)
PH	20	27	32	5	113.9
F4S6	28	39	44	16	45.75
F45S55	25	48	46	21	35.73

To: Onset temperature; Tf- Offset temperature; Tm- Peak temperature; Tr- Range of temperature; ΔHm- Melting enthalpy

Analyses of fatty acids by using Gas Liquid Chromatography

In accordance with the IUPAC technique [14], fatty acid methyl esters were made from total lipids using a fast approach. Using gas-liquid chromatography, fatty acid methyl esters of fat were measured. Through comparison with the established standard library (IUPAC), individual methyl esters were found. The constituents of the samples were examined. **Table 2** displays the main constituents of the samples. Gas-liquid chromatography was used to quantify the fatty acid methyl esters of fully hydrogenated soybean oil (FH), as well as the fatty blend of completely hydrogenated soybean oil and soybean oil with varying ratios (40:60 and 45:55) (F4S6 and F45S55) in comparison with partly hydrogenated soybean oil (PH). A trans-fat is produced when the unsaturated fat undergoes partial hydrogenation, which changes some of the cis-double bonds into trans-double bonds through an isomerization process with the hydrogenation catalyst [22, 23].

- The data illustrated that F4S6 and F45S55 had no trans fatty acid of C18:1T compared with the control (PH), which had around 16.976. However, F4S6 and F45S55 had very small amounts of trans fatty acid of C18:2T compared with control (PH), which recorded 0.0896, 0.096, 0.013 and 0.499 %w, respectively [24].
- The GC data represented the highest value of C18:I found in FH, followed by PH, F45S55, and then F4S6 85.073, 23.637, 14.128, and 13.352 %w, respectively. The results agree with [25, 26], who stated that shortening trans-fat free produced from fully hydrogenated soybean oil.
- C18:0 was recorded to be 40.840, 37.159, 28.818, and 0 %w, respectively, for F45S55, F4S6, PH, and FH, respectively. The GC profile shows that PH had a high amount of C16:0 followed by FH, F4S6, and F45S55, which were 16.298, 12.332, 11.692, and 11.348 %w, respectively. Meanwhile, the C18:2 value was 29.113, 31.208, 0.207, and 6.782%w, respectively, for F45S55, F4S6, FH, and PH, respectively [27-30].

Table 2. GC profile of all samples (fatty acid %w)

	Samples			
Fatty acids	**F45S55%**	**F4S6%**	**FH%**	**PH%**
C14:0	0.072	0.000	0.087	0.287
C16:0	11.348	11.692	12.332	16.298
C16:1	0.053	0.061	0.007	0.052
C17:0	0.206	0.202	0.332	0.135
C17:1	0.038	0.039	0.018	0.062
C18:0	40.841	37.159	0.000	28.818
C18:1T	0.000	0.000	0.074	16.976
C18:1	13.352	14.128	85.073	23.637
C18:2T	0.096	0.090	0.012	0.499
C18:2	29.114	31.208	0.207	6.782
C18:3n6	0.000	0.173	0.000	0.205
C18:3n3	3.911	4.257	0.156	0.449
C20:0	0.475	0.445	0.625	0.457
C20:1	0.132	0.103	0.000	0.092
C22:0	0.363	0.349	0.383	0.338
C22:1	0.000	0.000	0.005	0.000
C24:0	0.000	0.958	0.111	0.106

Fatty Acids composition of all samples under this study (%w)

Organoleptic properties

Data presented in **Table 3** shows the sensory evaluation of the blended shortening F4S6 and F45S55 compared with the control. The data revealed that F4S6 gained the highest panelist score in taste, followed by F45S55, which ended with the control. This may be the return that the more soybean oil is added to the blended mixture of soybean oil and fully hydrogenated soybean oil, the more lipid taste should appear. However, there is no significant difference between all treatments and control in color. It was also clear from the same Table that texture was quite high for F4S6 and the same for F45S55 and control. In general, the blended fatty from soybean oil and fully hydrogenated soybean oil (F4S6 and F45S55) improved sample sensory properties compared with the control.

Table 3. Panelist scores of different ratios of blended shortening (F4S6 and F45S55) compared with the control (PH)

Tests	**Blended fatty**			
	F4S6	**F45S55**	**Control**	**LSD**
Taste(10)	8.28±0.18[a]	7.85±0.26ab	7.28±0.42b	0.84
Color(10)	8.71±0.28a	8.85±0.26a	8.28±0.28a	0.76
Odor(10)	8.00±0.37a	8.28±0.47a	6.85±0.34b	1.11
Texture(10)	8.71±0.28a	8.57±0.20a	8.57±00.3a	0.81
Overall acceptability	8.42±0.23a	8.39±0.23a	7.75±0.14b	0.57

Means ± standard division of triplicate trials

Within a column means having the same superscript letters are not significantly different at 1%level

Conclusion

Regarding the thermal properties of different samples (SO, F4S6, F45S55), the results observed that melting occurs at approximately 27, 39, and 48 for soybean oil as a control sample, F4S6, and F45S55, respectively. Therefore, the addition of fully hydrogenated soybeans increases the melting point of oil. For the melting enthalpy ΔHm, the lowest was for PH, and the highest ΔHm was for F4S6. The GC data represented the highest value of C18: I found in FH followed by PH, F45S55, and then F4S6 85.07, 23.64, 14.13 and 13.35 %w, respectively. The data illustrated that F4S6 and F45S55 had no trans fatty acid (TFA) of oleic acid (C18:1T) compared with the control (PH), which had around 16.976. However, F4S6 and F45S55 had very small amounts of trans fatty acid of linoleic acid (C18:2T) compared with control (PH), which recorded 0.09, 0.09, 0.01 and 0.49 %w, respectively. In general, the blended fatty from soybean oil and fully hydrogenated soybean oil (F4S6 and F45S55) improved sample sensory properties compared with the control.

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Conflict of interest: None

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Ethics statement: None

References

1. Pande G, Akoh CC, Shewfelt RL. Production of trans-free margarine with stearidonic acid soybean and high-stearate soybean oils-based structured lipid. J Food Sci. 2012;77(11):C1203-10.
2. O'Brien RD. Fats and oils: formulating and processing for applications. Second edition: 2000 N. W. Corporate Blvd., Boca Raton, FL 33431, USA; CRC Press LLC, 2004.
3. Ribeiro AP, Basso RC, Grimaldi R, Gioielli LA, Goncalves LA. Effect of chemical interesterification on physicochemical properties and industrial applications of canola oil and fully hydrogenated cottonseed oil blends. J Food Lipids. 2009;16(3):362-81.
4. Acevedo NC, Marangoni AG. Characterization of the nanoscale in triacylglycerol crystal networks. Cryst Growth Des. 2010;10(8):3327-33.
5. Kummerow FA. The Effects of Hydrogenation on Soybean Oil. InSoybean-Bio-Active Compounds. IntechOpen. 2013;353:373. doi:10.5772/52610

6. Food and Drug Administration, HHS. Food labeling: trans fatty acids in nutrition labeling, nutrient content claims, and health claims. Final rule. Fed Reg. 2003;68(133):41433-506.
7. Lemaitre RN, King IB, Mozaffarian D, Sootodehnia N, Siscovick DS. Trans-fatty acids and sudden cardiac death. Atheroscler Suppl. 2006;7(2):13-5.
8. Lee J, Claro da Silva R, Gibon V, Martini S. Sonocrystallization of interesterified soybean oil: Effect of saturation level and supercooling. J Food Sci. 2018;83(4):902-10.
9. Roche HM. Fatty acids and the metabolic syndrome. Proc Nutr Soc. 2005;64(1):23-9.
10. Acevedo NC, Marangoni AG. Engineering the functionality of blends of fully hydrogenated and non-hydrogenated soybean oil by addition of emulsifiers. Food Biophys. 2014;9(4):368-79.
11. Mazzanti G, Marangoni AG, Idziak SH. Modeling phase transitions during the crystallization of a multicomponent fat under shear. Phys Rev E. 2005;71(4):041607.
12. Foon CS, Liang YC, Dian NL, May CY, Hock CC, Ngan MA. Crystallisation and melting behavior of methyl esters of palm oil. Am J Appl Sci. 2006;3(5):1859-63.
13. Tan CP, Che Man YB. Differential scanning calorimetric analysis of edible oils: comparison of thermal properties and chemical composition. J Am Oil Chem Soc. 2000;77(2):143-55.
14. IUPAC. Stander Methods for the Analysis of Oils, Fats, and Derivatives, 7th ed. International Union of Pure and Applied Chemistry, Oxford, Great Britain. 2000.
15. CoStat. CoHort Software, version 6.311. 798 Lighthouse Ave. PMB 320 Monterey, CA, 93940, USA. 2005.
16. Steel RG, Torrie JH. Principles and procedures of statistics. 2nd ed. (pp-120) MC Graw- Hill, New York, USA. 1980.
17. Gomez KN, Gomez AA. Statistical Procedures for Agricultural Research. John Wiley and Sons, New York, 2nd ed. 1984.
18. dos Santos MT, Viana IS, Ract JN, Le Roux GA. Thermal properties of palm stearin, canola oil and fully hydrogenated soybean oil blends: coupling experiments and modeling. J Food Eng. 2016;185:17-25.
19. Devi A, Khatkar BS. Thermo-physical properties of fats and oils. Int J Eng Tech Res. 2017;7(2):2454-69.
20. Szydłowska-Czerniak A, Karlovits G, Lach M, Szłyk E. X-ray diffraction and differential scanning calorimetry studies of β′→ β transitions in fat mixtures. Food Chem. 2005;92(1):133-41.
21. De Graef V, Foubert I, Smith KW, Cain FW, Dewettinck K. Crystallization behavior and texture of trans-containing and trans-free palm oil based confectionery fats. J Agric Food Chem. 2007;55(25):10258-65.
22. Ahmadi L, Marangoni AG. Functionality and physical properties of interesterified high oleic shortening structured with stearic acid. Food Chem. 2009;117(4):668-73.
23. Ye X, Li P, Lo YM, Fu H, Cao Y. Development of novel shortenings structured by ethylcellulose oleogels. J Food Sci. 2019;84(6):1456-64.
24. Zulkurnain M, Balasubramaniam VM, Maleky F. Effects of lipid solid mass fraction and non-lipid solids on crystallization behaviors of model fats under high pressure. Molecules. 2019;24(15):2853.
25. List GR, Byrdwell WC, Steidley KR, Adlof RO, Neff WE. Triacylglycerol structure and composition of hydrogenated soybean oil margarine and shortening basestocks. J Agric Food Chem. 2005;53(12):4692-5.
26. Bordi Jr PL, Snyder KS, Hessert Jr SW. Comparing the Sensory Characteristics of Doughnuts Made with Trans-Fat-Free Canola Shortening, Trans-Fat-Free Palm Shortening, and Trans-Fat Vegetable/Soybean Shortening. J Culin Sci Technol. 2010;8(1):57-72.
27. Andrikopoulos NK. Triglyceride species compositions of common edible vegetable oils and methods used for their identification and quantification. Food Rev Int. 2002;18(1):71-102.
28. Alonso L, Fraga MJ, Juárez M, Carmona P. Fatty acid composition of Spanish shortenings with special emphasis on trans unsaturation content as determined by Fourier transform infrared spectroscopy and gas chromatography. J Am Oil Chem Soc. 2002;79(1):1-6.
29. Allison DB, Egan SK, Barraj LM, Caughman C, Infante M, T Heimbach JA. Estimated intakes of trans fatty and other fatty acids in the US population. J Am Diet Assoc. 1999;99(2):166-74.
30. Gao J, Xiao H, Li J, Guo X, Cai W, Li D. N-3 polyunsaturated fatty acids decrease long-term diabetic risk of offspring of gestational diabetes rats by postponing shortening of hepatic telomeres and modulating liver metabolism. Nutrients. 2019;11(7):1699.