Crystal Morphology of Binary and Ternary Mixtures of Hydrogenated Fats and Soybean Oil

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ABSTRACT

The objective of this study was to verify the influence of temperature on crystallization of binary and ternary mixtures of two hydrogenated fats and soybean oil, by polarized light microscopy at temperatures of 30°C, 35°C, and 40°C. The types of crystals observed were spherulites type A, and B and the polymorphic forms were b, and b-prime. The soybean oil does not contribute statistically to total area or maximum diameter of the crystals. At 35°C the positive relative coefficients to the interactions presented, in general, absolute values higher than the negative ones, pointing that the crystals were larger than what could be expected, if there was no interaction among the components. At 40°C the negative relative coefficients revealed, in general, absolute values higher than the positive ones, indicating that the samples were close to the melting point, showing the presence of some small crystals.

Key words: Crystal morphology, Hydrogenated fat, Mixture, X-ray diffraction

INTRODUCTION

Triglycerides commodities undergo solid-to-liquid phase transitions between ambient and body temperatures. This reversible feature is highly desirable for many fats as functional food ingredients, providing such properties as structure, mouthfeel, flavor delivery, and barriers to moisture migration. Predicting the phase transitions of natural fats in complex mixtures depends on a precise understanding of the triglycerides molecules, the processes, and events of crystal formation, and the heterogeneity of crystal forms made possible by the rather large number of conformations possible in such large molecules (German and Simoneau, 1998).

From three general states of matter - solid, liquid, and gas - solids, in which molecular motion is restricted to oscillation about a fixed location, exhibit two states: amorphous, and crystalline. The crystalline state differs from the amorphous state as to regularity of the pattern of molecular arrangements.

A crystal results from the molecules arranged in a fixed pattern known as a lattice. The properties of crystals as solids arise from the strength of interactions between molecules in the lattice, and the relative uniformity of the interactions between the three faces defined by a solid. Their high degree of molecular complexity allows the same triglycerides to pack into not only but several relatively stable and different lattice structures. These different chain packing or polymorphic forms have quite discrete energies, lattice arrangements, and crystal habits. The property of reversible crystallization, and the development of plastic fats provide much of the desirable functionality of fats, such as shortening of doughs, texture in cakes, and confections, mouthfeel in frozen desserts, melting of chocolate, spreadability of margarine, butter, and spreads, snap of chocolate, and graininess, smoothness, mouthfeel, water binding, and emulsion stability of spreads (German and Simoneau, 1998; Marangoni and Hartel, 1998).

The macroscopic properties of a fat are influenced by a hierarchy of factors. The solid-like behavior in particular is influenced by the amount of solid fat present, the tipe of crystals, and the interaction amoung crystals leading to the formation of a fat

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crystal network. The importance of this hierarchy of factors should not be overlooked. Lipid composition, and crystallization conditions will influence crystal habit upon cristallization - different polymorphic forms, and crystal morphologies are possible. Crystals aggregate into larger structures which eventually form a continuous 3-dimensional network, largely responsible for the solid-like properties of fats (Marangoni and Hartel, 1998).

In order to obtain crystallization, it is necessary to increase the concentration of the solute to be crystallized above the saturated solution concentration at a given temperature. A crystal nucleous is the smallest crystal present in a solution submitted to a certain concentration and temperature. Once crystals have formed due to primary nucleation, secondary nucleation can occur. Secondary nuclei form whenever small pieces of crystal are removed from the growing crystal surface. If the pieces are smaller than the critical size, they redissolve; if larger, they act as nuclei, and grow to become crystals. Once a crystal nucleus has formed, it will start growing by the incorporation of other molecules. These molecules are taken from the adjacent liquid layer which is replenished continuously from the surrounding, supersaturated liquid. In the industrial crystallization of fats, stirring is the primary cause of secondary nuclei. Seeding is a form of secondary nucleation which is sometimes practiced in some chocolate industries (Timms, 1995).

In the industrial fractionation of fats, the crystallization must be given on slow cooling, and gentle stirring to provide large crystals, and stable polymorphic forms, which can be observed easily without any microscopy stand-by, and enable a simple filtration to effect the separation (Hoffmann, 1989; Timms, 1995). Vaisey-Genser *et al*. (1989) determined the threshold for crystal size of brick margarines consisted of canola oil which was detectable to the sensory panel. A physical stimulus which generates 50% of detection was considered as the threshold for a sensory perception. In that case, the threshold for crystal size detection as 22μm.

X-ray diffraction, differential scanning calorimetry, and microscopy are used for the study of the crystalline structure. The microscopy has been applied to explain the differences of texture in fats mixtures; to show the crystal types, and the morphologic alterations in the crystal growth; and to verify the transformations of the polymorphic forms (Berger *et al*., 1979; Meara, 1980).

The objective of this paper was to study the influence of temperature on crystallization of binary and ternary mixtures of hydrogenated fats, and soybean oil, by polarized light microscopy.

MATERIALS AND METHODS

Two commercial hydrogenated fats (Fatgill PF38, and Fatgill PF42 - Cargill Agrícola S.A., São Paulo, Brazil), and soybean oil (Liza - Cargill Agrícola S.A., São Paulo, Brazil) were used in order to prepare mixtures in proportions presented in Table 1.

Table 1 - Experimental design for mixtures of hydrogenated fats and soybean oil

Sample (No.)	Components (proportion w/w)		
	Soybean oil	Fatgill PF38	Fatgill PF42
1	1	0	0
2	0	1	0
3	0	0	1
4	1/2	1/2	0
5	1/2	0	1/2
6	0	1/2	1/2
7	1/3	1/3	1/3
8	2/3	1/6	1/6
9	1/6	2/3	1/6
10	1/6	1/6	2/3

X-ray diffraction: The data of X-ray diffraction were collected on a diffractometer with Cu radiation (Siemens, model DK 5000).

Polarized light microscopy: The samples were heated at 60 to 70°C in a microwave oven (Panasonic , São Paulo, Brazil) for complete melting of the crystals. A drop of molten hydrogenated fat was placed on a slide, and covered with a cover-glass, inclined at an angle of 45°. The slides were prepared at 50°C, and kept at 30, 35, and 40°C, for 24 h. Crystal morphology was studied under isothermal conditions with a polarized microscope (Olympus System Microscope, model BX 50 - Olympus America Inc.) with camera (Sony Color Video Camera

CCD-IRIS, model DXC-107A - Sony Co., Japan). The objective magnification was 4x (35 and 40°C), and 10x (30°C), and the ocular magnification was 10x. Temperature control was achieved with a platen specially built that was equipped with a heating/cooling system (Thermal Microscope Stage, TS-4 - Physitemp Instruments Inc.). The images show a typical field for each sample, preferentially at the center of the slide. The images were digitalized, and analyzed by the software Image Pro-Plus Version 1.3.2 for Windows (Media Cybernetics). The maximum diameter (μm), and the total area (μm^2) of the crystals were determined.

A multiple regression model was applied to some analytical responses - special cubic type - (Hare, 1974), represented by the following equation:

$$y = \beta 1x1 + \beta 2x2 + \beta 3x3 + \beta 12x1x2 + \beta 13x1x3 + \beta 23x2x3 + \beta 123x1x2x3$$

where:

y = variable

β = coefficients generated by multiple regression (Table 2)

x = proportion of the components (Table 1)

Statgraphics Statistical Graphics System (Maryland, USA) was used to generate coefficients for the model, besides presenting their significant levels, determination coefficients, and variance analysis. Contour curves are presented in triangular diagrams by using the Mixplot program (Barros Neto *et al*., 1996). These lines were obtained from the regression model (Braga Neto *et al*., 1995, Silva *et al*., 1993).

RESULTS AND DISCUSSION

Microscopic imaging techniques are the most appropriated approach for evaluating food structure since they provide results in the form of both images, and numerical data. Success in the measurements requires several stages of accomplishment - obtaining a truly representative image of material, analyzing that image properly, and, finally, interpreting the resulting data (Stanley *et al*., 1998). In their study, German and Simoneau (1998) described that polarized light microscopy allows the visualisation of crystals within a range of 0.5 to 100 μm. Nevertheless, in such a study the crystal size was observed up to 646μm. The crystallization on the slide occurs from the border to the center. The number of crystals increases and the crystal size falls on the border. On the center of the slide the number of crystals falls and the crystal size increases. Johansson (1995) has studied the influence of temperature on some interactions, and structures in semisolid fats by the polarized light microscopy verifying the crystal growth at room temperature. The results showed that fat crystals can be used as thickeners or gelling agents of triglyceride oils in various applications. It was possible to direct the thickening, by choice of phase composition, additives, tempering conditions and crystallization techniques.

Figures 1, 2, and 3 show the digitalized images for each sample, at the crystallization temperatures 30, 35, and 40°C, respectively. The types of crystals observed were: central core or nucleus of tightly-packed, rather broad, needles surrounded by radially-orientated, long, narrow, needle-like crystals (spherulites type A); and central core surrounded by small, closely-bound, randomly-orientated crystals (spherulites type B) (Meara, 1980; Berger *et al*., 1979).

The technique of polarized light microscopy can under certain circumstances differentiate the polymorphic forms of fats (α, β, and β-prime) (German and Simoneau, 1998). From Figures 1, 2, and 3, we can observe needle shaped crystals (β) and axial crossed crystals (β-prime) (Kleinert, 1970). From X-ray diffraction patterns, sample 2 showed short spacings at 3.9 and 4.2 A; and sample 3 showed short spacings at 3.9, 4.0 and 4.2 A. The short spacings at 3.9 A are characteristic for β form fats. On other hand, the short spacings at 4.0 and 4.2 A are characteristic for β-prime fats (Gunstone and Norris, 1983). These results indicated that the samples of hydrogenated fats showed both β and β-prime crystals. This was also observed from polarized light microscopy of the crystals. According to Wiedermann (1978) and Chrysam (1985), fats, and oils had been separated into β, and β-prime categories, according to crystal habit. Soybean oil was included into β type categorie.

Sample 2 Sample 3 Sample 4

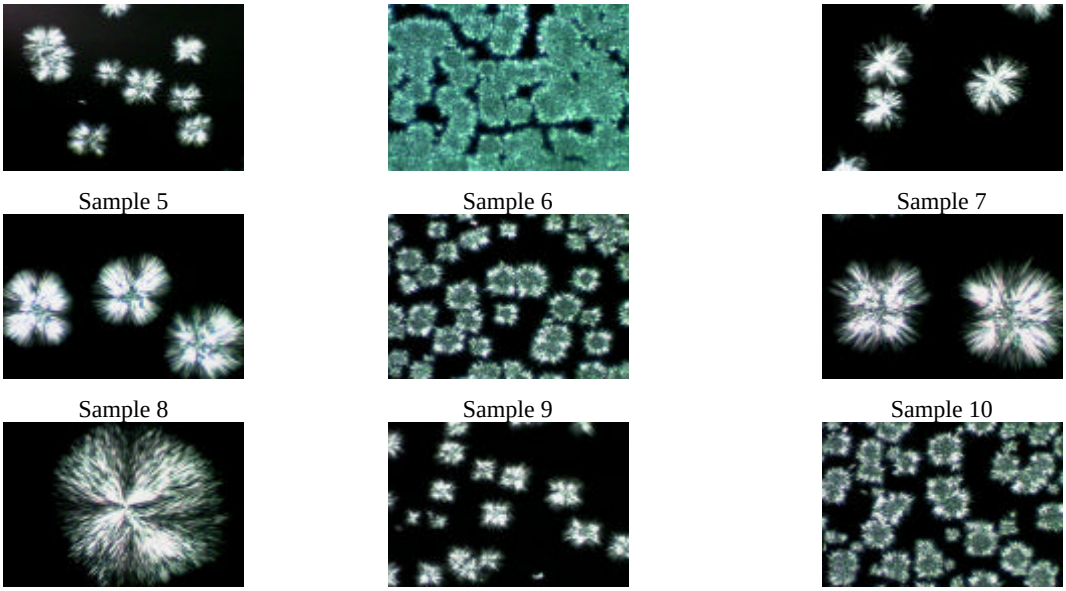


Figure 1 - Digitalized images of the crystals for the samples at a crystallisation temperature of 30°C.

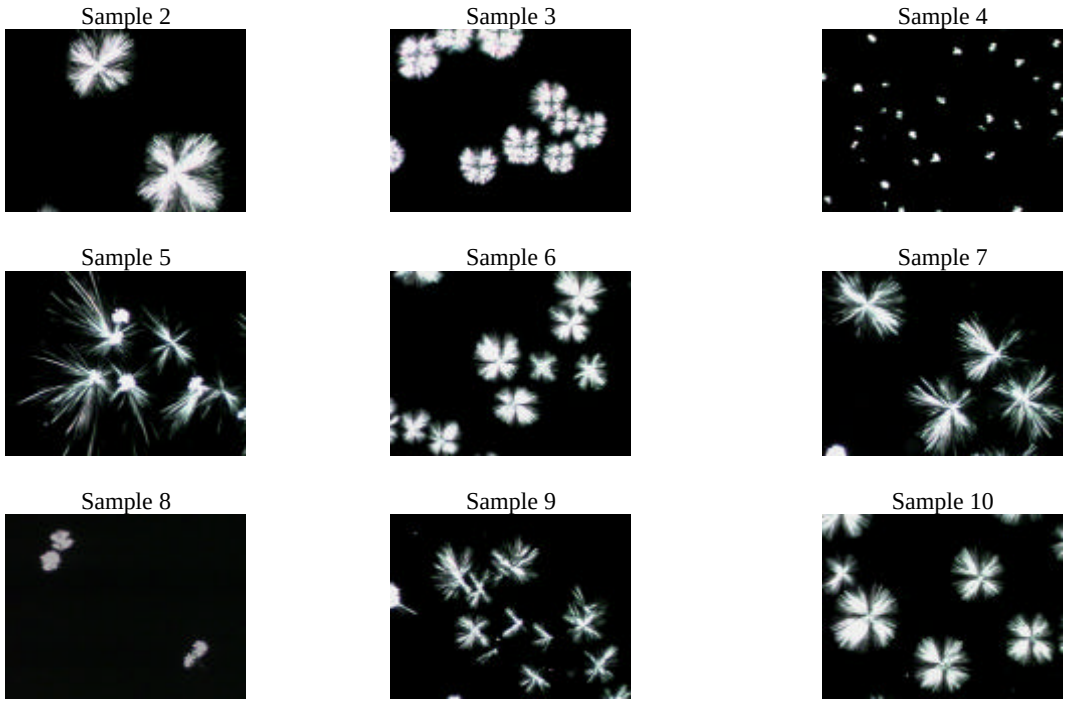


Figure 2 - Digitalized images of the crystals for the samples at a crystallisation temperature of 35°C.

Sample 2 Sample 3 Sample 5

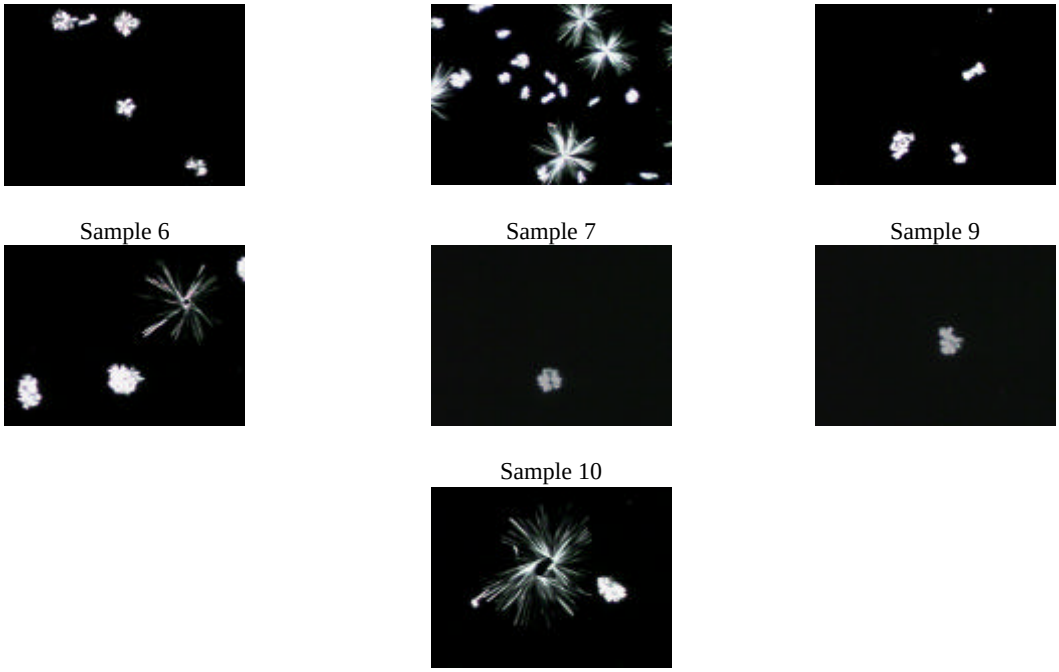


Figure 3 - Digitalized images of the crystals for the samples at a crystallisation temperature of 40°C.

Since nucleation rate increases at a roughly exponential rate with an increasing supersaturation whereas the growth rate is only proportional to supersaturation, the number of crystals increases and the crystal size falls when crystallization occurs at a lower temperature. Rapid cooling to a low temperature followed by intense stirring leads to the microscopically small crystals found in margarine. Conversely, slow cooling, and gentle stirring leads to large crystals which can be easily observed without any microscopy stand-by. With a gentle stirring, crystals can form agglomerates or clusters of spherulites with particle sizes of many hundred micrometers (Timms, 1995). Thus, an intermediary temperature of crystallization contributes to a formation process of larger crystals but in a smaller quantity. This justifies the values of the analyzed parameters, whose dimensions of the crystals, in general, increased from 30°C to 35°C and decreased from 35°C to 40°C, due to the proximity of the melting melt.

Table 2 - Coefficients calculated by multiple regression from experimental data.

Result	**Coefficients**							
	β1	**β2**	**β3**	**β12**	**β13**	**β23**	**β123**	**R^2**
35°C								
Total area	0.00	34,598	249,074	54,838	414,288	561,487	52,555	0.9801
Maximum diameter	0.00	537.17	261.96	-996.14	1,077.72	-583.75	4,722.37	0.9786
40°C								
Total area	3,490	47,789	152,375	-74,931	-214,672	-218,353	-253,705	0.9522
Maximum diameter	0.00	166.89	143.79	-319.67	323.88	346.37	443.41	0.9716

Table 2 presents the calculated coefficients from experimental data results of maximum diameter and total area ($p<0.10$). It can be observed that sample 1, which was liquid at the analysed temperatures, did not contribute statistically to any parameter analyzed at all temperatures. At 30°C the relative coefficients have not been significant. The relative coefficients to the samples 2, and 3 (β_2, and β_3) represented close values to such respective experimental determinations. When the

relative coefficients of the significant interactions were negative, they represent an effect on decreasing the analyzed parameter. On the other hand, as the relative coefficients of the significant interactions were positive, they indicated that the effect occurred in the sense of increasing the analyzed parameter.

Positive relative coefficients obtained at 35°C for the interactions presented, in general, absolute values higher than the negative ones. This meant that the crystals were larger than what could be expected, if there was no interaction among components. This confirmed the eutectic effect observed by Simões *et al*. (1997), and Gioielli and Oliveira (1998). The antagonistic effect for the properties of hardness were characteristic of eutectic interactions among triacylglycerols or between fats.On the other hand, at 40°C the negative relative coefficients for the interactions presented, in general, absolute values higher than the positive ones, indicating that the such samples have been close to the melting point, showing the presence of small crystals.

Figures 4, and 5 represent the triangular diagrams for the maximum diameter of the crystals at temperatures of 35, and 40°C, respectively. The maximum diameter at 35°C (Figure 4) showed the maximum values for compositions which has high proportion of component 2. The maximum diameter at 40°C (Figure 5) showed the maximum values for compositions which has equivalent amounts of components 2 and 3. Thus, the microscopic observation with image analysis included a key factor for the quantification and visualisation of the microstrucure of the fat crystal network.

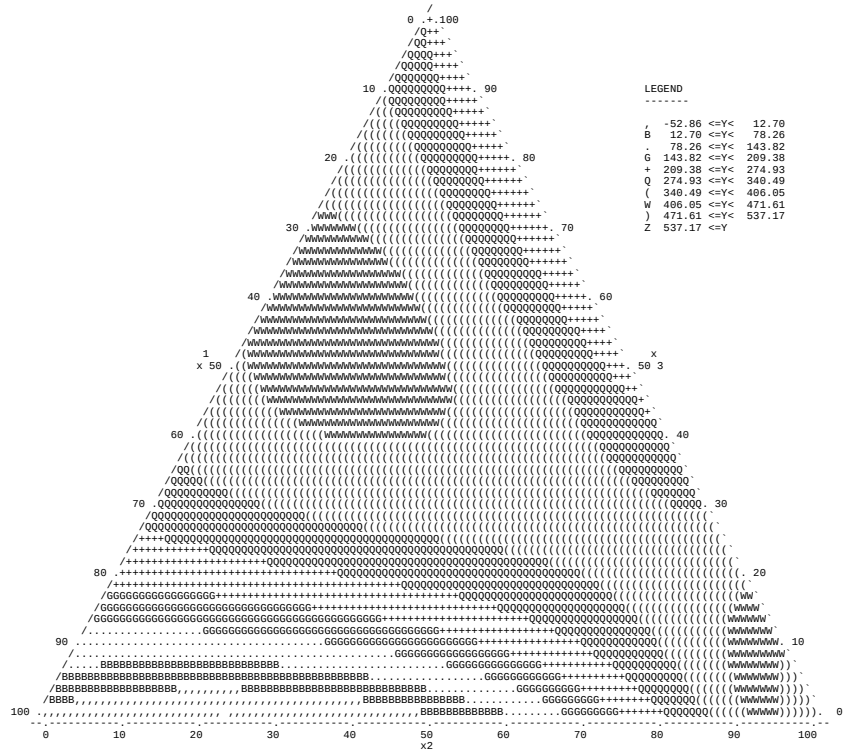
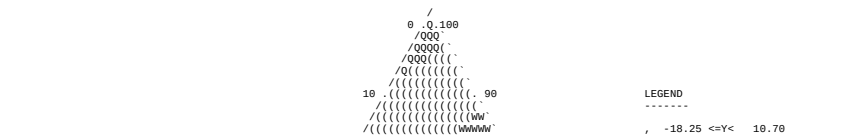


Figure 4 - Triangular diagram for maximum diameter (μm) of the crystals at 35°C.



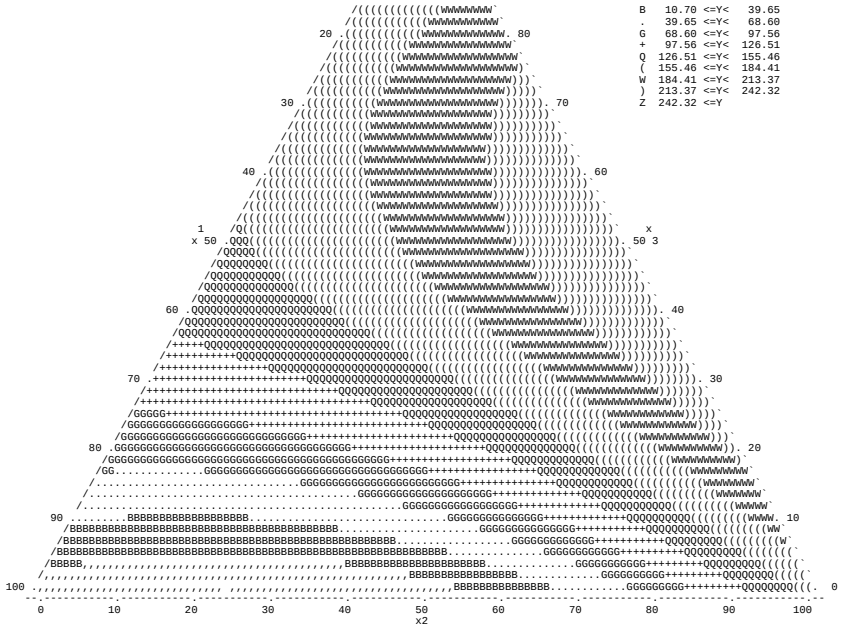


Figure 5 - Triangular diagram for maximum diameter (μm) of the crystals at 40°C.

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RESUMO

Os triacilgliceróis sofrem transições de fase sólido-líquido entre as temperaturas ambiente e corporal, e estas são altamente desejáveis para gorduras que atuam como ingredientes funcionais de alimentos. O objetivo foi estudar a influência da temperatura na cristalização de misturas binárias e ternárias de duas gorduras hidrogenadas e óleo de soja, por microscopia sob luz polarizada, às temperaturas de 30°C, 35°C e 40°C. Os tipos de cristais observados foram esferulitos A e B e as formas polimórficas eram β e β-prima. O óleo de soja não contribuiu estatisticamente para a área total ou para o diâmetro máximo dos cristais. A 35°C os coeficientes positivos relativos às interações entre os componentes apresentaram, geralmente, valores absolutos superiores aos negativos, indicando que os cristais eram maiores que o esperado se não houvesse interação. A 40°C os coeficientes negativos relativos às interações apresentaram, geralmente, valores absolutos superiores aos positivos, indicando que as amostras estavam próximas ao ponto de fusão e os cristais formados eram menores.

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