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THERMAL ANALYSIS OF ISOTHERMAL CRYSTALLIZATION KINETICS OF MILK FAT ENRICHED WITH GAMMA-LINOLENIC ACID

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ABSTRACT

Kinetics of isothermal crystallization of butter oil (BO) and interesterified butter oil (IEBO) with gamma-linolenic acid (GLA; $C_{18:3}$ ω-6) at 10 °C were evaluated by applying Avrami equation. Differential Scanning Calorimeter (DSC) analysis of Avrami parameters revealed significant differences ($P < 0.05$) between BO and IEBO samples obtained at 1, 8, and 24 h of interesterification. Results of crystallization rate constant (k), Avrami exponent (n), and half-time of crystallization ($t_{1/2}$) indicated different crystallization mechanisms for BO and IEBO samples. BO illustrated the lowest ($P < 0.05$) induction time of nucleation with a value of 8.93 min corresponding to its high peak melting temperature (T_p) of 30.54 °C which was converse to IEBO samples. The results were ascribed to alteration ($P < 0.05$) of fatty acids (FAs) composition of IEBO occurred by lipase-catalyzed acidolysis of BO and GLA.

Keywords: Avrami equation, milk fat, crystallization kinetics, gamma-linolenic acid, interesterification.

INTRODUCTION

Milk fat is one of the most complex lipid systems. Triacylglycerols comprise by far the greatest proportion of milk fat, making up 97-98% of the total composition. Over 400 different FAs have been identified in milk fat; however, only 20 individual FAs are account for (Jensen and Newburg, 1995). Based on all of the FAs identified, saturated fatty acids (SFAs) represent approximately 65% of the total composition, while unsaturated fatty acids represent the rest (Swaisgood, 1985).

Crystallization process consists of two steps: nucleation and crystal growth. However, before any crystallization can take place, supersaturation of the mother phase must be achieved as the thermodynamic driving force for the crystallization (Garside, 1987; Boistelle, 1988). Nucleation can be described as a process in which molecules come into contact, orient and interact to form highly ordered structures, called nuclei. Crystal growth is the enlargement of these nuclei. According to their environment, the crystals grow more or less regularly and exhibit different growth morphologies (Nawar, 1996). In fact, kinetics of fat crystallization, being dependent on the composition and on the processing conditions, is important for controlling operations in the food industry to produce the desired product melting characteristics (Metin and Hartel, 1998).

The most general approach for description of isothermal phase transformation kinetics is Avrami equation (Avrami, 1940). Isothermal Avrami kinetics is concerned with the overall crystallization process, including nucleation and growth. In other words, the equation describes an event in which there is an initial lag-period, where crystallization occurs very slowly,

and a subsequent rapid increase in crystal mass (Graydon *et al.*, 1994; Herrera *et al.*, 1999). Avrami equation is given as:

$$(1-X) = \exp(-k\,t^n) \quad [1]$$

where X is fraction of crystals transformed at time t during crystallization, k depends primarily on crystallization temperature and generally follows an Arrhenius-type temperature dependency, and n is a constant relating to the dimensionality of the transformation Toro-Vazquez,*et al.*, 2001; Rousset, 2002).

More comprehensively, k is a combination of nucleation and growth rate constants, and is a strong function of temperature. The numerical value of k is directly related to $t_{1/2}$, and therefore the overall rate of crystallization, which is given by the following equation:

$$(t_{1/2})^n = 0.693/k \quad [2]$$

n sometimes referred to as an index of crystallization, indicates the crystal growth mechanism. This parameter is a combined function of the time dependence of nucleation and the number of dimensions in which growth takes place. Nucleation is either instantaneous, with nuclei appearing all at once early on in the process, or sporadic, with the number of nuclei increasing linearly with time. Growth occurs as rods, discs, or spherulites in one, two, or three dimensions, respectively (Woldt, 1992)

Enhancing milk fat nutritional value by incorporating polyunsaturated fatty acids like GLA with all its therapeutic benefits, changes milk fat chemical composition (Fatouh *et al.*, 2009). Consequently, it is expected that crystallization behavior of enriched milk fat with GLA would be different. The aim of this work is to study the isothermal crystallization kinetics of milk fat enzymatically interesterified with GLA by applying Avrami equation.

MATERIALS AND METHODS

Enzymatic interesterification of BO and GLA was performed as previously described by Fatouh et al. (2009). IEBO obtained after 1, 8, and 24 h of interesterification using *Candida antarctica* immobilized lipase was chosen for this study. Samples studied were designated as an abbreviation, IEBO, followed by a number (1, 8 and 24). The number corresponds to the interesterification time (h) required to obtain the sample.

Isothermal analysis was conducted following the method of Metin and Hartel (1998) using a DSC (Model 7, Perkin Elmer, Norwalk, CT, USA). The DSC was calibrated with Indium (m.p.156.60 °C, ΔH_f 28.45 J/g) and Gallium (m.p.29.78 °C, ΔH_f 80.09 J/g). The system was purged with N_2 at 20 mL/min during the analysis, and liquid nitrogen was used as a refrigerant to cool the system. A sample of 9-10 mg was hermetically sealed in a 30 µL capacity aluminum pan (Perkin Elmer, Norwalk, CT, USA), with an empty sealed pan used as a reference. Changes in the heat flow during isothermal DSC operation at crystallization temperature (10 °C) were recorded. The following

temperature protocol was used: hold at 80 °C for 5 min, cool to 50 °C at a rate of 100°C/min, hold for 3 min, cool at 100°C/min to the crystallization temperature and then hold for 3 h. DSC thermograms were also analyzed for T_p.

Isothermal DSC data were used to evaluate kinetic parameters such as n, k, and $t_{1/2}$ by employing Equation 1. Equation 1 can be linearized by logarithmic transformation of [–ln (1-X)] and t to calculate k and n from the intercept and slope, respectively.

Fraction of crystals (X) or the relative amount of material crystallized, as a function of time, was calculated by integration of the isothermal DSC crystallization curves. The area under the exothermal crystallization curve corresponds to the total enthalpy of crystallization, ΔH_T. At a given time, X was approximated by the ratio of the partial enthalpy, ΔH_X, at that time to the total crystallization enthalpy as shown in Equation 3.

$$X = \Delta H_X / \Delta H_T \quad [3]$$

Average X value was taken from three replicates. The $t_{1/2}$ was calculated by employment of the isothermal crystallization data to Equation 2.

Experiments were triplicated, and triplicate analyses were performed on each replicate. Statistical analysis was performed by the SAS General Linear Method procedure (SAS, 1994). Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Isothermal crystallization kinetics were characterized in terms of induction time of neucleation and the empirical Avrami equation at crystallization temperature of 10 °C. Plots of X vs. time are given in Fig 1. BO started to crystallize after 10 min and crystallization was completed after 35 min. IEBO-1, 8, and 24 crystallized slower ($P < 0.05$) than BO. Crystallization behavior of both BO and IEBO is readily explained based on their FAs composition. For BO, SFAs dominated its chemical composition. Mmyristic ($C_{14:0}$, melting point (MP), 54.4 °C), palmitic ($C_{16:0}$, MP, 62.9 °C) and stearic ($C_{18:0}$, MP, 69.6 °C) represented 56.1 % of the total SFAs content of BO (Table 1). These FAs, with their high MP, crystallize very rapidly at low temperatures like 10 °C. On the other hand, FAs profile of IEBO samples (Table 1) contains a great portion of GLA. GLA is a polyunsaturated fatty acid that has a very low MP of (-12) - (-14) °C (Clough, 2001). That in turn reflected on T_p of IEBO samples (Table 3), conducive to increasing the crystallization time as GLA content of IEBO samples increased going from IEBO-1 to IEBO-24. The shape of IEBO-1, 8, and 24 curves was a typical sigmoid that consists of an induction period of crystallization, followed by an increase in X value associated with the acceleration in the rate of volume or mass production of crystals, and finally a metastable crystallization plateau is

reached. However, noticeable differences in the shape of crystals fraction vs. time curves between BO and IEBO samples suggest a different crystallization mechanisms (Wright *et al.*, 2000; Toro-Vazquez,*et al.*, 2001).

Table 1. Fatty acids profile of butter oil before and after acidolysis with GLA concentrate using *C. antarctica* immobilized lipase.

Fatty acid	BO	IEBO		
		1*	8*	24*
C6:0	1.30±0.09a	1.22±0.03a	0.74±0.01b	0.13±0.0c
C8:0	1.17±0.12a	1.09±0.01a	0.66±0.02b	0.09±0.1c
C10:0	2.68±0.02a	1.87±0.15b	1.58±0.08c	1.11±0.01d
C12:0	3.41±0.02a	2.70±0.03b	2.32±0.04c	2.00±0.03d
C14:0	11.62±0.04a	9.59±0.08b	8.44±0.10bc	7.47±0.27c
C14:1	0.97±0.01a	0.79±0.01b	0.68±0.02c	0.59±0.02c
C15:0	1.16±0.02a	0.97±0.01b	0.84±0.01c	0.74±0.03d
C16:0	31.82±0.06a	26.49±0.20b	22.02±0.17c	21.12±0.72c
C16:1	1.63±0.01a	1.33±0.02a	1.20±0.03b	1.05±0.04b
C18:0	12.66±0.04a	11.51±0.06a	9.78±0.06b	8.43±0.20b
C18:1	27.40±0.06a	23.02±0.14b	19.81±0.44c	18.10±0.57c
C18:2	3.65±0.01d	6.76±0.09c	8.31±0.05b	10.06±0.49a
GLA	ND	12.18±0.38c	23.17±0.24b	28.69±1.25a
C18:3	0.54±0.02a	0.48±0.01b	0.45±0.01bc	0.42±0.01c
SFAs	65.82±0.11a	55.44±0.31b	46.38±0.28c	41.09±1.24d
USFAs	34.19±0.21d	44.56±0.48c	53.62±0.33b	58.91±1.12a

Mean ± SD, n=3.
Different letters within the same row are significantly different (P<0.05).
BO, butter oil; IEBO, interesterified butter oil; GLA, gamma linolenic acid ($C_{18:3}$ ω-6); ND, not detected; SFAs, saturated fatty acids; USFAs, unsaturated fatty acids.
*** Represents interesterification time used (h).**

n and *k* values were calculated from the slope and y-intercept of the DSC curves, respectively and the data are given in Table 2. Significant differences ($P < 0.05$) were found between BO and IEBO samples regarding *n* value. For BO, *n* was 2, whereas for IEBO-1, 8 and 24, *n* values were 3, 4, and 4, respectively. A crystallization process with a polyhedral crystal growth from sporadic nuclei has *n* value of 4. The *n* of 3 indicates a spherulitic growth from an instantaneous nuclei, and *n* of 2 indicates a linear crystal growth mechanism from sporadic nuclei (Doremus, 1985; Marangoni, 1998; Toro-Vazquez, *et al.*, 2001). The results obtained can be ascribed to differences ($P < 0.05$) found earlier in the FAs composition (Table 1).

k value (Table 2) as mentioned previously, is a function of nucleation and growth rates, thus it can be used to provide a quantitative description of crystallization. Significant differences ($P < 0.05$) were found in *k* values between BO and IEBO-1, 8, and 24. Moreover, results presented in Table 2 reveal an inverse proportionate between *n* and *k* values. A similar relation was observed by Campos *et al.* (2002) for milk fat. Milk fat crystallized under fast cooling yielded a higher Avrami constant (*k*), and a lower Avrami exponent (*n*) than milk fat crystallized under slow cooling. Similar results for *n* and *k* values for milk fat were reported by Wright *et al.* 2000 and Fouberta *et al.*2002.

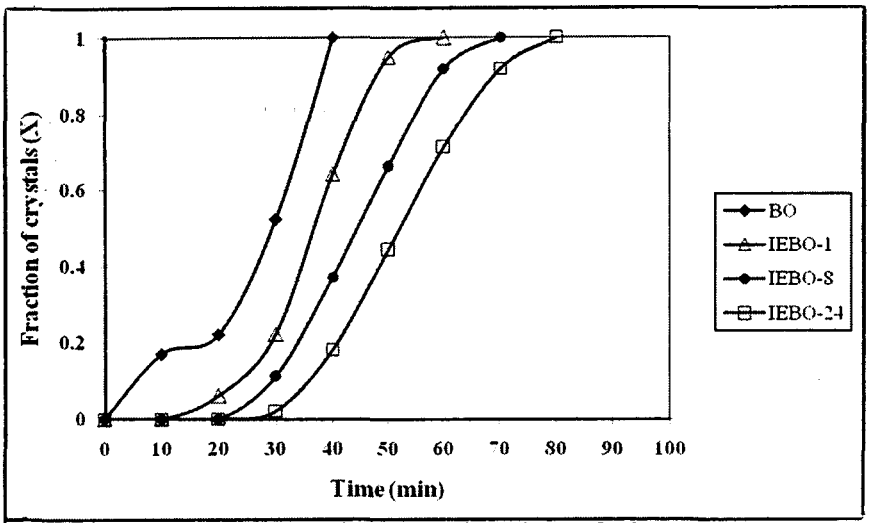

Fig 1. Fraction of crystals as a function of time for isothermal crystallization at 10 °C of butter oil (BO), interesterified butter oil with gamma-linolenic acid for 1 h (IEBO-1), interesterified butter oil with gamma-linolenic acid for 8 h (IEBO-8), and interesterified butter oil with gamma-linolenic acid for 24 h (IEBO-24).

Table 2. Avrami parameters of butter oil and interesterified butter oil with gamm-linolenic acid as crystallized isothermally at 10 °C.

Avrami parameter	BO	IEBO		
		1*	8*	24*
N	2.0±0.01c	3.0±0.02b	4.0±0.0a	4.0±0.01a
$K \times 10^{-3}$	2.26±0.03a	1.75±0.06b	1.08±0.01c	0.92±0.04d
$t_{1/2}$ (min)	17.52±0.22d	25.14±0.30c	33.12±0.74b	38.40±0.59a

Mean ± SD, *n*=3.
Different letters within the same row are significantly different (*P*<0.05).
BO, butter oil; IEBO, interesterified butter oil; *n*, Avrami exponent; *k*, crystallization rate constant; $t_{1/2}$, half time of crystallization.
*** Represents interesterification time used (h).**

$t_{1/2}$ is a combined function of *k* and *n*. Since $t_{1/2}$ is derived from the Avrami equation, it shows the time when crystal fraction reaches 50% (Sharples, 1966). $t_{1/2}$ values of BO and IEBO-1, 8 and 24 as a function of time, for isothermal crystallization at 10 °C are presented in Table 2. The results illustrate that, 50 % fraction of crystals (i.e., half of the crystallization) generally occurred halfway through crystallization, as determined from

Equation 2. This suggests that the crystallization peak occurred when about 50% of molten fat transformed into crystals under the isothermal conditions. $t_{1/2}$ values showed significant differences ($P < 0.05$) between BO and IEBO-1, 8 and 24 with BO has the highest $t_{1/2}$ and IEBO-24 has the lowest.

Induction time of nucleation (IT) is defined as the point where a fraction of crystals can be detected experimentally to be different from zero (Rousset, 2002). In lipid systems for a given rate of crystallization, as IT increases, the n gets higher in value (Metin and Hartel, 1996). Table 3 shows IT for nucleation of BO and IEBO-1, 8 and 24. Significant differences ($P < 0.05$) were found among the samples where BO exhibited a shorter IT for nucleation. Evidently, changes in FAs composition (Table 1) occurred by enzymatic acidolysis of BO and GLA were reflected on IT. The reaction caused a significant increase ($P < 0.05$) of unsaturated fatty acids (mainly GLA) on the account of SFAs. Crystallization, more specifically IT, of SFAs requires less time as compared to unsaturated fatty acids. The results obtained are in agreement with those of Metin and Hartel (1996) for milk fat and its fractions with different FAs profile crystallized isothermally at 15 °C.

Table 3. Induction time of nucleation and peak melting temperature of butter oil and interesterified butter oil with gamm-linolenic acid as crystallized isothermally at 10 °C.

Fat type	Induction time (min)	T_p(°C)
BO	8.93±0.17d	30.54±0.69a
IEBO-1	14.45±0.51c	21.05±0.37b
IEBO-8	19.06±0.93b	14.93±0.21c
IEBO-24	22.17±0.80a	11.02±0.08d

Mean ± SD, n=3.
Different letters within the same column are significantly different (P<0.05)BO, butter oil; IEBO, interesterified butter oil; T_p peak melting temperature of Differential Scanning Calorimeter thermograms. Numbers following abbreviation IEBO, represents interesterification time (h).

Abbreviations used

BO, butter oil; DSC, differential scanning calorimeter; FAs, fatty acids; GLA, gamma-linolenic acid; IEBO, interesterification butter oil; IT, Induction time; k, crystallization rate constant; MP, melting point; n, Avrami exponent; SFAs, saturated fatty acids; $t_{1/2}$, half-time of crystallization; T_p, peak melting temperature.

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التحليل الحرارى لخواص تبلور دهن اللبن المدعم بحامض الجاما-لينولينيك

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يعتبر دهن اللبن أحد أعقد الدهون الحيوانية من حيث التركيب الكيماوى ، حيث يدخل فى تركيبه مجموعة كبيرة ومتنوعة من الأحماض الدهنية إلا أن أهمها هى الأحماض التى يتراوح عدد ذرات الكربون بها ما بين ٤–١٨ ذرة . وتبلغ نسبة الأحماض الدهنية المشبعة فى دهن اللبن حوالى ٦٥% من إجمالى التركيب بينما تمثل الأحماض الدهنية غير المشبعة باقى التركيب. وينتج عن مثل هذا التركيب الكيماوى خواص تبلور مميزة لدهن اللبن عن الدهون الأخرى.

فى هذا البحث تم دراسة سلوك تبلور دهن اللبن المعدل بالأسترة الأنزيمية الداخلية مع حامض الجاما-لينولنيك (GLA, $C_{18:3}$ ω-6) من خلال تحليل منحنيات السلوك الحرارى وذلك لتقدير ثوابت التبلور لمعادلة Avrami. وتشير النتائج المتحصل عليها إلى وجود فروق جوهرية ($P<0.05$) فى قيم ثوابت التبلور ما بين عينة المقارنة وعينات الدهن الناتجة باستخدام الأسترة لمدة ١ ، ٨ و ٢٤ ساعة وذلك عند ١٠°م وهى الدرجة التى تم إجراء التبلور عليها.

بلغ الزمن اللازم لتكوين الأنوية لعينة المقارنة ٨,٩٣ دقيقة فى حين إزدادت هذه القيمة لتصل إلى ١٤,٤٥ ، ١٩,٠٦ و ٢٢,١٧ دقيقة لعينات الدهن الناتجة من عملية الأسترة لمدة ١ ، ٨ و ٢٤ ساعة على التوالى. أما الزمن اللازم لاتمام نصف عملية التبلور فقد كان ١٧,٥٢ دقيقة لعينة المقارنة فى حين تراوح ما بين ٢٥,١٤ – ٣٨,٤٠ دقيقة فى عينات دهن اللبن المدعم بـ GLA. كما أظهرت النتائج وجود علاقة عكسية ما بين محتوى الدهن من GLA وثابت معدل التبلور حيث بلغت قيمة هذا الثابت ٢,٢٦ × $١٠^{-٣}$ فى عينة المقارنة والخالية تماما من GLA و ٠,٩٢ × $١٠^{-٣}$ فى عينة الدهن الناتجة بعد ٢٤ ساعة من الأسترة والتى كانت تحتوى على ٢٨,٦٩% GLA. وقد تم تفسير النتائج المتحصل عليها على أساس التغير الجوهرى ($P<0.05$) الحادث فى تركيب الأحماض الدهنية لدهن اللبن نتيجة إجراء عملية الأسترة الأنزيمية الداخلية مع GLA.