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PRODUCTION OF A STRUCTURED DAIRY LIPID BY INCORPORATING GAMMA-LINOLENIC ACID INTO MILK FAT USING LIPASE-CATALYZED ACIDOLYSIS

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ABSTRACT

A structured dairy lipid was synthesized enzymatically via acidolysis of gamma-linolenic acid (GLA; $C_{18:3}$ ω-6), concentrated from borage oil (*Borago officinalis* L.), and butter oil (BO) using two different lipases. Two hundred international units of immobilized lipase from *Candida antarctica* or *Mucor miehei* were added and the reaction was carried out at 50 °C in an orbital shaking water bath at 250 rpm. Incorporation of GLA into BO was confirmed by gas chromatography. The greatest incorporation of GLA into BO was 28.69 % after 24 h incubation using lipase from *C. antarctica* lipase and 24.75 % after 3.5 h when using lipase from *M. miehei*. Thermal properties including peak melting temperature (T_p) and melting enthalpy (E_p) lowered ($P < 0.05$) as more GLA was incorporated. T_p and E_p of the inherent BO were 30.54 °C and 85.24 J/ g, respectively. By the end of the acidolysis, T_p values were 11.02 and 12.03 °C while E_p values had reached 38.85 and 40.29 J/ g when using *Candida antarctica* and *Mucor miehei* immobilized lipases, respectively. Solid fat content (SFC) corresponded ($P < 0.05$) to changes occurred in fatty acids (FAs) composition. BO possessed the highest SFC that downward progressively over the reaction course for both enzymes. However, at the late stages of the acidolysis, reduction in SFC became less pronounced. Enzymatic interesterification (EIE) by immobilized lipases affords an efficient tool for restructuring milk fat triacylglycerols while preserving the delicate flavor of milk fat to be used as an ingredient in functional food formulations.

Keywords: Enzymatic interesterification, milk fat, buter oil, Gamma-linolenic acid, structured lipids, Lipase, borage oil (*Borago officinalis* L.), acidolysis.

INTRODUCTION

Milk fat always has been an important component of the human diet through the consumption of dairy products. It is appreciated for being a fresh natural product with incomparable organoleptic properties. Nevertheless, recently a considerable decrease in the demand for milk fat has been observed. This decline in the market share is mainly due to the poor functional properties and negative health aspects that surround milk fat, especially its high content of saturated fatty acids (SFAs). More than 40 % of these SFAs are known to be hypercholesterolemic.

Gamma linolenic acid (GLA, *cis* 6*, cis* 9*, cis* 12-octadecatrienoic acid) is an essential fatty acid produced during the metabolism of linoleic acid (LA). Converting LA to GLA is a rate-limiting reaction due to the impaired activity of the Δ-6-desaturase enzyme (Sprecher, 1981). Several factors reduce the enzyme's activity of Δ-6-desaturase including: arthritis, diabetes, hypertension, smoking, *trans* fatty acids, and nutritional deficiencies of

vitamin B_6 and zinc (de Gomez *et al*., 1984; Ayala and Brenner, 1983). Once formed, GLA is elongated to dihomo-GLA (DGLA, 20:3 ώ-6) by the elongase enzyme, which is subsequently acetylated and incorporated into cell membrane phospholipids. DGLA can also be converted to arachidonic acid by the Δ-5-desaturase enzyme (Kapoor, 2005).

GLA has several therapeutic benefits: DGLA is the precursor of prostaglandin E_1 which exerts anti-inflammatory and vasodilator effects. It improves dysregulation of inflammation and provides immunity in atopic eczema (Henz *et al*. 1999; Goffman and Galletti 2001). Consumption of GLA is reported to be effective against hypercholesterolemia and other related clinical disorders that provide susceptibility to coronary atherosclerotic heart disease (Barre 2001). Patients with acute respiratory distress syndrome or patients with sepsis who received a diet enriched in GLA, demonstrated significant improvement (Gadek *et al*., 1999; Pontes-Arruda *et al*., 2006). Moreover, GLA is an anti-cancer agent because it possesses effective tumoricidal properties while not inducing damage to normal cells or creating harmful systemic side effects (Vartak *et al*., 1998; Menendez *et al*., 2004). Numerous *in vivo* and *in vitro* studies showed that GLA inhibits growth and metastasis of a variety of tumour cells, including breast, prostate, superficial bladder, and pancreas as well ashepatoma cells (Harris *et al*., 2002; Agombar *et al*., 2004; Vang, and Ziboh, 2005). GLA also revealed an anti-metastatic effect on endothelial cells and occlude vessels that feed tumors (Das, 2004).

EIE by immobilized lipases is an efficient tool for restructuring milk fat triacylglycerols (TAGs) while preserving milk fat delicate flavor. The mild reaction conditions applied, the specificity of the enzyme used, the full control of the overall process with complete ease of the end-product recovery and the ability of reusing the immobilized enzyme allow EIE to be the method of choice for modifying milk fat composition (Willis and Marangoni, 1999).

The objective of this research is to investigate the feasibility of replacing some of milk fat SFAs with GLA derived from borage oil in an attempt to improve the nutritional value of milk fat and to provide an ingredient for potential functional food applications.

MATERIALS AND METHODS

Borage (*Borage officinalis* L*)* oil was kindly donated by Bioriginal Food and Science Corp. (Saskatoon, Canada).

Butter was obtained from a local grocery store. Anhydrous BO was prepared by melting butter at 60 °C, decanting the top oil layer, filtering the oil through glass wool, and re-filtering it under vacuum (Whatman no.1 and 0.14 MPa) to obtain clear oil. The resultant BO was dried over anhydrous sodium sulfate (Amer *et al.*, 1985).

Immobilized lipase from *Candida antarctica* and *Mucor miehei* (Fluka, Denmark) were employed for the acidolysis reaction. All chemicals used were of analytical grade or better.

Preparation of GLA concentrate from borage oil was carried out according to the method of Spurvey and Shahidi (2000).

Preparation of free fatty acids from borage oil was carried by saponification of twenty five grams of borage oil (treated with 200 ppm hydroquinone) were saponified at 60 °C for an hour under reflux using a mixture of 5.57 g KOH, 11 mL distilled water and 66 mL 95 % ethanol, and the reaction was performed under an atmosphere of nitrogen. To the saponified mixture, 60 mL distilled water was added and the unsaponifiable matter was extracted into hexane (2 x 100 mL) and then discarded. The aqueous layer containing saponifiable matter was acidified to pH 1 with 3N HCl. The mixture was transferred to a separatory funnel and the liberated FAs were extracted into 50 mL hexane. The hexane layer containing free fatty acids (FFAs) was then dried over anhydrous sodium sulfate and the FFAs were recovered by evaporating the solvent at 40 °C using a rotary evaporator.

For the preparation of GLA concentrate by urea complexation ,ten grams of the FFAs were mixed with 50 mL of urea solution (20 % w/v in 95 % ethanol), and the mixture was heated at 60 °C with stirring until it turned into a clear homogenous solution. The urea-FAs mixture was allowed to crystallize at room temperature then at -15 °C/ 16h for further crystallization. The formed crystals (urea complex fraction) were separated from the liquid (nonurea complex fraction; NUCF) by suction filtration (Whatman # 4). The NUCF was diluted with an equal volume of distilled water and acidified to pH 4-5 with 6 N HCl. An equal volume of hexane was subsequently added and the mixture was thoroughly stirred for 1h, and then transferred to a separatory funnel. The hexane layer containing liberated FAs was separated from the aqueous layer and washed with distilled water to remove any urea residues and then dried over anhydrous sodium sulfate. Hexane was subsequently evaporated at 40 °C using a rotary evaporator.

Lipase-catalyzed acidolysis of BO with GLA concentrate was carried out by the method of Senanyake and Shahidi (2002). A 2:1 (v/v) mixture of BO and GLA was prepared in a screw capped test tube, and then lipase (200 IU) was added. The mixture was incubated in an orbital shaking water bath at 250 rpm and 50 °C for 24 h when using lipase from *C. Antarctica,* and 3.5 h when using lipase from *M. miehei*. Individual samples were taken for analysis and all reactions were performed in triplicate.

For the recovery of the end product, hexane (3 x 10 mL) was added to each tube and the contents were vortexed for 1 min. The enzyme was removed by filtration (Whatman No.1). The filtrate was placed in a 250 mL conical flask and 20 mL of acetone/ethanol mixture (1:1 v/v) were added. The reaction mixture was titrated with 0.5 N NaOH to a phenolphthalein endpoint. The mixture was transferred to a separatory funnel and phase separation was allowed to take place. The lower aqueous layer was separated and discarded. The upper hexane layer containing TAGs was passed through anhydrous sodium sulfate. The TAGs fraction was subsequently recovered following hexane removal at 40 °C using a rotary evaporator.

For the preparation of fatty acid methyl esters (FAMEs) ,

FAs profile of BO, borage oil, GLA concentrate, and BO enriched with GLA were determined after conversion of the FAs into their corresponding methylesters (Senanyake and Shahidi, 2002). Approximately 50 mg sample

were transferred to a 5 mL Reacti-vial (Pierce, Rockford, IL, USA) and its mass was accurately weighed. A 100 µL internal standard (20 mg heptadecanoic acid / mL hexane) was added to each Reacti-vial. The TAGs were then hydrolysed and derivatized with a transmethylation reagent consisting of 6 % concentrated H_2SO_4 in methanol. Two mL of the transmethylation reagent were added to each Reacti-vial which was then capped, vortexed for 1 min and incubated at 65 °C over night. The following day, samples were allowed to cool to room temperature. One mL of distilled water was added to each vial and the contents were vortexed for 30 s, and then extracted three times with 1.5 mL of pesticide-grade hexane. A few crystals of hydroquinone were added to each vial prior to extraction with hexane. The hexane layers were combined and washed twice with 1.5 mL deionized water. After the second wash, the hexane layer was removed and transferred to a new test tube. The hexane extract was dried under a stream of nitrogen and the resultant FAMEs were redissolved in 1.5 mL carbon disulfide. An aliquot was transferred to a GC autoinjector vial and then crimped.

An Agilent Technologies 6890N gas chromatograph (GC) (Agilent Technologies, Wilmington, DE, USA) equipped with a 7683B Series autoinjector module, 7683 autosampler tray module and GC ChemStation software was used for FAs profiling. Operating conditions included the following: the column was a J&W fused-silica DB-23 capillary column (60 m x 0.25 mm I.D. x 0.25µm film thickness); ultra high purity helium was the carrier gas at a flow rate of 2.7 mL/min; the inlet was set for split injection at a split ration of 1:50; the flame ionization detector was set at 250 °C; the oven temperature was initially set at 130 °C, and then ramped at 4 °C/min up to 240 °C and held at this temperature for an additional 2.5 min. Peaks were identified by comparing retention times with those of a standard mixture of FAMEs (Nu-Check-Prep, Inc., Elysian, MN, USA).

Thermal characteristics of BO and BO enriched with GLA were performed by Differential Scanning Calorimeter (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. AOCS method Cj-94 (AOCS, 1998) was followed. 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. The sample was rapidly heated (100°C/min) from room temperature to 80 °C and held at this temperature for 5 min before being cooled to -50°C at a rate of 10 °C/min. After 15 min holding at -50°C, the sample was heated to 70 °C at a rate of 10 °C/min. Thermograms were analyzed for T_p and E_p.

Solid fat content (SFC) was measured with a pulsed nuclear magnetic resonance spectrometer (Maran, Resonance Instruments, Whitney, UK) operating at 20MHz. SFC was determined according to the AOCS method Cd 16-81(AOCS, 1998).

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

The formation of complexes between urea and linear hydrocarbon templates is a well known separation technique for obtaining polyunsaturated fatty acids (PUFAs) concentrate (Wanasundara and Shahidi, 1999). Table 1 presents FAs composition of GLA concentrate prepared from borage oil by urea complexation. GLA comprised more than 75 % of the concentrate profile. The concentrate was completely void of any SFAs or monounsaturated fatty acids, except traces of oleic acid ($C_{18:1}$) and linolenic acid ($C_{18:3}$). Linoleic acid ($C_{18:2}$) was reduced by more than 15 % of its initial concentration in borage oil.

Table 1. Fatty acids profile of borage oil and GLA concentrate obtained by urea complexation.

Fatty acid	Borage oil	GLA concentrate
C 16:0	8.74 ± 0.05	ND
C 18:0	3.66 ± 0.03	ND
C 18:1 (ω-9)	18.49 ± 0.03	0.17 ± 0.02
C 18:2 (ω-6)	38.08 ± 0.07	20.39 ± 0.51
C 18:3 (ω-6; GLA)	22.25 ± 0.08	79.14 ± 0.63
C 18:3 (ω-3)	0.28 ± 0.01	0.24 ± 0.01
C 20:0	0.28 ± 0.0	ND
C 20:1	3.75 ± 0.02	ND
C 22:0	0.28 ± 0.01	ND
C 22:1	2.47 ± 0.03	ND
C 24:0	1.70 ± 0.03	ND

Mean ± SD, *n*=3.
GLA, gamma linolenic acid; ND, not detected.

In forming the urea complex, urea molecules associate together via hydrogen bonds building up a helical structural framework. Urea crystals are hexagonal. When preparing the urea complex, the compounds occupy the free space inside the hexagonal channels and are held there through different forces (e.g., van deer Waals) (Hayes *et al*., 1998). While SFAs with six carbon atoms or more are readily complexed, the presence of double bonds in the carbon chain increases the bulk of the molecule and reduces the likelihood of its complexation with urea. Monounsaturated fatty acids are more readily complexed compared to diunsaturated fatty acids, which, in turn, are more easily complexed than PUFAs (Swern, 1964).

Acidolysis is defined as the transfer of an acyl group between an acid and an ester. The process is used mainly to incorporate high concentrations of PUFAs into TAGs (Willis *et al*., 1998). Table 2 and Table 3 depict changes occurred in the FAs profile of BO during the enzymatic acidolyis using lipase from *C. antarctica* and *M. miehei*, respectively. For both enzymes, as the reaction proceeded, more GLA was incorporated into BO till reaching the reaction plateau. When *C. antarctica* lipase was used, the reaction plateau

was observed after 24 h where almost 29 % GLA was incorporated. For *M. miehei* lipase reaching the reaction equilibrium was faster requiring 3.5 h with 24 % GLA incorporatation. In terms of FAs, enzymatic acidolysis was a successful tool for improving nutritional value of milk fat. SFAs were decreased from 64.35 % in the inherent BO to 41.09 and 40.31 % in the final product when using lipase from *C. antarctica* and *M. miehei*, respectively. Most of this reduction was observed in the content of the hypercholesterolemic fatty acids like myristic ($C_{14:0}$), palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) (Katan *et al*., 1995).

The results obtained can be explained based on lipase enzymes specificity. Lipases are categorized into three groups: nonspecific, positional and substrate. *C. antarctica* lipase is a nonspecific one that shows no positional or fatty acid specifity during EIE. With this type of lipase, EIE gives a complete randomization of all FAs in all positions and gives the same product as chemical interesterification. *M. miehei* lipase is a *sn*-1,3 specific lipase that shows specificity toward ester bonds only in positions *sn*-1,3 of the TAG, results from an inability of the enzyme to act on position *sn*-2 due to steric hindrance. Steric hindrance prevents the fatty acid in position *sn*-2 from entering the active site, thus less reaction time as well incorporation of GLA as compared to *C. antarctica* lipase (Weete, 2002)

In accordance with our results, Garcia *et al*. (2000) who interesterified butterfat with conjugated linoleic acid (CLA; $C_{18:2}$ ω-6) using an immobilized lipase from *C. antarctica* and *M. miehei*. Their results showed that, both enzymes demonstrated the ability to increase CLA content of milk fat from the native value of 0.7 % to 17.3 and 13 % for lipase from *C. antarctica* and *M. miehei*, respectively. Similar observations were reported by Ronne *et al*. (2005) on the performance of using *C. antarctica* for lipase-catalyzed interesterification of butterfat and rapeseed oil (oleic, 60.7 %; linoleic, 20.1 % and linolenic, 10.2 %) in a packed bed reactor. Significant changes in FAs profile were also reported by Rousseau and Marangoni (1998 a) for EIE of butterfat and canola oil using *Rhizopus arrhizus* (*sn*-1, 3 specific) immobilized lipase.

AS with the thermal analysis ,Figs 1 and 2 show that, BO had the highest ($P < 0.05$) T_p value as compared to all interesterified products resulted from using either *C. antarctica* or *M. miehei*. As the reaction proceeded , more GLA was incorporated, thus T_p progressively decreased. Evidently, most of the significant changes ($P < 0.05$) in T_p took place in the early stages of the reaction. Tables 2 and 3 depict that more than 85 % of GLA incorporated was achieved after 12 h (out of 24 h total reaction time) in the case of *C. antarctica* lipase, while for *M. miehei* lipase, it was after 2 h (out of 3.5 h total reaction time). Moreover, considering key FAs affect T_p, a significant reduction ($P < 0.05$) was observed in the content of myristic ($C_{14:0}$, melting point (M.P.), 54.4 °C), palmitic ($C_{16:0}$, M.P., 62.9°C) and stearic ($C_{18:0}$, M.P., 69.6 °C) (Formo, 1979). Meanwhile, there was a corresponding increase ($P < 0.05$) in the amount of GLA incorporated. GLA is a PUFA that has a very low M.P. of (-12)-(-14) °C (Clough, 2001).

As the amount of GLA incorporated increased, E_p shifted to lower values ($P < 0.05$) as seen in Figs 3 and 4.

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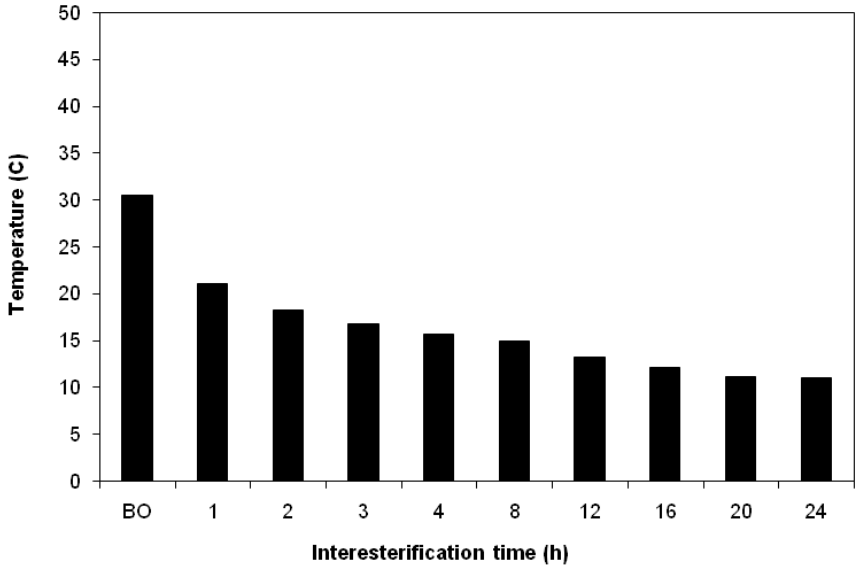

Fig. 1: Melting temperature (ºC) of non-interesterified butter oil (BO) and enzymatically interesterified butter oil with gamma-linolenic acid using *Candida antarctica* immobilized lipase as a function of interesterification time.

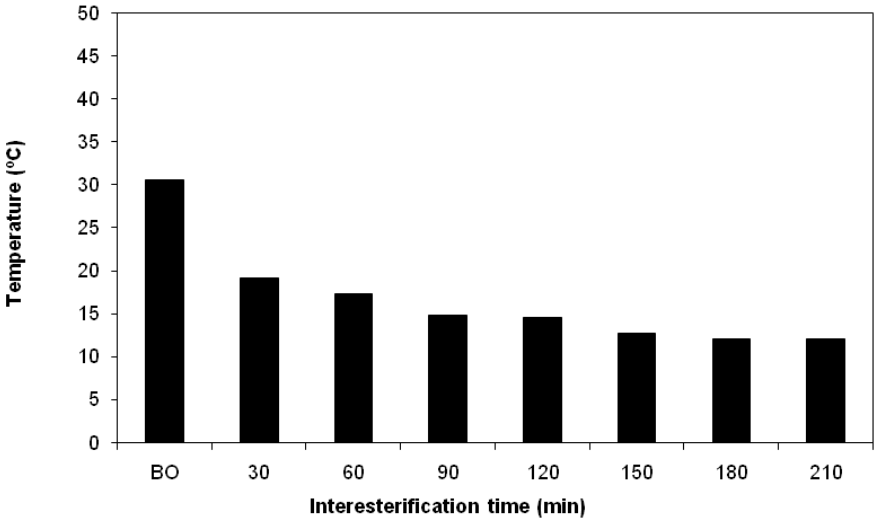

Fig. 2: Melting temperature (ºC) of non-interesterified butter oil (BO) and enzymatically interesterified butter oil with gamma-linolenic acid using *Mucor miehei* immobilized lipase as a function of interesterification time.

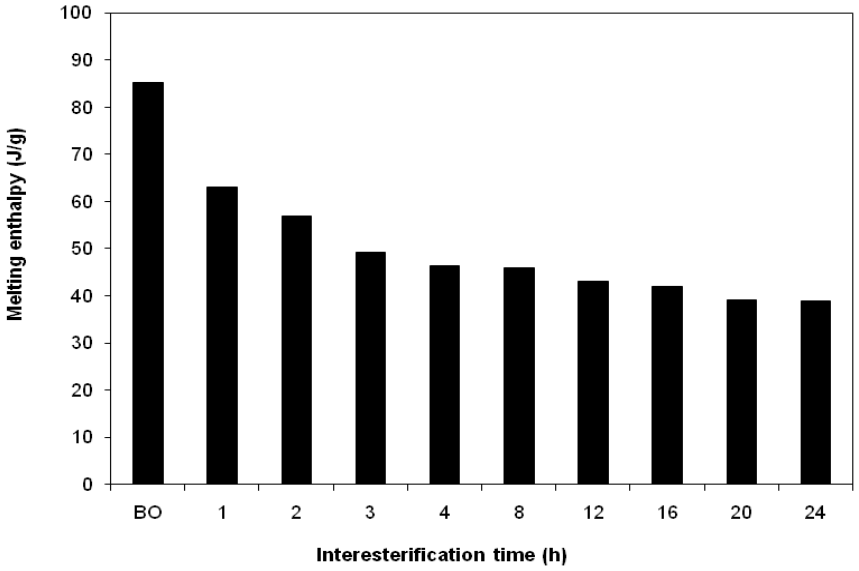

Fig. 3: Melting enthalpy (J/g) of non-interesterified butter oil (BO) and enzymatically interesterified butter oil with gamma-linolenic acid using *Candida antarctica* immobilized lipase as a function of interesterification time.

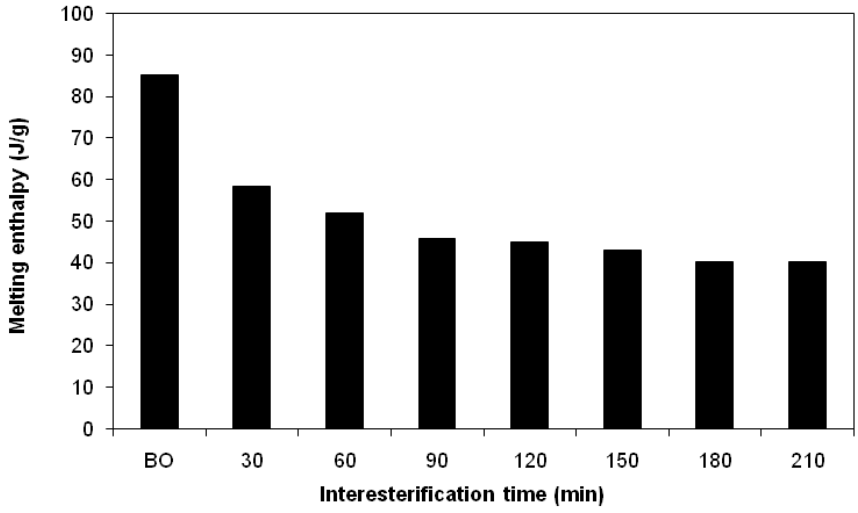

Fig. 4: Melting enthalpy (J/g) of non-interesterified butter oil (BO) and enzymatically interesterified butter oil with gamma-linolenic acid using *Mucor miehei* immobilized lipase as a function of interesterification time.

The highest ($P < 0.05$) E_p of 85.24 J/ g was observed for the control BO. By the end of the acidolysis, E_p was reduced by 46.39 and 44.95 J/g as compared to the control. The impact of increasing USFAs (mainly GLA) on the reduction of the thermal properties is relevant to the dilution effect caused by incorporating GLA which has a very low E_p of 1.22 J/g (Clough, 2001).

Our results are in agreement with previous works of Liew *et al*. (2001), who reported reduction in T_p when studied the physical properties of palm kernel olein-anhydrous milk fat mixture interesterified using lipase from *Rhizomucor miehei* (*sn*-1,3 specific). Peychii and Swaisgood (1997) came to a similar conclusion when observed a reduction of 3 °C in T_p of the interesterified BO as compared to the control (32.4 °C). They used *Pseudomonas fluorescens* (nonspecific) immobilized lipase to modify milk fat chemical and physical properties.

Solid not fat (SFC) as a function of temperature is shown in Figs 5 and 6. Lipase-catalyzed acidolysis using *C. antarctica* and *M. miehei* immobilized lipase lowered significantly ($P < 0.05$) SFC as compared to the native BO which in turn possessed the highest ($P < 0.05$) SFC till complete melting at 40 °C. When performing acidolysis using either *C. antarctica* or *M. miehei* lipase and as the measured temperature increased, SFC progressed downwards with no sharp turns, however, at higher temperatures (> 20 °C), SFC reduction became less pronounced. After reaching the reaction equilibrium no significant differences ($P < 0.05$) were observed in SFC profile which is ascribed to the similarity in FAs composition of the interesterified products (Tables 2 and 3).

SFC patterns correspond well with changes occurred in FAs profile. Referring to Tables 2 and 3, SFAs were replaced partially by USFAs mainly GLA. Acidolysis by immobilized lipases reduced SFAs by almost 25 % as compared to the inherent BO with a corresponding increase of the same value in USFAs. Similar to the data reported, Rousseau and Marangoni (1998 b) for EIE of butterfat and canola oil using *Rhizopus arrhizus* immobilized lipase (*sn*-1,3specific) and Peychii and Swaisgood (1997) for modification of milk fat physical properties by immobilized *Pseudomonas fluorescens* lipase (nonspecific). Both works showed that EIE progressively lowered SFC in the melting range of 5-40 °C.

Abbreviations used

BO, butter oil; DGLA, dihomo-GLA; DSC, differential scanning calorimeter; EIE, enzymatic interesterification; E_p, melting enthalpy; FAs, fatty acids; FFAs, free fatty acids; GLA, gamma-linolenic acid; IE, interesterification; LA, linoleic acid; m.p., melting point; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids; SFC, solid fat content; TAGs, triacylglycerols; T_p, melting temperature; USFAs, unsaturated fatty acids.

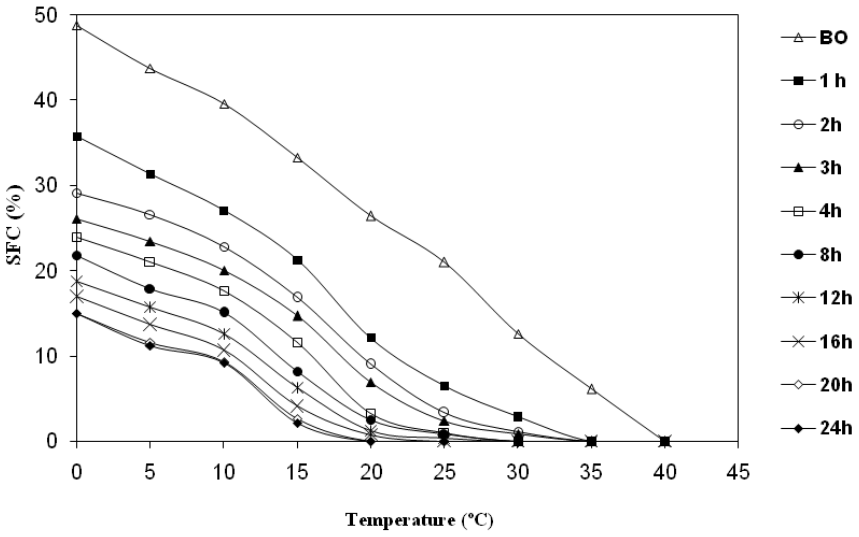

Fig. 5: Solid fat content (% SFC) of non-interesterified butter oil (BO) and enzymatically interesterified butter oil with gamma-linolenic acid using *Candida antarctica* immobilized lipase as a function of temperature.

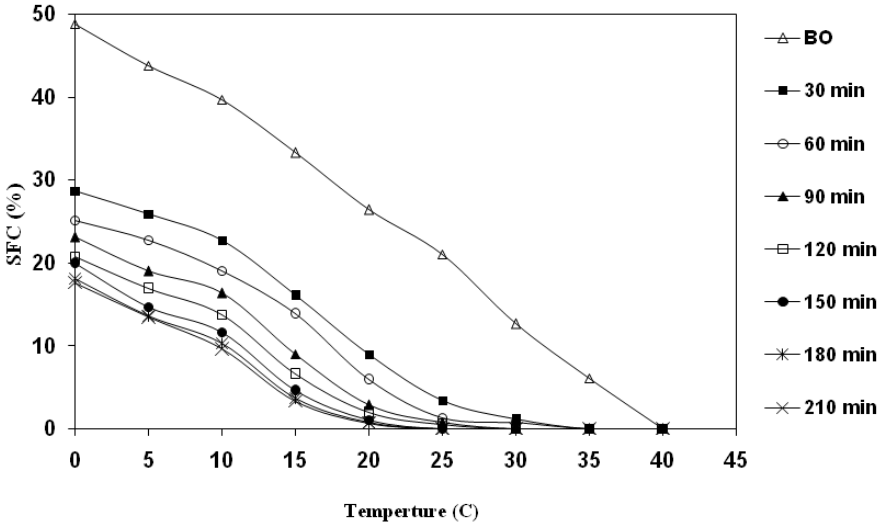

Fig. 6: Solid fat content (% SFC) of non-interesterified butter oil (BO) and enzymatically interesterified butter oil with gamma-linolenic acid using *Mucor miehei* immobilized lipase as a function of temperature.

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إنتاج دهن لبن ذو خواص وظيفية بإستخدام الأسترة الإنزيمية لدهن اللبن وحامض الجاما – لينولينيك

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**** قسم علوم الأغذية – كلية الزراعة والعلوم البيئية – جامعة جورجيا – ولاية جورجيا – الولايات المتحدة الأمريكية**

دهن اللبن هو أحد الدهون الطبيعية ذات الخواص الحسية عالية الجودة والمرغوبة لدى المستهلكين مما يجعله مكون هام فى العديد من الأغذية . إلا أنه فى الآونة الأخيرة ، إنخفض الطلب على دهن اللبن لمحتواه المرتفع من الأحماض الدهنية المشبعة والكوليسترول مما إستلزم معه تعديل تركيب دهن اللبن .

ويعتبر حامض الجاما – لينولينيك GLA ($C_{18:3}$ ω-6) من الأحماض الدهنية عديدة عدم التشبع والتى تشير العديد من الدراسات إلى فوائده العلاجية فى الكثير من الأمراض مثل إرتفاع ضغط الدم وبعض أمراض التنفس والأمراض الجلدية . كذلك فقد أثبتت الدراسات الدور الحيوى لـ GLA كأحد المواد المضادة لتكوين الأورام السرطانية .

وقد إستهدف هذا البحث تحسين الخواص الوظيفية لدهن اللبن بتدعيمه بالـ GLA وذلك من خلال الـ الأسترة بإستخدام إنزيمات الليبيز المحملة والمستخرجة من *Candida antarctica* و *Mucor miehei* . وقد تم الحصول على مركز الـ GLA بإستخلاص الأحماض الدهنية الحرة من زيت البوراج بعد إجراء التصبن له ثم فصل الـ GLA بإستعمال اليوريا ثم إجراء الأسترة مابين دهن اللبن والـ GLA على ٥٥°م ولمدة ٢٤ ساعة عند إستخدام ليبيز *C. antarctica* و ٣,٥ ساعة عند إستخدام ليبيز *M. miehei*.

ولقد أظهرت النتائج أن إستخدام ليبيز *C. antarcitica* قد أدى إلى دمج ٢٨,٦٩% GLA فى دهن اللبن أما عند إستخدام ليبيز *M. miehei* فقد بلغت تلك النسبة ٢٤,٧٥%. وقد إنعكس تغير التركيب الكيماوى لدهن اللبن على خواصه الفيزيائية مثل الحرارة الكامنة للإنصهار (Enthalpy) والتى إنخفضت جوهرياً ($P<0.05$) من ٨٥,٢٤ جول/جم فى عينة المقارنة إلى ٣٨,٨٥ و ٤٠,٢٩ جول/جم عند إستخدام ليبيز *C. antarctica* و *M. miehei* على التوالى . أما المحتوى من الدهن الصلب فقد تناسب عكسياً مع طول فترة الأسترة عند إستخدام كلا الإنزيمين مع عدم ظهور أى فروق جوهرية ($P<0.05$) فى هذه الخاصية فيما يتعلق بنواتج المراحل النهائية من التفاعل .

Table 2. Fatty acids profile of butter oil before and after acidolysis with GLA concentrate using lipase from *C. antarctica*.

Fatty acid	Acidolysis time (h)									
	BO	**1**	**2**	**3**	**4**	**8**	**12**	**16**	**20**	**24**
C6:0	1.30±0.09a	1.22±0.03a	1.18±0.02a	1.07±0.04ab	0.93±0.05b	0.74±0.01bc	0.51±0.01c	0.30±0.02c	0.24±0.01d	0.13±0.0d
C8:0	1.17±0.12a	1.09±0.01a	1.01±0.0a	0.90±0.01ab	0.81±0.03b	0.66±0.02c	0.43±0.0d	0.21±0.01d	0.15±0.02de	0.09±0.1e
C10:0	2.68±0.02a	1.87±0.15b	1.81±0.05b	1.74±0.03bc	1.69±0.06c	1.58±0.08cd	1.50±0.05d	1.43±0.10d	1.18±0.08e	1.11±0.01e
C12:0	3.41±0.02a	2.70±0.03b	2.66±0.03b	2.59±0.02bc	2.45±0.06c	2.32±0.04cd	2.27±0.01cd	2.12±0.03d	2.03±0.08de	2.00±0.03e
C14:0	11.62±0.04a	9.59±0.08b	9.42±0.11b	8.18±0.09cd	7.94±0.21d	8.44±0.10c	8.13±0.07c	7.95±0.11d	7.51±0.16e	7.47±0.27e
C14:1	0.97±0.01a	0.79±0.01b	0.77±0.01b	0.75±0.01b	0.71±0.02bc	0.68±0.02c	0.66±0.01c	0.63±0.01c	0.63±0.01c	0.59±0.02c
C15:0	1.16±0.02a	0.97±0.01b	0.94±0.01b	0.90±0.02bc	0.87±0.03c	0.84±0.01c	0.81±0.02cd	0.79±0.01d	0.78±0.01d	0.74±0.03d
C16:0	31.82±0.06a	26.49±0.20b	24.24±0.33bc	23.89±0.19c	22.57±0.60c	22.02±0.17c	21.72±0.25cd	21.37±0.4cd	21.25±0.26d	21.12±0.72d
C16:1	1.63±0.01a	1.33±0.02a	1.31±0.02ab	1.27±0.10b	1.24±.03b	1.20±0.03bc	1.14±0.02c	1.12±0.04c	1.10±0.03c	1.05±0.04c
C18:0	12.66±0.04a	11.51±0.06ab	11.19±0.14ab	10.90±0.07b	10.21±0.27bc	9.78±0.06c	9.33±0.16cd	9.09±0.22d	8.52±0.02e	8.43±0.20e
C18:1	27.40±0.06a	23.02±0.14b	22.62±0.30bc	21.04±0.20c	20.83±0.33c	19.81±0.44d	19.24±0.32d	18.82±0.73e	18.26±0.16e	18.10±0.57e
C18:2	3.65±0.01d	6.76±0.09c	6.75±0.19c	7.05±0.13c	8.04±0.17b	8.31±0.05b	8.85±0.10b	9.18±0.13ab	9.32±0.29a	10.06±0.49a
GLA	ND	12.18±0.38f	15.63±0.69e	18.25±0.35d	21.25±0.90c	23.17±0.24bc	24.97±0.53b	26.54±0.71b	28.59±0.77a	28.69±1.25a
C18:3	0.54±0.02a	0.48±0.01b	0.47±0.01b	0.47±0.01b	0.46±0.01bc	0.45±0.01c	0.44±0.0c	0.45±0.03c	0.44±0.01c	0.42±0.01c
SFAs	65.82±0.11a	55.44±0.31b	52.45±0.62c	50.17±0.34c	47.47±1.19d	46.38±0.28d	44.70±0.43d	43.26±0.61de	41.66±0.66e	41.09±1.24e
USFAs	34.19±0.21e	44.56±0.48d	47.55±0.55c	49.83±0.30c	52.53±1.32b	53.62±0.33b	55.30±0.31b	56.74±0.14ab	58.34±0.86a	58.91±1.12a

Mean ± SD, *n*=3.

Different letters within the same row are significantly different (*P*<0.05).

BO, butter oil; GLA, gamma linolenic acid ($C_{18:3}$ ω-6); ND, not detected; SFAs, saturated fatty acids; USFAs, unsaturated fatty acids.

Table 3. Fatty acids profile of butter oil before and after acidolysis with GLA concentrate using lipase from *M. miehei*.

	Fatty acid							
	Acidolysis time (min)							
	Butter oil	**30**	**60**	**90**	**120**	**150**	**180**	**210**
C6:0	1.30±0.09a	1.26±0.01a	1.19±0.05a	1.12±0.03ab	1.07±0.02b	1.00±0.0b	0.90±0.03c	0.82±0.0c
C8:0	1.17±1.12a	1.13±0.07a	1.09±0.01a	1.00±0.02ab	0.93±0.04b	0.85±0.01b	0.79±0.02c	0.68±0.01c
C10:0	2.68±0.02a	1.54±0.01b	1.57±0.06b	1.49±0.07b	1.37±0.27c	1.21±0.26d	1.35±0.04c	1.30±0.06c
C12:0	3.41±0.02a	2.60±0.02b	2.11±0.06b	2.05±0.16b	1.98±0.12c	1.70±0.06c	1.56±0.01d	1.47±0.05d
C14:0	11.62±0.04a	8.41±0.16b	7.90±0.11b	7.11±0.49bc	7.03±0.21bc	6.95±0.36c	6.75±0.03c	6.59±0.06c
C14:1	0.97±0.01a	0.58±0.01b	0.58±0.02b	0.46±0.05bc	0.41±0.02c	0.36±0.04c	0.27±0.01d	0.20±0.01d
C15:0	1.16±0.02a	0.81±0.01b	0.81±0.01b	0.79±0.03b	0.70±0.02b	0.52±0.02c	0.39±0.00d	0.31±0.01d
C16:0	31.82±0.06a	25.69±0.38b	24.59±0.41b	22.94±0.50c	22.67±0.36c	21.18±0.24cd	20.46±0.16d	20.19±0.71d
C16:1	1.63±0.01a	1.06±0.02b	1.06±0.02b	1.09±0.06b	1.03±0.05b	0.95±0.06bc	0.83±0.01c	0.64±0.01d
C18:0	12.66±0.04a	10.10±0.10b	10.09±0.03b	9.84±0.11c	9.68±0.11c	9.34±0.21c	9.08±0.16d	8.95±0.68d
C18:1	27.40±0.06a	20.86±0.12b	19.74±0.30b	19.50±0.39bc	19.06±0.33bc	18.83±0.43c	18.35±0.21c	18.27±0.73c
C18:2	3.65±0.01e	9.86±0.13d	10.35±0.07c	10.70±0.30c	11.76±0.21c	13.95±0.34b	14.57±0.02a	15.42±0.44a
GLA	ND	15.69±0.37e	18.52±0.65d	21.48±1.95bc	21.90±1.90bc	22.74±0.97b	24.30±0.52a	24.75±1.52a
C18:3	0.54±0.02a	0.41±0.01b	0.40±0.02b	0.43±0.02b	0.41±0.01b	0.42±0.02b	0.40±0.0b	0.42±0.01b
SFAs	65.82±0.11a	51.54±0.66b	49.35±0.62b	46.34±1.15c	45.43±0.82c	42.75±0.26d	41.28±0.33d	40.31±1.25d
USFAs	34.19±0.22e	48.46±0.54d	50.65±0.62d	55.66±1.19c	54.57±1.30bc	57.25±0.25b	58.72±0.35a	59.69±1.22a

Mean ± SD, *n*=3.
BO, butter oil; GLA, gamma linolenic acid ($C_{18:3}$ ω-6); ND, not detected; SFAs, saturated fatty acids; USFAs, unsaturated fatty acids.