Egypt. J. Food Sci.,**Vol. 34,** pp. 91-101 (2006)

Effect of Incorporating Buffalo Butter oil Fractions on the Physical Properties of Ice Cream

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BUFFALO butter oil was fractionated by multi-step dry method into low, medium and high melting fractions. The obtained fractions were used in the preparation of ice cream. The freezing points of the mixes were close. Ice cream mix made with high-melting fraction (slip melting point (SMP), 41.3°C) exhibited the highest viscosity and the lowest adsorbed protein and whipping ability among all mixes prepared. The use of low-melting fraction (SMP, 12.6°C) led to the production of ice cream with decreased melting resistance but an increased overrun and fat destabilization index. Ice cream made with middle-melting fraction (SMP, 29.4°C) revealed properties in between those made with low and high-melting fractions.

Keywords: Ice cream, Buffalo butter oil fractions, Freezing point, Whipping ability, Slip melting point, Viscosity, Overrun, Melting resistance.

Ice cream is a complex food system that consists of different components (Goff *et al.,* 1995). Milk fat is an important ingredient in this system for its contribution to the structure and mouthfeel. It contributes creamy rich flavor, produces a smooth texture by lubricating the palate and helps to give smooth body through its structure-forming properties (Marshall *et al.,* 2003). However, milk fat in its inherent form possesses limited functional properties like melting behavior. The complex nature of fatty acid composition of milk fat creates a wide melting range (Jensen *et al.,* 1991), which makes fractionation of milk fat into fractions with different characteristics is a useful tool in ice cream formulations.

Buffalo milk is a main source of milk and its products in several countries like Egypt, Italy, India and Iraq (Cockrill, 1989). However, no cited studies have reported the use of buffalo milk fat fractions in ice cream manufacture. The objective of this study was to investigate the impact of using buffalo butter oil fractions on the characteristics of ice cream.

Material and Methods

Dry fractionation

Butter oil (BO) fractions were obtained by multi-step dry fractionation procedure previously described by Fatouh *et al.* (2003). The resultant fractions were: low-melting fraction (LMF) (SMP, 12.6°C), middle-melting fraction (MMF) (SMP, 29.4°C) and high-melting fraction (HMF) (SMP, 41.3°C) .

Fractions characteristics

Triacylglycerol profile

BO and its fractions were analyzed for their triacylglycerol (TAG) composition using the analytical method developed by Lund (1988). About 1 μL of sample (10 mg fat/ 10 mL heptane) was injected into DB-1 fused silica capillary column (15 m x 0.25 mm ID x 0.1 μm) (J&W Scientific, Folsom, CA, USA). The column was installed in a HP5980 Series II Gas Chromatograph (Hewlett-Packard, San Fernando, CA, USA). The oven temperature was programmed in two stages: from 40 to 250 °C at 30°C/min then from 250 to 320°C at 4°C/min and was held at this temperature for 35 min. Nitrogen was the carrier gas at a flow rate of 20 ml/min. The injector and detector temperatures were 330 and 350°C, respectively. Identification of the main groups of triacylglycerol according to carbon number was made by comparing the retention times with those of standard mixtures from C_{24} to C_{54} (Alltech, State College, PA, USA).

Thermal analysis

Thermal analysis of BO and its fractions was performed on a 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). AOCS method Cj-94 (AOCS, 1998) was followed. Samples (9-10 mg hermetically sealed in aluminum pans) were heated to 80°C and held at this temperature for 5 min, and then cooled to -50°C at a rate of 10°C/min. After holding at -50°C for 15 min, the samples were heated again to 80°C at a rate of 10°C/min to obtain melting curves.

Slip melting point (SMP)

This was determined by the AOCS method Cc 3-25 (AOCS, 1998).

Ice cream processing

Ice cream mixes had the following composition: 10% fat, 11% milk solids not fat (MSNF), 14% sugar, and 0.2% stabilizer (Walstra *et al.,* 1999).They were prepared as outlined by Liew *et al.* (2001). Milk was skimmed using a cream separator (FT 15 Armfield, Hampshire, UK). The resultant skim milk was preheated to 60°C and dry ingredients(skim milk powder and sougr) were added. Melted BO or its fractions were then added and the mixture was homogenized (Gaulin 15MR-8TA, Everett, MA, USA) at 27.6 MPa. Ice cream mixes were pasteurized at 85°C for 15 sec, then cooled to 5°C and aged at this temperature for 16 hr. Batches (1.5 l) of ice cream mixes were flavored with vanilla , then frozen in a batch freezer (Taylor 104-12, Taylor Co., Rockton, IL, USA). The ice cream was drawn at -5°C, packaged into Styrofoam cups, hardened in a blast air freezer at -30°C for 24 hr, and finally stored at -20°C until all analyses were completed.

Analyses of ice cream mix

Freezing point : An automatic osmometer (OSMETTE A,Precision Systems, Inc., Natick, MA, USA) was used to determine freezing point of ice cream mixes (Baer and Czmowski, 1985). The osmometer was calibrated with NaCl standards and operated at the low range (0-2000 milliosmols / kg H_2O (mOsm / kg H_2O)). A 2 ml sample size was used. Osmometer readouts were converted to freezing point by using the following equation:

$$\text{Freezing point } (°C) = \text{mOsm / kg } H_2O \times (-0.001858)$$

Adsorbed protein : Determination of adsorbed protein on the surface of fat globules was performed by the procedure of Casiraghi *et al.* (2002). Total protein of the ice cream mix was determined by Kjeldahl method (AOAC, 2000 method 930.33) using a nitrogen to protein conversion factor of 6.38. An aged mix sample was centrifuged at room temperature (20-22°C) for 30 min then placed in a freezer at -30 °C for 45 min. The cream layer was removed, and the sedimented casein was resuspended in the serum phase that its content of protein was also determined. The percentage of AP was calculated using the following equation:

$$AP = \left(\frac{TP - SP}{TP} \right) \times 100$$

where AP: adsorbed protein.
TP: percentage of total protein.
SP: percentage of serum protein.

Viscosity measurements : Viscosity of aged mixes was measured using a controlled-stress dynamic rheometer (SR 5000, Rheometric Scientific, Piscataway, NJ, USA) at 5 °C with concentric cylinder geometry (32 mm ID cup and 29 mm diameter cylindrical probe). Viscosity was determined at shear rates ranging from 10 to 300 s^{-1}. Results were expressed as the apparent viscosity (mPa.s) determined at shear rate of 300 s^{-1}.

Whipping ability : Whipping ability of ice cram mixes was evaluated by measuring changes in the mix volume (350 ml) during whipping for 20 min at 5 min interval (Baer *et al.*, 1999).

Analyses of ice cream

Overrun : Overrun was calculated by the following equation (Adapa *et al.*, 2000):

% overrun = [(mass of unit volume of mix – mass of unit volume of ice cream) / mass of unit volume of ice cream] x 100

Meltdown test : Melting rate was determined by the method of Segall and Goff (2002). Ice cream samples were allowed to melt at room temperature (20-22°C), and the melted ice cream was collected and weighed every 10 min. A plot was constructed using % mass loss as a function of time. This plot was sigmoidal in shape with a linear region of maximal melting rate. The % mass loss / min in this linear region (slope) was used to compare the meltdown characteristics of the different samples.

Fat destabilization index : The absorbance of diluted solutions (1:500) of ice cream mix and melted ice cream samples at 540 nm was measured using deionized water as a blank. Fat destabilization index was calculated by applying the following equation (Goff & Jordan, 1989):

$$\text{Fat destabilization index} = [(A_{mix} - A_{ice\ cream}) / A_{mix}] \times 100$$

Where A_{mix} : the absorbance of diluted ice cream mix
$A_{ice\ cream}$: the absorbance of diluted melted ice cream

Replication and statistical analysis

Mixes preparing and freezing were triplicated and duplicate analyses were performed on each replicate. Analysis of variance was performed by the SAS General Linear Methods (SAS, 1994) and differences were considered significant at $P < 0.05$.

Results and Discussion

Fractions characteristics

Table 1 depicts triacylglycerol profile of BO and its fractions. With increasing SMP of the fractions, high-melting triacylglycerol (HMTAG) increased with a corresponding decrease in low-melting triacylglycerol (LMTAG). These compositional differences reflected on the fractions thermal profile. HMF exhibited (Table 2) the highest enthalpy (ΔH_f) followed by MMF then LMF.

Analyses of ice cream mix

Freezing point

Ice cream mixes made with different fractions showed no differences in their freezing point (Table 3) which can be attributed to similarity in mixes formulation. Baer and Czmowski (1985) reported a freezing point of - 2.83°C for an ice cream mix containing 10 % fat, 15 % sugar and 11 % MSNF .

TABLE 1. Triacylglycerol composition of buffalo butter oil and its fractions used in the manufacture of ice cream (mg/100mg)[1].

TAG[2] carbon number	BO[2]	LMF[2]	MMF[2]	HMF[2]
C_{24}	0.46±0.01^b	0.71±0.02^a	0.25±0.01^c	0.04±0.02^d
C_{26}	0.57±0.01^b	0.77±0.03^a	0.72±0.04^a	0.05±0.03^c
C_{28}	0.91±0.02^c	1.61±0.05^a	1.33±0.05^b	0.37±0.02^d
C_{30}	1.28±0.01^b	1.88±0.05^a	1.25±0.11^b	0.25±0.02^c
C_{32}	2.38±0.02^b	2.93±0.06^a	1.88±0.18^c	0.63±0.02^d
C_{34}	7.99±0.12^b	9.61±0.03^a	6.36±0.20^c	4.75±0.54^d
C_{36}	16.22±0.13^a	15.80±0.06^b	16.12±0.31^{ab}	12.70±0.69^c
C_{38}	18.40±0.42^a	18.64±0.11^a	20.58±0.41^b	16.60±0.45^c
C_{40}	12.20±0.04^b	14.04±0.03^a	14.25±0.31^a	11.9±0.39^b
C_{42}	5.56±0.11^b	5.36±0.12^{bc}	5.25±0.16^c	6.03±0.12^a
C_{44}	3.99±0.14^b	3.72±0.04^{bc}	3.52±0.13^c	5.29±0.39^a
C_{46}	5.08±0.12^b	4.15±0.25^c	4.90±0.10^b	7.13±0.15^a
C_{48}	6.93±0.19^b	5.61±0.12^c	5.74±0.18^c	10.19±0.31^a
C_{50}	8.10±0.05^b	5.67±0.08^d	7.18±0.27^c	11.16±0.55^a
C_{52}	7.57±0.04^b	6.19±0.08^c	6.97±0.36^b	10.67±0.67^a
C_{54}	2.36±0.18^c	3.31±0.03^b	3.70±0.26^a	2.24±0.10^c
C_{24}-C_{34}	13.59±0.13^b	17.51±0.23^a	11.79±0.47^c	6.09±0.51^d
C_{36}-C_{40}	46.82±0.53^c	48.48±0.07^b	50.95±0.25^a	41.2±1.00^d
C_{42}-C_{54}	39.59±0.51^b	34.01±0.29^d	37.26±0.67^c	52.71±1.22^a

Different letters within the same row are significantly different (P< 0.05).
[1] Mean ± S.D., *n*=3.
[2] TAG, triacylglycerol; BO, butter oil; LMF, low-melting fraction; MMF, middle-melting fraction; HMF, high-melting fraction.

TABLE 2. Enthalpy (ΔH_f) and slip melting point (SMP) of buffalo butter oil and its fractions used in the manufacture of ice cream[1].

	ΔH_f (j/g)	SMP (°C)
BO[2]	44.53±2.32^b	34.7±0.10^b
LMF[2]	15.81±0.94^d	12.6±0.59^d
MMF[2]	33.47±1.79^c	29.4±0.38^c
HMF[2]	61.21±1.50^a	41.3±0.61^a

Different letters within the same column are significantly different (P< 0.05).
[1] Mean ± S.D., *n*=3.
[2] BO, butter oil; LMF, low-melting fraction; MMF, middle-melting fraction; HMF, high-melting fraction.

TABLE 3. Characteristics of ice cream mixes made with buffalo butter oil and its fractions[1].

Characteristic	BO[2]	LMF[2]	MMF[2]	HMF[2]
Freezing point (°C)	-2.80±0.01[a]	-2.80±0.02[a]	-2.80±0.01[a]	-2.80±0.01[a]
Viscosity (mPa.s)	256.67±2.20[b]	168.20±3.72[d]	199.43±4.43[c]	296.50±3.79[a]
Adsorbed protein (%)	17.08±0.31[c]	22.28±0.36[a]	19.31±0.10[b]	13.61±0.28[d]

Different letters within the same row are significantly different (P < 0.05).
[1]Means ± S.D., *n*=3.
[2] BO, butter oil; LMF, low-melting fraction; MMF, middle-melting fraction; HMF, high-melting fraction.

Adsorbed protein

Table 3 shows the percentage adsorbed protein on the surface of fat globules in ice cream mixes. The lower the melting point of the fraction used, the higher the adsorbed protein. Differences among the mixes were significant (P<0.05). The results obtained may be ascribed to the differences in the TAG profiles of the fractions, which were substantially altered by the sequential crystallization process used for preparing these fractions (Fatouh *et al.*, 2003). Data presented in Table 1 reveal that, HMTAG (C_{42}-C_{54}) were concentrated in HMF representing more than 50% of the total composition. HMTAG gradually decreased going from MMF to LMF.

In the model of fat globule membrane in ice cream presented by Berger and White (1976), HMTAG form the shell that surrounds the liquid core (LMTAG). Adsorption of the serum proteins to the oil droplet surface is based on the presence of hydrophobic segments of the protein molecule, which penetrate the outer TAG layers of the oil droplet. The affinity of hydrophobic protein segments to lipids in the liquid state is much stronger than to crystallized lipids which can not dissolve protein (Krog, 1991). The trend found in the present study is in agreement with that of Abd El-Rahman *et al.* (1997).

Viscosity

Viscosity of ice cream mixes is illustrated in Table 3. The mix made with HMF revealed higher viscosity than mixes made with the rest of the fractions. This might be due to the presence of a greater amount of saturated fatty acids (>80% of the total composition) (data not shown) which solidifies at aging temperature (5°C) increasing the mix viscosity (Abd El-Rahman *et al.*, 1997). Im *et al.* (1994) reported higher viscosity values for ice cream mix made with milk fat only than that made with a milk fat-vegetable oils mix (canola and soybean oil) that is rich in unsaturated fatty acids.

Whipping ability

A comparison between volumes of ice cream mixes at different times of whipping (Table 4) exhibit significant differences (P<0.05) amongst the mixes.

A substantial increase of 97, 87and 77% in the initial volume (350 ml) of LMF, MMF and HMF mixes was observed after whipping for 5 min, respectively. As whipping continued, the increase in volume diminished, indicating decreased whipping ability. Marshall *et al.* (2003) reported that, as mix viscosity increases, the resistance to melting and the smoothness of texture increases, but the rate of whipping decreases. Mixes viscosity increased going from mix prepared with LMF to MMF and the highest viscosity was shown by mix prepared with HMF (Table 3).

TABLE 4. Whipping ability (mix volume (ml)) of ice cream mixes made with buffalo butter oil and its fractions[1].

Fraction	Time (min)				
	0	5	10	15	20
BO[2]	350±0.0[a]	640±5.0[c]	680±10.0[c]	700±10[c]	720±5.0[c]
LMF[2]	350±0.0[a]	690±10.0[a]	750±10.0[a]	785±10[a]	810±10.0[a]
MMF[2]	350±0.0[a]	655±10.0[b]	700±10.0[b]	735±5.0[b]	760±5.0[b]
HMF[2]	350±0.0[a]	620±5.0[d]	650±5.0[d]	670±5.0[d]	685±10.0[d]

Different letters within the same row are significantly different ($P < 0.05$).
[1]Means ± S.D., n=3.
[2] BO, butter oil; LMF, low-melting fraction; MMF, middle-melting fraction; HMF, high-melting fraction .

Analyses of ice cream

Overrun

Table 5 presents overrun values of the different ice creams. Overrun gradually decreased ($P<0.05$) with increasing SMP of the fraction used. Ice cream prepared with LMF exhibited the highest overrun (34.85%) among all ice creams, while ice cream prepared with HMF had the lowest (16.12%). These results may be attributed to mixes viscosity (Table 3). With increasing the mix viscosity, the ability of incorporating air cells in the mix tends to decreases, thus decreasing the overrun (Marshall *et al.*, 2003).

Melting resistance

Differences in fractions enthalpy (Table 2) led to change the meltdown characteristic of the ice creams (Table 5). Differences in the enthalpy were consistent with fractions chemical composition. HMTAG were abundant in HMF and their concentration gradually decreased in MMF and LMF, respectively, and vice versa LMTAG (C_{24}-C_{34}). The data suggest that, using HMF in ice cream manufacture would provide improved "stand up" properties of ice cream. The trend of the data confirmed other studies. Abu-Lehia *et al.* (1989) reported higher melting resistance for ice cream made with camel milk fat (SMP, 42.0°C) that made with bovine milk fat (SMP, 31.5°C). Im *et al.* (1994) found that, the higher enthalpy of milk fat than milk fat-vegetable oil mix led to increasing the melting resistance of ice cream made with the former fat than the later.

TABLE 5. Characteristics of ice cream made with buffalo butter oil and its fractions[1].

Characteristic	BO[2]	LMF[2]	MMF[2]	HMF[2]
Overrun (%)	24.39±1.34^{c}	34.85±1.48^{a}	29.46±1.27^{b}	16.12±1.54^{d}
Meltdown rate(% min^{-1})	1.15±0.01^{c}	1.60±0.04^{a}	1.37±0.01^{b}	0.9±0.02^{d}
Fat destabilization index	3.53±0.32^{b}	5.81±0.12^{a}	1.67±0.09^{c}	0.34±0.03^{d}

Different letters within the same row are significantly different (P < 0.05).
[1]Means ± S.D., *n*=3.
[2] BO, butter oil; LMF, low-melting fraction; MMF, middle-melting fraction; HMF, high-melting fraction.

Fat destabilization

Fat destabilization index of ice cream made with BO and its fractions is shown in Table 5. Fat destabilization index decreased (P<0.05) as SMP of the fraction used increased. Marshall *et al.* (2003) reported that, fat globules in ice cream mix are in a partially solidified state based on the broad melting range of TAG. HMTAG are crystalline in aged ice cream mix, while LMTAG are in the liquid form. Table 1 reveals that, the lowest content (P<0.05) of LMTAG was observed for HMF which, simultaneously, was enriched in HMTAG. The opposite was observed for LMF. Increased solidified fat in the milk fat globule reduces shear sensitivity (by increasing the rigidity of the globule) and thus results in less fat destabilization. Conversely, decreased solidified fat increases the susceptibility of the fat globule to be ruptured by the shear forces (from the blades) during freezing (Adleman and Hartel, 2002). The result found in this study in agreement with the trend reported by Abd El-Rahman *et al.* (1997).

Conclusion

The ice cream mix made with HMF revealed higher mix viscosity and lower fat destabilization than those made with the rest of the milk fat fractions. Such results impart a beneficial body to the ice cream and reduce iciness as indicated by increasing the melting resistance.

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(*Received* 2 / 8 / 2006;
accepted 7 / 11 / 2006)

تأثير إضافة شقوق دهن اللبن الجاموسي على الخواص الطبيعية للأيس كريم

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تم في هذا البحث تحضير شقوق دهن اللبن الجاموسي مختلفة نقطة الانصهار باستخدام طريقة البلورة متعددة المراحل في صناعة الأيس كريم ، وأشارت النتائج إلى :-

– وجود فروق معنوية ما بين لزوجة مخاليط الأيس كريم، حيث كانت أقل القيم المسجلة للمخلوط الذي تم تحضيره باستخدام الشقوق المنخفضة في نقطة الانصهار، بينما أعلى هذه القيم كان في المخلوط الذي تم تحضيره بالشقوق المرتفعة في نقطة الانصهار. وعلى النقيض من ذلك فقد انخفضت كل من كمية البروتين الممتص وكذلك القابلية للخفق تدريجيا وذلك بزيادة نقطة انصهار الشق المستخدم في تحضير المخلوط .

– معدل انصهار الايس كريم المصنع باستخدام الشقوق المنخفضة في نقطة الانصهار أعلى من ذلك المصنع باستخدام الشقوق المرتفعة والمتوسطة في نقطة الانصهار. كذلك لوحظ أن الريع قد تأثر عكسيا بلزوجة المخلوط بينما لم تتأثر صلابة الأيس كريم الناتج بعد التجميد ما بين الأنواع المحضرة بالشقوق المختلفة. أشارت النتائج أيضا إلى أن قيم عدم ثبات الدهن قد انخفضت تدريجيا بزيادة محتوي الشقوق المستخدمة في تحضير الأيس كريم من الأحماض الدهنية المشبعة. والخلاصة أن الأيس كريم المصنع من شق الدهن المرتفع في نقطة الانصهار أعطي أعلي لزوجة للمخلوط وأقل قيمة لمعامل ثبات الدهن. وهذه النتائج ذات تأثير ايجابي علي قوام الأيس كريم وتقلل من ظهور عيب التركيب البنائي الثلجي ، ويؤيد ذلك ملاحظة زيادة مقاومة الأيس كريم للانصهار.