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Application of low calorie hypocholesterolemic structured lipid as potential bakery fat

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Abstract

Low calorie structured lipid (SL1) containing essential fatty acids and having hypocholesterolemic activity was prepared by enzymatic interesterification of sunflower oil with ethyl behenate. DSC studies indicated the similarity in melting profile of SL1 with that of commercial bakery shortenings. The present study was designed to evaluate the effect of replacing bakery fat with SL1 in the preparation of biscuits and cakes on the sensory and acceptability of these products. The organoleptic and quality characteristics of the biscuits prepared with SL1 are comparable with those of biscuits prepared with commercial bakery fat. However, such replacement during the preparation of cake requires the addition of emulsifier to have the product indistinguishable from that prepared using traditional bakery fat. The bakery product prepared with SL1 is low in calorie and free from *trans* fats. Thus the utility of novel fat with lower calorific value and having hypocholesterolemic activity in product preparation is demonstrated.

Introduction

Structured lipids are beneficial for human nutrition as they can be tailor-made to target specific diseases and metabolic conditions (Haumann, 1997). They can also be tailored to provide reduced calorie by specifically positioning select fatty acids in the glycerol backbone. Such reduced calorie fats are normally designed by taking advantage of either limited absorption of long chain saturated fatty acids or lower caloric density of short chain saturated fatty acids (Wardlaw *et al*., 1995; Haumann, 1997). Several such low calorie structured lipids are reported in the literature; notable among them are Caprenin, Benefat, MCT, Captex (Gunstone, 1998; Osborn and Akoh, 2002). Behenic acid, a long chain saturated fatty acid having 22 carbon atoms have been extensively used in designing many such structured lipids (Jandacek *et al*.,1993; Tynek *et al*., 2005). Behenic acid containing triacylglycerols are considered as digestive retardant (Arishima *et al*., 2009). However, most of these reported structured lipids contain unnatural fatty acids and are mostly devoid of essential fatty acids. Nutritionally improved structured lipids (SL1) containing behenic acid and essential fatty acids was prepeared by enzymatic interesterification of sunflower oil with ethyl behenate, whose calorific value was estimated to be 5.36 kcal/g (Kanjilal *et al*.,1999). Thus, SL1 contained 28-29% behenic acid and 38-40% linoleic acid (LA). The metabolic studies conducted on rats indicated the beneficial effects of this type of structured lipid on serum lipid parameters, lowering cholesterol, LDL cholesterol and triglycerides in rats, given normal diets as well as atherogenic diets (Kanjilal *et al*., 2013). Moreover, lipid accumulation in arteries of rabbits given atherogenic diets with SL1 was also reduced significantly. Thus the low calorie structured fats have the additional benefit of reducing serum and liver lipids which are considered to be risk factor for cardiovascular diseases.

Fat is an important ingredient in cake- and biscuit-making. Bakery fats normally contain approximately 15 – 30% of *trans* fatty acids, which are deleterious to health (Hunter, 2005). *Trans* fatty acids are produced during hydrogenation. Interesterification is an ideal method to replace hydrogenation and is a useful tool to prepare *trans*-free plastic fat for bakery fat application (Ahmadi and Marangoni, 2009). Interesterified structured lipids with improved

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nutritional and functional properties are often used in the confectionery products (Ghotra *et al.*, 2002; Tynek and Ledochowska, 2005). They can also be used in the preparation of bakery products if they have properties of bakery fats. In the present study, melting profiles of SL1 was evaluated and compared with commercial bakery fat for studying their suitability in the preparation of biscuits and cakes. These two bakery products were prepared by standard procedures using conventional bakery fats or by fully replacing bakery fat with SL1. The products were then evaluated for sensory and organoleptic properties in comparison to those prepared from traditional bakery fat. The final products, cake and biscuit will not only be *trans*-free but also will have additional health promoting parameters such as low in calorie and hypocholesterolemic properties. Thus the utility of a novel low calorie hypocholesterolemic fat in the preparation of cake and biscuit is demonstrated in this study.

Materials and Methods

Materials

Refined sunflower oil of well-known brands was purchased from the local market. The lipase, lipozyme TL IM was purchased from Novozymes A/S, (Bagsvaerd, Denmark). Ethyl behenate was a generous gift of M/s VVF (Mumbai, India). All the solvents needed for the preparations as well as analysis are of AR grade and purchased from Ranbaxy Fine Chemicals Ltd. (New Delhi, India). All the general ingredients required for the preparation of biscuit and cake was obtained from the local market. Glycerol monostearate was procured from Biocon India Pvt. Ltd. (Bangalore, India). Shortening (Margarine) was obtained from Hindustan Lever Ltd. (Mumbai, India).

Preparation and physicochemical characterization of structured lipid

Structured lipid (SL1) was prepared according to the procedure reported earlier (Kanjilal *et al.*, 1999; Kanjilal *et al.*, 2013). The acid value (Method Cd 3d-63), peroxide value (Method Cd 8-53), iodine value (Method Cd 1-25), saponification value (Method Cd 3-25), and slip melting points (Method Cc 3-25) of SL1 were determined following AOCS methods (Firestone, 2004). The fatty acid composition of SL1 was determined by GC (Kanjilal *et al.*, 2013)

Differential scanning calorimetry (DSC)

Mettler (Zurich, Switzerland) DSC-30 was used to obtain the melting and crystallization characteristics of the structured lipids. The heat flow of the instrument was calibrated using Indium. The PT-100 sensor was calibrated using indium, zinc and lead. To ensure homogeneity and to destroy all crystal nuclei, the samples were heated to 60°C. About 15 mg of molten sample was accurately weighed into a standard crucible and that was crimped in place. An empty crucible with a pierced lid was used as a reference. For melting characteristics the samples were stabilized according to the IUPAC method (Paquot and Hautfenne, 1987) which includes keeping the samples at 0°C for 90 min, 26°C for 40 h and then again at 0°C for 90 min prior to introduction into the DSC cell. Thermograms were recorded by heating at the rate of 2°C per minute from -5 to 60°C. The peak temperatures, heats fusion (ΔH), and % liquid at various temperatures were recorded directly using a TC-10A data processor and STARe programme. The solid fat content (SFC) was calculated from percent liquid and the melting profiles were drawn by plotting SFC against temperature.

Preparation of bakery products

Bakery products namely biscuits and cakes were prepared by standard procedures using conventional bakery fats (Marvo and margarine) or using SL1 according to the method described in the literature (Manohar and Rao, 1999; Sowmya *et al.*, 2009).

Preparation of biscuits

Biscuits were prepared using a method reported by Manohar and Rao (1999). The ingredients used in the preparation of biscuits are: flour (100 g); sugar powder (30 g); skimmed milk powder (2 g); glucose (2 g); salt (1 g); ammonium bicarbonate (1 g); sodium bicarbonate (0.5 g); vanilla flavour (0.2 ml) and water (as per the requirement). The control biscuits contained bakery shortenings at 20 g level, which was fully replaced by SL1 in experimental biscuits.

Preparation of cake

Formulations used for making cakes is: wheat flour (100 g); sugar (100 g); egg (120 g); calcium propionate (0.5 g); baking powder (0.5 g); salt (0.5 g); acetic acid (0.2 g); water (10 g) and vanilla essence (0.4 g). The control cakes contained bakery shortening at 66 g level which was fully replaced by SL1 in experimental cakes. Formulations were made with or without glycerol monostearate as emulsifier at 0.5% level and cakes prepared following standard protocol (Sowmya *et al.*, 2009).

Physical and sensory evaluation

The physical and sensory characteristics of cake and biscuit were carried out as described earlier (Manohar and Rao., 1999; Ashwini *et al*., 2009; Sowbhagya *et al*., 2011). Sensory analysis of biscuits was carried out by a panel of six experienced judges assigning scores as follows: crust color: 1= pale/dark brown and 10 = golden brown; surface characteristics: 1 = rough and 10 = smooth; crumb color: 1 = brownish and 10 = cream white; texture: 1 = soft/hard/brittle and 20 = crisp; taste: 1 = foreign and typical = 10; mouth feel: 1 = doughy/lumpy and 15 = no residue and those for cakes are based on crust colour: 1= dull brown, 10 = golden brown; crust shape: 1= flat, uneven, 10 = normal; crumb color: 1 = dull, 10 = bright white; crumb grain size: 1 = very coarse, 10 = very fine; texture: 1 = very firm, 20 = very soft; mouthfeel: 1 = gummy/dry, 20 = no residue in mouth. The overall quality score of biscuits and cakes separately was taken as the combined score of all the attributes

Statistical analysis

Data on quality characteristics of cakes and biscuits were statistically analyzed using Duncan's new multiple range tests with different experimental groups appropriate to the completely randomized design with four replicates each as described by Steel and Torrie (1960). The significant level was established at $P \leq 0.05$.

Results

The structured lipid, SL1 was synthesized by enzymatic interesterification of sunflower oil with ethyl behenate (Kanjilal *et al*., 1999; Kanjilal *et al*., 2013). The fatty acid composition (in wt%) of the interesterified product was found to be: 4.2% of palmitic acid, 2.4% of stearic acid, 25.8% of oleic acid, 37.8% of linoleic acid, 1.0% of arachidic acid and 28.8% of behenic acid. The acid value, peroxide value, iodine value and saponification value of SL1 are found to be 0.15, 2.2, 87.0 and 197.4 respectively. The slip melting points of SL1 was found to be in the range of 38-40°C.

Melting characteristics of structured lipids by DSC

Structured lipid, SL1 showed long plastic (melting) range, being soft at normal ambient temperature and containing some solids even at 40°C (Figure 1). Extending melting range of SL1 is due to presence of behenic acid and also presence of heterogeneous types of triacylglycerols (TAG). The melting profile or solid fat content (SFC) of SL1 is similar to that of commercial bakery shortening. However, the SFC of this type of structured lipid was found to be different from that of cocoa butter (CB), which has a very short melting range with high solids at ambient temperature and with no solids at body temperature. This indicated the role of homogeneous and heterogeneous TAGs present respectively in CB and SL1 on their melting characteristic. SL1 showed long plastic range required for usage in bakery. Commercial bakery shortenings contain *trans* fatty acids, whereas SL1 is devoid of *trans* fatty acids. Based on results from DSC studies, it can be predicted that SL1 can be used as a replacement for bakery fat.

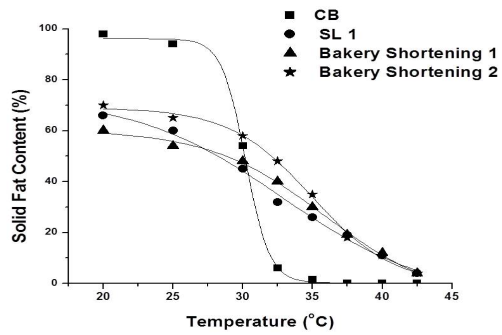

Figure 1. Melting profiles of structured lipid (SL1), commercial bakery shortenings (1 and 2) and cocoa butter (CB)

Preparation of biscuits

SL1 was evaluated in making biscuits which was prepared by standard procedures using conventional bakery fats or by fully replacing bakery fat with SL1. The appearance of biscuits prepared using two different fats was indistinguishable. The organoleptic and quality characteristics of the biscuits prepared with SL1 as compared to that prepared with bakery fat (control) is shown in Table 1. There was no significant difference in the physical as well as sensory parameters of biscuits prepared with bakery fat and those prepared with SL1.

Preparation of cakes

Structured lipid, SL1 was also used for the preparation of cake. The control cake contained bakery shortenings, which was fully replaced by SL1 in the experimental cake. These products were then evaluated for sensory and physical properties. The physical characteristics of cake prepared with bakery shortening (control) and SL1 (experimental) showed that the replacement of bakery fat with SL1 made the batter heavier indicating less air incorporation in the batter. This reflected on the volume of the cake with SL1 which was reduced and the texture was harder

Table 1. Physical and sensory characteristics of biscuits prepared using commercial bakery shortening (control) in comparison to biscuits, prepared using structured lipid, SL1 (experimental)

Parameters	control	experimental
*physical characteristics**		
weight (g)	7.4±0.20 [a]	7.1±0.15 [a]
diameter, D (mm)	56.5±0.31 [a]	56.3±0.25 [a]
thickness, T (mm)	5.50±0.22 [a]	5.25±0.21 [a]
spread ratio (D/T)	10.3±0.45 [a]	10.7±0.35 [a]
breaking strength (g)	1260±5.5 [a]	1180±4.5 [a]
*sensory characteristics***		
surface colour (10)	9±0.11[a]	9±0.13[a]
surface character (10)	9±0.15[a]	9±0.17[a]
crumb colour (10)	8±0.20[a]	8±0.22[a]
texture (20)	17±0.18[a]	17±0.21[a]
taste (10)	9±0.14[a]	8±0.16[a]
mouth feel (20)	18±0.20[a]	17±0.18[a]
total score(80)	70±0.25[a]	68±0.27[a]

[#] measured with an overall score of 80; maximum scores for each parameter is given in parenthesis
Values are means ± standard deviation (n=6).
Values in a row with the same letter in superscript are not significantly different from each other at P ≤ 0.05.

Table 2. Quality characteristics of cake prepared using commercial bakery shortening (Control) in comparison to cake prepared using structured lipid, SL1 with emulsifier (Experimental)

group	batter specific gravity (g/cc)	weight (g)	volume (cc)	specific volume (cc/g)	texture (g, force)
control	0.90 ±0.01 [a]	291 ±0.25 [a]	805 ±3.5 [a]	2.8 ±0.1 [a]	870 ±5.5 [a]
experimental	0.92 ±0.02 [a]	294 ±0.35 [a]	790 ±2.5 [a]	2.7 ±0.1 [a]	865 ±6.5 [a]

Values are means of three replicates ± standard deviation.
Values in a column with the same letter in superscript are not significantly different from each other at $P \leq 0.05$.

than control cake. The sensory characteristics of cake prepared with SL1 are found to be poorer than the control group. In order to improve the quality of cake prepared with SL1, specific emulsifier (glycerol monostearate) was added in the formulation. The experimental cake is found to be indistinguishable from the control cake. The physical and sensory characteristics of experimental cake are at par with those of control cake (Tables 2 and 2).

Discussion

With increasing consumer awareness of the risks associated with high fat intake for chronic diseases and for obesity, the market for reduced calorie fat is substantial (Akoh, 1995; Osborn and Akoh, 2002). Currently carbohydrate- and protein-based fat replacers are available. But these have inherent problems when exposed to high temperatures (Mela, 1996; Osborn and Akoh, 2002,). To overcome this, lipid-based fat substitutes are preferred, which can be used in cooking and deep fat frying applications. Reduced calorie fats are normally designed by taking advantage of limited absorption of long chain saturated fatty acids and/or lower caloric density of short chain saturated fatty acids (Wardlaw *et al*., 1995; Haumann, 1997). Caprenin, Salatrim, MCT, Captex etc are some of the popular low calorie structured fats (Wardlaw *et al.*, 1995; Gunstone 1998; Osborn and Akoh, 2002; Webb and Sanders, 1991). One of the drawbacks of these low calorie fat substitutes is that they lack essential fatty acids. This may lead to essential fatty acid deficiency if consumed over a long period of time. In addition to triglyceride based structured lipids, low calorie fats have also been prepared as carbohydrate esters. These include sucrose fatty acid polyester known as olean or olestra developed by Procter and Gamble Company (Peters *et al*, 1997), sorbitol fatty acid polyester known as Sorbestrin developed by Pfizer (Akoh and Swanson, 1989).

To overcome the drawbacks associated with low

Table 3. Sensory Characteristics of cake prepared using commercial bakery shortening (Control) in comparison to cake prepared using SL1 with emulsifier (Experimental)

sensory characteristics #	control	experimental
	crust	
color (10)	9 ± 0.2 [a]	9±0.4 [a]
shape (10)	9±0.2 [a]	9±0.3 [a]
	crumb	
color (10)	8±0.5 [a]	7±0.3 [a]
grain (10)	9±0.3 [a]	9±0.4 [a]
texture (10)	9±0.2 [a]	9±0.5 [a]
mouthfeel (10)	9±0.4 [a]	9±0.2 [a]
overall quality (60)	53±1.2 [a]	52±1.5 [a]

#Maximum scores for each parameter are given in parenthesis
Values in a row with the same letter in superscript are not significantly different from each other at $P \leq 0.05$

calorie structured fats, novel structured lipid (SL1) is developed in our laboratory (Kanjilal *et al*., 1999; Kanjilal *et al*., 2013). SL1 contains not only long chain saturated fatty acid, like behenic acid (C22:0) but also contains essential fatty acids (EFA), like linoleic acid (C18:2). Such EFA-enriched low calorie structured fat is not available in the market. The major objective of this investigation is to find a suitable application of such low calorie structured fat, developed from natural oil using biotechnological tools such as lipase catalyzed interesterification. The choice of behenic acid was based on the observations that long chain saturated fatty acids are poorly absorbed and hence will not enter into system through digestion (Tynek and Ledochowska, 2005; Arishima *et al*., 2009). Earlier work carried out by us on SL1 indicated its calorific value to be 5.36 kcal/g (Kanjilal *et al*., 1999; Kanjilal *et al*., 2013). The studies using rats on restricted diet intake and ad libitum feeding studies indicated no adverse effects on growth and food intake. Moreover, the nutritional studies of this type of structured lipids, conducted on rats and rabbits fed on normal and atherogenic diet indicated their hypocholesterolemic activity. Thus the novel low calorie structured fats have the additional benefit of reducing serum and liver lipids which are considered to be risk factor for cardiovascular diseases (Kanjilal *et al*., 2013).

The evaluation of SL1 for physical properties using DSC indicated the similarity in melting profile of SL1 with that of commercial bakery shortenings. Bakery shortenings are hydrogenated fats and contain deleterious *trans* fatty acids. SL1 is devoid of *trans* fatty acids but has melting characteristics similar to that of bakery shortenings (Figure 1). The organoleptic and quality characteristics of the biscuits prepared with SL1 are compared with those of biscuits prepared with bakery fat. There was no significant difference in the quality and sensory parameters of biscuits prepared with bakery fat and those prepared with SL1. However, such replacement during the preparation of cake made the batter heavier indicating less air incorporation. The volume of the cake with SL1 was reduced and the texture was harder than control cake. The sensory characteristics of cake with SL1 were also not up to the mark. This was also overcome by the addition of specific emulsifier and the product obtained from such formulation is practically indistinguishable from the one prepared from traditional bakery fat.

Conclusions

Low calorie structured lipid (SL1) containing behenic acid was studied for their applicability as a replacement for bakery fat. The DSC studies of SL1 indicated its similarity in thermo physical properties with bakery fats. Therefore SL1 was used for making bakery products such as cakes and biscuits. The organoleptic and quality characteristics of the biscuits and cakes prepared with SL1 is practically indistinguishable from the one prepared from traditional bakery fat. This indicates that the bakery fats can be fully replaced by the studied structured lipid to prepare *trans*-free low calorie cakes and biscuits. Thus the utility of a novel low calorie fat in product preparation is demonstrated.

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