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Formulation of a Functional Fat Spread Stabilised by Natural Antioxidants and Emulsifiers

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ABSTRACT

Introduction: Essential fatty acids (EFAs) play a vital role in the human body and need to be taken through a regular diet. EFAs are susceptible to autoxidation, hence the stability of the EFAs and their products is a matter of concern. **Methods**: Margarine containing sunflower oil as a carrier of EFAs was prepared and the effects of water content, incorporation of EFAs, emulsifiers and antioxidants on the physical properties of margarine, that is, slipping point, dropping point and spreadability were studied. The oxidative stability of the formulated margarine was also evaluated after incorporation of EFAs and antioxidants. **Results:** The incorporation of EFAs in the form of sunflower oil resulted in improved physical properties especially spreadability. The study revealed that up to 45% sunflower oil can be incorporated using glycerol monostearate as an emulsifier with total fat to water ratio of 85:15. Lecithin imparted better spreadability and grainy structure but is known to be susceptible to microbial attack. The capsicum oleoresin showed good activity as an antioxidant. Further addition of kalonji seeds ethanol extract (KEE) as well as curcuminoids resulted in improved spreadability but showed a decrease in oxidation stability. **Conclusion:** A stable and nutritional margarine was developed with the addition of natural antioxidants. Consumers can avail the benefits of both the EFAs and natural antioxidants in the margarine.

Keywords: Margarine, antioxidants, capsicum oleoresin, curcuminoids, kalonji seeds extract

INTRODUCTION

The nutritional quality of food can be improved by increasing its concentrations of nutrients, and by adding or improving the bioavailability of a particular component (Roberfroid, 2002). The nutritive content of margarine has been enhanced by incorporating essential fatty acids (EFAs) through the use of vegetable oils like sunflower oil, soybean oil and safflower oil as a source of EFAs. However, owing to the tendency of EFAs to undergo autoxidation, their stability becomes a matter of concern in product development. Consequently, the addition of antioxidants at proper concentrations has become an important step when designing a food product containing EFAs. Synthetic antioxidants such as tertiary butylhydroquinone (TBHQ),

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butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are used to stabilise the products containing EFAs. However, these antioxidants may be toxic (Gharavi, Haggarty & El-Kadi, 2007). Thus, research has diverted towards the use of natural compounds as a source of antioxidants.

Capsicum (Nag, 2000), curcuminoids (Jayaprakasha, Rao & Sakariah, 2006) and kalonji seeds (Singh *et al*., 2005), commonly used as food ingredients are known to have antioxidant effects. These products are also believed to confer medicinal benefits. Capsicum possesses various therapeutic actions including anti-inflammatory and antiseptic effects (Mason *et al*., 2004) and treatment of cutaneous allergy and neurological disorders (Palevitch & Craker, 1995). Curcumin, a major component of curcuminoids has medicinal properties against various diseases such as anorexia, coughs, diabetes, hepatic disorders, rheumatism and Alzheimer disease (Wang *et al*., 2008). Likewise, the seeds of kalonji are known to have several therapeutic uses (Machmudah *et al*., 2005) such as anticarcinogenic, antibacterial, anti-inflammatory, analgesic and antipyretic benefits.

In the present study, margarine was formulated using partially hydrogenated fat, sunflower oil, water and emulsifier by simultaneous additions of capsicum oleoresin, curcuminoids and kalonji seeds extract at various concentrations and combinations. Both EFAs and antioxidants are known to affect physical properties like slipping point, dropping point and spreadability. In this regard, a study of oxidative stability and physical properties of the formulated margarine were carried out.

METHODS

Fat base

Refined sunflower oil without antioxidant (RSFO) was received from M/s Cargill India Pvt. Ltd., New Delhi. The hydrogenated fat (K-care; melting point 38.8°C, Iodine Value 47.5% I_2/g and *trans* fatty acid less than 1%) was procured from Kamani Oil Industries Pvt. Ltd., India. Glycerol monostearate (GMS) was procured from Fine Organics, India. Soy lecithin was procured from M/s V.R. Chemicals, Mumbai. Capsicum oleoresin and curcuminoids powder were obtained from Kancor India Ltd., Angamaly South, India. Kalonji seeds were procured from a local vendor. All other chemical reagents and solvents were obtained from s.d. fiNE-CHEM LiMiTEd, Mumbai.

Extraction of kalonji seeds

Kalonji seeds were crushed and 50 g of crushed seeds were extracted with 250 mL ethanol in a Soxhlet extractor. The kalonji seeds ethanol extract (KEE) was obtained after removal of solvent using rotary vacuum evaporator.

Analysis of capsicum oleoresin

The presence of capsaicinoids was checked for Capsicum oleoresin in isopropanol (Gonzalez & Tamirano, 1973; Markey *et al*., 2007) and by UV spectrometry (Shimadzu UV-1601).

IR spectrophotometry

The functional groups present in capsicum and KEE were confirmed by infrared spectrometry (Shimadzu 8400S FTIR spectrometer).

Preparation of margarine

Fat base (80% or 85% w/w) and emulsifiers (1% to 3% w/w) were gradually heated to melt at 50 ± 5°C on a water bath, and continuously stirred with a three bladed stirrer at 200 rpm to dissolve the emulsifier; further, a known quantity of warm water containing 1 g of common salt was added drop wise for 5 min with stirring continued for the next 20 min. The mixture was cooled for about 5 min (till pourable consistency) using a cold water bath at 15°C. The antioxidants were added to the fat base prior to preparation of margarine during the study.

Study of antioxidant activity

The various blends of margarine containing a combination of capsicum oleoresin (1.5%, 2%, 2.5% w/v), curcuminoids (50 ppm) and KEE (2% w/v) were prepared. The oxidative stability of margarine was checked after 30 days at 10°C according to the AOCS Official Methods for peroxide value (AOCS, 2011). All the experiments were carried out in triplicate and the values were expressed as arithmetic mean of the experiments.

The antioxidant activities of natural antioxidants were compared with a control sample and TBHQ (200 ppm) under the same conditions. The relative antioxidant activities were compared using Oxidative Factor (OXF) for antioxidants using the following formula (Ghada & Vassiliki, 2007):

$$OXF = \frac{(PV_{final} - PV_{initial})\ antioxidant}{(PV_{final} - PV_{initial})\ control}$$

where PV indicates the mean values of all triplicate determinations of the peroxide value.

Physical properties of margarine

The physical properties of margarine (slipping point, dropping point and spreadability) were determined after 24 hours of preparation (Rege *et al.*, 2012). Slipping point (SP) was determined by using an aluminium tube (6 mm *i.d.* X 7 mm, 2 mm thickness) open at both ends, filled with margarine and suspended in cold water using a thin wire. The temperature was slowly raised while stirring the water. Slipping point was noted as the temperature at which the column slips from the tube. Dropping point (DP) was determined by using the glass tube (7 mm *i.d.*, with 1 mm thickness) open at both ends, filled with margarine and suspended in a beaker with lid. The beaker was kept in a water-bath with the temperature slowly raised while stirring the water. Dropping point was noted as the temperature at which the column dropped from the tube. Spreadability was determined by spreading 1 g sample uniformly in the circle of 2 cm diameter on a glass plate. Another glass plate (weighing 244.26 g) was placed on the circle and the increase in area in 60 sec was measured as spreadability of margarine.

RESULTS AND DISCUSSION

Analysis of raw materials

The presence of capsaicinoids in capsicum oleoresin was confirmed by the peak at 281 nm in the UV spectrum, given by the solution of capsicum oleoresin in isopropanol (Gonzalez & Tamirano, 1973; Markey *et al.*, 2007). The FTIR of capsicum oleoresin showed the presence of phenolic –OH group (3433 cm^{-1}), alkyl chains (2924 & 2852 cm^{-1}) and amide group (1741 cm^{-1}). The FTIR spectrum of KEE showed the presence of phenolic –OH group (3358 cm^{-1}), alkyl chains (2924 & 2854 cm^{-1}) and carbonyl group (1713 cm^{-1}).

Effect of hydrogenated fat: water ratio and emulsifiers on physical properties of margarine

Margarine can be considered as a network of solid emulsion, in which voids are filled with liquid fat and water. The slower crystallisation process leads to a decrease in solid fat content and hardness, as well as the aggregation of small crystalline particles into larger ones (Acevedo, Peyronel & Marangoni, 2011).

The crystallisation rate was higher for the samples formed by rapid cooling. As the cooling rate increases, the crystallisation takes place in a shorter period of time affecting the nucleation and crystal growth mechanisms. With rapid crystallisation of higher melting fat components, solid clusters within the liquid phase are formed at a faster rate (Toro-Vazquez *et al.*, 2001) that is accompanied by a rapid increase in viscosity, severely limiting mass transfer and ultimately forming mixed crystals (Campos, Narine & Marangoni, 2002). The forces which stabilise a fat crystal network are van der Waals forces (Acevedo *et al.,* 2011) along with possibly other forces such as steric, coulombic and solid bridges (Sato, 2001). The number of possible interactions between crystal particles will be different as the number of interactions is proportional to the number of particles present. Consequently, in a network composed of large particles, the attractive forces will be weaker. On the other hand, rapidly crystallised samples have a greater number of interactions between crystal particles and are therefore harder (Campos *et al.,* 2002). Emulsion tightness is a function of processing, emulsifier content and formulation of the aqueous phase (Chrysan, 2005).

The effect of type and concentration of emulsifiers on the physical properties of margarine with hydrogenated fat: water ratio of 80:20 and 85:15 is given in Table 1 and Table 2 respectively. The type and amount of emulsifiers affected the rheological properties of margarine. Margarine containing lecithin showed slightly higher SP and DP than that containing GMS with higher spreadability and sandy appearance. The time taken to get product of pourable consistency was much longer in the case of lecithin. This can be explained by the crystal inhibition action of lecithin that reduced the rate of crystallisation resulting in better spreadability and grainy texture. However, when the mixture of GMS and lecithin was used, SP and DP were increased and spreadability was decreased. The improvement was due to better wetting properties of GMS and lecithin as the emulsifier improves the wetting property of the solid fat clusters. Lecithin, being an anti-splattering agent, has an additional advantage for frying application whereas it is not required for spreads. Nevertheless, the products prepared using lecithin are susceptible to microbial attack. Hence, in further work, the

Table 1. Effect of emulsifier concentration on the properties of margarine containing hydrogenated fat: water in the ratio of 80: 20

Emulsifier (w/w)	Dropping point† (°C)	Slipping point† (°C)	Spreadability† (cm^2)
GMS (1.5%)	45.3 ± 0.2	43.5 ± 0.2	2.37 ± 0.11
Lecithin (1%)	46.0 ± 0.1	44.6 ± 0.2	3.81 ± 0.21
Lecithin (1%) & GMS (1.5%)	46.5 ± 0.2	45.5 ± 0.2	3.24 ± 0.14
Lecithin (0.5%) & GMS (0.75%)	46.0 ± 0.2	43.1 ± 0.1	2.91 ± 0.09

† The values given are means of three consecutive experiments ± standard deviations

Table 2. Effect of emulsifier concentration on the properties of margarine containing hydrogenated fat: water in the ratio of 85: 15

Emulsifier (w/w)	Dropping point† (°C)	Slipping point† (°C)	Spreadability† (cm^2)
GMS (1.5%)	42.9 ± 0.3	44.0 ± 0.2	1.87 ± 0.09
Lecithin (1%)	43.5 ± 0.1	44.4 ± 0.2	3.35 ± 0.19
Lecithin (1%) & GMS (1.5%)	44.2 ± 0.3	43.8 ± 0.2	1.97 ± 0.13
Lecithin (0.5%) & GMS (0.75%)	45.2 ± 0.2	44.0 ± 0.2	1.87 ± 0.10

† The values given are means of three consecutive experiments ± standard deviations

products were formulated only with GMS (1.5%) as an emulsifier.

The margarine containing 20% water allowed for better spreadability with higher SP, DP than that containing 15% water. The trend is observed irrespective of the emulsifier used. The water enhanced coarse crystal formation leading to more rigid texture of margarine with closer crystal packing. However, 85:15 (fat: water) ratio was used in the subsequent studies to incorporate a maximum amount of essential fatty acids.

Effect of using EFAs from RSFO in place of hydrogenated fat on physical properties of margarine

As margarine is a non-nutritional food, the solid fat can be replaced with RSFO rich in EFAs. However, the use of RSFO may also affect the physical properties of the formulated product. Hence, the physical properties of margarine were analysed at different proportions of hydrogenated fat and RSFO as given in Table 3. The results indicated that spreadability was increased with an increase in RSFO content or more specifically EFAs with the finest spreadability at hydrogenated fat: RSFO: water ratio of 45: 40: 15. These physical properties (i.e., spreadability, graininess or smoothness of margarine) are originated by the characteristics of the crystal network formed due to its lipid composition (Narine & Marangoni, 2002). The crystallites formed due to early nucleation, associate into micron-range particles that further aggregate into larger clusters to form a continuous three-dimensional network (Marangoni & Rousseau, 1999; Narine & Marangoni, 1999; Marangoni, 2002). The existing voids are filled with EFAs rich liquid fat to stabilise the network by van der Waals force. The solid material forms a three-dimensional network that traps the liquid component and ultimately imparts plasticity and rigidity to the material (Metzroth, 2005). The higher the amount of solids, the more the solid-like behaviour of fat.

The use of RSFO provided increased liquidity that eventually reduced the crystallisation rate. The slower crystallisation finally led to the formation of fine crystals resulting in lower SP, DP and increased spreadability. Thus, the use of RSFO improved the physical properties of margarine besides imparting nutritive value. The RSFO can be used up to a maximum of 45%, above which liquid oil oozed out of the solid crystal network of the margarine.

Table 4 gives the effect of GMS (2%, 2.5% and 3%) on the physical properties of margarine containing 40% RSFO. As the concentration of GMS was increased, the emulsion tightness also increased. This resulted in a higher crystallisable solid content over the temperature range with an increase in hardness and a decrease in spreadability.

Table 3. Effect of replacing hydrogenated fat with RSFO on the properties of margarine containing total fat: water in the ratio of 85: 15 with 1.5% GMS

Hydrogenated fat (% w/w)	RSFO (% w/w)	Dropping point† (°C)	Slipping point† (°C)	Spreadability† (cm²)
75	10	41.2 ± 0.2	43.1 ± 0.2	2.48 ± 0.13
65	20	42.1 ± 0.2	42.5 ± 0.1	3.93 ± 0.17
60	25	40.0 ± 0.2	41.9 ± 0.2	4.17 ± 0.15
55	30	40.8 ± 0.1	41.3 ± 0.1	4.41 ± 0.14
50	35	41.0 ± 0.2	40.9 ± 0.1	4.90 ± 0.09
45	40	41.1 ± 0.3	40.0 ± 0.3	6.76 ± 0.25

† The values given are means of three consecutive experiments ± standard deviations

Table 4. Effect of concentration of GMS as an emulsifier on the properties of margarine containing hydrogenated fat: RSFO:water in the ratio of 45: 40: 15

GMS (% w/w)	Dropping point† (°C)	Slipping point† (°C)	Spreadability† (cm^2)
2	40.1 ± 0.1	41.3 ± 0.2	5.67 ± 0.19
2.5	42.9 ± 0.2	42.6 ± 0.1	4.65 ± 0.23
3	45.0 ± 0.3	44.0 ± 0.1	3.93 ± 0.18

† The values given are means of three consecutive experiments ± standard deviations

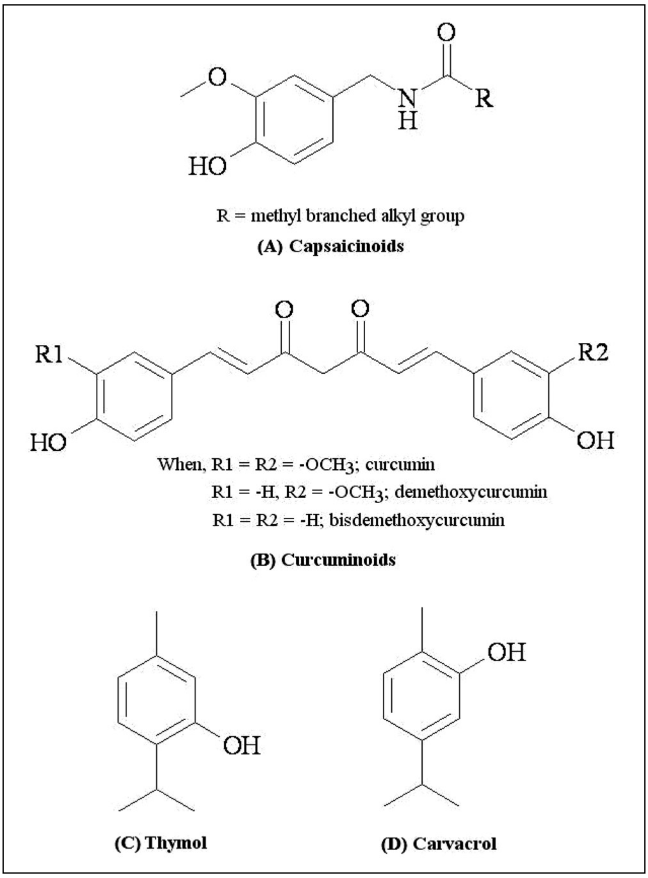

Figure 1. Chemical structures of antioxidants

Oxidative stability of margarine

The oxidation of lipids mainly takes place by autoxidation and involves formation of radicals that continue to absorb oxygen by chain reaction to form peroxides. Hence, the rate of oxidation depends on both radical generation and supply of oxygen. Anti-oxidants act by two mechanisms. The phenolic hydroxyl group is normally responsible for deactivating radicals. Another way is to reduce the oxygen supply by adsorbing on the surface of air droplets. In the case of emulsions, the activity of antioxidant decreases in the presence of water due to hydration of polar groups.

Capsaicinoids present in capsicum contain polar groups like ether, hydroxyl and amide group (Figure 1A) that can form

Table 5. Effect of natural antioxidants on oxidative stability (at 10°C) and physical properties of margarine containing hydrogenated fat: RSFO: water in the ratio of 45: 40: 15

Antioxidants added	Dropping point† (°C)	Slipping point† (°C)	Spreadability† (cm^2)	OXF
TBHQ	42.2 ± 0.1	41.5 ± 0.2	5.94 ± 0.19	0.65
1.5% capsicum oleoresin	42.8 ± 0.3	40.9 ± 0.5	5.28 ± 0.24	0.51
2% capsicum oleoresin	40.9 ± 0.3	39.5 ± 0.6	5.54 ± 0.25	0.44
2.5% capsicum oleoresin	41.4 ± 0.4	39.6 ± 0.5	7.32 ± 0.09	0.29
1.5% capsicum oleoresin + 50 ppm curcuminoids	40.4 ± 0.6	39.3 ± 0.3	5.67 ± 0.11	0.71
2% capsicum oleoresin + 50 ppm curcuminoids	41.2 ± 0.3	38.5 ± 0.3	6.21 ± 0.24	0.65
2.5% capsicum oleoresin + 50 ppm curcuminoids	42.5 ± 0.2	38.7 ± 0.5	7.61 ± 0.45	0.36
1.5% capsicum oleoresin + 2% KEE	39.9 ± 0.3	38.0 ± 0.5	7.91 ± 0.07	0.65
2% capsicum oleoresin + 2% KEE	39.5 ± 0.5	38.1 ± 0.3	8.05 ± 0.26	0.52
2.5% capsicum oleoresin + 2% KEE	38.4 ± 0.6	37.5 ± 0.3	9.12 ± 0.25	0.50

† The values given are means of three consecutive experiments ± standard deviations

hydrogen bonding. Hence, capsicum oleoresin formed a rigid monolayer with GMS at interface of emulsion. This hindered the diffusion of oxygen resulting in higher oxidative stability. This can be observed by an increase in oxidative stability (Table 5) of margarine after incorporation of capsicum oleoresin. The oxidative stability was better than TBHQ that has only two phenolic hydroxyl groups capable of forming hydrogen bonds. The stability was increased with an increase in concentration of capsicum oleoresin from 1.5% to 2.5%, as can be seen from the decrease in OXF. The trend remained in the presence of either curcuminoids (50 ppm) or KEE (2%) with capsicum oleoresin.

However, the addition of curcuminoids (50 ppm) and KEE (2%) resulted in a decrease in oxidative stability with an increase in OXF. Curcuminoids (Figure 1B), thymol (Figure 1C) and carvacrol (Figure 1D) present in KEE had adsorbed into the monolayer thereby decreasing its rigidity by hindering the interaction between GMS and capsaicinoids. Due to higher influx of oxygen, oxidative stability was decreased in the presence of curcuminoids and KEE.

Physical properties of margarine

The addition of capsicum oleoresin resulted in a non-significant change in DP and SP but marginal increase in spreadability (Table 5). There was no remarkable change in DP, SP and spreadability on the addition of curcuminoids along with capsicum oleoresin. On the other hand, addition of KEE to capsicum oleoresin resulted in a decrease in DP, SP and an increase in spreadability. The antioxidants present in KEE (Singh *et al.*, 2005) affected the rheological properties of margarine, that is, they increased the flexibility of the monolayer of GMS and capsaicinoids resulting in a lower energy being required to slide one layer above the other. The decrease in hydrogen bonding between GMS and capsaicinoids inhibited the crystallisation. This ultimately led to an increase in spreadability and a decrease in SP.

CONCLUSION

Margarine was prepared with the addition of essential fatty acids and a natural antioxidant namely, capsicum oleoresin. The addition of kalonji seeds ethanol extract

(KEE) along with capsicum oleoresin improved its physical properties such as slipping point and spreadability.

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