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Use of lycopene as a natural antioxidant in extending the shelf-life of anhydrous cow milk fat

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ABSTRACT

Oxidative rancidity in anhydrous cow milk fat leads to reduction in its shelf life. Use of synthetic antioxidants is prevalent in dairy industry to prevent the development of rancidity. Keeping in view the increasing demand for natural additives, the present study was carried out to explore the potential of lycopene as a natural antioxidant in anhydrous cow milk fat. Lycopene at five different levels (30, 60, 90, 120 and 150 ppm) and butylated hydroxyl anisole (200 ppm), were incorporated in anhydrous cow milk fat. Potential of lycopene extract to enhance the shelf life of anhydrous cow milk fat was evaluated by measuring Free Fatty Acids, peroxide value, Thiobarbituric Acid value and color value during 12 months of storage at ambient conditions (30 °C). Lycopene significantly ($p < 0.05$) prevented the development of oxidative rancidity. Lycopene containing samples scored significantly higher in terms of sensory attributes as compared to control.

1. Introduction

Lycopene is a red colored pigment, apolar and acyclic carotenoid, which is abundantly found in red colored fruits and vegetables such as tomatoes, gac fruit, papaya, carrots, pink grapefruit, pink guava and watermelon (Mangels, Holden, Beecher, Forman, & Lanza, 1993). It exhibits a range of unique and distinct biological properties owing to its acyclic structure, hydrophobicity and large array of conjugated double bonds. Recently, its antioxidant properties have gained substantial interest by consumers demanding healthy and natural foods. Among all naturally occurring carotenoids, lycopene is the most efficient quencher of singlet oxygen (Britton, 1995). The antioxidant activity of lycopene has been extensively evaluated based on its ability to scavenge free radicals in systems, or to protect cell components against oxidative damage in cell culture models, or in animal models (Agarwal & Rao, 1998; Kravchenko et al., 2003). Evidences are sufficient to demonstrate the antioxidant property of lycopene. Moreover, being highly accepted as a food additive and as a natural antioxidant, it is in great demand (Vita, 2007). *In vitro* antioxidant potential of lycopene and its role in lowering the risk of cardiovascular diseases and cell oxidative stress owing to its radical scavenging activity have been extensively evaluated by several workers (e.g., Porrini et al., 2005; Sesso, Buring, Norkus, & Gaziano, 2004). But few studies have focused on its potential as an antioxidant in foods for extending their shelf-life (Osterlie & Lerfall, 2005; Sahin, Davis, & Dezman, 1990).

Anhydrous milk fat commonly known as *ghee* in India means the pure clarified fat derived solely from milk, curd, cooking butter, or cream (FSSR, 2011). Anhydrous cow milk fat is one of the most widely consumed dairy products in India. Oxidative rancidity in milk fat is one of the major factors in limiting its shelf-life (Mehta, 2006; Puravankara, Boghra, & Sharma, 2000). Oxidative degradation causes major changes in quality parameters such as color, flavor, aroma and nutritive value which ultimately affect consumer acceptability (Nerin, Tover, & Salafranca, 2008). Synthetic antioxidants such as butylated hydroxyanisole (BHA), propyl gallate and tertiary butyl hydroquinone (TBHQ) are often used in anhydrous milk fat to prevent oxidative deterioration (Pawar, Arora, Bijoy, & Wadhwa, 2012). Though BHA is widely used in milk fat yet it is suspected to induce tumour formation in animals (Clayson, Iverson, Nera, & Lok, 1993; Hocman, 1988), while intake of lycopene from tomato is comparatively safe (Bhowmik, Kumar, Paswan, & Srivastava, 2012).

Consumers' awareness of the health risks of synthetic antioxidants and demand for natural food ingredients (Dua, Singh, & Mahajan, 2015; Iqbal, Bhanger, & Anwer, 2007; Sivam, Sun-Waterhouse, Siew, & Perera, 2010) has resulted in extensive

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research on naturally occurring antioxidants. Keeping in view the benefits of natural antioxidants such as lycopene and awareness of consumers towards its use as a natural antioxidant, the present study was carried out to assess the effects of incorporation of lycopene extract from tomatoes on the sensory attributes and shelf-life enhancement of anhydrous cow milk fat during storage.

2. Materials and methods

2.1. Materials

Fresh red ripe tomatoes were purchased from local market, blanched in boiling water for 5–7 min and separated into three different fractions, *viz*., skin, pulp and seeds manually. The seed fraction was discarded; the pulp and skin fractions were collected in separate glass petriplates and frozen immediately at −20 °C for 24 h. Frozen samples were freeze-dried for 72 h using a Hetto PowerDry LL1500 freeze dryer at a condenser temperature of −90 °C and reduced pressure of 0.7 torr. Dried fractions were crushed into powder, and stored at −18 °C in amber colored air tight glass bottles until lycopene was extracted. All chemicals used were of analytical grade. Fresh cow cream of 45–50% fat content was obtained from the Experimental Dairy of National Dairy Research Institute, Karnal, India and kept at 8–10 °C in a refrigerator for 12–24 h.

2.2. Extraction of lycopene

Extraction of lycopene was carried out according to the procedure of Ranganna (1986) from the mixture of freeze dried pulp and skin fractions at a ratio of 3:2. Five grams of dried powder was taken in a pestle and mortar and extracted with acetone using three times 150 ml until the residue was colorless. The three acetone fractions containing lycopene were transferred into a 1 l separating funnel containing 40–50 ml of petroleum ether and mixed gently. Water (30 ml) was added to separating funnel for phase separation. Four to five washings were carried out using distilled water to remove acetone. Lower acetone layer was discarded while upper layer of petroleum ether containing lycopene was collected. Dry extract was obtained by using a rotary vacuum evaporator. All sample preparations and experiments were performed under dim light conditions to minimize light-induced degradation and isomerization.

2.3. Antioxidant activity of lycopene extract

Free radical scavenging activity of the lycopene extract was determined by ABTS method (Re et al., 1999) which involved the direct production of the $ABTS^{\cdot+}$ (2,2-azinobis(3-ethylbenzothiazoline-6-sulfonate) chromophore through the reaction between ABTS and potassium persulfate. Trolox equivalent antioxidant capacity (TEAC) measured the relative ability of lycopene to scavenge the $ABTS^{\cdot+}$ in comparison to the antioxidant capacity of standard amounts of Trolox (6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid). In the presence of antioxidants, the absorbance at 734 nm decreased and the extent of decolorization as percentage inhibition of radical cation was determined as a function of concentration and time. A standard curve was prepared by plotting concentration (500–5000 μM) of Trolox (*X*-axis) against per cent inhibition (*Y*-axis). One ml of ABTS working solution was added to a 1 ml microcuvette and absorbance adjusted to 0.70 ± 0.02 at 734 nm against the phosphate buffer saline (pH 7.4). Ten microlitre of sample (lycopene extract dissolved in dichloromethane) was added to ABTS working solution as well as in the blank. The contents were mixed for 5 s and change in absorbance at 734 nm was recorded over 10 min using SPECORD-200 double beam spectrophotometer (Analytik Jena AG, Analytical Instrumentation, Jena, Germany). Based on the per cent inhibition of absorbance of sample, Trolox equivalent was determined from standard curve using equation as follows: $y = 0.014x + 13.23$ where;

$$Y(\%\ \text{inhibition}) = \frac{(\text{Initial absorbance} - \text{Final absorbance after 10 min})}{\text{Initial absorbance}} \times 100$$

x is the μM concentration of Trolox

2.4. Preparation of anhydrous cow milk fat

Anhydrous cow milk fat, i.e., cow *ghee* was prepared using creamery butter method. Cow cream was kept overnight at refrigeration temperature (8–10 °C) and processed in a hand churner with the addition of 1–2 l cold water and ice cubes to maintain cream at 5 °C. Churning of cream proceeded until fat globules adhered, forming larger and larger masses, and finally a complete separation of butter granules and buttermilk occurred. Butter granules were washed with chilled potable water (4–5 °C) and then heated to a temperature of 110–115 °C in a stainless steel vessel. When temperature reached 110 °C, a slight caramelization of curd particles was observed. Heating was discontinued as soon as curd particles attained the desired golden yellow or brown color. The contents of the vessel were left undisturbed till the residue settled down. The clarified fat (anhydrous cow milk fat) was decanted off and anhydrous cow milk fat residue was separated using cheese cloth.

2.5. Addition of lycopene and BHA in anhydrous cow milk fat

Lycopene extract was added to anhydrous cow milk fat at levels of 30, 60, 90, 120 and 150 ppm. The concentration of lycopene extract to be added in anhydrous cow milk fat was decided on the basis of antioxidant activity and its effect on sensory scores for color. Small quantity of anhydrous cow milk fat was melted (60–70 °C) and the weighed amount of crude lycopene extract was added in anhydrous cow milk fat and mixed properly. BHA was added in hot anhydrous cow milk fat (60–70 °C) at a level of 0.02% (200 ppm) and mixed properly.

2.6. Storage study

Anhydrous cow milk fat samples containing lycopene and BHA along with control sample were packaged in airtight glass bottles of 500 ml capacity and stored for a period of 12 months at 30 °C in BOD incubator wrapped in aluminum foil to protect from light. During storage, all samples were evaluated for physico-chemical characteristics at a regular interval of 2 months and for sensory attributes at a regular interval of 4 months.

2.7. Physico-chemical analysis

Photometric color value of anhydrous cow milk fat samples was measured using a spectrophotometric method as described in IS: 3508 (1966) by taking the absorbances at 460, 550, 620 and 670 nm. Color was also measured by colorimetric method (Assawarachan & Noorhorm, 2010) using a Colorflex Colorimeter supplied by Hunterlab (Hunter Associates Laboratory, Inc., Reston, VA) along with the software version 4.10 and the results were expressed in CIE Lab system. The FFA content of anhydrous cow milk fat samples was measured by the method described in Indian Standard (IS: 3508-1966) and expressed as per cent oleic acid. Peroxide value represents the primary reaction products of lipid