Effect of clove bud and curry leaf essential oils on the anti-oxidative and anti-microbial activity of *burfi*, a milk-based confection

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Revised: 3 August 2018 / Accepted: 4 September 2018 / Published online: 12 September 2018
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Abstract With scientific advancement in the field of food science and technology, there has been an increased availability of innovative ingredients that can be utilized towards value addition, quality enhancement, natural preservation, shelf life enhancement and adding novelty to traditional Indian dairy products. Here a scientific attempt has been made to increase the anti-oxidative and anti-microbial potential of *burfi*, a popular confection of Indian sub-continent with low shelf life using herbal essential oils (EOs) (natural preservative, antioxidant and antimicrobial) such as curry leaf (CRYF) (0.05–0.15 ppm) and clove bud (CLVB) (0.15–0.25 ppm) EO. Samples were subjected to physico-chemical, sensory, anti-oxidant and microbiological analysis and the results revealed that increasing the herbal EOs levels in *burfi* led to increase in anti-microbial and anti-oxidative attributes but simultaneously decreased the sensory attributes. Physico-chemical attributes remained unaffected upon EOs incorporation. Principal component analysis revealed 81.5% relation between the *burfi* samples and its quality attributes (DPPH activity, ABTS activity, total phenolic content, sensory attributes, standard plate count, yeast and mould count, moisture content, water activity, lightness, L^* value, redness, a^* value and yellowness, b^* value). Herbal EOs i.e. CRYF@0.10 ppm and CLVB@0.20 ppm on *khoa* basis were found optimum for incorporation into *burfi* for

enhancing storage stability without compromising the sensory acceptability.

Keywords Essential oil · Curry leaf · Clove bud · Herb · Antioxidant · *Burfi* · Anti-oxidant activity · Anti-microbial activity · Principal component analysis

Introduction

Burfi, the most popular, heat desiccated milk (*khoa*)-based confection, has its own distinguished niche in Indian sub-continent diets during festive seasons as well as in day-to-day life due to its delicious and nutritious nature. Several varieties of *burfi* could be prepared depending on the additives used such as coconut, besan, fruits, chocolate, nut, palm, groundnut, saffron, doda, soy, low calorie and probiotics (Arora et al. 2007; Chetana et al. 2010; Chawla et al. 2015). *Burfi* is prepared by heating a mixture of concentrated milk solids (*khoa*) and sugar to a near homogenous consistency followed by cooling and cutting into small cuboids. Beating and whipping operations prior to cooling are sometimes practiced to obtain a product with smooth texture and closely knit body (Chetana et al. 2010). The economic significance of all traditional Indian dairy products can be realized from the fact that they have huge market size estimated at ₹75,000 crores (Parekh 2013). *Burfi* produced and sold in the traditional manner has a shelf life of about 1 week at room temperature. High moisture content is mainly responsible for the proliferation of micro-organisms and surface mould growth is the most common defect encountered in spoilage during storage. Further, the manufacturing conditions, handling and packaging as well as type of packaging materials also influence the shelf life of the product. With the increasing

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demand of ethnic milk products in India and abroad, there has been a great need for increasing their quality and shelf life. Various preservation methods have been tried over the decades either in single or in combination to extend the shelf life of *burfi* such as chemical preservatives like propyl gallate and dodecyl gallate, sorbic acid and its salts, sodium and potassium bisulphites, synthetic antioxidants, natural preservatives like saffron, citric acid, cardamom and various packaging materials such as parchment paper, tin cans, aluminum boxes, single layer pouches, laminate pouches, vacuum packaging (Sarkar et al. 2002; Palit and Pal 2005; Vijayalakshmi et al. 2005; Shobha and Bharati 2007).

In the recent years, consumers have been demanding a reduction in the use of chemicals in their food and beverages because of the associated potential health risks. Especially the industrialized societies appear to experience the trend towards green consumption, desiring fewer synthetic food additives and products with a low impact on the environment. Keeping this in view, herbs or their extracts are being frequently used as food ingredients. Herbs are bactericidal and have a broad-spectrum activity against Gram-positive and Gram-negative bacteria and their essential oils have proved to be potentially useful sources of antimicrobial and antioxidant compounds (Abdelfadel et al. 2015), which can thus be used as natural preservative agents. These herbal essential oils have attained GRAS (Generally Recognized as Safe) status and are considered as safe for human consumption without limitations on intake and commonly accepted by consumers. Numerous studies have been carried out for incorporation of herbs and their essential oils into traditional dairy products such as *ghee*, *paneer*, *sandesh*, *shrikhand*, *khoa*, *burfi* and *peda* (Bandyopadhyay et al. 2007a, b; Landge et al. 2011; Parmar et al. 2013; Singh et al. 2014; Gavhane et al. 2014; Sivakumar et al. 2014). In view of the broad spectrum antimicrobial activity and antioxidant activities of herbs or their extractives and compatibility with milk and milk products, the present work was carried out to optimize the levels of curry leaf and clove bud essential oils in *burfi* to obtain a high quality product with maximum antimicrobial and antioxidant activities.

Materials and methods

Ingredients and chemicals

Fresh pooled buffalo milk was received from the Experimental Dairy of the ICAR-National Dairy Research Institute, Karnal (India). Commercially available superfine sulphurless cane sugar (TrustTM Classic) manufactured by M/s Simbhaoli Sugars Ltd., Hapur (UP) was procured from the local market. Curry leaf and clove bud essential oils

were generously provided by M/s Plant Lipids (P) Limited, Kolenchery, Cochin, Kerala. The media for different microbiological analyses such as plate count agar, potato dextrose agar and violet red bile agar were procured from M/s HiMedia Laboratories Pvt. Limited, Mumbai. Rest all the chemicals used for the preparation of different reagents was of Analytical Grade (AR) and procured from standard companies.

Preparation of herbal *burfi*

Herbal *burfi* was prepared using the standard batch method of manufacture as proposed by Aneja et al. (2002) with some modifications, by desiccation of standardized buffalo milk (fat 6%, SNF 9%) into *khoa* in a double jacketed stainless steel kettle and adding sugar (30 g 100 g⁻¹ of milk) and herbal essential oils (EOs) at the final stages of processing. Based on preliminary experiments, curry leaf essential oil (CRYF) and clove bud essential oil (CLVB) were incorporated into *burfi* as given in Table 1. *Burfi* samples without herbal essential oils were treated as control.

Antioxidant capacity

The antioxidant capacity of *burfi* was determined by free radical scavenging methods using DPPH (2,2-diphenyl-1-picryl hydrazyl) and ABTS (2,2'-azino-bis(3-ethyl benzothiazoline)-6-sulfonic acid) and total phenolic content. The antioxidant capacity based on DPPH radical of herbal *burfi* was determined as per the method of Brand-Williams et al. (1995) using trolox as standard. For this, 0.1 mL of *burfi* extract was mixed with 2.9 mL of 0.1 mM DPPH solution and incubated in dark for 30 min at 37 °C. The absorbance of the solution was measured at 517 nm against methanol using a spectrophotometer. For blank determination, 0.1 mL methanol was taken instead of sample and absorbance was measured immediately against methanol. In the second method, antioxidant capacity of herbal *burfi* was determined based on free radical scavenging using ABTS as given by Re et al. (1999) using trolox as standard. The ABTS reagent was prepared by mixing 7 mM ABTS with 2.45 mM potassium persulphate in equal ratio. After the mixture was kept in the dark at room temperature for 16 h to allow the completion of radical generation, it was diluted with methanol so that its absorbance was adjusted to 0.70 ± 0.02 at 734 nm. To determine the scavenging activity, 3.9 mL ABTS reagent was mixed with 0.1 mL of appropriate diluted sample and the absorbance was measured at 734 nm after 6 min of reaction at room temperature, using methanol as a control. In the third method, the total phenolic content of control and herbal *burfi* was analyzed by Folin Ciocalteu's method of Zhang et al.

Table 1 Details of treated samples containing essential oils

Sample code	Type of essential oil incorporated	Level of essential oil incorporated (mL kg ⁻¹)
Control	–	–
CRYF005	Curry leaf	0.05
CRYF010	Curry leaf	0.10
CRYF015	Curry leaf	0.15
CLVB015	Clove bud	0.15
CLVB020	Clove bud	0.20
CLVB025	Clove bud	0.25

(2006) using gallic acid as standard. For this, the extract (0.2 mL) was mixed with 0.1 mL of 0.2 N Folin–Ciocalteu's phenol reagent. After 2–5 min, 0.8 mL of 20% sodium carbonate solution was added to the mixture and incubated for 10 min at room temperature. The mixture was centrifuged at 150×g for 8 min and the absorbance of the supernatant was measured at 730 nm against distilled water as blank. The total phenolic content was expressed in terms of µg gallic acid equivalent (GAE) per gram of *burfi*. All the measurements were carried out in triplicates.

Microbiological quality

The total viable bacterial or standard plate count, yeast and mold count and coliform count in the *burfi* samples were enumerated by Indian standard procedure (IS: SP-18 (Part-XI) 1981) using plate count agar, acidified potato dextrose agar and violet red bile agar, respectively. Samples for microbial assay were drawn aseptically to obtain error free results.

Physico-chemical and textural properties

Moisture content of herbal *burfi* was determined by gravimetric method as described in Indian Standards (IS: SP-18 1981). Water activity was determined at 25 °C using Aqua Lab (Model Series 3 TE) water activity meter (M/s Decagon Devices, WA, USA). Colour measurements were conducted using Color Flex (M/s Hunter Associates Laboratory, Inc., Reston VA, USA) system equipped with dual beam xenon flash lamp and universal software (version 1.72). The instrument was calibrated prior to sample measurements with standard black, white and green tiles as prescribed by the supplier. The results were expressed in terms of L^* , a^* and b^* , where L^* indicates lightness (0 for black and 100 for white), a^* indicates redness (0–60) or greenness (0 to – 60) while b^* indicates yellowness (0–60) or blueness (0 to – 60). Texture profile of the herbal *burfi* was studied using a TA-XT2i Texture Analyzer (M/s

Stable Micro Systems, UK) equipped with a load cell of 25 kg which was calibrated using a 5 kg standard dead weight prior to use. Cylindrical samples of *burfi* (1 cm height and 1 cm in diameter) tempered at 25 °C, were subjected to uniaxial compression to 80% of the initial sample height using a 75 mm diameter compression plate (P-75). The textural attributes (hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience) were calculated from the force–time deformation curve obtained using Texture Expert Exceed software (version 2.55). All the measurements were carried out in triplicate. However, for textural properties 10 replicates were considered.

Sensory evaluation

Control as well as herbal *burfi* samples were subjected to sensory evaluation by a panel of nine discriminative and communicative judges selected from the faculty and research scholars of the Dairy Technology Division using a 25-point scale score card. The panel members were selected based on their experience in judging of traditional Indian dairy products. Sensory attributes such as flavour (10), body and texture (10), colour and appearance (5) and overall acceptability (25) were evaluated.

Statistical analysis

Data obtained from various experiments was recorded as mean ± standard error (SE) and subjected to statistical analysis to arrive at valid and meaningful inferences. Data obtained was analyzed using one way analysis of variance (ANOVA) followed by Tukey's comparison test to establish the significance of differences among the means values at 5% level of significance ($P < 0.05$). Principal component analysis (PCA) was also carried out using SAS Enterprise Guide software (version 5.3). The principal components were extracted based on the eigen values which are greater than 1 and the Varimax method was used to rotate the solutions. Normalization of the data was done by Kaiser normalization method.

Results and discussion

Effect of herbal essential oils on the antioxidant activity of *burfi*

The values obtained for DPPH, ABTS (free radical scavenging activities) and total phenolic content of essential oils (EOs) incorporated *burfi* are shown in Fig. 1. It was

observed that the antioxidant activity of *burfi* increased with increasing concentration of EOs. The total phenolic content in curry leaf EO incorporated *burfi* increased from 1.07 to 5.09 $\mu\text{g g}^{-1}$, DPPH and ABTS activities increased from 0.63 to 2.12 and 0.15 to 0.30 $\mu\text{mol g}^{-1}$, respectively as the concentration was increased from 0.05 to 0.15 mL kg^{-1} of *khoa*. This may be attributed due to the presence of high amounts of carbazole alkaloids such as mahanimbine, koenigine, mahanine, koenine, etc. in curry leaf, which are associated with anti-oxidative (aryl hydroxyl group of alkaloids) activity (Ganesan et al. 2013). Similar effects of incorporation of curry leaf have been reported in *sandesh* (Bandyopadhyay et al. 2007a) and *ghee* (Patel and Rajorhia 1979). Similarly, increasing trend in the antioxidant activity was observed due to the addition of CLVB to *burfi*, which may be attributed to the presence of eugenol (phenolic hydrogen atom), the main constituent of this essential oil. Samaddar et al. (2015) also reported that incorporation of eugenol essential oil (0.005%) increased the antioxidant activity of flavoured milk. Phenolic compounds, which are biologically active, are the main agents that can donate hydrogen (electron) to free radicals and thus break the chain reaction of lipid oxidation at the initiation step. This high potential of phenolic compounds to scavenge radicals may be explained by their phenolic hydroxyl groups. The observations in the present study very well correlated with the work reported by Bandyopadhyay et al. (2007b) who assessed the antioxidant activity of *sandesh* containing either beetroot (*Beta vulgaris*), mint (*Mentha spicata* L.) and ginger (*Zingiber officinale* L.) alone or in combination and found that ginger alone and combination of ginger with mint resulted in highest total antioxidant activity. Further, it was reported that use of synthetic antioxidants (BHA and BHT) can be substituted by natural sources (beet, mint, ginger). Hala et al. (2010) reported that supplementing cheese with rosemary extract proportionally increased the total phenol content and ferric reducing antioxidant power (FRAP) values of the resultant fresh cheese with the increase in the additive concentration. It was reported that the rate of increase in FRAP values of fresh UF cheese samples supplemented with 1 and 5% extract was 87 and 222%, respectively. Similarly, Sivakumar et al. (2014) assessed the effect of betel leaves (*Piper betel* L.) extract on the antioxidant properties of *khoa* made from cow's milk which was stored at room temperature and observed that *khoa* with 0.5% aqueous extract of betel leaves restricted the production of free fatty acids compared to control, due to antioxidant property of betel leaves. Srivastava et al. (2015) used different levels of ginger extract and beet root extract to produce herbal yoghurt from cow, buffalo and goat milks and reported that the antioxidant activity of goat milk yoghurt containing 2% each ginger and beet root

Fig. 1 Effect of different levels of curry leaf and clove bud essential oils on the antioxidant activity of *burfi*. **a** Total phenolic content, **b** DPPH value, **c** ABTS value. *Please refer Table 1 for code description/detail

extracts and cow milk yoghurt containing 2% ginger extract had highest antioxidant activities as measured by DPPH and FRAP methods.

Effect of herbal essential oils on the microbiological quality of *burfi*

The microbial profile of *burfi* samples incorporated with herbal EOs at three different levels is presented in Fig. 2. The control sample was found to have maximum total viable bacterial (4.15 log cfu/g) and yeast and mould counts (1.60 log cfu/g). However, the counts were within the limits as specified by Food Safety and Standards Regulations of India (FSSR 2011) Rules, which specifies that *khoa* and *khoa* based products shall possess maximum 75,000 cfu/g aerobic plate count, maximum 50 cfu/g yeast and mould count and 100 cfu/g coliform count. In the present study, no coliform counts were observed in the control or in the treated samples, indicating hygienic processing and no post-process-contamination of the product.

Fig. 2 Effect of different levels of curry leaf and clove bud essential oils on the total viable bacterial and yeast and mould counts of *burfi*. **a** Total viable count, **b** yeast and mould count. *Please refer Table 1 for code description/detail

Maximum reduction in the total viable bacterial and yeast and mould count were found at highest level of essential oil incorporation namely 0.15 mL kg⁻¹ (CRYF015) and 0.25 mL kg⁻¹ (CLVB025). Similar results were observed by Smith et al. (2001), where higher inhibitory activity was observed when 1% clove oil was used in cheese slurry. Landge et al. (2011) reported that SPC and yeast and mould count of *shrikhand* decreased from 27.32×10^6 to 23.00×10^6 and 54 to 40 cfu/g, respectively on addition of 0.7% Ashwagandha (*Withania somnifera* L.) powder in *shrikhand*. Gavhane et al. (2014) observed that the average standard plate count of fresh *peda* samples were in the range of 3000–8000 cfu g⁻¹ for different levels (0–6%) of ginger powder incorporation. The antimicrobial activity of herbal *burfi* in the present study may be attributed to the presence of antimicrobial activities of active compounds such as carbazole alkaloids (such as mahanine, mahanimbicine and mahanimbine) in curry leaf essential oil and eugenol in clove bud essential oil. It is well known that essential oil phenolic compounds can denature proteins and react with cell membrane phospholipids changing their permeability and inhibiting a great number of Gram-negative and Gram-positive bacteria as well as different types of yeast (Chaib et al. 2007; Ganesan et al. 2013). Eugenol, a phenolic compound is known to have antibacterial property by the inhibition of extracellular enzymes synthesis and disruption of the cell wall structure resulting in lack of cytoplasm, cytoplasm granulation, cytoplasm hyperacidity, and depletion of intracellular ATP pool (Diaz et al. 2002).

Effect of herbal essential oils on physico-chemical, instrumental colour and textural properties of *burfi*

The physico-chemical, instrumental colour and textural properties of control and herbal *burfi* samples are presented in Table 2. Similar results were reported in *burfi* by several researchers in the literature (Palit and Pal 2005; Vijayalakshmi et al. 2005; Arora et al. 2007; Chawla et al. 2015). It can be seen from the Table 2 that no significant effect ($P > 0.05$) on the physico-chemical, instrumental colour and textural properties of the product was observed due to the addition of essential oils. This can be attributed to addition of low concentration of essential oils in the product. Olmedo et al. (2013) reported no significant difference in colour between flavoured cheese (0.2%) prepared with cream cheese base using either oregano or rosemary essential oils and control samples. Anurag and Chawla (2016) observed that with an increase in the proportion of bottle gourd shreds to *khoa*, the proximate parameters such as fat, protein, titratable acidity and ash contents of bottle gourd *burfi* decreased. However, moisture content of the product increased, which can be

Table 2 Effect of different levels of herbal essential oils on the physico-chemical, instrumental colour and textural attributes of *burfi*

Attribute	Control	Herbal <i>burfi</i> samples					
		CRYF005	CRYF010	CRYF015	CLVB015	CLVB020	CLVB025
Moisture content (%)	14.67 ± 0.10 ^a	14.33 ± 0.19 ^a	15.08 ± 0.25 ^a	14.27 ± 0.18 ^a	14.34 ± 0.26 ^a	14.38 ± 0.08 ^a	14.38 ± 0.18 ^a
Water activity	0.79 ± 0.02 ^a	0.75 ± 0.01 ^a	0.77 ± 0.01 ^a	0.76 ± 0.01 ^a	0.75 ± 0.01 ^a	0.75 ± 0.01 ^a	0.76 ± 0.01 ^a
<i>Instrumental colour</i>							
Lightness, <i>L</i> [*] value	66.6 ± 0.09 ^a	66.9 ± 0.16 ^a	66.5 ± 0.25 ^a	66.4 ± 0.29 ^a	65.6 ± 0.32 ^a	65.8 ± 0.67 ^a	65.8 ± 0.67 ^a
Redness, <i>a</i> [*] value	1.05 ± 0.08 ^a	1.05 ± 0.21 ^a	1.13 ± 0.06 ^a	0.99 ± 0.06 ^a	1.21 ± 0.04 ^a	1.13 ± 0.02 ^a	1.15 ± 0.05 ^a
Yellowness, <i>b</i> [*] value	24.7 ± 0.16 ^a	24.5 ± 0.13 ^a	24.5 ± 0.21 ^a	25.4 ± 0.28 ^a	25.1 ± 0.26 ^a	25.4 ± 0.17 ^a	25.0 ± 0.28 ^a
<i>Texture profile analysis</i> ^b							
Hardness (N)	51.0 ± 1.78 ^a	53.4 ± 2.27 ^a	54.0 ± 2.05 ^a	49.2 ± 1.95 ^a	49.9 ± 1.48 ^a	51.9 ± 1.85 ^a	49.2 ± 1.67 ^a
Adhesiveness (N s)	− 1.20 ± 0.04 ^a	− 1.19 ± 0.06 ^a	− 1.22 ± 0.04 ^a	− 1.17 ± 0.06 ^a	− 1.36 ± 0.02 ^a	− 1.32 ± 0.05 ^a	− 1.33 ± 0.01 ^a
Springiness	0.08 ± 0.01 ^a	0.09 ± 0.01 ^a	0.06 ± 0.01 ^a	0.07 ± 0.01 ^a	0.07 ± 0.01 ^a	0.07 ± 0.01 ^a	0.08 ± 0.01 ^a
Cohesiveness	0.13 ± 0.01 ^a	0.12 ± 0.01 ^a	0.13 ± 0.01 ^a	0.12 ± 0.01 ^a	0.12 ± 0.01 ^a	0.12 ± 0.01 ^a	0.12 ± 0.01 ^a
Gumminess	5.02 ± 0.24 ^a	4.92 ± 0.22 ^a	4.86 ± 0.14 ^a	4.92 ± 0.15 ^a	5.01 ± 0.31 ^a	4.83 ± 0.23 ^a	4.91 ± 0.17 ^a
Chewiness	0.52 ± 0.02 ^a	0.48 ± 0.02 ^a	0.53 ± 0.02 ^a	0.48 ± 0.02 ^a	0.49 ± 0.01 ^a	0.54 ± 0.01 ^a	0.53 ± 0.01 ^a
Resilience	0.06 ± 0.01 ^a	0.05 ± 0.01 ^a	0.06 ± 0.01 ^a	0.06 ± 0.01 ^a	0.06 ± 0.01 ^a	0.06 ± 0.01 ^a	0.06 ± 0.01 ^a

^aMean values with same superscript within the same row indicates no statistically significant difference ($P > 0.05$)

^bMean ± SE (n = 10), Mean ± SE (n = 3)

attributed to addition of high amount (greater than 10%) of bottle gourd shreds to *khoa*. Similar results were recorded in bitter gourd *burfi* by Srivastava and Saxena (2012) and in ash gourd *peda* by Sirsat et al. (2013).

Effect of herbal essential oils on the sensory attributes of *burfi*

The sensory scores of *burfi* with and without essential oils are presented in Fig. 3. In terms of body and texture and colour and appearance, herbal *burfi* resembled the control and possessed similar desirable attributes. This can be correlated with no significant ($P > 0.05$) changes in instrumental colour and texture parameters of *burfi* (Table 2). However, the flavour and overall acceptability scores significantly decreased ($P < 0.05$) in both the cases with an increase in the level of incorporation of respective EO as can be observed from Fig. 1. This trend in sensory scores may be attributed due to the presence of certain phytochemicals such as eugenol in CLVB (Wenqiang et al. 2007) and 3-carene and caryophyllene in CRYF (Chowdhury et al. 2008), which might have played an important role in causing undesirable organoleptic properties of the product. Similarly, Bandyopadhyay et al. (2007a) reported lower acceptance of *sandesh* fortified with curry leaf

extract (10%) as compared to control samples. Gavhane et al. (2014) observed that the overall acceptability score of *peda* prepared with different levels of ginger powder (0–6% *khoa* weight basis) ranged between 6.89 and 8.49, with decreasing scores at higher levels of ginger powder incorporation. Sawale et al. (2015) reported that as the concentration of *Pueraria tuberosa* extract was increased from 0.2 to 0.5% in milk, a significant drop ($P < 0.05$) in all the sensory attributes was observed. Anurag and Chawla (2016) observed that with an increase in the proportion of bottle gourd shreds to *khoa*, the color and sweetness scores of bottle gourd *burfi* increased, whereas the scores of flavor, texture and overall acceptability decreased. Similar observations were made by Srivastava and Saxena (2012) in bitter gourd *burfi*.

Principal component analysis

In the present study, similarities and differences among the physico-chemical, instrumental colour, sensory, antioxidant and antimicrobial attributes of herbal *burfi*, was evaluated based on the correlation matrix of the 14 independent variables. On the basis of eigen values more than 1.0, three principal components (PC) were obtained and their eigen-vector loadings revealed that the first three PCs

Fig. 3 Effect of different levels of curry leaf and clove bud essential oils on the sensory attributes of *burfi*. **a** Flavour score, **b** body and texture score, **c** colour and appearance score, **d** overall acceptability score. *Please refer Table 1 for code description/detail

(PC1, PC2 and PC3) with eigen values of 6.62, 4.78 and 1.36 were able to explain 47.3, 34.2 and 4.6% of the total variation, respectively. Cumulatively the three components explained 81.5% of the total variance. It can be seen from Fig. 4a, that different herbal *burfi* samples got differentiated into four different quadrants. Also a two-dimensional plot (Fig. 4b) was obtained showing all the attributes of different herbal *burfi* samples, in which PC1 was characterized by yellowness value, total phenolic content, ABTS and DPPH activity and opposed by overall acceptability score, flavour score, lightness, standard plate count and yeast and mould count. PC2 was defined by water activity, colour and appearance and body and texture scores and opposed by moisture content. The loadings plot depicts the distance between the location of any two attributes which is proportional to the degree of difference or similarity between them. The characteristics of prime importance i.e. total phenolic content and DPPH activity were found

loaded in herbal *burfi* samples having 0.20 and 0.25 mL/kg of CLVB. While flavour and overall acceptability scores and standard plate count loaded in control *burfi* and herbal *burfi* containing CLVB at the rate of 0.15 mL per kg *burfi*.

Factor analysis (Table 3) revealed that PC1 was positively and significantly loaded with yellowness (0.907), total phenolic content, DPPH and ABTS activity (0.966, 0.981 and 0.761, respectively) whereas negatively loaded with lightness value (− 0.714), flavour score (− 0.689) and overall acceptability score (− 0.714), along with standard plate count (− 0.892) and yeast and mould count (− 0.927). PC2 was positively and significantly loaded with water activity (0.801), body and texture (0.896), colour and appearance (0.975) and overall acceptability scores (0.638) whereas negatively loaded with moisture content (− 0.819). PC3 having eigen value of 1.36 was only positively loaded with redness value (0.885).

Fig. 4 PCA of physico-chemical, instrumental colour, sensory, antioxidant and antimicrobial attributes of *burfi* subjected to natural preservatives (EOs). **a** Preference mapping of *burfi*, **b** biplots from the

Table 3 Factor loadings for quality attributes of herbal *burfi* (EOs individually) employing all attributes

Attributes	Factor 1	Factor 2	Factor 3
Moisture content (%)	0.197	− 0.819	− 0.279
Water activity	0.097	0.801	− 0.289
Lightness value	− 0.714	− 0.566	0.124
Redness value	− 0.174	− 0.319	0.885
Yellowness value	0.907	0.307	0.044
Flavour score	− 0.689	0.540	0.468
Body and texture score	0.287	0.896	0.091
Colour and appearance score	0.089	0.975	0.167
Overall acceptability score	− 0.714	0.638	− 0.011
ABTS activity ($\mu\text{mol g}^{-1}$)	0.760	− 0.592	0.180
DPPH activity ($\mu\text{mol g}^{-1}$)	0.981	0.094	0.041
Total phenolic content ($\mu\text{g g}^{-1}$)	0.966	0.137	0.023
Standard plate count ($\log \text{cfu g}^{-1}$)	− 0.892	0.222	− 0.321
Yeast and mould count ($\log \text{cfu g}^{-1}$)	− 0.927	− 0.274	− 0.079

Values in bold correspond to correlation coefficient for each variable under each factor which is more than 0.60

Conclusion

The antioxidant activity increased and total viable bacterial and yeast and mould counts decreased significantly with increase in herbal essential oil addition in *burfi*. Also, herbal essential oil incorporation into the *burfi* had no significant effect on the product's physicochemical attributes, viz. instrumental colour and texture attributes and chemical composition. With regard to sensory attributes,

1st and 2nd principal components of PCA. *Please refer Table 1 for code description/detail

the samples containing either curry leaf or clove bud essential oil above 0.15 mL/kg and 0.25 mL/kg, respectively were not liked by sensory panelists. PCA of the physico-chemical, instrumental colour, sensory, antioxidant and antimicrobial attributes of *burfi* revealed that PC1, PC2 and PC3 explained 81.5% relation between the *burfi* samples and its quality attributes. Thus, based on above results, *burfi* incorporated with 0.15 mL/kg and 0.25 mL/kg of CRYF and CLVB, respectively showed acceptable sensory scores along with appreciable antimicrobial and antioxidant effect and thus was suggested for successful preparation and further studies on herbal *burfi*.

Acknowledgements The author acknowledge the Director, ICAR-National Dairy Research Institute, Karnal for extending facilities for pursuing this study and ICAR-NDRI Institutional fellowship for the first author. Also, M/s Plant Lipids (P) Limited, Kolenchery, Cochin, Kerala for generously providing samples of herbal essential oils for the study.

References

- Abdelfadel MM, Khalaf HH, Sharoba AM, Assous MTM (2015) Effect of extraction methods on antioxidant and antimicrobial activities of some spices and herbs extracts. *Int J Adv Res* 3:165–179
- Aneja RP, Mathur BN, Chandan RC, Banerjee AK (2002) Technology of Indian milk products. Dairy India Publication, New Delhi
- Anurag, Chawla R (2016) Studies on preparation of composite dairy food-bottle gourd *burfi*. *Asian J Dairy Food Res* 35:196–200
- Arora S, Singh VP, Yarrakula S, Gawande H, Narendra K, Sharma V, Wadhwa BK, Tomer SK, Sharma GS (2007) Textural and microstructural properties of *burfi* made with various sweeteners. *J Texture Stud* 38:684–697

- Bandyopadhyay M, Chakraborty R, Raychaudhuri U (2007a) Incorporation of herbs into *sandesh*, an Indian sweet dairy product, as a source of natural antioxidants. *Int J Dairy Technol* 60:228–233
- Bandyopadhyay M, Chakraborty R, Raychaudhuri U (2007b) A process for preparing a natural antioxidant enriched dairy product (*Sandesh*). *LWT Food Sci Technol* 40:842–851
- Brand-Williams W, Cuvelier ME, Berset CLWT (1995) Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci Technol* 28:25–30
- Chaib K, Hajlaoui H, Zmantar T, Kahla-Nakbi AB, Rouabhia M, Mahdouani K, Bakhouf A (2007) The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzigium aromaticum* L. Myrtaceae): a short review. *Phytother Res* 21:501–506
- Chawla R, Singh AK, Patil GR (2015) Shelf life enhancement of functional *doda burfi* (Indian milk cake) with biopreservatives application. *Int J Res Sci Technol* 5:2249–2254
- Chetana R, Ravi R, Reddy SY (2010) Effect of processing variables on quality of milk *burfi* prepared with and without sugar. *J Food Sci Technol* 47:114–118
- Chowdhury JU, Bhuiyan MNI, Yusuf M (2008) Chemical composition of the leaf essential oils of *Murraya koenigii* (L.) Spreng and *Murraya paniculata* (L.) Jack. *Bangladesh J Pharmacol* 3:59–63
- Diaz TL, Gonzalez CJ, Moreno B, Otero A (2002) Effect of temperature, water activity, pH and some antimicrobials on the growth of *Penicillium olsonii* isolated from the surface of Spanish fermented meat sausage. *Food Microbiol* 19:1–7
- FSSR (2011) Food safety and standards act, rules and regulations. Akalank Publications, New Delhi
- Ganesan P, Phaiphon A, Murugan Y, Baharin BS (2013) Comparative study of bioactive compounds in curry and coriander leaves: an update. *J Chem Pharm Res* 5:590–594
- Gavhane MS, Kamble NS, Desale RJ, Ghule BK, Mule PR (2014) Studies on preparation of *peda* with ginger powder. *Int J Food Agric Vet* 4:64–68
- Hala MF, Ebtisam ED, Sanaa I, Badran MA, Marwa AS, Said ME (2010) Manufacture of low fat UF-soft cheese supplemented with rosemary extract (as natural antioxidant). *J Am Sci* 6:570–579
- IS: SP: 18-(Part XI) (1981) Handbook of food analysis—dairy products (Part XI). Bureau of Indian Standards, Manak Bhavan, New Delhi
- Landge UB, Pawar BK, Choudhari DM (2011) Preparation of *shrikhand* using *ashwagandha* powder as additive. *J Dairy Foods Home Sci* 30:79–84
- Olmedo RH, Nepote V, Grosso NR (2013) Preservation of sensory and chemical properties in flavoured cheese prepared with cream cheese base using oregano and rosemary essential oils. *LWT Food Sci Technol* 53:409–417
- Palit C, Pal D (2005) Studies on mechanized production and shelf life extension of *burfi*. *Indian J Dairy Sci* 58:12–16
- Parekh JV (2013) Innovations in traditional Indian dairy foods. Global dairy industry and food security. In: Souvenir, 41st dairy industry conference and IIDE, IDA (west zone), Mumbai, pp 21–30
- Parmar P, Khamrui K, Devaraja HC, Singh RRB (2013) The effects of alcoholic extract of Arjuna (*Terminalia arjuna* Wight and Arn.) bark on stability of clarified butterfat. *J Med Plants Res* 7:2545–2550
- Patel RS, Rajorhia GS (1979) Antioxidative role of curry (*Murraya koenigi*) and betel (*Piper betel*) leaves in *ghee*. *J Food Sci Technol* 16:158–160
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 26:1231–1237
- Samaddar M, Ram C, Sen M (2015) Assessment of storage stability of essential oil enriched flavoured milk. *Indian J Dairy Sci* 68:357–363
- Sarkar K, Ray PR, Ghatak PK (2002) Effect of sodium and potassium metabisulphites on shelf life of cow milk *burfi*. *Indian J Dairy Sci* 55:79–82
- Sawale PD, Singh RRB, Arora S (2015) Stability and quality of herb (*Pueraria tuberosa*)-milk model system. *J Food Sci Technol* 52:1089–1095
- Shobha D, Bharati P (2007) Preparation of *burfi* from ber: a value addition. *Karnataka J Agric Sci* 20:448–449
- Singh RR, Singh R, Shakya BR (2014) Impact of turmeric addition on the properties of *paneer*, prepared from different types of milk. *Int J Curr Eng Sci Res* 4:1874–1883
- Sirsat AB, Shinde AT, Korake RL (2013) Studies on preparation of ash gourd *peda*. *Indian J Dairy Sci* 66:213–217
- Sivakumar GM, Dhanalakshmi B, Nareshkumar C, Pugazhenthir TR (2014) Antioxidant activity of herbal extract on *khoa*. *Indian J Vet Anim Sci Res* 43:436–444
- Smith PA, Stewart J, Fyfe L (2001) The potential application of plant essential oils as natural food preservatives in soft cheese. *Food Microbiol* 18:463–470
- Srivastava T, Saxena DC (2012) Optimization of total polyphenol content and antioxidant activity on preparation of novel bitter gourd sweet. *IRACST Eng Sci Technol Int J (ESTIJ)* 2:861–874
- Srivastava P, Prasad SGM, Ali MN, Prasad M (2015) Analysis of antioxidant activity of herbal yoghurt prepared from different milk. *Pharma Innov J* 4(3):18–20
- Vijayalakshmi NS, Indiramma AR, Viswanath P, Dattatreya A, Kumar KR (2005) Extension of the shelf-life of *burfi* by packaging. *J Food Qual* 28:121–136
- Wenqiang G, Shufen L, Ruixiang Y, Shaokun T, Can Q (2007) Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and other three traditional extraction methods. *Food Chem* 101:1558–1564
- Zhang Q, Zhang J, Shen J, Silva A, Dennis DA, Barrow CJ (2006) A simple 96-well microplate method for estimation of total polyphenol content in seaweeds. In: Eighteenth international seaweed symposium. Springer, Netherlands, pp 219–224