Physicochemical, antioxidant and in-vitro release behaviour of *burfi* added with curcumin as a source of functional ingredient

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Received: 19 December 2019 / Accepted: 17 April 2020 / Published online: 12 July 2020
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Abstract: The present study was conducted to explore the benefits of curcumin and to enhance the antioxidant potential of *burfi*. Three different forms of curcumin *viz.* native curcumin, curcumin emulsion, and dried curcumin encapsulate powder were added to *burfi*. Addition of curcumin in *burfi* resulted in significant ($P < 0.05$) increase in the antioxidant potential. A highest antioxidant potential with minimal decrease in the sensorial and colour attributes was observed in dried encapsulate added at patting stage. The functional properties were enhanced with the extent of dried curcumin encapsulate in *burfi*. The in-vitro study revealed that encapsulated curcumin was the most suitable form for addition in *burfi* as 63% of curcumin retention was observed after intestinal digestion. Curcumin encapsulate added *burfi* was found effective against fungal spoilage-causing microorganisms due to the reduction of 0.35 Log CFU/ml *Aspergillus flavus* and 0.47 Log CFU/ml *Aspergillus niger*. The available curcumin restricts the growth of spoilage-causing microorganisms and thus extends the shelf life of curcumin encapsulate added *burfi* by 3 days when stored at $30 \pm 1^\circ\text{C}$. Thus, curcumin in the form of dried encapsulate was found to be effective for enhancement of antioxidant potential and shelf life of *burfi*.

Keywords: Antioxidant, *Burfi*, Curcumin, Shelf life, Sensory attributes

Introduction

Turmeric (*Curcuma longa* L.) is a herbal plant that belongs to the ginger family, originated and grown in the Indian subcontinent. Curcumin is the bioactive component present in turmeric, comprise of about 3-5% of total rhizome and is responsible for the yellow colour (Jayaprakasha et al. 2006). It has a wide array of functional attributes *viz.*, antioxidant, antimicrobial, antifungal, neuroprotective and anticarcinogenic activity and over the past few years, there has been an increasing interest in curcumin due to its medicinal properties (Maheshwari et al. 2006). Curcumin possess Generally Recognized As Safe (GRAS) status by the FDA for food applications. Despite of many benefits, the lipophilic nature of curcumin limits its application in many of the food products, thus restricts the exploration of all benefits of this bioactive compound. In addition to its solubility, the lower bioavailability, another critical issue which hinders its application in food products (Kakkar et al. 2011).

To explore the functional benefits of curcumin, extensive research has been carried out to enhance its water solubility and stability to make it available for food application. Many novel technologies like complexation, emulsion, and encapsulation were used to protect curcumin from harsh processing conditions, makes it more stable, mask undesirable taste and enable it for food application (Madene et al. 2006). Encapsulation is the promising technology to protect bioactive components and it is extensively used in dairy as well as in the food industry. The various phytochemicals have been successfully encapsulated using nanoemulsions (Garti, 2003). Spray-drying is considered as an effective technique for encapsulating curcumin in milk system as being a fat soluble substance, curcumin gets entrapped with fat globules inside a solid mix of lactose and protein and thus protected against harsh processing condition during food application (Neves et al. 2019). Despite of many successful preparations of curcumin emulsion and encapsulates, only a few researchers have studied the actual behaviour of prepared curcumin emulsion in dairy products. Lodh et al. (2018) reported that *ghee* with enhanced antioxidant property and overall sensorial acceptability could be prepared by incorporation of curcumin.

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Burfi is one of the most popular traditional *khoa* based sweet prepared by heat desiccation of milk followed by the addition of sugar. However, it is highly susceptible to the chemical as well as microbial spoilage (Prasad et al. 2017b). With increased demand, the lower shelf life imparts an additional burden on the dairy industry. Thus, addition of naturally occurring bioactive compounds such as curcumin to improve functionality and shelf life. The incorporation of curcumin in *burfi* not only enhances its shelf life but also contributes to better human health of consumer due to the beneficial health attributes of curcumin. Thus, the present work has been undertaken with the objective of application of encapsulated curcumin as a source of functional ingredient in *burfi* to enhance its antioxidant potential and shelf life and its comparison assessment with native curcumin added *burfi*.

Materials and Methods

Materials

Fresh milk was procured from the Experimental Dairy of the ICAR-National Dairy Research Institute, Karnal, India and was standardised to 6% fat and 9% SNF using Pearson's square method. Curcumin powder with 93.03% total curcuminoids was procured from M/s Plant Lipids Pvt. Ltd. Kolenchery, Cochin, Kerala. Whey protein-80 (WPC- 80) was procured from M/s Globex Enterprises, Delhi, India. Maltodextrin (maximum 20.00% reducing sugar) and Gum acacia were procured from M/s HiMedia Laboratories Pvt. Ltd., Mumbai. Butteroil was prepared from cream containing 80% fat and 2.3% SNF obtained from Experimental Dairy, National Dairy Research Institute, Karnal using direct evaporation method (De, 1989). Partially concentrated skimmed milk with 13.85% total solids was taken from Experimental Dairy, National Dairy Research Institute, Karnal.

Preparation of oil-in-water (O/W) curcumin emulsion

The curcumin emulsion (O/W) was prepared in three stages using the method described by Meena (2018). In the first stage, the core phase was prepared by solubilisation of curcumin in butteroil with continuous stirring at 60°C for 10 min with the help of magnetic stirrer. In second stage, the wall phase was prepared by mixing 1:2 parts of protein (WPC-80) and carbohydrate (maltodextrin and gum acacia in 90:10 proportions) in partially concentrated skimmed milk. In third stage, the core material (oil phase) and the wall material were properly mixed using a blender and the emulsion was prepared by high-speed homogenisation using Ultra-Turrax mixer at 10,000 rpm at 60°C for 10 min. In the final stage to dried curcumin encapsulate was prepared by spray drying the emulsion at 180° and 85°C inlet and outlet air temperature, respectively.

Effect of different processing conditions on emulsion stability

The stability of curcumin emulsion was tested against diverse processing conditions like pH, ionic concentration, and heat treatment using the method described by Sari et al. (2015) with certain modifications. For effective heating, 10 ml emulsion was taken in test tubes and subjected to different heat treatments viz., pasteurization (63°C for 30 min) and boiling (95°C for 10 min). The effect of pH was assessed by adjusting the pH (3, 6 and 9) of emulsion using either 0.1 N HCl or 0.1 N NaOH solution. A different concentration of salt (0.35M, 0.70M, and 1M NaCl) was added to prepare the emulsion with different ionic concentration. After exposing to the above mentioned treatment, the stability of curcumin emulsion was evaluated on the basis of particle size distribution, viscosity and zeta potential using Master sizer 3000 version 3.30 (Malvern Instruments Ltd, Malvern, Worcs WR14)).

Preparation of *burfi*

The control *burfi* was prepared using the standard batch method as proposed by Bhatele (1983). During the preparation of curcumin *burfi*, different forms of curcumin viz., native curcumin (free form), curcumin emulsion, and dried curcumin encapsulate powder in quantity equivalent to 100 ppm free curcumin (100 ppm native curcumin, 25% liquid emulsion, and 10% dried curcumin encapsulate on *khoa* basis) were added separately at different stages of *burfi* preparation (initially in standardized milk, after the first boil, initially in milk along with sugar, and at patting stage).

Antioxidant potential and total phenolic content

The antioxidant potential of control and curcumin added *burfi* was evaluated by DPPH (2, 2 diphenyl-1-picryl hydrazyl) method given by Brand-Williams et al. (1995). 0.1 mL of prepared methanolic extracted sample solution was mixed with 2.9 mL of freshly prepared DPPH working solution in amber coloured test tube and was properly vortexed and incubated in dark at 37°C for 30 min. After incubation, absorbance of the solution was measured spectrophotometrically at 517 nm. For blank, 0.1 mL methanol was taken instead of the sample.

ABTS [2, 2'-azinobis (3 ethyl benzothiazoline) -6-sulfonic acid] free radical scavenging activity of control as well as curcumin added *burfi* was evaluated by the method given by Re et al. (1999). 0.1 mL of prepared sample solution was mixed with 3.9 mL of ABTS working solution and incubated in dark at 37°C for 6 min. After incubation, absorbance of the solution was measured spectrophotometrically at 734 nm. For blank, 0.1 mL methanol was taken instead of the sample.

$$\text{Inhibition (per cent)} = \frac{(\text{Blank absorbance} - \text{Sample absorbance})}{\text{Blank absorbance}} \times 100$$

Total phenolic content of *burfi* samples (control and curcumin *burfi*) were analysed by Folin-Ciocalteu's method of Maizura et al. (2011) with slight modification. 0.4 mL of methanolic extracted sample solution was mixed with 2 mL of 0.2N Folin Ciocalteu's phenol reagent. After 3 minutes, 1.6 mL of sodium carbonate solution (7.5%) was added into it and incubated at 37°C for 2 hr. The absorbance was measured spectrophotometrically at 730 nm.

Estimation of curcumin retention in *burfi*

The curcumin retention in *burfi* was estimated according to the method of ASTA (1985) with slight modification. Exactly, 1 g of *burfi* sample was mixed with 10 mL of 95% ethyl alcohol and mixed thoroughly using pestle and mortar to facilitate colour extraction. The solution was subjected to centrifugation at 4000 rpm for 30 minutes followed by filtration through 0.22µ syringe filter. The absorbance of filtrate was measured at 425 nm.

Colour analysis

Colour measurement of *burfi* samples was estimated using Hunter-Lab model Colour Flex® (Mini Scan XE plus, Hunter Associates Laboratory Inc. Reston, Virginia, U.S.A.) and the results were expressed in terms of CIE-LAB (L*, a* and b*) system.

Sensory evaluation

Control and curcumin added *burfi* samples were organoleptically evaluated on a 9-point hedonic scale by a panel of semi-trained judges selected from the Faculty of Dairy Technology Division.

Texture analysis

The texture profile analysis of *burfi* sample (1 cm height and 1 cm in diameter) was evaluated using TA-XT2i Texture Analyzer (M/s Stable Micro Systems, UK) using P75 Probe, test speed of 1 mm/s, Compression of 80 %, 25 ± 1°C testing temperature, Load cell 25 kg.

In vitro release kinetics of encapsulated curcumin added *burfi*

In-vitro release kinetics of encapsulated curcumin *burfi* was evaluated under simulated gastrointestinal conditions as per protocol given by Herrero-Barbudo et al. (2009).

Microbial analysis

For microbial analysis, Total plate count, coliform count, yeast and mould count were determined by the standard procedure given by Indian Standards IS: SP: 18(Part XI)-1981.

Physicochemical analysis

The free fatty acids (FFA) content of *burfi* was estimated using the method of Deeth and Fitz-Gerald (1975). Total Hydroxy Methyl

Furfural (HMF) content in *burfi* was determined by the method recommended by Keeney and Bassette (1959).

Antifungal activity

Fungal cultures NCDC-224 (*Aspergillus niger*) and NCDC- 226 (*Aspergillus flavus*) were subjected for proper activation by three subculturing. Curcumin *burfi* (11 g) was properly mixed in 99 mL saline solution added with 100µL fully activated fungal culture and allowed to react with curcumin present inside *burfi* for 4 h. The subsequent plating was performed by standard protocol given in Indian Standards IS: SP: 18 (Part XI)-1981.

Statistical analysis

Data obtained from various experiments were recorded as mean ± standard error (SE) and were subjected to analysis of variance (ANOVA) using Tukey's post hoc comparison test to establish the significance of differences among the mean values at 5 per cent level of significance by employing SPSS statistical package.

Results and Discussion

Characterization and process stability of curcumin nanoemulsion

The effect of process conditions on emulsion stability was evaluated in terms of particle size distribution, zeta potential, and viscosity and the results are presented in Table 1.

Effect of pH

The particle size and zeta potential were significantly affected with change in pH of emulsion, while the viscosity showed a non-significant difference between all the pH adjusted emulsions. The emulsion adjusted to pH 3 was completely destabilized with increased particle size to 1603.67 nm (Figure 1) and reduction in zeta potential to -0.313 mV. This could be due to coagulation and aggregation of milk proteins with the decrease in pH caused due to the shifting of electrical charge on particles that result in the reduction of electrostatic repulsive forces between protein molecules. A similar trend was observed by Sari et al. (2015) in curcumin nanoemulsion adjusted to pH 3 where the zeta potential was significantly reduced from - 6.9 to + 2.03 mV. Rao and McClements (2011) studied the effect of processing conditions on nano/microemulsions and found destabilization and aggregation of emulsion at lower pH values, which was attributed to the reduction of electrostatic repulsive forces.

Effect of heat treatment

Heat treatment (63°C/30 min) caused a slight change in the particle size and zeta potential, but the destabilization effect was significantly higher in extremely heated emulsions (95°C/10 min) with increased particle size to 1099.83 (Figure 2) while the zeta

potential decreased to -16.84 mV. A tremendous increase in viscosity from 16.57 to 1623.33 mPa.s was observed in boiled emulsion (95°C/10 min). The destabilizing effect was associated with the denaturation and aggregation of whey proteins due to intense heating that destroyed the native structure of the emulsion. Sari et al. (2015) reported a slight increase in the particle size of curcumin nanoemulsion after pasteurization (63°C/30 min) that increased with increased heat treatment (95°C/10 min).

Effect of ionic concentration

A non-significant difference in the viscosity of curcumin emulsion was observed in comparison to control emulsion due to variation in ionic concentration, but the zeta potential and particle size distribution were significantly affected. The average particle size was increased to 394.43 nm at 1M NaCl concentration (Figure 3). A non-significant difference in zeta potential was observed in control curcumin emulsion (-20.97 mV) and emulsion added with 0.35 M NaCl (-20.20 mV), but with increased ionic concentration to 1 M NaCl a significant decrease in the zeta potential to -17.73 mV was observed. This could be due to the alteration in the repulsive forces between the proteins at higher salt concentration. Sari et al. (2015) reported a significant alteration in the zeta potential of curcumin nanoemulsion with changes in ionic concentration.

Effect of a different form of curcumin and stages of addition on free radical scavenging activity (ABTS and DPPH) and total phenolic content of *burfi*

The results of the antioxidant activity and total phenolic content of *burfi* added with different forms of curcumin at various stages of preparation are presented in Table 2. The ABTS and DPPH free radical scavenging activity of *burfi* was significantly increased with the addition of curcumin. Antioxidant activity of both emulsion and dried encapsulate added *burfi* was significantly higher than native curcumin added *burfi*. The increase in antioxidant potential of encapsulation was observed as compared with native curcumin added *burfi*, with an approximate 25 to 30

µg Trolox equivalent (depends on the stage of addition). The different stages of curcumin addition had a significant effect on antioxidant potential with the highest activity observed in *burfi* added with curcumin encapsulate at patting stage. The total phenolic content in curcumin added *burfi* was significantly affected by different forms of curcumin. The highest phenolic content was found in *burfi* added with liquid curcumin emulsion at patting stage. The lowest antioxidant potential and phenolic content of native curcumin added *burfi* could be due to the interactive effect of curcumin and milk constituents that decreased the curcumin concentration in *burfi*. The hydrophilic and hydrophobic interactions were the binding force behind the complexation of polyphenolic compounds and caseins (Pan et al. 2014). Encapsulation protects curcumin and restricts its interaction with casein, thus enhances the potential functional benefits of curcumin. Addition of curcumin encapsulate at patting stage caused less destabilization of curcumin encapsulate (due to heating and stirring) thus results in minimal interaction with milk solids. Pan et al. (2014) observed the interaction of curcumin with casein micelles that limits the actual functional benefits of curcumin when added in milk system. Bourassa et al. (2013) also reported the interaction of casein (α -casein and β -casein) with curcumin and other polyphenolic compounds when added in the milk system.

Effect of a different form of curcumin and stages of addition on curcumin stability in *burfi*

The effect of different form of curcumin and stages of addition on curcumin stability in *burfi* were compared and listed in Table 2. Emulsion and dried curcumin encapsulate added *burfi* retained significantly higher amount of curcumin (approximately 90%) than native curcumin added *burfi*. Among all the stages, the highest curcumin retention was observed at patting stage. The results indicate the resistance of encapsulation against the interaction of curcumin and milk components due to the shielding effect of the coating material. A same shielding effect of the coating material against the interaction of curcumin and milk components was observed by Maurya (2012), who prepared curcumin-

Table 1 Effect of processing conditions on emulsion stability

Treatment	Average particle size distribution (nm)	Zeta potential (mV)	Viscosity (centipoises)
Untreated emulsion (pH- 6.5)	155.70±10.51 ^a	-20.97±0.44 ^b	16.57±0.78 ^a
pH -3	1603.67±16.90 ^c	-0.31±0.04 ^c	13.03±0.29 ^a
pH -6	307.00±31.97 ^{ab}	-17.90±0.46 ^{cd}	11.63±0.90 ^a
pH -9	505.80±47.35 ^c	-24.50±0.26 ^a	12.57±0.09 ^a
Ionic concentration 0.35 M NaCl	382.03±34.69 ^{bc}	-20.20±0.57 ^b	12.37±0.32 ^a
Ionic concentration 0.70 M NaCl	334.06±37.20 ^{bc}	-19.33±0.30 ^{bc}	9.34±0.16 ^a
Ionic concentration 1 M NaCl	394.43±34.98 ^{bc}	-17.73±0.52 ^{cd}	10.15±0.74 ^a
Heat treatment (63°C/30 min)	286.77±21.47 ^{ab}	-19.60±0.40 ^{bc}	20.97±0.75 ^a
Heat treatment (95°C/10 min)	1099.83±54.54 ^d	-16.84±0.29 ^d	1623.33±8.82 ^b

Values are Mean ± standard error (n=3)

a,b,c,d,e: Mean with different superscripts are significantly different within the column (p<0.05)

Table 2 Effect of a different form of curcumin and stages of addition on antioxidant activity, total phenolic content, curcumin stability and hunter colour profile of *burfi*

Form of curcumin	Stages of addition	Antioxidant free radical scavenging activity (μg Trolox equivalent/g sample)			Total phenolic content (μg Gallic acid equivalent/g sample)	Curcumin stability and retention in <i>burfi</i> (ppm)	Hunter colour profile		
		ABTS	DPPH				L*	a*	b*
Native curcumin (free form)	Initially in standardized milk	127.435 $\pm$ 3.40 ^{Bb}	88.717 $\pm$ 3.66 ^{Bbc}		35.883 $\pm$ 0.32 ^{Ab}	76.224 $\pm$ 2.51 ^{Ab}	67.57 $\pm$ 0.09 ^{Bc}	1.45 $\pm$ 0.01 ^{Ca}	55.51 $\pm$ 0.05 ^{Bc}
	After the first boil	127.072 $\pm$ 4.42 ^{Bb}	89.009 $\pm$ 4.44 ^{Bb}		36.517 $\pm$ 0.47 ^{Ab}	77.628 $\pm$ 2.11 ^{Aa}	64.39 $\pm$ 0.21 ^{Bb}	1.64 $\pm$ 0.01 ^{Ca}	53.41 $\pm$ 0.03 ^{Bb}
	Initially along with sugar	131.633 $\pm$ 1.22 ^{Bb}	92.848 $\pm$ 5.72 ^{Bb}		35.667 $\pm$ 0.22 ^{Ab}	81.630 $\pm$ 0.32 ^{Abc}	65.73 $\pm$ 0.05 ^{Bb}	2.53 $\pm$ 0.04 ^{Ca}	52.93 $\pm$ 0.04 ^{Bb}
	Patting stage	136.087 $\pm$ 1.39 ^{Bc}	95.431 $\pm$ 2.14 ^{Bc}		36.033 $\pm$ 0.20 ^{Ab}	82.497 $\pm$ 0.30 ^{Ac}	58.31 $\pm$ 0.04 ^{Ba}	3.40 $\pm$ 0.04 ^{Cb}	49.43 $\pm$ 0.21 ^{Bb}
	Initially in standardized milk	140.194 $\pm$ 1.08 ^{Cb}	112.684 $\pm$ 2.16 ^{Cbc}		44.500 $\pm$ 0.62 ^{Cb}	89.635 $\pm$ 0.60 ^{Cab}	69.22 $\pm$ 1.17 ^{Bc}	2.66 $\pm$ 0.05 ^{Ba}	65.70 $\pm$ 1.08 ^{Dc}
Curcumin emulsion	After the first boil	146.754 $\pm$ 2.70 ^{Cb}	107.548 $\pm$ 1.37 ^{Cb}		44.617 $\pm$ 1.11 ^{Cb}	89.795 $\pm$ 0.79 ^{Ca}	65.16 $\pm$ 0.87 ^{Bb}	4.04 $\pm$ 0.12 ^{Ba}	56.51 $\pm$ 0.72 ^{Db}
	Initially along with sugar	144.759 $\pm$ 3.63 ^{Cb}	106.094 $\pm$ 2.65 ^{Cb}		41.567 $\pm$ 0.53 ^{Cb}	91.895 $\pm$ 0.59 ^{Cbc}	63.84 $\pm$ 0.89 ^{Bb}	3.28 $\pm$ 0.09 ^{Ba}	58.59 $\pm$ 0.89 ^{Db}
	Patting stage	155.234 $\pm$ 3.65 ^{Cc}	120.760 $\pm$ 2.70 ^{Cc}		43.700 $\pm$ 1.69 ^{Cb}	90.685 $\pm$ 0.46 ^{Cc}	58.19 $\pm$ 0.62 ^{Ba}	3.55 $\pm$ 0.06 ^{Bb}	59.33 $\pm$ 0.64 ^{Db}
	Initially in standardized milk	145.930 $\pm$ 1.51 ^{Cb}	110.143 $\pm$ 1.53 ^{Cbc}		40.800 $\pm$ 0.23 ^{Bb}	86.689 $\pm$ 0.61 ^{Bab}	67.87 $\pm$ 0.84 ^{Bc}	4.25 $\pm$ 0.06 ^{Aa}	60.93 $\pm$ 1.11 ^{Cc}
	After the first boil	144.488 $\pm$ 2.64 ^{Cb}	109.745 $\pm$ 1.02 ^{Cb}		40.400 $\pm$ 0.29 ^{Bb}	84.178 $\pm$ 0.46 ^{Ba}	65.65 $\pm$ 0.93 ^{Bb}	3.16 $\pm$ 0.08 ^{Aa}	59.16 $\pm$ 0.31 ^{Cb}
Dried curcumin encapsulate powder	Initially along with sugar	148.509 $\pm$ 2.29 ^{Cb}	108.720 $\pm$ 1.39 ^{Cb}		40.217 $\pm$ 0.22 ^{Bb}	85.639 $\pm$ 0.42 ^{Bbc}	65.51 $\pm$ 0.54 ^{Bb}	3.57 $\pm$ 0.06 ^{Aa}	56.93 $\pm$ 0.57 ^{Cb}
	Patting stage	158.994 $\pm$ 1.80 ^{Cc}	118.295 $\pm$ 2.15 ^{Cc}		41.717 $\pm$ 0.15 ^{Bb}	87.397 $\pm$ 0.64 ^{Bc}	63.83 $\pm$ 0.85 ^{Ba}	1.34 $\pm$ 0.06 ^{Ab}	55.46 $\pm$ 1.21 ^{Cb}
	Control	112.707 $\pm$ 1.70 ^{Aa}	71.780 $\pm$ 0.88 ^{Aa}		35.180 $\pm$ 0.60 ^{Aa}	-	60.79 $\pm$ 1.53 ^{Aa}	6.27 $\pm$ 0.75 ^{Dc}	27.23 $\pm$ 0.49 ^{Aa}

Values are mean $\pm$ standard error (n=3)

a, b, c: Mean with

different superscripts are significantly different within stage of addition (p<0.05)

A, B, C, D: Mean with different superscripts are significantly different within form of curcumin (p<0.05)

Fig. 1 Particle size distribution of curcumin emulsion of varied pH (3, 6 and 9)

Fig. 2 Particle size distribution of curcumin emulsion with varied heat treatment (63°C/30 min and 95°C/10 min)

Table 3 Effect of a different form of curcumin and stages of addition on sensory attributes of *burfi*

Form of curcumin	Stages of addition	Flavour	Texture	Colour and appearance	Overall acceptability
Native curcumin (free form)	Initially in standardized milk	7.20±0.27 ^{Aa}	7.30±0.15 ^{Aa}	7.23±0.37 ^{Aa}	7.31±0.35 ^{ABab}
	After the first boil	7.20±0.18 ^{Aa}	7.12±0.16 ^{Aa}	7.24±0.04 ^{Aa}	7.22±0.12 ^{ABa}
	Initially along with sugar	7.23±0.09 ^{Aa}	6.99±0.07 ^{Aa}	7.48±0.08 ^{Aa}	7.21±0.15 ^{ABa}
	Patting stage	7.28±0.15 ^{Aab}	7.13±0.19 ^{Aa}	7.37±0.19 ^{Aa}	7.36±0.07 ^{ABab}
Curcumin emulsion	Initially in standardized milk	6.90±0.26 ^{Aa}	7.26±0.06 ^{ABa}	7.48±0.29 ^{Aa}	7.18±0.16 ^{Aab}
	After the first boil	7.22±0.02 ^{Aa}	7.18±0.04 ^{ABa}	7.20±0.22 ^{Aa}	7.03±0.09 ^{Aa}
	Initially along with sugar	7.10±0.06 ^{Aa}	7.16±0.23 ^{ABa}	6.88±0.51 ^{Aa}	6.92±0.19 ^{Aa}
	Patting stage	7.53±0.15 ^{Aab}	7.29±0.05 ^{ABa}	7.18±0.31 ^{Aa}	7.08±0.20 ^{Aab}
Dried curcumin encapsulate powder	Initially in standardized milk	7.37±0.20 ^{Aa}	7.19±0.18 ^{ABa}	7.30±0.05 ^{Aa}	7.20±0.09 ^{Aab}
	After the first boil	7.19±0.11 ^{Aa}	7.26±0.13 ^{ABa}	7.13±0.14 ^{Aa}	7.17±0.11 ^{Aa}
	Initially along with sugar	7.13±0.13 ^{Aa}	7.51±0.07 ^{ABa}	7.13±0.07 ^{Aa}	7.08±0.04 ^{Aa}
	Patting stage	7.45±0.23 ^{Aab}	7.51±0.07 ^{ABa}	7.47±0.08 ^{Aa}	7.49±0.20 ^{Aab}
	Control	7.83±0.03 ^{Bb}	7.55±0.13 ^{Bb}	7.73±0.16 ^{Aa}	7.69±0.15 ^{Bb}

Values are mean ± standard error (n=3)

a,b: Mean with different superscripts are significantly different within stage of addition (p<0.05)

A,B: Mean with different superscripts are significantly different within form of curcumin (p<0.05)

cyclodextrin complex and reported 85 to 90% of total curcumin retention in *lassi*.

Effect of a different form of curcumin and stages of addition on hunter colour values of *burfi*

The lightness (L*), redness-greenness (a*) and yellowness-blueness (b*) index of the control and curcumin added *burfi* were presented in Table 2. The L* value of all the curcumin added

burfi samples varied from 58.19 to 69.22 which was significantly higher than control *burfi*. The lightness value of *burfi* was associated with the extent of heat treatment which mainly depends upon the stage of addition. The initial fortification in standardized milk showed the highest lightness index with L* value of 69.22 whereas fortification at patting stage showed the least (58.19). The a* value of control sample was 6.27 which was significantly higher than all curcumin added *burfi* and ranged from -4.25 to

Fig. 3 Particle size distribution of curcumin emulsion with different ionic concentration (0.35 M NaCl, 0.70 M NaCl, and 1M NaCl)

Fig. 4 *In-vitro* release kinetics of curcumin encapsulated *burfi*

+3.55. Both (different forms of curcumin and the stage of addition) were responsible for decreased a^* value of *burfi*. The b^* value (yellowness-blueness index) of control sample was 27.23 that significantly increased to 65.70 in curcumin emulsion added *burfi* at the initial stage of addition. The emulsion and encapsulate curcumin added *burfi* showed significantly higher b^* value than native curcumin added *burfi*. This could be due to the lower retention of curcumin in *burfi* when added in native form. The change in the colour value of *burfi* was due to the addition of encapsulated curcumin. Meena (2018) has observed the L^* , a^* , and b^* value of dried curcumin encapsulate to be 87.44, -7.49, and 72.02, respectively that further decreased with storage period. The addition of curcumin in milk at the initial stage of *burfi* preparation resulted in higher destabilization of curcumin thus released more curcumin due to the higher exposure of heat and scrapping that affected the colour values of curcumin added *burfi*.

Effect of a different form of curcumin and stages of addition on sensorial attributes of *burfi*

The average flavour score of control *burfi* was 7.83, which was reduced to 6.90 upon addition of curcumin (Table 3). A significant difference was observed in *burfi* samples fortified with different forms of curcumin added at various stages during preparation. The decreased flavour score of curcumin *burfi* may be attributed to the bitter taste of curcumin that mask the flavour of *burfi*. The body and textural scores of all the curcumin added *burfi* were

significantly less than the control sample. A non-significant difference was observed in the colour and appearance score of curcumin added *burfi* and control sample. The intense yellow coloured *burfi* was least accepted by the sensory panellists than light yellow coloured *burfi*. The overall acceptability scores of curcumin added *burfi* ranged from 6.92 to 7.49 which were significantly less than control *burfi* with a mean score of 7.69. The bitter after taste and intense yellow colour decreased the overall acceptability of curcumin added *burfi*. The bitter taste was more pronounced in *burfi* added with native curcumin which was reflected in the flavour score. *Burfi* added with dried curcumin encapsulate at patting stage was highly acceptable with average overall acceptability score of 7.49 than other curcumin added *burfi* but comparatively less than the control sample. Similar, results of bitterness in *burfi* added with turmeric essential oil that decreased the consumer acceptability were reported by Prasad et al. (2017a). Maurya (2002) reported a decreased overall acceptance of curcumin fortified *lassi* due to intense yellow colour. Based on above observation the dried curcumin encapsulate was the most suitable form for incorporation in *burfi* that enhance the antioxidant potential but the enhancement was much higher during the patting stage of preparation.

Optimization of the level of addition of dried curcumin encapsulate in *burfi*

To optimize the level of addition, the *burfi* was incorporated with dried curcumin encapsulate at levels of 5%, 7.5%, 10%, 12.5%,

Fig. 5 Antifungal activity of curcumin encapsulate added *burfi*

and 15% and the responses were measured in terms of antioxidant potential, hunter colour value and sensorial attributes.

Effect of level of dried curcumin encapsulate on antioxidant activity and total phenolic content of *burfi*

The antioxidant potential of *burfi* samples incorporated with different levels of dried curcumin encapsulate were analyzed by ABTS and DPPH free radical scavenging method and compared with the positive control sample containing 200 ppm BHA. The ABTS and DPPH free radical scavenging activity of curcumin encapsulate added *burfi* was significantly less than the positive control but increased with the extent of curcumin (Table 4). Moreover, the highest antioxidant potential was obtained at 15% level of dried curcumin encapsulate. In addition, the *burfi* added with 15% level of dried curcumin encapsulate was observed to have highest phenolic content of 48.317 µg gallic acid equivalent/g sample. Polyphenolic compounds are naturally present in many

plants and are potential source of antioxidants (Soong and Barlow, 2004; Balasundram et al. 2006). A similar trend of increased antioxidant potential was observed in *burfi* with the addition of different polyphenol-rich herbs (Prasad et al. 2017a). The increase in the antioxidant potential of bread with incorporation of turmeric was observed by Lim et al. (2011).

Effect of level of dried curcumin encapsulate on Hunter colour profile of *burfi*

Burfi samples with different concentrations of curcumin encapsulate were evaluated for colour profile using Hunter lab colorimeter and the results were presented in Table 4. A non-significant difference in L* value was observed in *burfi* with highest lightness (63.268) value in 10% curcumin encapsulate *burfi*. However, the a* (redness-greenness) value of *burfi* showed a significant decrease with increased level of curcumin

encapsulate. With the increased level of curcumin encapsulate a significant increase in the b^* (yellowness-blueness) value was observed with the highest yellowness (58.174) in *burfi* with 15% curcumin encapsulate. The oversaturation of yellowness at 15% curcumin encapsulates exhibits unnatural appearance of *burfi*, thus less accepted. Cheese curcumin showed higher yellowness index while yoghurt curcumin showed a slight greenish-yellow colour (Marcolino et al. 2011). Manoharan et al. (2012) observed increased yellowness of ice cream with addition of curcumin.

Effect of level of dried curcumin encapsulate on sensorial attributes of *burfi*

The sensory scores of *burfi* in terms of flavour, texture, colour and overall appearance perceived by sensory panellists, were reported in Table 5. The level of curcumin encapsulate showed a non-significant effect on the sensory attributes of *burfi* and the scores decrease beyond 10 % level of curcumin encapsulates due to the over saturation of yellowness. At higher concentration curcumin encapsulate induced intense yellow colour and bitter taste in *burfi* thereby, limiting its overall acceptance. Kumar et al.

(2016) observed a non-significant difference in sensory analysis on 9 point hedonic scale of control and curcumin emulsion ice cream. The overall acceptability of bread was observed to be decreased with the increase in the curcumin concentration due to intense yellow crumb colour (Lim et al. 2011).

Based on antioxidant and sensory analysis, 10% level of curcumin encapsulate was found to be effective for incorporation in *burfi* and hence, was considered to be the optimized product with chemical composition of 14.72% moisture, 21.23% fat, 14.82% protein, 18.09% lactose, 2.81% ash and 28.33% sucrose in the final product.

In-vitro release kinetics of curcumin encapsulate added *burfi*

Functional benefits of optimized *burfi* primarily depends on the release of curcumin from encapsulate which was evaluated in simulated gastro-intestinal conditions. A 33% of total curcumin from encapsulate present in *burfi* was released in salivary fluid which was further increased to around 83% in gastric fluid. The alkaline conditions in the intestinal fluid caused around 20%

Table 4 Effect of level of dried curcumin encapsulate on antioxidant activity (ABTS and DPPH), total phenolic content and hunter colour profile of curcumin encapsulate added *burfi*

Level of addition curcumin encapsulates (%)	Antioxidant free radical scavenging activity (μg Trolox equivalent/g sample)		Total phenolic content (μg Gallic acid equivalent/g sample)	Hunter colour profile		
	ABTS	DPPH		L*	a*	b*
Control	112.707 $\pm$ 1.70 ^a	71.780 $\pm$ 0.88 ^a	35.183 $\pm$ 0.60 ^a	60.787 $\pm$ 1.53 ^a	6.269 $\pm$ 0.75 ^b	27.230 $\pm$ 0.49 ^a
BHA (200 ppm)	200.515 $\pm$ 3.46 ^f	195.304 $\pm$ 1.20 ^g	34.633 $\pm$ 0.59 ^a	58.910 $\pm$ 1.92 ^a	4.121 $\pm$ 0.24 ^{ab}	49.508 $\pm$ 1.75 ^b
5 %	119.182 $\pm$ 1.96 ^a	103.148 $\pm$ 0.78 ^b	41.167 $\pm$ 1.11 ^b	62.210 $\pm$ 0.47 ^a	4.424 $\pm$ 0.34 ^{ab}	53.698 $\pm$ 0.71 ^{bc}
7.5 %	134.922 $\pm$ 2.38 ^b	111.909 $\pm$ 0.95 ^c	42.017 $\pm$ 0.62 ^b	63.268 $\pm$ 0.48 ^a	2.727 $\pm$ 0.26 ^a	56.201 $\pm$ 0.92 ^c
10 %	150.634 $\pm$ 1.75 ^c	125.439 $\pm$ 1.41 ^d	43.667 $\pm$ 0.57 ^{bc}	59.600 $\pm$ 1.53 ^a	3.822 $\pm$ 0.64 ^a	55.971 $\pm$ 1.05 ^c
12.5 %	168.404 $\pm$ 2.12 ^d	140.746 $\pm$ 2.27 ^e	45.300 $\pm$ 0.29 ^{cd}	58.762 $\pm$ 0.79 ^a	3.766 $\pm$ 0.37 ^a	58.174 $\pm$ 0.91 ^c
15 %	183.439 $\pm$ 2.61 ^e	152.410 $\pm$ 1.93 ^f	48.317 $\pm$ 0.39 ^d			

Values are mean $\pm$ standard error (n=3)

a,b,c,d,e,f,g: Mean with different superscripts are significantly different within the level of curcumin encapsulates ($p < 0.05$)

Table 5 Effect of varied levels of curcumin encapsulate on sensory attributes of *burfi*

Level of addition curcumin encapsulates (%)	Flavour	Texture	Colour and Appearance	Overall acceptability
Control	7.81 $\pm$ 0.04 ^a	7.73 $\pm$ 0.02 ^a	7.79 $\pm$ 0.09 ^a	7.72 $\pm$ 0.06 ^{ab}
5 %	8.01 $\pm$ 0.07 ^a	7.71 $\pm$ 0.11 ^a	7.73 $\pm$ 0.04 ^a	7.70 $\pm$ 0.06 ^{ab}
7.5 %	7.99 $\pm$ 0.20 ^a	7.79 $\pm$ 0.15 ^a	7.76 $\pm$ 0.18 ^a	7.79 $\pm$ 0.10 ^{ab}
10 %	7.88 $\pm$ 0.25 ^a	8.12 $\pm$ 0.07 ^a	7.84 $\pm$ 0.22 ^a	7.93 $\pm$ 0.12 ^b
12.5 %	7.69 $\pm$ 0.04 ^a	7.66 $\pm$ 0.22 ^a	7.47 $\pm$ 0.06 ^a	7.45 $\pm$ 0.06 ^a
15 %	7.84 $\pm$ 0.05 ^a	7.89 $\pm$ 0.21 ^a	7.30 $\pm$ 0.03 ^a	7.45 $\pm$ 0.04 ^a

Values are mean $\pm$ standard error (n=3)

a,b: Mean with different superscripts are significantly different within the level of curcumin encapsulates ($p < 0.05$)

Fig. 6 Changes in the antioxidant activity of control and encapsulated curcumin *burfi* during storage

degradation of free curcumin released from encapsulate and the remaining 63% free curcumin was found to be stable at the end of intestinal digestion (Figure 4). Sari et al. (2015) determined the *in-vitro* release behaviour of curcumin nanoemulsion and observed that the emulsion had excellent stability in gastric fluid with the release of only 8.48 % of total curcumin, but undergoes destabilization in intestinal digestion with the release of 77.75% curcumin from the emulsion. The release of curcumin from encapsulated added *burfi* in gastric fluid is based on the behaviour of whey protein during the processing of *burfi*. The heat treatment results in the denaturation of whey protein (WPC-80) known for higher digestibility, readily digested in gastric condition and thus released the curcumin present inside encapsulate.

Antifungal activity of curcumin encapsulate added *burfi*

The curcumin encapsulate added *burfi* was evaluated for its antifungal activity against two major milk sweet spoilage causing moulds *viz.*, *Aspergillus flavus* and *Aspergillus niger* and with 0.35 Log CFU/ml and 0.47 Log CFU/ml reduction in the *Aspergillus flavus* and *Aspergillus niger*, the curcumin encapsulate present inside the *burfi* was found to be effective against fungal spoilage (Figure 5). Wang et al. (2009) studied the inhibitory effect of microcurcumin encapsulate against spoilage microorganisms. The highest inhibition was found in *Aspergillus niger* and thus indicates the efficacy of curcumin against fungal microorganisms.

Storage stability and shelf life of curcumin encapsulate added *burfi*

Changes in the antioxidant activity of control and curcumin encapsulate added *burfi* during storage

The ABTS and DPPH free radical scavenging activity of curcumin encapsulate added *burfi* was increased up to 6 days followed by continuous decrease throughout the storage period as depicted in Figure 6. A non-significant difference in the antioxidant activity of curcumin encapsulate *burfi* was observed with storage irrespective of storage temperature. The initial increase in the antioxidant potential of curcumin encapsulate *burfi* was due to the release of remaining curcumin from encapsulate with the storage. This released curcumin scavenges free radicals with

storage period thus, caused further reduction in the antioxidant activity. A similar finding of control release of curcumin from hydrogel during storage was observed by Nakagawa et al. (2013).

Changes in the physicochemical parameters of control and curcumin encapsulate added *burfi* during storage

Control and the curcumin encapsulate added *burfi* were analysed for the physicochemical changes *viz.*, free fatty acid (FFA) content, hydroxymethylfurfural (HMF), thiobarbituric acid (TBA) value, water activity and acidity during storage. The FFA content was significantly increased with storage period and higher FFA content was observed at $30\pm 1^\circ\text{C}$ than $4\pm 1^\circ\text{C}$ storage temperature (Figure 7). At $30\pm 1^\circ\text{C}$, the initial FFA content in control and curcumin encapsulate added *burfi* was 3.68 and 4.6 μ equivalent oleic acid/g *burfi* sample respectively which was significantly increased to 6.88 and 8.12 μ equivalent oleic acid/g *burfi* sample within 9 days of storage. The HMF content indicates the extent of Maillard browning reaction during storage. The HMF formation was significantly higher in control and curcumin encapsulate *burfi* stored at $30\pm 1^\circ\text{C}$ than the samples at $4\pm 1^\circ\text{C}$ storage. At $30\pm 1^\circ\text{C}$, HMF content of curcumin encapsulate *burfi* reached to 28.98 $\mu\text{moles}/100\text{g TS}$ in 12 days of storage. The non-significant difference in the HMF content of control and the curcumin encapsulate added *burfi* indicates a similar extent of browning reaction in both samples. The lipid oxidation (measured in terms of TBA value) was observed to be higher at $30\pm 1^\circ\text{C}$ temperature as compared with the oxidation at $4\pm 1^\circ\text{C}$ storage. The water activity showed a non-significant difference throughout the storage period with initial decrease in the activity of *burfi* samples up to 3 days of storage. A significant increase in acidity of control and curcumin encapsulate *burfi* was observed with increased storage period at both temperatures. The results are in line with the observations of Chawla et al. (2015) who reported a significant increase in the FFA content, HMF and acidity with decreased water activity throughout the storage period in Doda *burfi*.

Changes in the sensory attributes of control and curcumin encapsulate added *burfi* during storage

Fig. 7 Changes in the free fatty acids, hydroxymethylfurfural, thiobarbituric acid, water activity and acidity of control and encapsulated curcumin *burfi* during storage

As depicted in Figure 8, the flavour score of control and the curcumin encapsulate added *burfi* stored at $4 \pm 1^\circ\text{C}$ was significantly decreased from 7.55 and 7.60 to 6.85 and 6.65 within 18 and 21 days of storage respectively. A significant variation in the body and texture score of samples was observed during the storage period and was decreased to as low as 6.50 in curcumin encapsulate added *burfi* after 21 days, while the control *burfi* had scored 6.70 at the end of 18 days of storage. Moreover, a similar trend in colour, appearance score and overall acceptability was also observed throughout the storage period. The overall acceptability score declined from 7.60 to 6.80 in control *burfi* after 18 days of storage, whereas it was reduced to 6.65 after 21 days of storage in the *burfi* added with curcumin encapsulate. A non-significant difference was observed in all the sensorial attributes of control and curcumin encapsulate added *burfi* during the entire storage period at $30 \pm 1^\circ\text{C}$. The decrease in the sensorial attributes of *burfi* samples during storage was due to the evaporative loss of moisture and the oxidative and physical-

chemical changes. Prasad et al. (2017b) observed a decrease in the sensorial attributes of herbal *burfi* during storage due to the excessive hardness of sample in the refrigerated condition. The results observed are in agreement with the findings of Chawla et al. (2015) in Doda *burfi*.

Changes in the instrumental textural attributes of control and curcumin encapsulate added *burfi* during storage

The results revealed that the hardness of control and curcumin encapsulate added *burfi* stored at $4 \pm 1^\circ\text{C}$ was significantly increased from 54.58 N and 46.78 N to 73.87 N and 80.25 N in 18 and 21 days of storage respectively as shown in Figure 9. A similar increased trend was observed in adhesiveness, cohesiveness, gumminess and chewiness while springiness was found to be decreased throughout the storage. The resilience showed a non-significant difference in *burfi* sample stored at different temperatures. These textural changes were less

Fig. 8 Changes in the sensorial attributes of control and encapsulated curcumin *burfi* during storage

Fig. 9 Changes in the textural attributes of control and encapsulated curcumin *burfi* during storage

pronounced in *burfi* stored at $30\pm 1^\circ\text{C}$ as compared to sample kept at refrigerated temperature

($4\pm 1^\circ\text{C}$). The textural change observed in *burfi* samples was due to the evaporation of moisture from the product. Arora et al.

Fig. 10 Changes in the hunter colour profile of control and encapsulated curcumin *burfi* during storage

Fig. 11 Changes in the microbiological count of control and encapsulated curcumin *burfi* during storage

(2007) reported a significant increase in the hardness of low-calorie sweetener added *burfi* from 62.21 N to 89.18 N during one week storage under refrigerated temperature. Chawla et al. (2015) observed a significant variation in hardness, adhesiveness, cohesiveness and gumminess of Doda *burfi* kept at $30\pm 1^\circ\text{C}$ storage temperature.

Changes in the hunter colour profile of control and curcumin encapsulate added *burfi* during storage

The results presented in Figure 10 revealed that the lightness value (L^*) of the control and curcumin encapsulate added *burfi* decreased significantly with storage. The extent of decrease in lightness value was more pronounced at $30\pm 1^\circ\text{C}$ as compared to $4\pm 1^\circ\text{C}$ storage temperature. The a^* value of curcumin encapsulate

added *burfi* was significantly increased from 3.38 and 3.31 to 4.52 and 4.32 within 12 and 21 days of storage at $30\pm 1^\circ\text{C}$ and $4\pm 1^\circ\text{C}$ temperature respectively. The b^* value was found to be increased during storage period. The changes in *burfi* samples may be due to Maillard browning reactions. Arora et al. (2010) observed a decreased lightness value of artificial sweetener added *burfi* with increased storage period. The increase in a^* value with increase in storage temperature and time have been reported by Gothwal and Bhavdasan (1991) and Arora et al. (2010) in *khoa* and artificial sweetener added *burfi*, respectively.

Changes in the microbiological count of control and curcumin encapsulate added *burfi* during storage

The changes in the microbiological counts *i.e.*, total plate count (TPC), coliform count, yeast and mould count of *burfi* samples were evaluated throughout the storage period as revealed in Figure 11. A rapid microbial growth was observed in the control *burfi* sample as compared to the curcumin encapsulate added *burfi*. A similar total plate count was observed in control (after 9 days) and curcumin encapsulate added *burfi* (after 12 days) at $30\pm 1^\circ\text{C}$ storage. The visible yeast and mould growth was also observed after 9 and 12 days of storage at $30\pm 1^\circ\text{C}$ in control and curcumin encapsulate added *burfi*. The coliform count was observed to be increased to 1.30 Log CFU/g in curcumin encapsulate added *burfi* after 12 days of storage but no count was seen upto 6 days of storage at $30\pm 1^\circ\text{C}$. Thus, the results revealed that the antimicrobial and antifungal activity of curcumin restricts or delays the growth of spoilage-causing microorganisms and thus extends the shelf life of curcumin encapsulate added *burfi* upto 3 days as depicted in Figure 11. Hosny et al. (2011) observed the curcumin addition in *Karish* cheese could be an effective mean to protect it from spoilage and pathogenic microorganisms.

Conclusions

In this work, different forms of curcumin were added in *burfi*. The study concluded that the antioxidant activity and total phenolic content of curcumin added *burfi* were higher than the control. The highest antioxidant activity was observed in *burfi* sample added with curcumin encapsulate at patting stage. It was also observed that the curcumin addition affected the hunter colour profile of *burfi* with the least colour variation observed at patting stage. The dried curcumin encapsulate added *burfi* was highly accepted by the sensory panel. Thus, the dried curcumin encapsulate added at patting stage of *burfi* was selected for incorporation due to higher antioxidant potential. The curcumin encapsulate added at different concentrations showed increased b^* value with extent of addition. The overall acceptability of curcumin encapsulate added *burfi* significantly decreased beyond 10% addition and thus, the addition of curcumin encapsulates at 10% level was selected for incorporation in *burfi*. The encapsulated curcumin *burfi* was found to be effective

against fungal spoilage-causing microorganisms. The in-vitro release behaviour revealed that 63% curcumin was found to be stable after the end of intestinal digestion. The free radical scavenging activity of encapsulated curcumin *burfi* was increased upto 6 days of storage. The encapsulated curcumin added *burfi* was observed with a shelf life of 12 days at $30\pm 1^\circ\text{C}$. The storage life was observed to be 18 and 21 days for control and curcumin encapsulated *burfi* at $4\pm 1^\circ\text{C}$.

Acknowledgements

Director, ICAR-NDRI (Karnal), is duly acknowledged for providing all the research facilities. Authors are also thankful to M/s Plant Lipids Pvt. Ltd. Kolenchery, Cochin, Kerala (India) and M/s Globex Enterprises, Delhi, India for providing curcumin and whey protein-80, respectively.

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