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Preparation and Stability Evaluation of Curcumin Fortified *Lassi*, a Fermented Dairy Beverage

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Abstract

Complexing curcumin with β-cyclodextrin increases water solubility and thus functional activity of curcumin. However, application of this complex in traditional fermented products has rarely been explored for curcumin delivery. The present study was undertaken to enhance the curcumin supplementation in *lassi* by using β-cyclodextrin. Starter culture's activity was not affected by curcumin addition. β-cyclodextrin addition increased curcumin retention in *lassi* from 50 to 90%. Curcumin (@ 250 ppm) and its conjugation with β-cyclodextrin at 1:4 ratio was found optimum for supplementation in *lassi*. The developed product had a shelf-life of 20 days at 4±1 °C and 90-95 % RH, when packaged in low density polyethylene pouches or in poly ethylene terephthalate bottles, whereas the control *lassi* had storage stability of 12 days.

Keywords: *Lassi*, Curcumin, β-Cyclodextrin, Storage stability

Alzheimer's disease (AD) is a neuro-degenerative disorder which progresses from mild dementia to widespread neurological impairment. Facts from the World Health Organization reveal that about 60 percent of people living with AD worldwide are from developing country and the number of affected people are projected to reach 82 million in 2030 and 152 million in 2050, (Alzheimers-statistics, 2020). Currently, there is no medical cure for AD except some medications that control its symptoms. Studies in transgenic mouse model containing human familial Alzheimer's disease (AD) gene has demonstrated that low dose (160 ppm) of chronic dietary curcumin effectively disaggregates as well as prevents formation of β-amyloid plaques responsible for AD, supporting the effect of curcumin in preventing and treating the disease (Yang *et al.* 2005). Curcumin also possess anti-microbial activity against many microorganisms with minimum inhibitory concentration between 0.09 and 0.67 μM against gram-positive cocci and gram-negative bacilli (Singh *et al.* 2010). However, the very low (<0.005 wt.%) water solubility of curcumin (Ahmed *et al.* 2012) limit its application in food products having water as the continuous phase. Studies pertaining to enhancing water solubility and hence bio-accessilbity of curcumin mention about binding it with proteins through hydrophobic interactions (Mohammadian *et al.* 2019). On similar lines, encapsulation has also been reported to increase the bio-accessilbity of

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curcumin (Tonnesen *et al.* 2002). Among the different ingredients, β-cyclodextrin has been reported to be an efficient encapsulating agent because of its cyclic structure (Szejtli, 1998). Further, its extremely low toxicity and cheaper cost of production enables it to use in food products (Horvath *et al.* 2008). Complex formed between β-cyclodextrin and curcumin has reported to enhance curcumin delivery in prostate cancer cells (Yallapu *et al.* 2010), anti-cancer effects of curcumin (Zhang *et al.* 2016), etc. However, application of these β-cyclodextrin and curcumin complex in food matrix as delivery vehicle is yet to be explored in greater details.

Lassi is a traditional fermented milk beverage prepared by dilution of curd. It is ready to serve, nutritious, refreshing, delicious and easily digestible beverage which can serve as an excellent medium to carry functional ingredients. Those persons who are unable to take milk due to lactose intolerance, for them *lassi* is an alternative to relish the nature's wonder food milk (Husain *et al.* 2015). Because of intense flavour characteristics (as evident by high acidity and low pH), it could be explored as a vehicle for delivery of a variety of physiologically active compounds. Considering this, it appears that *lassi* could be explored as a delivery vehicle for curcumin in different forms. Hence, the present study was envisaged to develop prepare curcumin fortified *lassi* and evaluation of its storage stability in different packaging materials. The study was undertaken in different stages, viz., effect of curcumin on growth of starter culture for curd (*dahi*) preparation, utilization of β-cyclodextrin for enhancing curcumin retention in *lassi* by varying curcumin level and curcumin: β-cyclodextrin ratio and storage stability of curcumin fortified lassi in different packaging materials.

MATERIALS AND METHODS

Fresh cow whole milk and cream, LDPE packaging material (thickness 70µm) and cane sugar (crystallized sucrose) was obtained from Experimental Dairy of the National Dairy Research Institute, Karnal, India. Cow milk on an average contained 4.00 ± 0.20 percent fat and 8.70 ± 0.20 percent solids not fat. The titrable acidity of fresh cow milk varied between 0.16 to 0.17 percent lactic acid. Cow cream contained 55 ± 0.5 fat. The cream was stored at about 4±1°C till it was used for standardization of milk intended for production of *lassi*. Poly ethylene terephthalate (PET) bottles were procured from Ashoka Containers, Karnal were used for the packaging of the product. Each packet/ containers contained approximately 200 ml product. Tween- 80 was procured from Merck Specialties Pvt. Ltd. Mumbai, India. β- Cyclodextrin was procured from Mengzhou Hauxing Biochemistry Co. Ltd., China. Alfanso mango flavour was obtained from Bush Boake Allen (P) Ltd., Chennai, India. Dehydrated media, *viz*.. potato dextrose agar (PDA), lactic agar and violet red bile agar (VRBA) for microbiological analysis, curcumin (assay 97%) and all other chemicals used during the study was procured from Hi Media Laboratories Pvt. Ltd., Mumbai, India.

Mesophilic mixed strain *dahi* culture NCDC-167, Yoghurt culture NCDC 260 and NCDC 263 were obtained from National Collection of Dairy Cultures (NCDC) of Dairy Microbiology Division, National Dairy Research Institute, Karnal. DVS culture (DH 111) for fermented non-yoghurt products was obtained from DSM Food Specialties, Holland. Starter culture obtained from the NCDC was maintained in sterilized skim milk. The fresh skim milk (100 ml) was taken into 250 ml conical flasks and plugged with non-absorbent cotton plugs. The flasks were transferred in autoclave and sterilized at 15 psi pressure for 15 min. The culture was propagated by inoculating sterilized skim milk @ 1.5% using laminar air flow chamber. The flasks were incubated at 30°C for 12 h and stored at 6±1 °C. The propagation of the culture was done at regular intervals to maintain culture activity. DVS cultures were maintained at -10 °C.

Preparation of *lassi*

Whole cow milk standardized to 4.5% fat and 8.5% SNF was heated to 90°C for 10 minutes, cooled, inoculated with different cultures (1%) and incubated at respective temperatures for setting to obtain *dahi*. For *lassi* preparation, *dahi* was added with cane

sugar at the rate of 13% (w/w) of milk was dissolved in equal quantity of hot (70°C) potable water and filtered through clean and dry muslin cloth. The sugar syrup thus obtained was heated to 90 °C and cooled to room temperature. Curcumin was added to *lassi* at different levels. When β-cyclodextrin was used as a carrier material, the required amount of curcumin and β-cyclodextrin were weighed and mixed using mortar and pestle. Mango essence was then added @ 1 ml/kg and the freshly prepared *lassi* was then cooled to 4 ± 1°C, packaged and stored under refrigerated condition (Fig. 1).

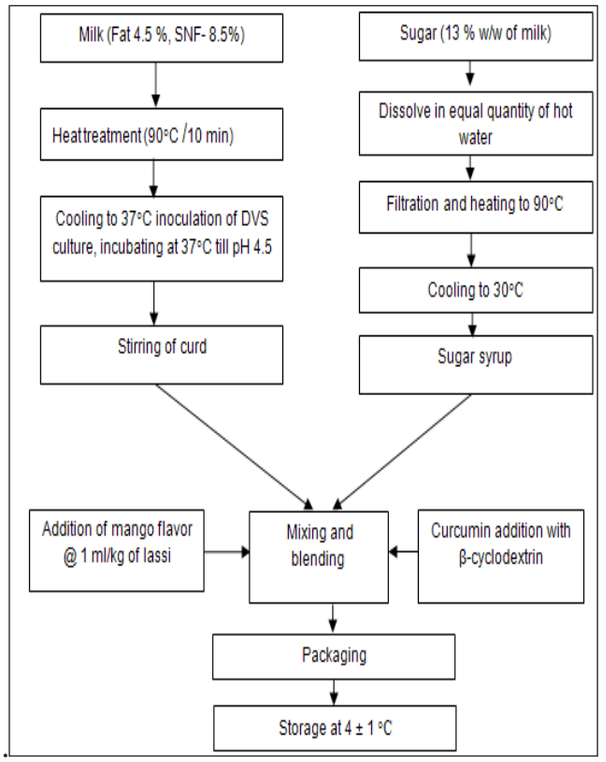

Fig. 1: Flow diagram for preparation of curcumin fortified *lassi*

Storage studies of the product

The curcumin fortified *lassi* samples along with control samples without addition of curcumin and the carrier material were stored at 4 ± 1°C, 90-95% RH. The stored *lassi* samples were analyzed for their sensory, physico-chemical, curcumin retention and microbiological quality at a regular interval of 5 days.

Analytical Methods

Sensory evaluation

Lassi samples were evaluated for their acceptability during the process of standardization as well as storage studies. Sensory evaluation was carried out by presenting approximately 100 ml of *lassi* samples (10±1°C) to seven semi-trained panelists selected from the faculty of Dairy Technology Division. Panel members were requested to judge each sample on the basis of taste, smell, consistency and colour & appearance and to indicate the score on a 100-point scale.

Physico-chemical analysis

Gross compositional analysis (*viz*., fat, SNF, total solids and ash content) and titratable acidity in all the samples was determined as per the method provided by Hussain *et al.* (2015). pH meter (Model No PHS 25 CW, SDFCL, Mumbai) was used for pH determination of all the samples. Total nitrogen content in *lassi* was determined by Micro Kjeldahl method (AOAC, 1992) and extent of proteolysis (tyrosine value) by the method provided by Maji *et al*. (2018). Viscosity of the *lassi* sample was determined at 25°C using Viscostar Plus viscometer. The viscosity was measured at shear rate of 100/s and the results were obtained in mPa.S. Free fatty acid content of the stored *lassi* samples was estimated using the method described by Deeth & Fitzgerald (1976).

Wheying off of *lassi* was measured by centrifugation method. Ten ml of sample was filled in 15 ml centrifuge tubes and centrifugated in a Remi centrifuge at 2000 rpm for 10 minutes. The volume of clear whey measured and expressed as ml of whey off per 10 ml of sample.

Determination of curcumin content

Curcumin content in the samples was estimated according to method of ASTA (1985) with slight modification. In brief, one gram of well mixed sample was weighed accurately in 25 ml volumetric flask. After weighing volume was make up using 95% ethyl alcohol. The mixture was mixed thoroughly

to facilitate colour extraction. After the colour extraction, the mixture was decanted in centrifuged tubes and subjected to centrifugation for 10 minutes. After centrifugation supernatant was filtered through Whatman filter paper No 42. The first few milliliters of the filtrate were discarded and absorbance was measured at 425 nm using similarly prepared extract of control sample as a blank. Results were expressed as ppm of curcumin.

Retention of curcumin in the product was determined by multiplying the reading of curcumin concentration corresponding to sample OD reading in standard curve by dilution factor.

Microbiological analysis

Lassi samples were analysed for the lactic count, coliform count and yeast and mould count in accordance with method described by Hussain *et al*. (2015).

Statistical analysis

The results were statistically analysed using SPSS software (Version 20, USA) and data were expressed as means ± standard error (S.E.). Analysis of variance (ANOVA) test was employed to identify significant differences ($p < 0.05$) between data sets.

RESULTS AND DISCUSSION

Selection of culture for *lassi* preparation

Experiments were conducted with four different cultures for obtaining shortest possible time of fermentation. The pH reduction pattern of cultures namely NCDC 167, NCDC 260, NCDC 263 and DVS culture (DH-111) are presented in Fig 2. Lactic cultures NCDC 167, NCDC 260, NCDC 263 and DH-111 took 9, 8, 8 and 5 hours respectively to decrease the pH of milk for setting of curd to a end point pH of 4.5. As the DVS culture DH 111 took shortest time this culture was selected in this investigation.

Effect of curcumin on the growth of lactic acid bacteria

To study the effect of curcumin on the growth characteristic of the selected starter culture, curcumin was added in milk with two different levels *i.e*., 305 ppm and 490 ppm before inoculation of culture and pH profiles were studied along with control sample. The pH reduction pattern of these two different level of curcumin added milk and control milk (without addition of curcumin) using DVS culture (DH 111) are shown in Fig 3. Addition of curcumin up to 490 ppm had no effect on the growth of culture organisms, as evidenced by no change in the pH reduction pattern of milk. The observed results are contrary to Singh *et al*. (2010), who reported bactericidal activity of curcumin because of its structural similarity with the bacterial cell wall. This could be attributed to difference in bacterial strains and level of curcumin studied. Further, as evident from Table 1, about 50% of the added curcumin was retained in the product and most of the retained curcumin was present in bound to different milk constituents and hence the added curcumin could not exhibit its anti-bacterial activity in the present activity. The obtained results are in agreement with Khanji *et al*. (2018) who added curcumin in milk during yoghurt preparation and reported that curcumin addition had no effect on the culture's growth and acidification rate of milk.

Table 1: Sensory scores and curcumin retention in *lassi* fortified with different ratio of curcumin : β-cyclodextrin

Parameters	Curcumin: β-Cyclodextrin ratio*		
	1:2	**1:3**	**1:4**
Taste score	21.08±0.41	22.00±0.45	21.79±0.31
Smell score	22.46±0.40	22.57±0.27	22.93±0.28
Consistency score	25.48±0.60	26.29±0.47	26.0±0.40
Colour score	17.46±0.38	18.07±0.28	17.79±0.30
Total score score	86.38±1.11	88.93±0.82	88.50±0.60
Maximum acceptance (% of all panellists)	—	67	33
Curcumin retention (ppm)	$86.66±0.88^{c}$	$130.44±1.80^{a}$	$120.15±0.84^{b}$
Curcumin retention (%)	53	81	75

**Values are mean ± SE (n=3); means with different superscripts within column for same parameter differ significantly (p<0.05)*

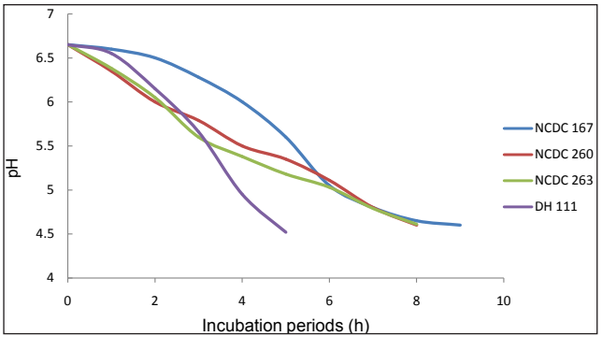

Fig. 2: pH reduction pattern of milk by different cultures during incubation
Values are mean (n=3)

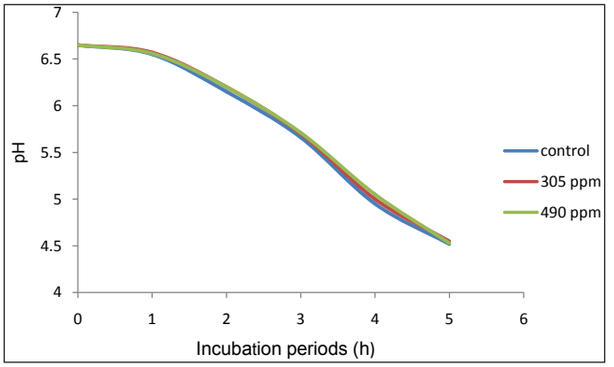

Fig. 3: pH reduction pattern of curcumin added milk during incubation
Values are mean (n=3)

Preparation of curcumin fortified *lassi*

Preliminary studies were conducted to investigate the retention of curcumin in *lassi* by adding curcumin directly into it. It was found that 79 ppm of curcumin (*i.e.*, approximately 50%) was retained in *lassi*. Lower retention of curcumin could be attributed to its highly hydrophobic nature (Stohs *et al.* 2020). Retention of curcumin could be because of its binding with different milk constituents through hydrophobic interactions and hydrogen bonding (Sneharani *et al.* 2010). Curcumin has been reported to form complex with whey proteins (Mohammadian *et al.* 2019) and casein (Hudson *et al.* 2019). In addition, Khanji *et al.* (2018) reported curcumin binding activity of lactic acid bacteria (*Lactobacillus delbrueckii* and *Streptococcus thermophilus*).

Considering the fact that only 50% of the added curcumin is retained in the product, in order to further increase the curcumin retention in *lassi*, experiments were conducted with β-cyclodextrin to act as suitable carrier at different ratios with curcumin.

Selection of curcumin: β-cyclodextrin ratio

To find out the optimum ratio of curcumin: β -cyclodextrin (β-CD) for maximizing the retention of curcumin in *lassi*, three different ratios *i.e.*, 1:2, 1:3 and 1:4 of curcumin to β-CD were tried. The required minimum level of curcumin for the desired health benefits especially its anti-Alzheimeric effect is 160 ppm (Yang *et al.* 2005), so the *lassi* was fortified with 160 ppm curcumin with three different ratios of binding material (i.e., 1:2, 1:3 and 1:4 of curcumin to β-CD) and subjected to evaluation of sensory attributes and curcumin retention (Table 1). It was found that there was no significant effect ($p>0.05$) of different ratios of cyclodextrin on sensory attributes of *lassi* fortified with curcumin. Further, the panellists were asked to mark the most acceptable sample and it was observed that the *lassi* prepared by incorporating curcumin:β-cyclodextrin (of 1:3 and 1:4) was acceptable to all the panellist. Sample having curcumin: β-cyclodextrin of 1:3 was adjudged as best (most acceptable) by 67% of the judges and the product having ratio of 1:2 was not adjudged best by anyone among the sensory panellists. Lower overall acceptability of *lassi* prepared by incorporating curcumin: β-cyclodextrin in the ratio of 1:4 was probably due to slight 'unnatural' taste contributed by higher amount of β-cyclodextrin to the product.

Curcumin retention significantly increased ($p<0.05$) with the addition of β-cyclodextrin and was affected by the amount of β-cyclodextrin in curcumin-β-cyclodextrin ratio. This was probably due to enhancement of water solubility of curcumin by curcumin - cyclodextrin complex formation (Kelanne *et al*. 2019) because of the presence of hydrophibic moieties at the core of β-cyclodextrin. This finding was also in agreement with the observation of Tonnesen *et al.* (2002), who reported that curcumin-cyclodextrin complex formation resulted into an

increase in water solubility of curcumin by a factor of at least 10^4 at pH 5, *i.e.*, close to the pH of *lassi*. Further, Kelanne *et al*. (2019) reported that cyclodextrin forms stoichiometric complexes with flavonoids at about 1:1 ratio, but this primarily depends upon the structural aspects of flavaniod such carbon chain length, and number and types of functional groups present.

On the basis of retention of curcumin and overall sensorial acceptability of the product, the *lassi* samples having curcumin and β-cyclodextrin in the ratio of 1:3 and hence was selected for further studies.

Selection of curcumin level in *lassi*

In order to optimize the curcumin level, *lassi* was fortified with three different levels of curcumin *i.e.*, 160, 250 and 400 ppm with β-cyclodextrin as carrier material in the ratio of 1:3 (curcumin: β-cyclodextrin), and subjected to sensory evaluation and curcumin retention (Table 2).

Table 2: Sensory scores and curcumin retention in *lassi* fortified with different levels of curcumin

Parameters	Curcumin level*		
	160 ppm	**250 ppm**	**400 ppm**
Taste score	20.25±0.52^b	22.14±0.31^a	21.64±0.31^a
Smell score	21.92±0.55^a	22.71±0.26^a	22.27±1.40^a
Consistency score	25.48±0.54^a	25.71±0.41^a	25.09±0.49^a
Colour score	18.14±0.26^a	18.00±0.27^a	16.18±0.60^b
Total score	84.62±1.47^b	88.71±0.74^a	85.18±1.31^b
Curcumin retention (ppm)	131.05±1.80^a	231.52±1.81^b	385.18±2.94^c
Curcumin retention (%)	81	92	96

**Values are mean ± SE (n=3); means with different superscripts within column for same parameter differ significantly (p<0.05).*

It was found that with an increase in the curcumin level from 160 to 250 ppm, taste score increased significantly ($p<0.05$) from 20.25 to 22.14; however, no such significant increase ($p>0.05$) was observed at 250 and 400 ppm of curcumin. Better taste score of *lassi* sample fortified with higher level of curcumin was probably due to higher concentration of curcumin and β-cyclodextrin in them, which contributed to a 'balanced' sweet taste. As it could be seen that the acceptability of *lassi* added 640 ppm of β-cyclodextrin (curcumin: β-cyclodextrin :: 1:4, table 1) had slight 'unnatural' flavour perception, while no such effect was observed at 750 and 1200 ppm of β-cyclodextrin (table 2). As regards to the colour of curcumin fortified *lassi* samples, at the levels of 160 ppm and 250 ppm the colour scores did not vary significantly ($p>0.05$); however, at 400 ppm level colour score decreased significantly ($p<0.05$) to 16.18. The decrease in colour score was probably due to intensification of the yellow colour of the product because of the color of curcumin. Overall sensory score of *lassi* samples fortified with 250 ppm was significantly ($p<0.05$) higher than that of *lassi* sampled that contained 160 and 400 ppm curcumin, which could be respective color of these samples.

Curcumin retention increased significantly ($p<0.05$) with increase in curcumin and β-cyclodextrin concentration. Further, it was observed that highest curcumin retention (about 96%) was observed in case of sample having highest level of curcumin (400 ppm) and lowest curcumin retention (about 81%) in case of sample having lowest level of curcumin (160 ppm). On the other hand, when the samples had 160 ppm of curcumin, highest curcumin retention (about 81%) was obtained at curcumin: β-cyclodextrin of 1:3, and curcumin retention decreased on either side of this ratio (Table 1). This reveals that complex formation between curcumin and β-cyclodextrin is both concentration and ratio dependent. At curcumin: β-cyclodextrin ratio of 1:3, curcumin retention increased from 81% to 96% upon increase in the curcumin concentration from 160 ppm to 400 ppm.

It could be concluded that taste, colour and overall sensorial score of *lassi* fortified with 250 ppm curcumin was numerically better than that of the remaining two levels. Besides, addition of 250 ppm curcumin with β-cyclodextrin as carrier material in the ratio of 1:3 (curcumin: β-cyclodextrin) meet the target of 160 ppm in the product. Hence this level of addition of curcumin was selected for further studies.

Chemical composition of the product

The optimized product was evaluated for gross compositional attributes. The product contained 3.48±0.04 % fat, 2.73±0.02 % protein, 13.8±0.08 % total carbohydrates, 0.52±0.01 % ash, 233.52±1.81 ppm curcumin and 20.56±0.08 % total solids. Chemical composition of optimized product was in agreement with Ramana (1994) who found *lassi* contained 3.0% fat, 21.84% total solids, 11.7 % sugar, when it was prepared using 4 % fat and 15 % sugar (w/w of milk).

Evaluation of storage stability

To evaluate the storage stability, the product added with 250 ppm curcumin and 750 ppm β-cyclodextrin (*i.e*., curcumin: β-cyclodextrin :: 1:3) was packaged in low density polyethylene (LDPE) pouches and poly ethylene terephthalate (PET) bottles and stored at 4 ± 1 ºC and 90-95% RH. Control *lassi* (having no added curcumin), and *lassi* fortified with 250 ppm curcumin without the carrier material *i.e*., β-cyclodextrin was also prepared and packaged in LDPE pouches and PET bottles. These *lassi* samples are also stored in same conditions. During the study, no significant effect ($p>0.05$) was obtained between the samples packaged in different packaging materials; however, curcumin addition (with and without β-cyclodextrin) had significant effect ($p<0.05$) on the storage stability of *lassi*.

Changes in sensory attributes

Sensory attributes of *lassi* were significantly affected (p <0.01) by different forms of curcumin addition. Consistency scores of *lassi* fortified with curcumin along with β-cyclodextrin as a carrier material was significantly ($p<0.05$) higher than the remaining two samples (Fig. 4 A). This could be due to stabilizing action of β-cyclodextrin which in turns increased the viscosity of the sample, thus obtaining better consistency scores than the remaining samples. The sensory score for smell of curcumin fortified *lassi* was significantly ($p<0.05$) better than control *lassi*. Sample containing curcumin along with β-cyclodextrin and control sample had significantly higher ($p<0.05$) color scores than the sample containing only curcumin (Fig. 4 B). This could be due to uniform yellow color of curcumin and β-cyclodextrin fortified samples, because of the action of β-cyclodextrin which led to better retention of curcumin resulting in uniform yellowness in the sample. *Lassi* fortified with curcumin without carrier material obtained lesser score, which was probably due to non-uniform yellowness of the product.

All the sensory attributes decreased significantly ($p<005$) during storage. Significant decrease in taste (Fig. 4 C), smell (Fig. 4 D) and overall acceptability (Fig. 4 E) score was observed after 8 days; consistency score (Fig. 4 A) after 12 days of storage. It was observed that scores of control *lassi* decreased sharply after 12 days of storage, while gradual decrease in the scores of curcumin fortified *lassi* samples was noticed. This could be attributed to faster growth of spoilage microorganisms especially yeast and mould, which imparted unnatural taste and off flavours. On 16th day, control *lassi* was criticised for alcoholic fermentation, high acid and unnatural taste. On the other hand, curcumin fortified samples were acceptable even on the 24th day of storage. This could be attributed to anti-bacterial and anti-fungal activity of curcumin. These results are in agreement with the findings by Hosny *et al.* (2011) that Karish cheese fortified with curcumin enhanced its organoleptical properties, flavour and taste in particular which was much appreciated by the sensory panellists. Similarly, Maji *et al*. (2018) prepared herbal *lassi* by adding turmeric extract and reported increase in the sensory attributes and storage stability of *lassi*.

Changes in physico-chemical properties

The physico-chemical characteristics studied during the storage of the *lassi* included titratable acidity (Fig. 4 F), pH (Fig. 4G), viscosity (Fig. 4 H), wheying off (Fig. 4 I), tyrosine value (Fig. 4 J) and free fatty acids (Fig 4 K) and curcumin stability (Fig. 4 L). All the physico-chemical characteristics were significantly affected ($p<0.05$) by curcumin fortification and storage period.

Upto 8th days of storage, no marked variation in pH among different samples was observed. After 8th day

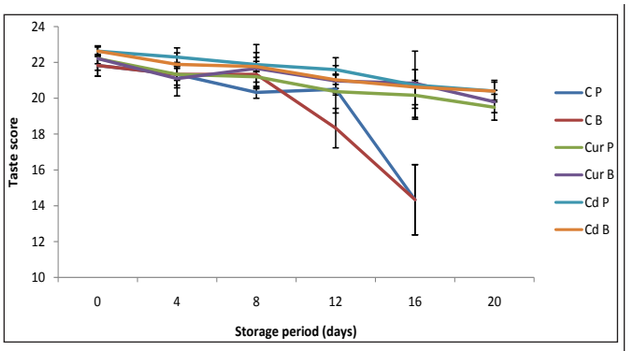

Fig. 4A

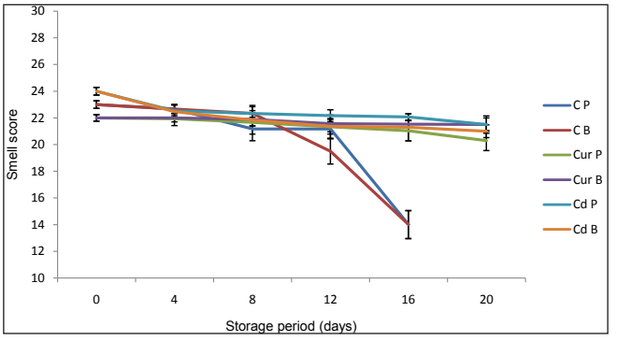

Fig. 4B

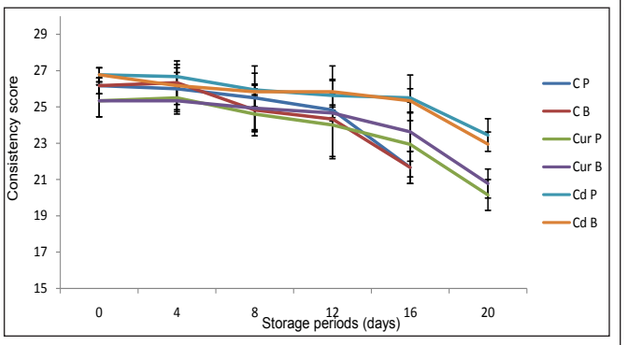

Fig. 4C

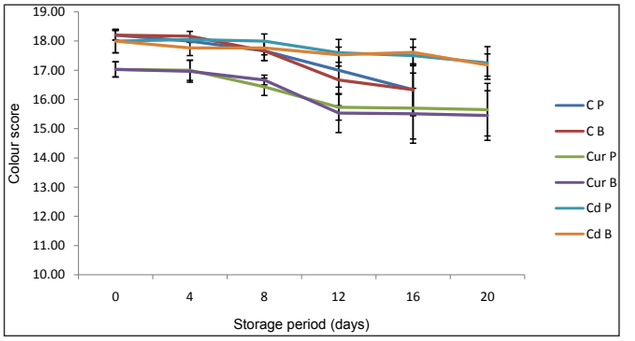

Fig. 4D

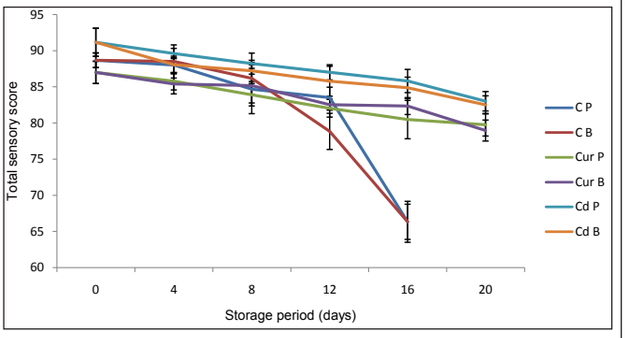

Fig. 4E

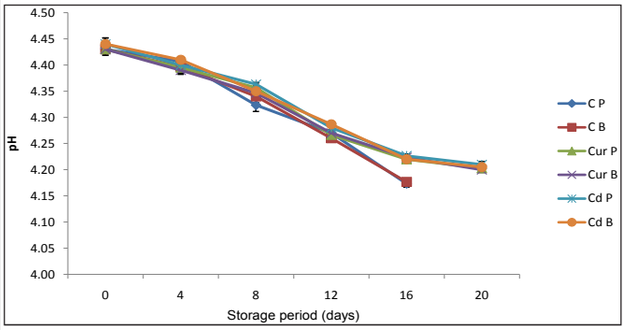

Fig. 4F

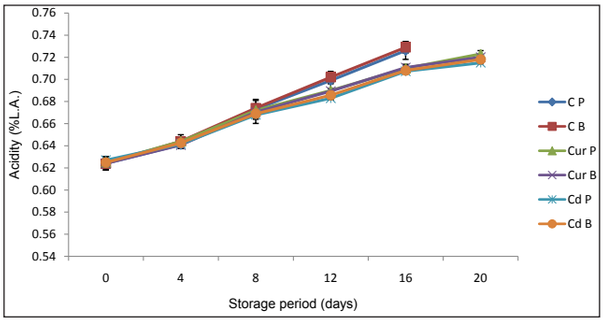

Fig. 4G

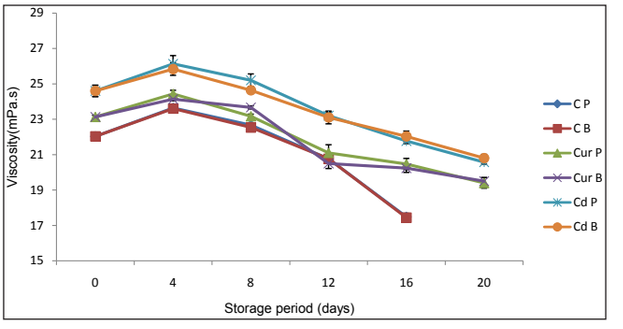

Fig. 4H

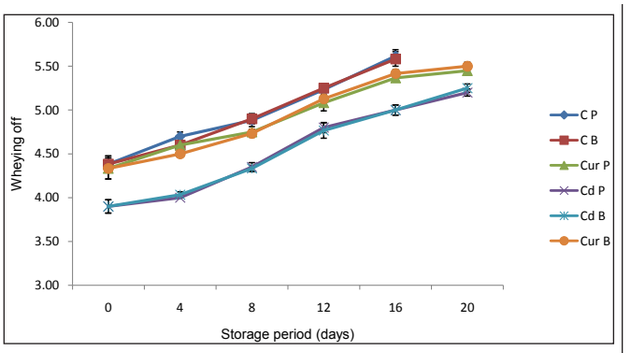

Fig. 4I

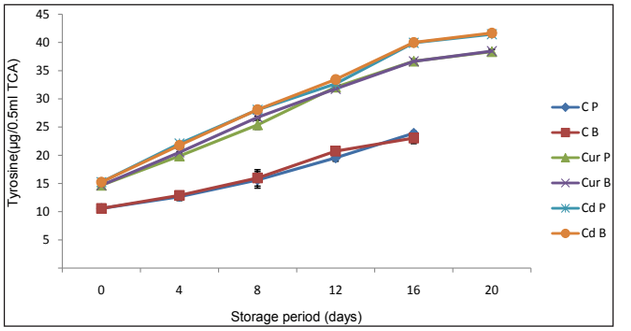

Fig. 4J

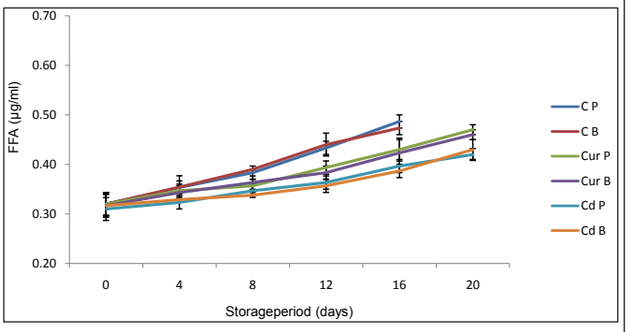

Fig. 4K

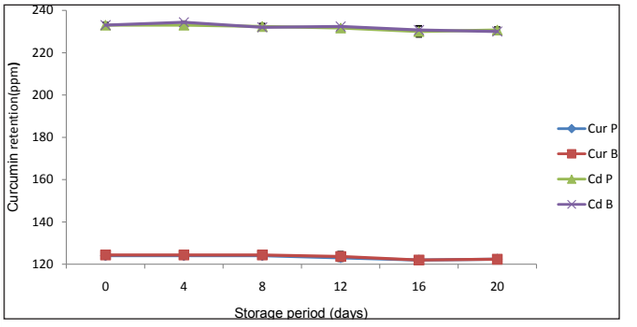

Fig. 4L

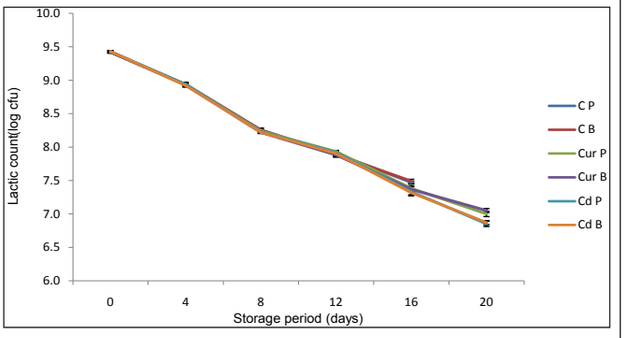

Fig. 4M

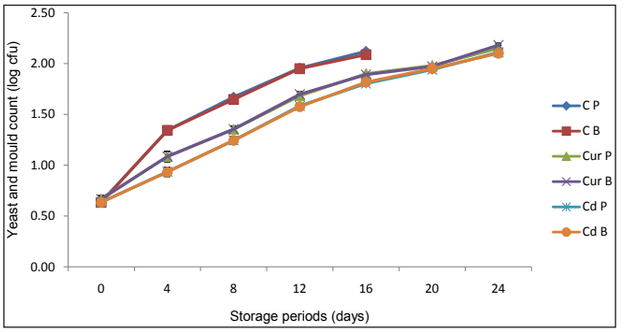

Fig. 4N

C P - Control lassi packed in poly pouch; C B - Control lassi packed in PET bottle; Cur P- Lassi fortified with 250 ppm curcumin packed in poly pouch; Cur B- Lassi fortified with 250 ppm curcumin packed in PET bottle; Cd P- Lassi fortified with 250 ppm curcumin with β-cyclodextrin added in 1:3 ratio packed in poly pouch; Cd B - Lassi fortified with 250 ppm curcumin with β-cyclodextrin added in 1:3 ratio packed in PET bottles

Fig. 4: Changes in quality attributes of lassi during storage

the extent of decrease in pH (Fig. 4 G) was higher in control samples than curcumin fortified samples. Samples containing curcumin had no significant difference ($p>0.05$) in acidity and pH throughout the storage period. Delay in the extent of reduction of pH in curcumin fortified samples during storage could be ascribed to antimicrobial activity of curcumin against a number of spoilage microorganisms.

Viscosity of all the samples increased up to 4th day of storage and then decreased gradually (Fig. 4 H). Later

with further increase in storage period (in case of control sample after 12th day of storage) an undesirable decline in viscosity (thinning of *lassi*) was observed. On the other hand, whey separation increased in all the *lassi* samples irrespective of the treatments. Initially the extent of increase was relatively lower, which could be attributed to the action of high heat treatment during processing. However, during later stages of storage rapid increase in whey separation was observed (Fig. 4 I). Changes in viscosity and whey separation could be due to the growth of spoilage microorganism like yeast and mould that hydrolyze the casein network resulting into decrease in viscosity and serum separation (Sandhya *et al.* 2018). Further, *lassi* fortified with curcumin with β-cyclodextrin as a carrier material was significantly ($p<0.05$) higher viscosity (Fig 4 H) and lower whey separation (Fig 4 I) than the remaining samples. This could be attributed to the interaction between protein and curcumin, which allows curcumin to remain in close proximity of protein and contribute to its stability (Fatima *et al.* 2009), besides of the inhibitory effect of curcumin against spoilage microorganisms and stabilizing action of β-cyclodextrin by the hydrophilic moieties.

Free fatty acid content and tyrosine value was determined in the samples to measure the extent of lipolysis and proteolysis, respectively. Lipolysis and proteolysis could be attributed to the activity of lipolytic and proteolytic enzymes produced by starter and other microflora present in *lassi*, respectively. These changes during storage might become detrimental to the shelf-life of product by imparting off flavours. A gradual increase in lipolysis and proteolysis was observed in all the samples with increase in storage period. It was also observed that the lipolytic activity was relatively lesser in *lassi* samples fortified with curcumin (Fig. 4 K), but opposite was obtained in case of proteolysis (Fig. 4 J). Difference in free fatty acid content (lipolysis) and tyrosine value (proteolysis) was probably due to presence of curcumin in the product. Samples fortified with curcumin with β-cyclodextrin as a carrier material has lowest FFA due to higher retention of curcumin in *lassi*. Sharma *et al.* (2012) reported highly significant difference in FFA content in turmeric powder treated stored chicken mince. Maurya *et al.* (2010) also observed that turmeric showed a significant effect in controlling oxidative rancidity of fat of *carabeef pastirma* - a buffalo meat product. Higher degree of proteolysis in curcumin fortified *lassi* samples might be due to protein digestive action of the component and enhancement of activity of proteolytic enzyme. These finding are also in correlation with observation by Yang *et al*. (2005) that curcumin breaks the β-Amyloid plaques in brain which are made of fibrous protein aggregates. Mukhopadhyay *et al.* (2002) suggested that curcumin represses cyclin D1 expression by promoting proteolysis. Significantly higher proteolysis in *lassi* fortified with curcumin with β-cyclodextrin as a carrier material than only curcumin fortified samples were probably due to higher retention of curcumin in *lassi*. Platel and Srinivasan (1996) reviewed that curcumin, when incorporated in the diet stimulates trypsin activity by as much as 120-165%.

To know the stability of curcumin in *lassi* samples during storage, curcumin was estimated throughout the storage period. Curcumin content was significantly affected ($p<0.05$) by different treatments. Packaging material and storage period had not any significant effect on curcumin retention of stored *lassi*. No significant loss ($p<0.05$) of curcumin was observed during storage period (Fig. 4 L), which indicates that curcumin is stable throughout the storage period. Higher retention of curcumin in *lassi* fortified with curcumin along with carrier material was probably due to formation of inclusion complex between β-cyclodextrin and curcumin.

Changes in microbiological counts

To know the general distribution of bacteria in the *lassi* samples and their growth pattern during storage the changes in lactic count (Fig. 4 M), yeast and mould count (Fig. 4 N) and coliform count was observed during storage. All the microbiological counts were significantly affected ($p<0.05$) by different treatments and storage period. Packaging material had no

significant effect ($p>0.05$) on microbiological quality of stored *lassi*.

Lactic count of all *lassi* samples decreased significantly ($p<0.05$) throughout the storage period. Curcumin fortified *lassi* with β-cyclodextrin as a carrier material significantly ($p<0.05$) affected the growth of lactic acid bacteria once the storage period reached 16th day. This is contradictory to the results obtained during early stages of our study (Fig. 2), during which no bactericidal activity of curcumin was obtained. This indicates of the fact that there exists a time lag between the exposure of curcumin into the product and its anti-bacterial activity. Similar results has been reported by Hosny *et al.* (2011) that lactic acid bacteria count in Karischum cheese was slightly lower during end of storage period in samples made with curcumin than in control cheese.

Yeast and mould count of all *lassi* samples increased gradually throughout the storage period in all the treatments, however the extent of proliferation of yeast and mould was higher in control samples (Fig. 4 N). In control samples, yeast and mould growth is significantly (*p<0.05*) higher than curcumin fortified samples. Further, curcumin fortified sample with carrier material had significant (*p<0.05*) inhibitory effect on yeast and mould than only curcumin fortified samples. This was probably due to highest retention of curcumin in samples with carrier material than without carrier material.

Coliforms are an indicator of hygienic practices employed during product manufacture. It was found that coliforms were absent throughout the storage period in all the samples. Absence of coliform could be due to high heat treatment given to the milk and subsequent hygienic handling of the product.

CONCLUSION

Curcumin addition had no significant effect ($p<0.05$) on growth profile of culture during *lassi* preparation. More than 90% of curcumin was retained, when it was added at 250 ppm level and curcumin: β-cyclodextrin ratio of 1:3, besides of having higher sensory acceptance. The developed product had a shelf life of 20 days at 4 ± 1°C and 90-95% RH, when packaged in LDPE pouches or PET bottles with no significant loss of curcumin during the storage. The developed product possesses great potential as a functional *lassi* with increased shelf life through natural preservation.

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