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Process optimization for the production of ready-to-cook *carrot halwa*

Arvind, Pavan Choudhary, Shikha Pandhi, Dinesh Chandra Rai and Veena Paul

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Abstract: Increasing prevalence of nuclear family among the population as well as changing lifestyles have subjected consumers towards the adoption of instant food at the global level. Increasing popularity of ready-to-cook (RTC) foods amongst the consumers owing to convenience in their preparation, handling as well as their longer shelf life had encouraged the development of a variety of instant foods by various food processors. The term 'ready-to-cook' refers to simple, quick, and convenient food, which is easy and quicker to cook apart from being hygienic and free from contamination. The present study aims at the optimization of the recipe for the development of ready-to-cook *carrot halwa* based on color, texture, and sensory characteristics such as appearance, odor, taste, and overall acceptability. The sample (H₄) with desirable red color, best organoleptic properties, and least hardness was selected for the proximate analysis.

Keywords: *Carrot halwa*, Convenience, Instant, Ready-to-cook

Introduction

Escalating affluence and changing lifestyles as a function of accelerating technology has become one of the most pervading trends in today's scenario (Mansoor et al. 2013). Fast-paced life

and changing consumer needs have shown a paradigm shift towards processed foods that are easy and fast to cook. Ready-to-cook products are one of the modern alternative solutions to these growing needs to ease the process of cooking (Munjal and Noida, 2019). Ready-to-cook foods are convenience foods that require less time and energy for the final food preparation. Indian traditional foods symbolize the wealth of cultural heritage that is gaining a lot of popularity in the world. *Carrot halwa* is also one amongst those traditional Indian sweetmeats that are highly relished in northern and western parts of India. It is a dense source of nutrients and calories generally prepared by cooking carrot shreds with milk and sugar in moderate frying oil and added with grated *khoa*. *Carrot halwa* forms an essential part of festivals, marriages, feasts, religious functions as well as of daily menus. But its preparation takes several hours depending on the number of raw materials used that makes the whole process laborious and cumbersome.

It is preferred to make during winters season due to availability of raw material at a reasonable price (Mathpal et al. 2017). Advancement of technological interventions has created an opportunity to enhance the availability of seasonal commodities round the year. Ready-to-cook food products are one such intervention to provide food formulations that are associated with constraints of seasonal availability with prolonged shelf life. Dehydration of fruits and vegetables is an efficient method for extending the shelf life by evaporating water while preserving the taste. Dehydration reduces the weight and space required to store and transports the products that remain stable in ordinary storage conditions.

The present study aims at optimization of the process for the development of ready-to-cook *carrot halwa* using blanched dried carrot shreds with different combinations of *khoa* powder and sugar. The study was based on optimization of parameters like rehydration time, rehydration ratio, volume expansion, density, and textural properties of dried carrot shreds for the production of *carrot halwa* and then the optimized blanched dried carrot shreds were mixed with a different amount of the *khoa* powder and sugar.

Department of Dairy Science and Food Technology
Institute of Agricultural Sciences
Banaras Hindu University
Varanasi-221 005, Uttar Pradesh, India

Arvind (✉)
Department of Dairy Science and Food Technology
Institute of Agricultural Sciences
Banaras Hindu University
Varanasi-221 005, Uttar Pradesh, India
Phone: +91-9793583702
Email: arvind00000@gmail.com

Materials and Methods

Carrots, *khoa*, and sugar powder was purchased from the local market of Varanasi. All chemicals used in this study were of analytical grade and procured from Hi-Media Laboratories Pvt. Ltd., Mumbai, India; Fisher Scientific, Mumbai, India; Merck Specialties Pvt. Ltd., Mumbai, India.

Optimization of blanched carrot shreds

Carrot shreds were blanched for different time intervals as a pre-treatment before dehydration with sugar and potassium metabisulphite (KMS). The experimental design for the blanching of the carrot shreds has been shown in Table 1. Six samples were obtained by subjecting carrot shreds to six different blanching times. The process for the blanching and drying of the carrot shreds has been described in Figure 1. The six samples thus obtained were optimized based on five different parameters such as:

Bulk density (ρ_B)

Bulk density is defined as the ratio of mass per unit volume. It is an important physical property that reveals the extent of porosity in a given sample. It is generally given by

$$\text{Density} = \frac{\text{mass}}{\text{volume}}$$

Higher bulk density corresponds to less number of pores. Lower bulk density corresponds to a more porous structure that indicates efficient dehydration and facilitates the process of rehydration (Sasmitaloka et al. 2019).

Rehydration time

Rehydration time is defined as the minimum time required by the dehydrated sample to re-absorb water and acquire a homogenous texture. Porosity is one important factor that affects the rehydration ability of the sample. The product with a more porous structure takes lesser time for rehydration as pores facilitate water penetration (Sasmitaloka et al. 2019).

Volume expansion

Volume expansion is the increase in volume caused by the absorption of water during the process of rehydration. Porosity and water absorption both act synergistically and facilitate volume expansion. It is the ratio of the volume of the sample after rehydration to the volume of the sample before rehydration.

$$\text{Volume expansion} = \frac{V_1}{V_2}$$

Where V_1 = Volume of the sample after rehydration; V_2 = Volume of the sample before rehydration

Higher porosity and more water penetration correspond to high volume expansion on rehydration (Sasmitaloka et al. 2019).

Rehydration ratio

The rehydration ratio is the ratio of the weight of the rehydrated sample to the weight of the sample after drying. It is a key quality aspect of dehydrated foods. The high value of the rehydration ratio indicates good quality of the dried product because the pores allow water to re-enter the cells more adequately.

$$\text{Rehydration ratio} = \frac{W_1}{W_2}$$

Where W_1 = Weight of the sample after rehydration; W_2 = Weight of the sample after drying

Texture analysis

Textural parameters of a product like hardness, springiness, chewiness, gumminess and cohesiveness were analyzed using a texture analyzer (TA.XT plus texture profile analyzer, Stable Micro Systems, UK). The rehydrated carrot shreds samples were first brought to room temperature and then analyzed for various textural parameters using back extrusion rig with a 35mm disc with pre-test and test speed of 2mm/sec. For ready-to-cook *carrot halwa* samples 1 cm of cube was cut from the central portion of the RTC *halwa* cake and analyzed for textural parameters using an 80 mm diameter cylindrical plunger at a crosshead speed of 50 mm/min at 90° of compression and the time gap between the two compressions varied between 6 and 7 sec. The trigger force was 5g with the post-test speed of 5mm/sec. Hardness, cohesiveness, springiness, gumminess, and chewiness, were calculated from the texture profile analysis as described by Bourne (1978).

Khoa powder making

The process for *khoa* powder making has been described in Figure 2.

Experimental design

The different variation in the ingredients level has been shown in Table 2. Optimization of ready to cook *carrot halwa* was done based on sensory evaluation, textural attributes, and color analysis.

Sensory evaluation

The *carrot halwa* samples were subjected to sensory evaluation using a 9- point hedonic scale. The sensory evaluation was

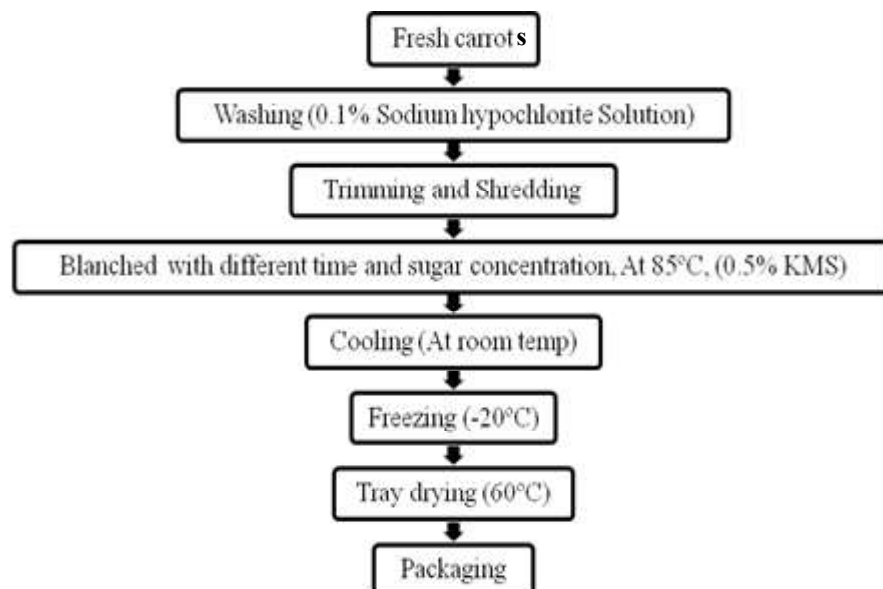


Fig. 1 Flowchart for the blanching and drying of carrot shreds

performed by a panel of 10 semi-trained judges (aged 25-35 years) from the Department of Dairy Science and Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. The samples were served in odorless plastic trays. Water was used for mouth rinsing between the evaluations of two consecutive samples. All the analyses were performed in triplicate. The sensory evaluation was carried out at 25°C and 60% relative humidity (Ranganna, 2001). The sensory parameters that were studied include color, body and texture, flavor, and overall acceptability. The 9-point hedonic rating scale was arranged such that: Like extremely-9, Like very much-8, Like moderately-7, Like slightly-6, Neither like nor dislike-5, Dislike slightly-4, Dislike moderately-3, Dislike very much-2, Dislike extremely-1 (Amerine et al. 1965).

Hunter color estimation

The color coordinates L^* , a^* , b^* (L^* -lightness/darkness, a^* -redness/green, b^* - yellowness/blue) were measured using D-65 illuminant, with a spectral range of 400-700nm and a spectral resolution of 10nm (color flex, CFLX-45-2, Hunter lab, Reston, USA). The photosensor was standardized using standard black and white color tiles before the analysis and the sample color values were read and recorded using Easy match QC software (Hunter Lab Reston, USA). The values of the sample color were taken at different intervals and were measured. The samples were tested under illuminate C, view angle 2°, fast speed with slit width 2.0nm following calibration with black and white standard tiles, samples of known weight and thickness were placed into a standard glass container and read an opponent color scales L , a^* and b^* were measured. The Hunter L , a , b color space is a three-dimensional rectangular color space based on the opponent color theory. L^* (Lightness) axis indicates Lightness vs darkness, where a low number (0-50) indicates the dark and high number

(51-100) indicates light. a^* indicates a red-green axis where a positive number indicates red and a negative number indicates green. b^* indicates blue to the yellow axis where positive values are yellow; negative values are blue and 0 is neutral.

Proximate analysis of the optimized product

Proximate analysis of the optimized product was carried out for various parameters such as % protein, % fat, % ash, % moisture, and reducing sugar content as per the method prescribed by AOAC (2000).

Statistical analysis

All the experiments were performed in triplicate. The data of the analysis were pooled and the mean and standard deviation were calculated using MS excel software. Experiments were laid out in a completely randomized block design with three replications. Data on hardness, springiness, cohesiveness, gumminess, chewiness, density, rehydration time, rehydration ratio, and volume expansion were subjected to an analysis of variance (one way ANOVA). The difference at $p < 0.05$ was considered significant. For the computation of data, a software application program like Microsoft excel was used.

Results and Discussion

Optimization of cooking properties of the blanched dried carrots shreds

The data about the cooking properties of the blanched dried carrot shreds were presented in Table 1. The physicochemical properties like density, rehydration time, rehydration ratio, and volume expansion were calculated for the differently treated dried

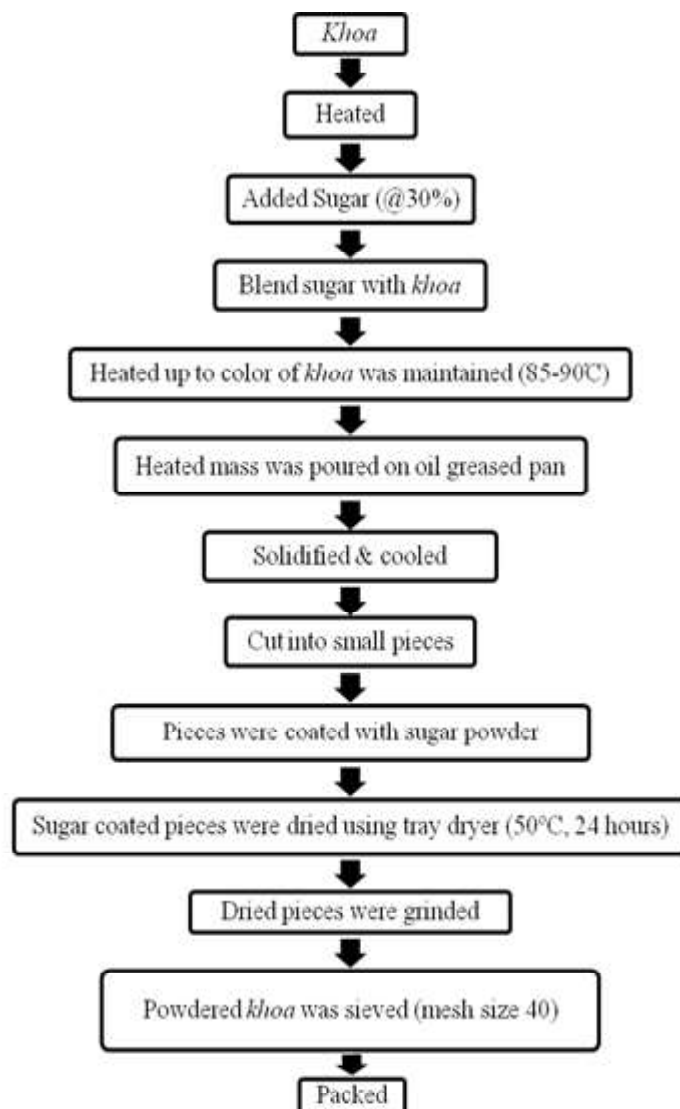


Fig. 2 Flowchart for the preparation of *khoa* powder

carrot shreds. The rehydration time in sec varies from 29.2 to 32.7 sec. There was no significant difference in the rehydration time in samples T_1 , T_2 , and T_3 . But there was a significant difference in rehydration time of the treatment T_6 . This could be because of the more blanching time and sugar concentration of the solution used for the blanching of the shredded carrots. The rehydration ratio tells about the amount of water uptake of the dried products. The maximum and minimum value for the rehydration ratio of the differently treated samples was 5.74 and 6.93. There was no significant difference between T_1 , T_2 , T_3 , and also in between T_5 , T_6 . But there was a significant difference between T_1 , T_4 , and T_6 . This could be because of the difference in the blanching time of the different samples. Blanching leads to the oozing out of some of the constituents of the food products depending on the extent of treatment time and results in a difference in rehydration ratio. Higher rehydration ratio refers to better rehydration due to efficient

moisture penetration in the dried sample. In a study, the effect of different drying temperatures on the quality of dehydrated carrot shreds was studied and showed that the rehydration ratio varies from 5.8 to 10.0 when subjected to different temperatures and time intervals (Qurtulanea et al. 2015). Volume expansion is another important parameter that deals with the tendency of the dried matter to change its shape, size, and volume when boiled in hot water. The maximum and minimum value of the volume expansion is 2.30 and 1.30. There was no significant difference between volume expansion of T_1 , T_2 , T_3 , and T_4 , T_6 . But there was a significant difference between T_3 , T_5 , and T_6 . This could be because of the variation in blanching time, drying temp, drying time, and the final moisture content of the dried products that greatly affect the structure, porosity, and water-absorbing capacity of dried samples. Based on these parameters, T_3 was found to be most effective treatment that gives moderate values

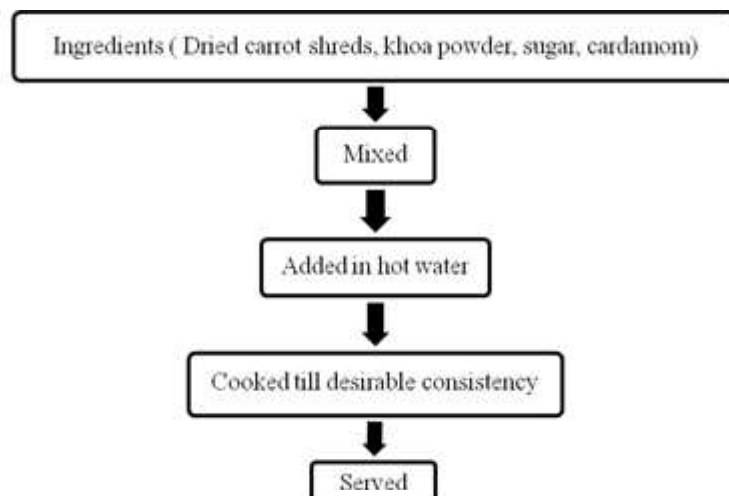


Fig. 3 Flowchart for the preparation of RTC carrot halwa

Table 1 Effect of blanching time and sugar concentration on cooking properties of dried carrot shreds

Sample	Blanching Time (min)	Sugar Concentration	KMS (%)	Bulk Density	Rehydration Time (Sec)	Rehydration Ratio	Volume Expansion
T ₁	2	8	0.5	0.61±0.02 ^c	29.5±5.23 ^a	5.74±0.56 ^a	2.30±0.23 ^c
T ₂	5	8	0.5	0.38±0.06 ^b	29.9±2.19 ^a	5.86±0.63 ^a	2.21±0.36 ^c
T ₃	10	8	0.5	0.38±0.03 ^b	29.2±1.93 ^a	5.96±0.96 ^a	2.29±0.63 ^c
T ₄	2	12	0.5	0.22±0.03 ^a	30.6±2.36 ^b	6.22±1.23 ^b	1.60±0.29 ^b
T ₅	5	12	0.5	0.21±0.06 ^a	30.5±3.12 ^b	6.79±0.83 ^c	1.30±0.39 ^a
T ₆	10	12	0.5	0.27±0.08 ^a	32.7±2.45 ^c	6.93±1.29 ^c	1.69±0.65 ^b

Values are mean ± standard deviation, n=3, Values with different superscript letters in column indicate a significant difference (p<0.05).

Table 2 Experimental design with different level of ingredients and its effects on the color of ready-to-cook carrot halwa

Sample	Carrot (g)	Khoa Powder (g)	Sugar (g)	Cardamom (g)	L* (Lightness)	a* (Red-green)	b* (Blue-yellow)
H ₁	15	20	12.8	1	31.56±0.34 ^a	19.89±0.14 ^c	13.92±0.02 ^a
H ₂	15	20	28.2	1	32.28±0.26 ^b	20.17±0.17 ^d	14.33±0.14 ^b
H ₃	15	25	19.2	1	32.98±1.66 ^b	19.25±0.57 ^c	14.14±0.15 ^b
H ₄	15	25	28.8	1	33.27±1.85 ^c	19.21±0.81 ^c	14.11±0.17 ^b
H ₅	15	30	19.6	1	33.96±0.19 ^c	19.11±0.02 ^c	13.99±0.02 ^a
H ₆	15	30	29.4	1	34.22±0.27 ^d	18.02±0.23 ^b	14.75±0.22 ^b
H ₇	15	40	18.6	1	35.87±0.05 ^e	17.43±0.22 ^a	13.95±0.26 ^a

Values are mean ± standard deviation, n=3, Values with different superscript letters in column indicate a significant difference (p<0.05).

of bulk density, rehydration ratio and volume expansion relating to desirable porous texture of shreds with lowest rehydration time.

Texture profile analysis of carrot shreds

The data about the textural properties of the blanched dried carrot shreds were presented in Table 3. Hardness, springiness, cohesiveness, gumminess, and chewiness were analyzed using force-time textural curve. Hardness (N) is the force necessary to

attain a given deformation of the material or it is the force required to bite through the sample with molars. Rehydrated carrot shreds with a low value for hardness means soft and smooth in texture and vice-versa. Cohesiveness is related to work required to overcome the internal bonding or maximum degree to which the sample holds together in a mass while chewing. Springiness is described as the rate at which a deformed material recovers to its original form under formed conditions after the deforming force was removed. Rehydrated carrot with higher springiness possesses higher elasticity. The rehydrated carrot with higher

chewiness was stiffer and harder to eat. The middle portion of the rehydrated carrot was analyzed for textural parameters because it had a homogeneous texture. Textural parameters were influenced by parameters like cooking time, blanching time, drying temp, drying time, and the final moisture in the dried products. Out of all the differently treated samples, T₃ has the minimum hardness and sample T₁ has the maximum hardness followed by T₅. But there is no significant difference in the hardness other samples such as T₂, T₄, and T₆.

The average springiness of the rehydrated carrot was found to be around 8.0 to 8.4. T₂ and T₄ show the maximum and minimum springiness respectively which is 9.34±0.32 and 6.95±0.64. The highest value for cohesiveness was noticed in T₁ (0.63±0.03) and the least values in other T₂ and T₃. Gumminess i.e. force required to disintegrate a semisolid sample for swallowing. Again T₄ showed the highest value (8.72±1.62 N) and least for T₆ (5.84±1.69 N). Based on these results, T₃ was selected as it gives moderate values for hardness, springiness, gumminess, cohesiveness and chewiness.

Sensory evaluation of carrot halwa

The sensory quality of the carrot halwa prepared using different levels of ingredients was evaluated by a panel of judges. The data about the sensory properties of the ready-to-cook carrot halwa were presented in Table 4.

The sensory score for color and appearance, body and texture, flavor, and overall acceptability for all the seven variants was

Table 3 Texture profile analysis of the differently treated dried carrot shreds

Treatment	Hardness(N)	Springiness	Cohesiveness	Gumminess	Chewiness
T ₁	82±2.36 ^d	9.92±0.73 ^d	0.633±0.03 ^a	6.13±1.02 ^b	5.98±0.65 ^a
T ₂	65±3.25 ^b	9.34±0.32 ^d	0.383±0.06 ^b	7.62±0.46 ^c	7.92±0.46 ^c
T ₃	42±2.36 ^a	7.63±0.36 ^b	0.376±0.05 ^b	6.88±0.96 ^b	5.05±0.96 ^a
T ₄	61±1.36 ^b	6.95±0.64 ^a	0.559±0.03 ^c	8.72±1.62 ^d	6.05±1.36 ^b
T ₅	76±3.36 ^c	8.28±0.25 ^c	0.514±0.09 ^c	6.72±1.63 ^b	5.50±1.46 ^a
T ₆	65±1.65 ^b	7.15±0.35 ^b	0.520±0.05 ^c	5.84±1.69 ^a	6.25±1.82 ^b

Values are mean ± standard deviation, n=3, Values with different superscript letters in column indicate a significant difference (p<0.05).

Table 4 Sensory evaluation of ready-to-cook carrot halwa

Sample	Color & Appearance	Body and Texture	Flavor	Overall acceptance
H ₁	6.75±0.46 ^b	6.75±0.65 ^a	7.42±0.56 ^b	6.89±0.70 ^a
H ₂	7.64±0.94 ^c	7.21±0.69 ^b	7.07±0.73 ^b	7.38±0.78 ^b
H ₃	7.28±0.75 ^c	7.42±0.53 ^b	7.21±0.81 ^b	7.35±0.74 ^b
H ₄	7.83±0.51 ^c	8.33±0.51 ^c	7.93±0.98 ^b	7.83±0.54 ^b
H ₅	6.91±0.81 ^b	6.75±0.61 ^a	7.08±0.66 ^b	6.91±0.64 ^a
H ₆	5.83±1.32 ^a	6.41±1.28 ^a	7.16±1.16 ^b	6.47±1.13 ^a
H ₇	6.16±1.16 ^b	6.58±0.91 ^a	6.51±1.04 ^a	6.41±0.93 ^a

Values are mean ± standard deviation, n=3, Values with different superscript letters in column indicate a significant difference (p<0.05).

found to be significantly different from each other (p<0.05). The maximum sensory score for color and appearance, body and texture, flavor, and overall acceptability was found for variant H₄ as shown in Table 4. The factors that mainly contributed to the sensory parameters of the carrot halwa were the amount of carrot shreds and khoa powder. The amount of carrot shreds and khoa powder in the premix contributed to brighter color and more acceptable body and texture. The addition of khoa powder affected the flavor of the carrot halwa. In the case of overall acceptability, all the samples had been given average scores that correspond to descriptors “like moderately to like very much”.

Color analysis of carrot halwa

The color parameters for developed ready-to-cook carrot halwa were studied using Color flex hunter colorimeter. The data about the color parameters for ready-to-cook carrot halwa was presented in Table 2. The variation in L* value was basically because of the different amount of khoa powder and sugar used for the preparation of the carrot halwa. The maximum value of L is 35.87±0.05 and a minimum value of L* is 31.56±0.34. The value of L* was maximum in the case of H₇ which could be because of the highest amount of khoa powder used in the preparation. There was a gradual increase in the lightness value from H₁ to H₇ which could be because of the increasing amount of khoa powder and sugar. There was a significant difference in L value between H₁ and H₇. A positive a* value indicates the intensity of the red color of the product while the negative value indicates the green

Table 5 Textural profile analysis of ready-to-cook *carrot halwa*

Treatment	Hardness (N)	Springiness	Cohesiveness	Gumminess	Chewiness
H ₁	2.11±0.36 ^a	0.57±0.03 ^c	0.65±0.02 ^d	1.14±0.23 ^a	2.07±0.83 ^c
H ₂	4.22±0.63 ^b	0.95±0.03 ^c	0.39±0.03 ^b	3.32±0.13 ^c	3.14±0.73 ^d
H ₃	4.02±1.02 ^b	0.99±0.06 ^c	0.43±0.03 ^c	2.56±0.16 ^b	2.54±0.62 ^c
H ₄	2.85±1.23 ^a	0.68±0.05 ^d	0.37±0.06 ^b	1.07±0.18 ^a	0.73±0.65 ^a
H ₅	4.50±1.63 ^b	0.51±0.08 ^c	0.21±0.05 ^a	2.85±0.21 ^b	3.27±0.68 ^d
H ₆	5.32±1.24 ^c	0.42±0.05 ^b	0.19±0.05 ^a	1.28±0.25 ^a	1.53±0.52 ^b
H ₇	4.26±0.35 ^b	0.33±0.02 ^a	0.15±0.03 ^a	1.04±0.19 ^a	1.35±0.58 ^b

Values are mean ± standard deviation, n=3, Values with different superscript letters in column indicate a significant difference (p<0.05).

color of the product. Higher the magnitude of the value greater will be the intensity of the particular color. The value of a* for the carrot *halwa* varies from 17.43 to 20.17. For H₂ the a* value was more as compared to another sample this could be because of the less amount of *khoa* powder used in the preparation. In other formulation, the amount of *khoa* powder was more which would have masked the red color of the carrot. A positive value of b* indicates the intensity of the yellow color in the product while the negative value indicates the magnitude of the blue color of the *halwa*. In the product, the positive value of b* is because of the carrot but the color intensity is very less, and not much significant difference was observed amongst the different samples. The highest value of b* is 14.75±0.22 and the minimum value is 13.92±0.02.

Texture profile analysis of ready-to-cook *carrot halwa*

Texture analyses of seven samples of RTC *halwa* were performed and various parameters were measured such as hardness, cohesiveness, springiness, gumminess, and chewiness using force-time textural curve. The RTC *halwa* variant with a low value for hardness means soft and smooth in texture and vice-versa. RTC *halwa* with higher springiness possesses higher elasticity. Therefore, RTC *halwa* with higher chewiness is stiffer and harder to eat. The middle portion of the RTC *halwa* was analyzed for textural parameters because it had a homogeneous texture. Textural parameters were influenced by the type of carrots used in the base material of the *halwa*. From table 5 it was observed that sample H₄ exhibited the least hardness whereas maximum hardness was observed in the case of H₆. The highest gumminess and chewiness were found in sample H₂. Cohesiveness was found to be highest in sample H₁. From the table, it can be concluded that sample H₄ was found to have the least values for chewiness and hardness and moderate values for springiness, cohesiveness, and gumminess. On the following basis, it can be concluded that treatment H₄ can be adopted reasonably for further processing.

Proximate analysis of ready-to-cook *carrot halwa*

Proximate composition of ready-to-cook *carrot halwa* premix was found to be 7.41% protein, 5.84% fat, 3.11 % ash, 67 % carbohydrate, and 13.35 % moisture.

Conclusions

Ready-to-cook *carrot halwa* that require minimal preparation time was successfully developed keeping in mind the consumers escalating demand for instant foods. T₃ was optimized as the best treatment for dehydration of carrot shreds that offers desirable porous structure to the shreds. Further, the variant H₄ stood out to be best amongst all the variants of *carrot halwa* based on sensorial, textural, and color parameters. The developed ready-to-cook *carrot halwa* takes 4-5min to prepare with sensory, textural, and color characteristics similar to the traditionally prepared product. The overall acceptability of optimized product was 7.83±0.54. The developed RTC *carrot halwa* can be relished at any time with minimal preparation and provide an effective solution to constraints related to seasonal availability of raw materials.

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Comparison of nitrogen and carbon dioxide in MAP packaging for shelf life extension of *Cham-Cham*

Rohit G Sindhav¹, Tanmay Hazra¹, Ankit J Thesiya¹ and PS Prajapati²

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Abstract: *Cham-Cham* is a traditional Indian milk product prepared by cooking the chhana balls in sugar syrup. The final product has a pleasant sweet taste due to absorption of syrup during frying. However, shelf life of *Cham-Cham* is low due to a number of factors. For increasing its market one has to focus more on enhancement of its shelf life. For enhancement of shelf life, modified atmosphere packaging of nitrogen and carbon dioxide was adopted. The moisture, pH, acidity, sensory attributes and microbial analysis were carried out during storage of sample at 20°C. The sensory attributes like color and appearance, flavor, body and texture and overall acceptability scores of *Cham-Cham* were recorded on 9-point hedonic scale. It was observed from the results that as storage progressed, the score for overall acceptability decreased irrespective of gases tried. However, modified atmospheres inside the package, controlled the growth of microbes which increased the shelf life of the product up to 8 days as compared to 4 days in normal packaging at 20°C.

Keywords: *Cham-Cham*, Carbon dioxide, Shelf life, Modified atmosphere packaging, Nitrogen,

Introduction

India is the highest milk producing country in the world, producing 176.4 million tonnes milk with the annual growth rate of 6.7 % (NDDB, 2017-2018). According to the latest annual report of DAHD (Department of animal husbandry and dairying, 2017-18),

almost 46 % milk consumed in household without any processing, rest 34 %, 10 % and 10 % utilized by unorganized sector, cooperative and private sectors, respectively. However, due to sufficient milk production, a huge portion of milk is converted into Traditional Indian Dairy Products (TIDP) by organized and unorganized dairy sectors or sweet makers. Unfortunately, low shelf life is a major factor for marketability of TIDP as compared to western dairy products. The choice of TIDP varies from region to region and the product range is very wide indeed. In India, various packaging modifications have been adopted to increase the shelf life of TIDP in market. However, during storage several biochemical changes occur that make the product unsuitable or unacceptable for consumer (Londhe et al. 2012). Hence, shelf life issue is a major problem for TIDP, so adoption of advanced packaging technology including MAP is a better option for enhancement of shelf life of TIDP. In MAP, replacement of head space gases with suitable gases (i.e. N₂, CO₂ or combination of both) or flushing of gases is carried out.

CO₂ gas is a natural anti-microbial agent and is also effective against psychrotrophic bacteria (Hotchkiss and Chen, 1996), it has also been granted GRAS (Generally Recognized as safe) status (Mermelstein, 1997). Recently, studies reported that shelf life of fresh cheese increased by modified atmospheres packaging, containing CO₂ (Brown et al. 2018). In a different study Jha et al. (2015) applied MAP with combination of gases (i.e. N₂: CO₂ = 70:30) that increase the shelf life of TIDP like *lal peda*.

The usefulness of MAP for shelf-life extension depends on several factors such as mixture of gases, packaging material, temperature of storage, handling of packed product, type of food etc. (Belay et al. 2019). Singh et al. (2012) found effectiveness of CO₂ during packaging for delaying the oxidation of food. Some researchers also reported the beneficial effect of MAP for powder product as well (An et al. 2018). Because of microbial spoilage caused by certain gram-negative bacteria and yeasts, products like cheese and yogurt are packed in packages that have inside atmosphere of CO₂ that retard the growth of microorganisms (Ganguly et al. 2017). Chowdhury et al. (2017) concluded that khoa packed in MAP had a shelf life of 8 days as compared to khoa packed in normal packaging, having only 4 days of shelf life itself when stored at same conditions (27±2°C with 65 % Rh).

¹College of Dairy Science, Kamdhenu University, Amreli, Gujarat, India

²SMC College of Dairy Science, AAU, Anand, Gujarat, India

Rohit G Sindhav (✉)

¹College of Dairy Science, Kamdhenu University, Amreli, Gujarat, India

Email: rohitsindhav7@gmail.com

So, application of MAP both in TIDP or western dairy products proved to be very effective for extension of shelf life than normal packaging.

Cham-Cham is a popular traditional dairy product very popular in Bangladesh (Mesbahuddin Md et al. 2013) as well as in India especially in West-Bengal and Orissa. It is made by kneading of chhana into pasty mass and forming oval shaped pieces which are cooked in sugar syrup. Lastly, the cooled product is transferred to sugar syrup solution for particular time period followed by drainage of excess syrup (Aneja et al. 2002). Till date in India, *Cham-Cham* has been mainly produced by local sweet makers, so shelf life used to be very less that adversely affected the marketability of such a delicious sweet-meat. No work has been reported about the applications of modified atmosphere packaging for *Cham-Cham*. Therefore, the present study was contemplated to evaluate the effect of MAP on sensory quality, moisture, acidity, pH and microbial quality of *Cham-Cham* during storage.

Materials and Methods

Preparation of *Cham-Cham*

Fresh milk (mixed milk) was procured from Anubhav dairy of AAU, Anand. *Cham-Cham* was prepared according to the method suggested by Puri et al. (2016b) (Figure 1). However, during processing of *Rasogolla* the channa ball use to be immersed in sugar syrup until consumption, whereas for manufacturing of *Cham-Cham* only 2-3 hours soaking in sugar syrup is required.

Packaging of *Cham-Cham*

The pet-polyester/polyfilm pouches (thickness: ~ 85 μ) were procured from Vidya dairy, Anand. The PVC trays were purchased from Industrial plastic forming co., Mumbai. Prepared *Cham-Cham* was divided into three batches, one batch was used for normal packaging (control) and remaining two batches were used for MAP (one for N₂ and one for CO₂). After preparation, the product was transferred into PVC trays and these trays were inserted into pouches under hygienic condition. For sterilization of trays and pouches, both were placed in UV radiation chamber before use. Before heat sealing of packages flushing with N₂ and CO₂ process was carried out with gas flushing machine. While flushing, care was taken for damage of product and head space O₂ level in range of 2-4%. Tray and pouch used were sufficient to contain ~500g product i.e. ~ 24 pieces of *Cham-Cham* weighing ~18-20 g each.

Storage of Samples

The study comprised of the assessment of fresh and stored *Cham-Cham* in two different environments for moisture, acidity, pH, standard plate count (SPC), yeast and mold count and sensory characteristics at regular intervals of storage. The packages were

kept at 20±2°C with 65% RH. The samples stored at 20±2°C were evaluated at regular intervals of 0, 2, 4, 6, 8, 10 days. The samples where surface mold growth observed were discarded.

Analysis of Samples

Moisture of *Cham-Cham* samples was determined using Mojonnier Milk Tester Model-D as per the process prescribed in the Laboratory Manual (1959). It was expressed as % of moisture content. All the measurements were taken in triplicate for each sample. Whereas, titratable acidity in terms of percent lactic acid for all the samples was determined in triplicate by procedure described in BIS (IS: 1166, 1968) for condensed milk. The pH of *Cham-Cham* was measured by using the calibrated Systronic digital pH meter (model: 335). The method described by O'Keeffe et al. (1976) for cheddar cheese was used to determine pH of samples in triplicate. Microbiological analysis of *Cham-Cham* samples during storage was carried out for SPC and yeast and mold as per the standard methods described in BIS (IS: 5550, 1970).

Sensory evaluation of fresh and stored samples of *Cham-Cham* was done by subjective test based on 9-point hedonic scale. Panelists were selected from faculty and technical staff of SMC College of dairy science, Anand. 9-point hedonic scale system was used with individual scores from 1 to 9 (1 indicate Dislike extremely whereas, 9 indicate like extremely) (Ranganna, 1987).

The data were represented as mean value with standard deviation and subjected to Factorial Completely Randomized Design with 5% levels of significance (Steel and Torrie, 1980). The data of microbiological analysis were converted into log values.

Results and Discussion

The proximate analysis of fresh *Cham-Cham* is show in Table 1. It was observed from Table 1 that for fresh *Cham-Cham* the % of moisture, fat, protein, total carbohydrate and ash were 39.15, 12.84, 11.54, 35.19 & 1.28, respectively. Earlier Singh et al. (2013) reported preparation of *Cham-Cham* from buffalo milk having 5% fat. However, in our current study we used mixed milk having 4.5% fat and found soft and smooth texture. Our product was at par with earlier findings of Singh et al. (2013) who reported the texture of *Cham-Cham* depended on the quality and fat percentage of milk. In our present study, moisture content in *Cham-Cham* was found to be 35.15% that supported the earlier observation of Puri et al. (2016b), who reported the moisture content in *Cham-Cham* as varying from 36.56 to 62.56% and that it depended on the soaking time of sugar syrup during preparation of *Cham-Cham*.

Effect of storage on quality and sensory attributes of *Cham-Cham*

Sensory attributes

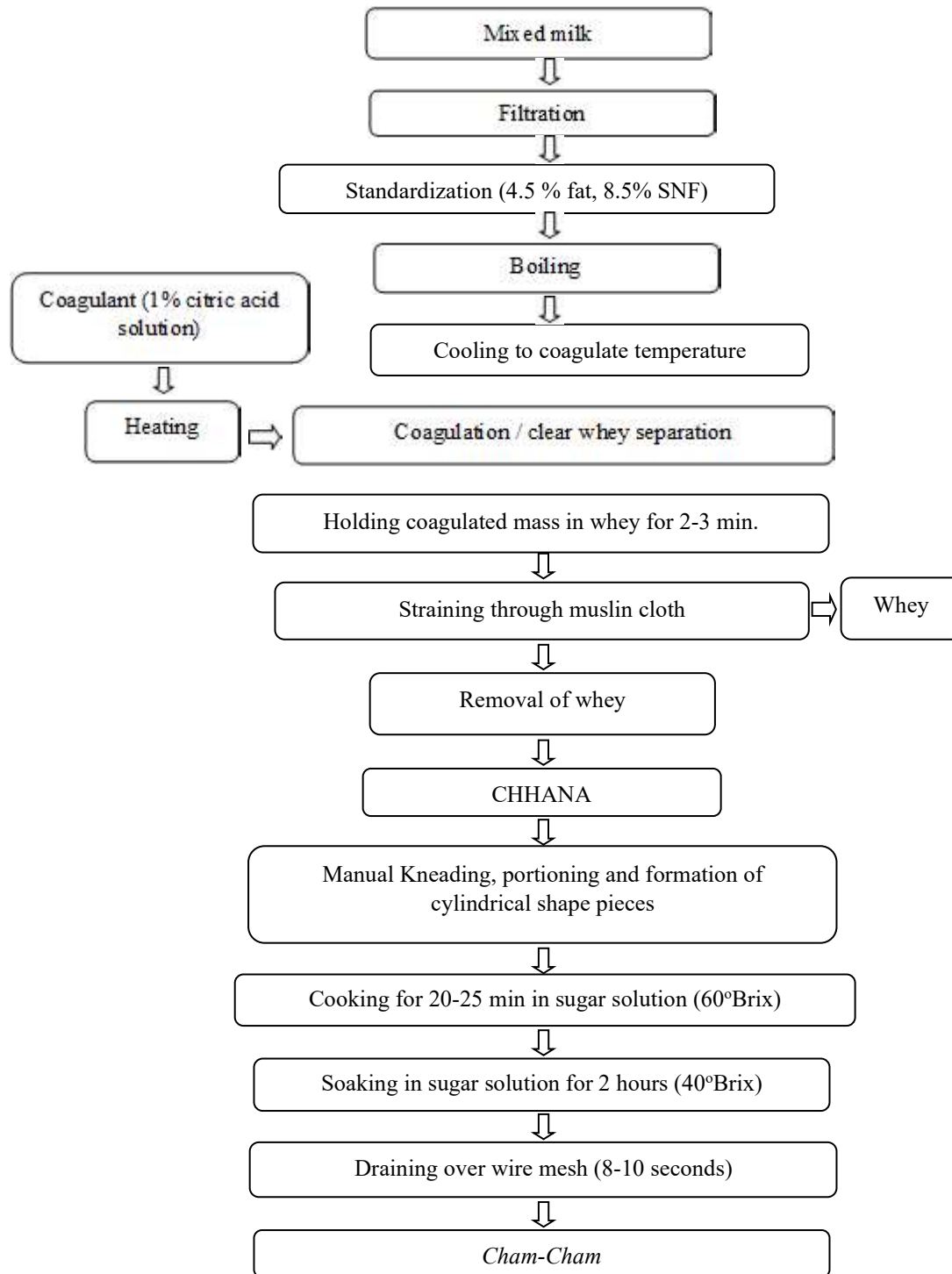


Fig 1. Flow diagram for the preparation of *Cham-Cham*

The average sensory score of fresh as well as MAP *Cham-Cham* (like flavor, colour and appearance, overall acceptability etc.) during storage (20°C) were represented in Figure 2 to 4.

Flavor

It was observed that during storage the average flavour score decreased significantly ($p < 0.05$) with advancing storage period at $20 \pm 2^\circ\text{C}$ (Figure 2). Initially, on the 0th day all the treatments secured nearly equal flavor score or no significant effect of packaging was observed on 0th day of storage among the three

Fig 2. Effect of storage period at $20\pm 2^\circ\text{C}$ on the Flavour score of *Cham-Cham* packed in different packaging systems

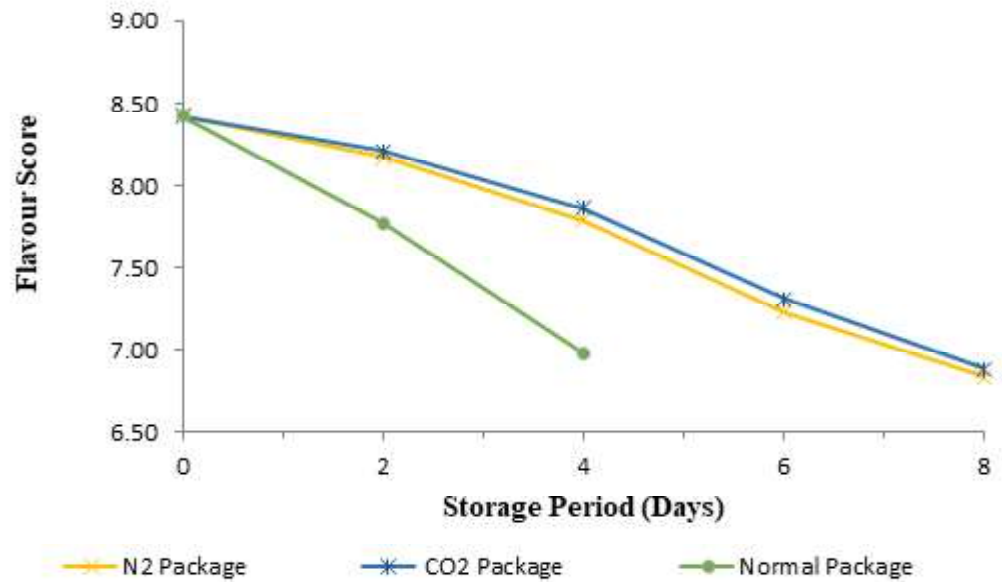
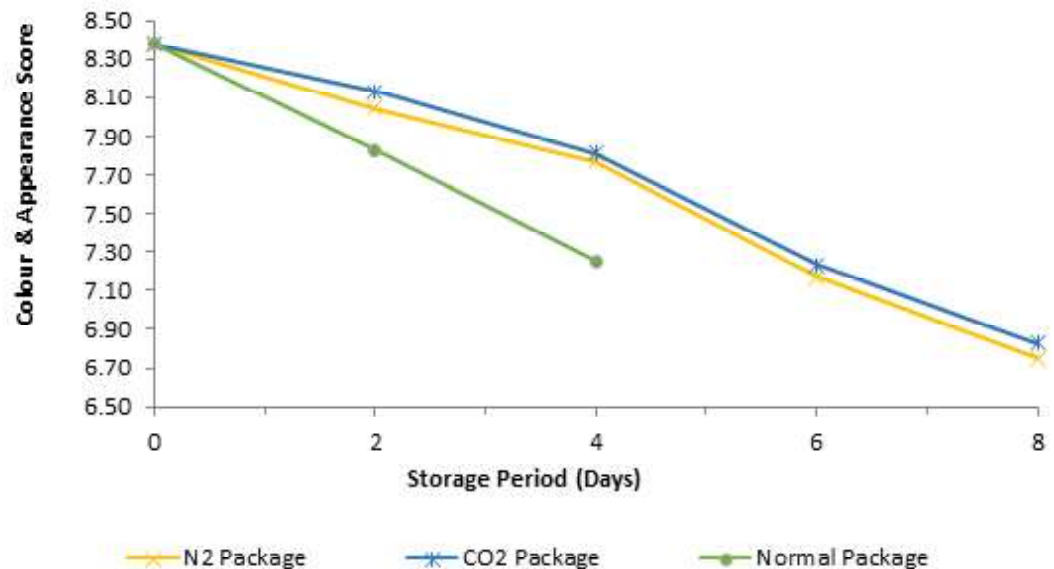


Fig 3. Effect of storage period at $20\pm 2^\circ\text{C}$ on the Colour & Appearance score of *Cham-Cham* packed in different packaging systems



samples. Samples packed in normal package, CO_2 package and N_2 package were found to be unacceptable after 4, 8 and 8 days of storage, respectively. After fourth day of storage, visible mold growth was observed for normal packed *Cham-Cham* so for that sample the study was discontinued and the study was further continued with MAP packed *Cham-Cham* samples.

It was observed that during entire storage period of 8 days maximum flavor score was obtained by samples packed in CO_2 package. However, flavor deterioration was greater in normal package than N_2 package and CO_2 package, respectively. The present observation was similar to early observation of

Chowdhury et al. (2017) for khoa stored in different modified atmospheric packaging (10% CO_2 + 90% N_2 with and without preservatives) and those authors also reported the reduction in flavour score of *Ujani basundi* during storage at 30°C and 5°C (Gaikwad and Hembade, 2013). However, Puri et al. (2016b) reported that the packaging material directly affects the shelf life of *Cham-Cham* during storage at 30°C and 5°C .

Colour & appearance

Color and appearance is one of the most crucial attributes of *Cham-Cham* that directly influences the consumer acceptability

Fig 4. Effect of storage period at $20\pm 2^{\circ}\text{C}$ on the Overall Acceptability score of *Cham-Cham* packed in different packaging systems

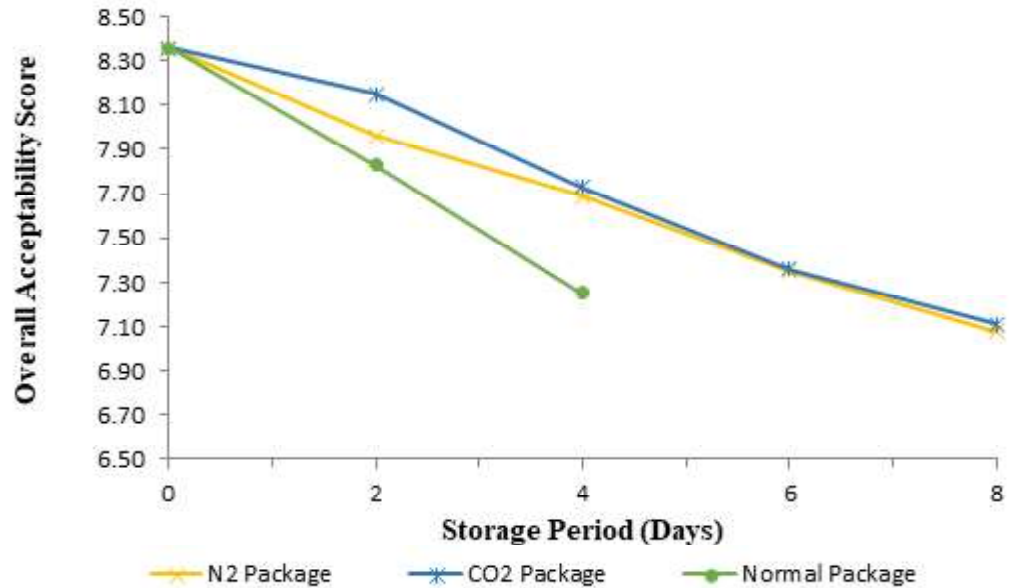
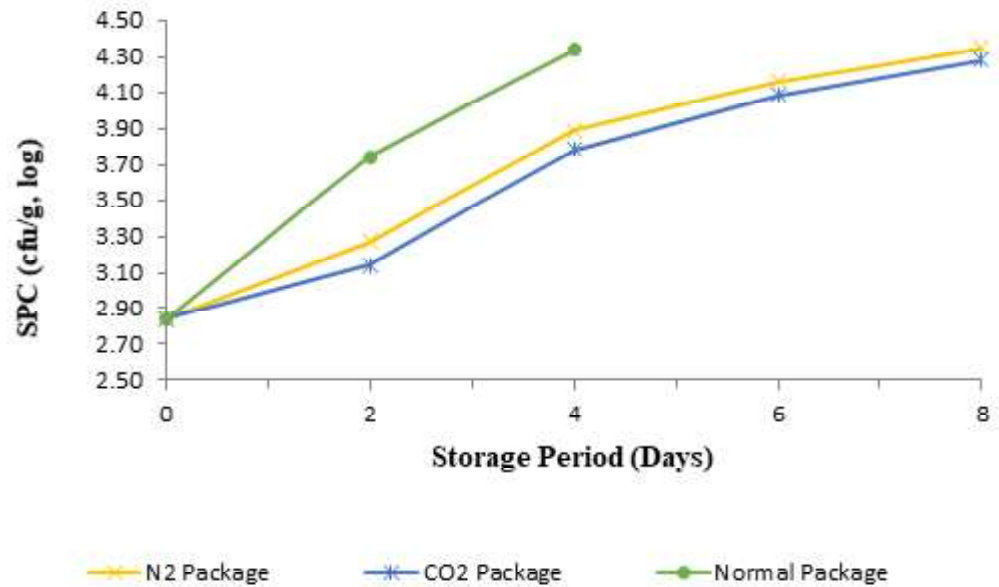


Fig 5. Effect of storage period at $20\pm 2^{\circ}\text{C}$ on the SPC of *Cham-Cham* packed in different packaging systems



of the sweet (Puri et al. 2016a). Initially it was observed that colour and appearance score for the three sets of *Cham-Cham* was 8.38. However, it is revealed from Figure 3 that the mean value of colour & appearance score decreased significantly ($P < 0.05$) during advancing of storage period; this might be due to evaporation of moisture from the product. During entire storage period, the colour & appearance score was high for CO_2 package followed by N_2 package and normal package, respectively. Similar observation was also recorded by Chawla et al. (2015), during storage of *doda Burfi*.

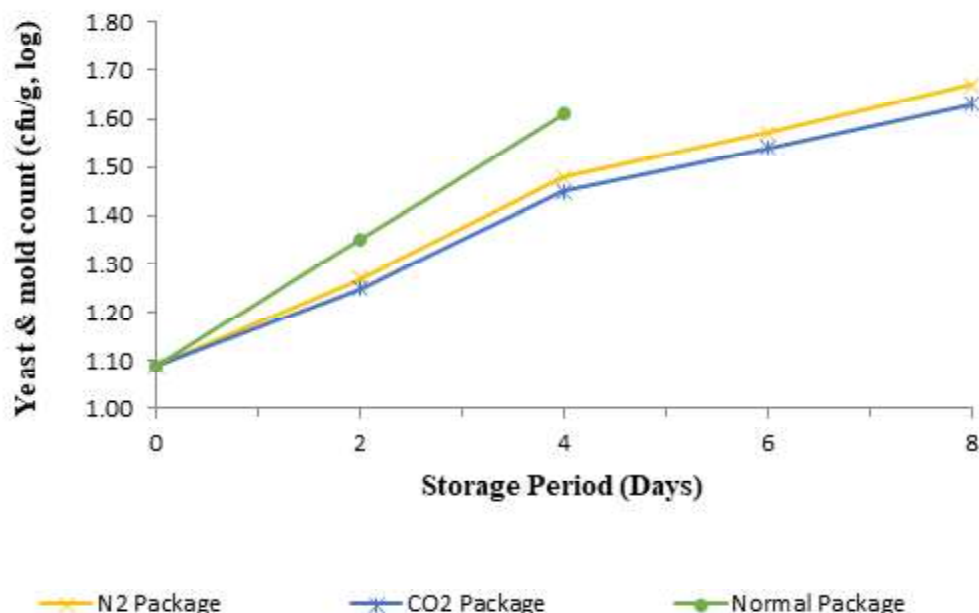
Puri et al. (2016a) reported that the colour of *Cham-Cham* was produced due to non-enzymatic browning during soaking of *Cham-Cham* in sugar syrup. However, Sutariya and Rao (2017)

reported that packaging material (Polystyrene tubs, Low Density Polyethylene, Laminates, Multilayer pouch) and condition directly affect the sensory attributes of *Thirattupal* during storage at 30°C .

Overall acceptability

During storage overall acceptability showed a significant ($P < 0.05$) declining trend. Mean overall acceptance score indicated that changes were faster in normal package followed by N_2 package and CO_2 package samples (Figure 4). Due to decrease in the scores of other sensory attributes, its effect also reflected in overall acceptability score.

Fig 6. Effect of storage period at 20±2°C on the Yeast & mold count of Cham-Cham packed in different packaging systems



Desai et al. (2013) & Puri et al. (2016a) reported that representation of ideal sensory attributes is crucial to develop a product which meets the consumer expectations as the sensory quality is driving force for consumer acceptance and demand. However, different processing aspects directly influenced the acceptability of Cham-Cham (Singh et al. 2013). In the present study, it was clearly identified that MAP packaging directly influenced the overall acceptability of Cham-Cham and more over this technique increased the shelf life of Cham-Cham than normal packaging.

Physico-chemical changes of Cham-Cham samples during storage (20°C)

Table 1 Proximate composition of fresh Cham-Cham

Compositional Attribute	Value (%)
Moisture	39.15±0.05
Fat	12.84±0.05
Protein	11.54±0.02
Total carbohydrate	35.19±0.03
Ash	1.28±0.01

Table 2 Influence of storage period at 20±2°C on the Moisture content of Cham-Cham packed in different packaging systems

Storage days	0 day	2 day	4 day	6 day	8 day	Average for Package
Package	Moisture (%)					
N ₂ Package (Pa1)	39.15±0.05	38.17±0.52	37.34±0.47	36.71±0.41	36.06±0.38	37.48
CO ₂ Package (Pa2)	39.15±0.05	38.31±0.51	37.66±0.43	36.87±0.33	36.42±0.38	37.68
Average of Period	39.15	38.24	37.50	36.79	36.24	
Normal Package	39.15±0.05	37.37±0.11	37.05±0.31			
	SEM		CD (0.05)		CV%	
Package (Pa)	0.100		NS			
Period (P)	0.158		0.47		1.04	
Pa*P	0.224		NS			

Each observation is a mean ± Standard deviation

Changes of moisture during storage

During storage period loss of moisture content from all samples was observed. The moisture content plays significant role in the microbial quality of the product during storage as bacterial activity; especially that of yeast and mold affects the shelf life of the product. It is evident from Table 2 that during storage of Cham-Cham moisture loss was observed steadily. However, between both MAP systems (N₂ and CO₂) there was no significant (P<0.05) effect on moisture loss during entire storage period (Table 2). However, for all the samples loss of moisture occurred significantly (P<0.05) during increment of storage period.

The observation was in line with Londhe et al. (2012) who prepared brown peda and reported reduction in moisture content of samples during storage at 30°C. Sachdeva and Rajorhia, (1982) also reported loss in moisture of Burfi when stored at different temperature. However, the observation was similar to result of Sharma et al. (2003) who reported that malai peda samples packaged in MAP showed less moisture loss as compared to control during storage at 32°C and 11°C.

Effect of pH on storage

The changes in pH value cannot be directly correlated with change in acidity; still it was found that with the increase in acidity, the pH was decreasing. The pH value was found to be

significant between packaging materials (Polystyrene tubs, Low Density Polyethylene, Laminates, Multilayer pouch) during storage of *Thirattupal* at 30°C (Sutariya and Rao, 2017). The values regarding pH of *Cham-Cham* revealed that with the increased storage period, the pH of the *Cham-Cham* samples stored at 20±2°C decreased significantly (P<0.05) in both packages containing N₂ and CO₂ (Table 3). However, as the storage period progressed, the samples showed a decrease in the mean pH content from 6.33 to 6.12 on 8 day of storage at 20±2°C; this may be due to the microbial changes, resulting in the decreased pH of *Cham-Cham*. After storage of 8 days, the product was found with visible mold growth on surface. Further, non-significant (P<0.05) effect was observed in the pH of *Cham-Cham* whether packed in N₂ or CO₂ containing MAP package during storage. However, for normal package pH decreased rapidly and on fourth day of storage the pH was 5.19 and visible yeast and mould growth was observed. The antimicrobial effect of CO₂ is already well known and it is considered as GRAS by US-FDA. In this work, better shelf life of CO₂ packaging reflected the antimicrobial effect that retarded the growth of spoilage micro-organisms of *Cham-Cham* than normal packaging.

Earlier different researchers reported decline of pH during storage of *Rasogolla* and *peda* (Arora et al. 1995, 1996, Singh et al. 2007 and Kumar et al. 1997). The decrease in pH value could be due to the action of microorganisms as well as production of organic acids during processing and storage. Recently, Sutariya and Rao (2017) reported that pH of the product (*Thirattupal*- A South Indian traditional Product) used to be decreased during storage due growth of microorganisms in spite of using different packaging material (PS, LDPE, Laminates, Multilayer pouch).

Change of acidity during storage

Mean acidity values of fresh *Cham-Cham* increased significantly (P<0.05) from 0.345 (% LA) to 0.420 (% LA) when stored at 20±2°C temperature (Table 4). The mean acidity contents of 0.382 and 0.387 (% LA) were observed in N₂ and CO₂ packages respectively. These observations imply that the acidity of *Cham-Cham* packed in N₂ and CO₂ package was affected significantly (P<0.05) by the package selected. The interaction effect of package and storage period was found statistically non-significant. However, for normal package *Cham-Cham*, acidity decreased rapidly and on fourth day of storage acidity of normal packaged *Cham-Cham* was 0.612 (% LA). However, visible yeast and mould growth was observed. Acidity development may also indicate the extent of microbial fermentation in product (Prasad et al. 1989). The effect of storage period on acidity is a normal biochemical process in dairy products due to increase of acidity as a result of degradation

Table 3 Influence of storage period at 20±2°C on the pH of *Cham-Cham* packed in different packaging systems

Storage daysPackage	0 day	2 day	4 day	6 day	8 day	Average for Package
N ₂ Package (Pa1)	6.33±0.02	6.26±0.03	6.19±0.02	6.17±0.03	6.13±0.03	6.22
CO ₂ Package (Pa2)	6.33±0.02	6.25±0.02	6.17±0.03	6.14±0.02	6.11±0.03	6.20
Average of Period	6.33	6.25	6.18	6.15	6.12	
Normal Package	6.33±0.02	6.16±0.01	5.19±0.01			
	SEm		CD (0.05)		CV%	
Package (Pa)	0.006		NS			
Period (P)	0.009		0.03		0.37	
Pa*P	0.013		NS			

Each observation is a mean ± Standard deviation

Table 4 Effect of storage period at 20±2°C on the Titratable acidity of *Cham-Cham* packed in different packaging systems

Storage daysPackage	0 day	2 day	4 day	6 day	8 day	Average for Package
N ₂ Package (Pa1)	0.345±0.004	0.368±0.008	0.381±0.006	0.399±0.007	0.414±0.008	0.382
CO ₂ Package (Pa2)	0.345±0.004	0.373±0.008	0.387±0.005	0.405±0.006	0.426±0.004	0.387
Average of Period	0.345	0.371	0.384	0.402	0.420	
Normal Package	0.345±0.004	0.483±0.003	0.612±0.002			
	SEm		CD (0.05)		CV%	
Package (Pa)	0.0016		0.005			
Period (P)	0.0025		0.007		1.59	
Pa*P	0.0035		NS			

Each observation is a mean ± Standard deviation

of lactose or protein. Prasad et al. (2017) showed that irrespective of packaging material and storage temperature, the extent of increase in acidity and microbial load in the *Burfi* was significant.

Increase in acidity values on storage in different packaging materials was also reported by Sachdeva and Rajorhia (1982), Sharma et al. (2003) and Londhe et al. (2012) for *Burfi*, *peda* and brown *peda*, respectively.

Change of SPC during storage

SPC includes all types of microorganisms including post contamination, therefore it presents the overall microbiological quality of product. It was observed that SPC of three different *Cham-Cham* samples increased significantly ($P < 0.05$) during progress of storage period at $20 \pm 2^\circ\text{C}$ (Figure 5). The initial mean SPC of normal packaged *Cham-Cham* stored at $20 \pm 2^\circ\text{C}$ was 2.84 at 0 day which increased significantly ($P < 0.05$) to 3.20 on 2nd day, 3.84 on 4th day, 4.12 on 6th day and 4.32 on 8th day of storage in MAP. However, the two different MAP systems individually had statistically ($P < 0.05$) significant difference on the SPC. Although increase of SPC was visible in both the cases during advancement of storage period, this trends followed entire storage period of the study.

Increase in SPC of *Burfi* on storage at 30°C was observed by Sachdeva and Rajorhia (1982). Similar type of observation was also reported by Garg and Mandokhot (1987) & Misra and Kuila (1988) for *Burfi*. Londhe et al. (2012) reported increased SPC of brown *Peda* stored at 30°C and packed using different packaging techniques including Cardboard box, MAP and Vacuum packaging. It was observed that during storage of *Cham-Cham*, SPC increased. However, adoption of MAP technique directly affected the microbial populations that seem the increase shelf life of the stored product.

Yeast and mould count

In dairy products, especially traditional products mold growth is a major problem and often most important single factor limiting their shelf life.

The effect of storage period at both the storage temperature on the yeast & mold count of *Cham-Cham* stored in N_2 and CO_2 gas flushed packages is depicted in Figure 6. The values revealed that as the storage period enhanced, the package atmosphere had significant ($P < 0.05$) effect on the yeast & mold count of the *Cham-Cham* stored in both packages containing N_2 and CO_2 . However, as the storage period progressed the samples showed an increase in the mean yeast & mold count from zero to 1.63 on 8th day of storage. After storage of 8 days, the product was not analyzed due to visible mold growth on surface. The interaction effects of package and storage period were statistically non-significant ($P < 0.05$) at $20 \pm 2^\circ\text{C}$.

Microbial growth was well co-related with acidity and pH increment of *Cham-Cham* sample during storage. It was observed that on fourth day of storage highest acidity and low pH was observed in normal packed *Cham-Cham*; so that significantly higher microbial count was observed for normal packed *Cham-Cham*.

For *Pedha*, *Burfi* and *Kalakand* almost similar observations were recorded by various researchers. (Biradar et al. 1985, Dwarkanath and Srikanta, 1977). Sachdeva and Rajorhia (1982) observed increased yeast and mold count during storage of *Burfi* at 30°C and 5°C .

Conclusions

Cham-Cham is one of the traditional products with wide market opportunity and acceptability. However, MAP using two different conditions increased the shelf life of *Cham-Cham* to 8 days that is almost double as compared to normal package. However, adoption of MAP was proved to be very suitable to increase the shelf life of TIDP.

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Determination of functional, textural and colour properties of market Mozzarella cheese

Lakshmana N¹, Pradyuman Barnwal¹, Ankit Deep¹, Bhavesh Chavhan¹, Yogesh Khetra², Vivekanand N Sukre¹

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Abstract: Mozzarella cheese is one of the most popular members of pasta-filata family cheeses that originated in Italy. There is increasing demand of Mozzarella cheese for preparation of varieties of food products. The various properties such as functional, textural and colour properties of Mozzarella cheese play a vital role in its marketing and consumer acceptance. In the present study, these properties of four brands of market Mozzarella cheese were determined. The stretchability, flowability and meltability of market Mozzarella cheese samples were found statistically significantly different ($p < 0.01$). The average values for stretchability, flowability and meltability ranged (minimum to maximum) between 14.67 ± 4.16 to 76.67 ± 2.06 cm, 20.47 ± 6.39 to 40.67 ± 1.01 % and 1.67 ± 0.23 to 10.2 ± 1.35 cm, respectively. All the textural and colour properties of market Mozzarella cheese were found statistically highly significantly different between the brands ($p < 0.001$). The values of the hardness, adhesiveness, springiness, cohesiveness, gumminess and chewiness of market Mozzarella cheese (minimum to maximum) varied from 29.51 ± 4.48 to 35.64 ± 0.16 N, -0.02 ± 0.02 to -0.67 ± 0.32 Ns, 0.32 ± 0.06 to 0.89 ± 0.05 , 0.28 ± 0.03 to 0.56 ± 0.05 , 10.11 ± 1.56 to 28.91 ± 4.16 and 3.29 ± 1.06 to 25.80 ± 4.52 , respectively. The L^* , a^* , b^* , chroma value, hue angle and whiteness index values of Market Mozzarella cheese varied from 70.30 ± 0.10 to 73.77 ± 0.15 , -1.14 ± 0.19 to 1.60 ± 0.12 , 19.88 ± 0.27

to 25.55 ± 0.34 , 19.91 ± 0.28 to 25.60 ± 0.33 , -1.51 ± 0.00 to 1.51 ± 0.01 radian and 60.79 ± 0.27 to 66.22 ± 0.33 , respectively. Colour and functional characteristics of Mozzarella cheese play a vital role for market acceptance along with textural characteristics for development of process equipments for Mozzarella cheese making.

Keywords: Mozzarella cheese, stretchability, springiness, whiteness index

Introduction

Mozzarella cheese is a prominent member of the pasta filata or stretched curd cheeses that originated in Italy. Pasta filata cheese is a high volume production cheese used in pizza and other Italian foods. Pasta filata cheeses are distinguished by a unique plasticizing and kneading treatment of the fresh curd in hot water, which imparts the finished cheese, with characteristic fibrous structure, melting and stretching properties (Kindstedt and Fox, 1993).

Traditionally Mozzarella cheese was mainly produced from milk of water buffaloes in Italy. In other European countries and U.S.A, Mozzarella cheese is produced by using cow's milk with some required modifications (Ghosh et al. 1990). Mozzarella cheese has soft texture, white colour with glossy appearance and it is mainly valued for its stretchable property which helps in preparation of lasagna, veal cutlet and pizza (Kosikowski, 1982; Jana, 2001). An increase in pizza consumption by people specially youngsters, resulted into increase in the demand for Mozzarella cheese. In general, Mozzarella cheese is used as pizza topping due to its stretchability (Kindstedt et al. 1989).

Although there is widespread popularity and acceptability of Mozzarella cheese in the Indian market, the consumption of the cheese is limited to major cities only. Mozzarella cheese production is mostly confined to large-scale industry. In rural or semi urban area, its usage is very limited. There are many reasons for this such as lack of importance of Mozzarella cheese and its application, limited production and availability at rural and semi urban area, etc. The various properties of some market dairy products are available in published literature e.g. Kradi cheese

¹Dairy Engineering Division
²Dairy Technology Division
ICAR-National Dairy Research Institute (Deemed University),
Karnal-132 001, Haryana, India

P Barnwal (✉)
Dairy Engineering Division
ICAR-National Dairy Research Institute (Deemed University)
Karnal-132 001, Haryana, India
Phone: +91-184-2259419(O), +91-8397833349 (M)
Fax: +91-184-2250042
Email: pbarnwal@rediffmail.com; psbarnwal@gmail.com

(Punoo et al. 2018), Indian cottage cheese i.e. Paneer (Wangdare et al. 2017), cheese and cheese products (Jayadevan, 2013), KheerMohan (Meena et al. 2014), Rasogolla (Srinivasa et al. 2017). Therefore, considering above-mentioned information, attempts were made to determine the functional, textural and colour properties of four brands of market Mozzarella cheese in present study.

Materials and Methods

Collection of Mozzarella cheese samples

There are different brands of Cheese manufacturers producing good quality Mozzarella cheese and also available throughout the country. Due to high demand of Mozzarella cheese in pizza making, some local cheese manufacturers are also producing good quality Mozzarella cheese to fulfill the local demand. For the present study, four brands of Mozzarella cheeses (two national, one regional and one local brand) were collected from Delhi and local market of Karnal. The collected different brands of Mozzarella cheese were designated as M1, M2, M3 and M4.

Functional properties of Mozzarella cheese

Meltability

Meltability was determined by the method described by (Obergruber et al. 1993). Fifteen gram (15 g) shredded cheese was filled into a Pyrex glass tube (30 mm diameter × 250 mm length) and gently tapped with a spatula until each of the samples occupied a length of about 35 mm. The one end of the tubes was sealed with stoppers (Silicon rubber) and the other with aluminum foil having a small hole to allow gases to escape. The tubes were tempered at 4°C (in Refrigerator) for 30 min in a vertical position and then placed horizontally in an oven at 100°C for 60 min. After cooling to room temperature, the length of melted cheese was measured.

Stretchability

Stretchability of Mozzarella cheese was measured on pizza by following the standard procedure (Catherine et al. 1998). Shredded cheese (0.25g/cm²) was evenly distributed on the pizza base that was pre-cut in half and it was placed in the oven at 280°C for 4 minute and then evaluated for Stretchability. One half of the pizza was clamped and the unclamped section was manually pulled until the extended string that joining the both section of pizza base completely broke. The stretch was recorded by measuring the distance between the pizza halves.

Flowability

Flowability of cheese on cooking was assessed by the 'Schreiber test'. A disk of cheese (44.5 mm diameter; 4.0 mm thick) from the freshly cut surface of the cheese block was taken and placed on a glass disc, which was marked with four intersecting diagonals.

The glass disc was placed in an electrostatically controlled hot air oven at 280°C for four minutes. Glass disc with cheese was removed from the oven and placed on a steel surface at 0°C and allowed to cool for 3 minutes. The diameter of the molten cheese disk was measured at four locations (i.e. along the four diagonals). The flowability is the change in dimension, expressed as a percentage of dimension in the unheated sample (Fox et al. 2017). Therefore, flowability was calculated by following equation (Fox et al. 2017):

$$\% \text{ Flowability} = \frac{(D_1 - D_0)}{D_0} \times 100$$

Where, D_1 = diameter of cheese disk after heating

D_0 = diameter of cheese disk before heating

Texture profile analysis of Mozzarella cheese

Market Mozzarella cheese samples were analysed by using texture analyser (Model TAXT2i, Stable Micro Systems U.K., double cycle compression) installed with texture expert exceed (version 2.55; English) software. The product was subjected to a typical two bite compression test using compression probe (P-75) attached to the texture analyser. The texture analyser was calibrated for probe and then for force of 5 kg, before starting the analysis. Mozzarella cheese was evaluated for various textural properties like hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience at 2mm/s probe speed and 80 % compression. Initially the product samples were cut into a cylinder of height and diameter of 1 cm each. Product sample was kept on the Texture Analyser platform under the load cell of 25 kg. Five samples of the product for selected parameters were analysed.

Colour properties

Colour values (L^* , a^* , b^*) of the Mozzarella cheese samples were determined by using a colour flex colorimeter (Hunter Associated Laboratory, Inc., Reston, VA USA). Before colour measurement, the instrument was calibrated with a standard black glass and white glass tiles as specified by the manufacturer. Mozzarella cheese samples were shredded using stainless steel grater, the shredded samples were transferred to sample plate and then colour was measured. Data were received from software in terms of L^* {lightness; range: 0 (black) to 100 (white)}; a^* {redness; range: +60 (red) to -60 (green)} and b^* {yellowness, range: +60 (yellow) to -60 (blue)}. The chroma, hue angle and browning index (BI) were calculated from the L^* , a^* and b^* values (Barnwal et al. 2015; Gupta et al. 2011) and used to describe the colour:

$$\text{Chroma} = \sqrt{(a^{*2} + b^{*2})}$$

$$\text{Hue angle} = \tan^{-1}\left(\frac{b^*}{a^*}\right)$$

Whiteness index (WI) of the Mozzarella cheese samples was calculated using the following standard relation (Siriwongwilaichat et al. 2014; Srinivasa et al. 2017):

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$$

Statistical analysis

Analysis of variance (ANOVA) for different characteristics of market Mozzarella cheese was carried out by PROC ANOVA procedure (SAS software, version 9.3).

Results and Discussion

Functional properties of market Mozzarella cheese

The stretchability, meltability and flowability of market Mozzarella cheese samples were statistically significant (p<0.01) as represented in Table 1. The stretchability of M2 and M4 were observed to be statistically similar. The functional properties values of market Mozzarella cheese as obtained were in the range of stretchability 14.67± 4.16 to 76.67± 2.06 cm, flowability 20.47± 6.39 to 40.67± 1.01 % and meltability 1.67±0.23 to 10.2±1.35 cm. The highest value of stretchability and meltability was observed in M3 sample and the highest flowability observed in sample M4. The lowest value of stretchability and meltability was observed in sample M4 and the lowest value of flowability was observed in M2 sample (Fig. 1).

Textural properties of market Mozzarella cheese

The hardness, springiness, gumminess, adhesiveness, chewiness, cohesiveness of market Mozzarella cheese samples were found highly significant (p<0001) as shown in Table 2. The hardness of M1 and M4 were observed to be statistically similar. The adhesiveness of M1 and M2 were observed to be statistically similar whereas M3 and M4 were statistically at par with each other. The cohesiveness of M1 and M3 were observed to be statistically similar. The values of the hardness, adhesiveness, springiness, cohesiveness, gumminess and chewiness of market Mozzarella cheese were ranges in between the values of 29.51±4.48 to 35.64±0.16 N, -0.02±0.02 to -0.67±0.32 Ns, 0.32±0.06 to 0.89±0.05, 0.28±0.03 to 0.56±0.05, 10.11±1.56 to 28.91±4.16 and 3.29±1.06 to 25.80± 4.52, respectively. The value of the hardness, adhesiveness, springiness, cohesiveness, gumminess and chewiness of market Mozzarella cheese were found maximum in sample M2 among other samples (Figs.2-4).

Colour properties of market Mozzarella cheese

Colour properties like L*, a*, b*, chroma value, hue angle and whiteness index of Market Mozzarella cheese were studied and found statically highly significant (p<0.0001) with each other (Table 3). The a* of M1 and M2 were observed to be statistically similar whereas M2 and M4 were statistically at par with each other. The b* of M2 and M4 were observed to be statistically similar. Chroma value of M2 and M4 were observed to be statistically similar. Hue angle (in radian) of M1, M2 and M4 were observed to be statistically similar. The b* stands for yellow-blue value, thus + b* stands for the increase of yellow and -b* for the increase of blue (Li et al. 2014). Whiteness index of M2 and M4 were observed to be at par with each other. The values of

Table 1 ANOVA table for functional properties of market Mozzarella cheese

Market Mozzarella cheese	Strechability (cm)	Flowability (%)	Meltability (cm)
DF	3	3	3
SS	6950.33	3222.853	121.64
MS	2316.78	1074.284	40.55
F-Value	80.35	15.073	80.02
Prob	<.0001***	<.001***	<.0001***

***p<0.001; n=3

Table 2 ANOVA for textural properties of market Mozzarella cheese

Market Mozzarella cheese	Hardness, N	Adhesiveness, Ns	Springiness	Cohesiveness	Gumminess	Chewiness
DF	3	3	3	3	3	3
SS	1360.961	1.550	0.981	0.190	1083.447	1459.527
MS	453.654	0.517	0.327	0.063	361.149	486.509
F-Value	30.63	16.98	94.69	73.14	55.91	74.54
P	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***

***p<0.001; n=3

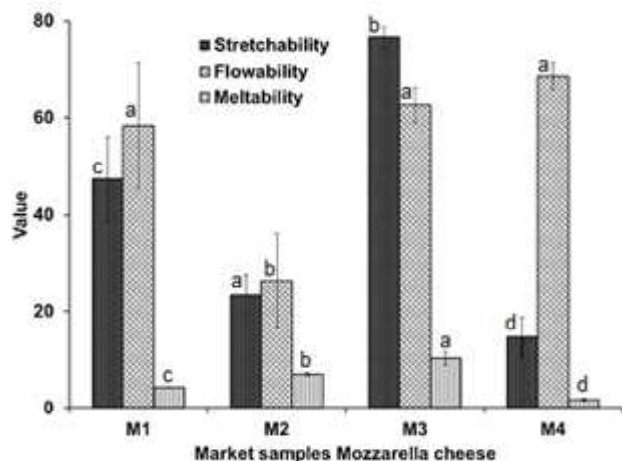


Fig. 1: Variation in stretchability, flowability and meltability of market Mozzarella cheese

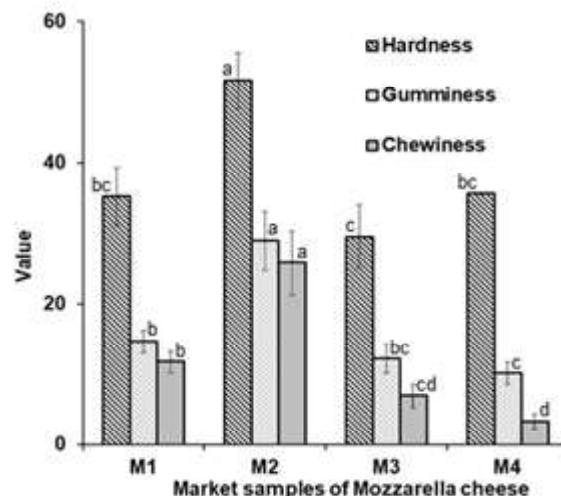


Fig. 2 Variation of hardness (N), gumminess and chewiness of Mozzarella cheese

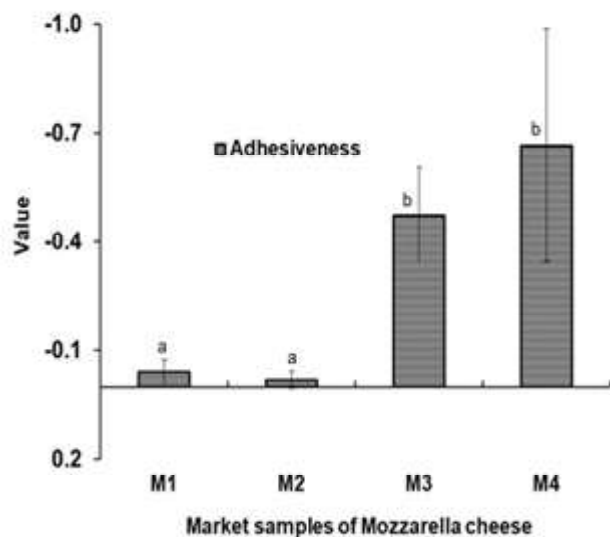


Fig. 3: Variation in adhesiveness (Ns) of market Mozzarella cheese

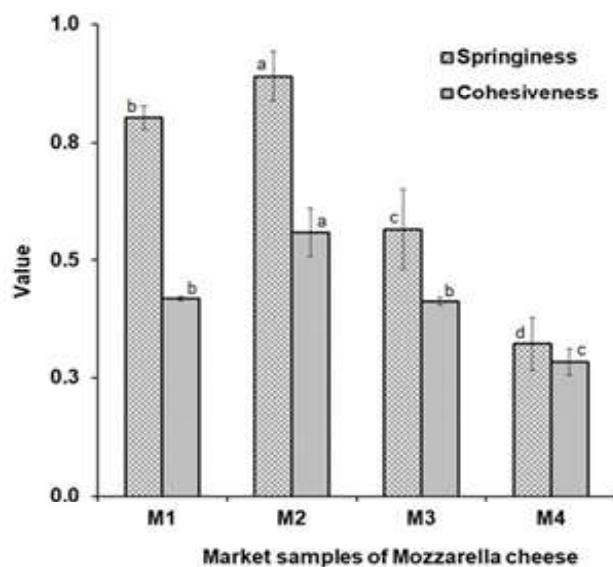


Fig. 4: Variation in springiness and cohesiveness of market Mozzarella cheese

Table 3 ANOVA for colour characteristics of market Mozzarella cheese

Market Mozzarella cheese	L^*	a^*	b^*	Chroma	Hue angle	WI
DF	3	3	3	3	3	3
SS	22.20	18.31	48.25	48.53	20.55	51.88
MS	7.40	6.10	16.08	16.18	6.85	17.29
F-Value	310.67	329.75	324.37	324.13	184276.00	314.49
P	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***

***p<0.001; n=3

L^* , a^* , b^* , chroma value, hue angle and whiteness index of Market Mozzarella cheese were ranged between 70.30 ± 0.10 to 73.77 ± 0.15 , -1.14 ± 0.19 to 1.60 ± 0.12 , 19.88 ± 0.27 to 25.55 ± 0.34 , 19.91 ± 0.28 to 25.60 ± 0.33 , -1.51 ± 0.00 to 1.51 ± 0.01 radians and 60.79 ± 0.27 to 66.22 ± 0.33 respectively. Imm et al. (2003) has observed L^* , a^* and b^* values for bovine mozzarella cheese ranging from

79.6 ± 1.74 (0 week) to 74.7 ± 1.84 (8 week), -4.3 ± 0.09 (0 week) to -5.9 ± 0.21 (8 week) and 17.4 ± 0.29 (0 week) to 22.1 ± 2.40 (8 week), respectively. The maximum L^* value observed in M2 sample. The M3 sample shows maximum value of a^* , b^* , chroma value and hue angle. The highest value of whiteness index observed in sample M1 (Fig. 5-6).

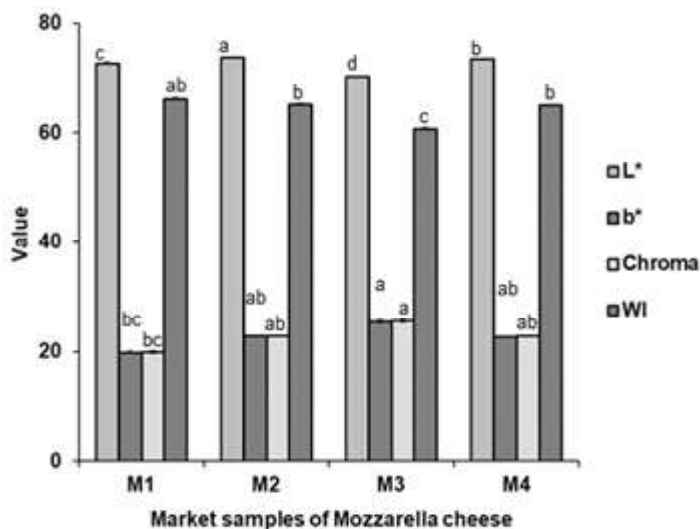


Fig.5 Variation of L^* , b^* , chroma and whiteness index of market Mozzarella cheese

Conclusions

The functional, textural and colour properties of four brands of market Mozzarella cheese (M1, M2, M3 and M4) were determined and found statistically significant among each other. The highest value of stretchability and meltability was observed in M3 sample, highest flowability observed in sample M4, lowest value of stretchability and meltability was observed in sample M4 and the lowest value of flowability was observed in M2 sample. Maximum L^* value was observed in M2 sample whereas M3 sample showed maximum value of a^* , b^* , chroma value and hue angle. The highest value of whiteness index was found in sample M1. In textural characteristics, the value of the hardness, adhesiveness, springiness, cohesiveness, gumminess and chewiness of market Mozzarella cheese were found maximum in sample M2 among other samples.

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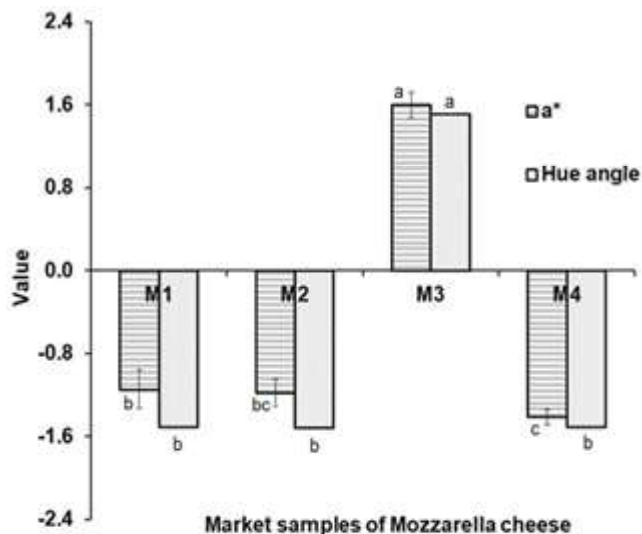


Fig. 6 Variation of a^* and hue angle of market Mozzarella cheese

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Utilization of exopolysaccharide producing lactic acid bacteria for improving the quality of low fat curd

Rosemol Jose, K Radha, CT Sathian and Binsy Mathew

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Abstract: An experiment was conducted to utilize exopolysaccharide producing lactic acid bacteria for the preparation of low fat curd. Exopolysaccharide producing cultures were isolated from 25 curd samples collected from different sources. From this samples 32 EPS producing cultures were isolated. Based on morphological, biochemical and sugar utilisation tests the isolates were grouped. According to results, two were *Streptococcus thermophilus*. Eight of the isolates were *Lactobacillus*. Remaining 22 isolates were identified as *Lactococcus*. All the 32 EPS producing isolates showed mucoid colonies with a slimy appearance when the isolates were streaked on MRS or M17 agar with 4 per cent sucrose which indicates the capability of EPS production. Exopolysaccharides produced by different isolates were purified by ethanol precipitation method followed by dialysis and they were quantified by phenol sulphuric acid method. The maximum EPS yield was observed in two isolates of *Lactococcus lactis* subsp *lactis* with 109 mg/L and 107.4 mg/L respectively. Low fat curd was prepared from skim milk by using these two EPS producing isolates. Physico - chemical parameters and sensory quality of the low fat curd were analysed by standard methods. Statistical analysis revealed that the whey separation and curd setting time of low fat curd decreased with the use of exopolysaccharide producing isolates. There was no significant difference in fat content but an increase in total solid content of curd samples prepared by EPS producing isolates was observed. Overall sensory scores were higher for low fat

curd prepared with EPS producing isolates when compared to control curd prepared with non EPS producing cultures.

Keywords: Exopolysaccharides, Low fat curd, Lactic acid bacteria, Sensory quality

Introduction

Curd is the most popular fermented milk product of India consumed in almost every household as a part of daily diet. Lactic acid bacteria are widely used in the manufacture of traditional fermented milk products. Apart from the production of lactic acid, flavouring compounds and bacteriocins several strains of lactic acid bacteria are capable of producing exopolysaccharides (EPS) (Behare et al. 2009). Exopolysaccharides are long chain polysaccharides produced extracellularly mainly by bacteria. They are made up of branched, repeating units of sugar or sugar derivatives. Lactic acid bacteria have the potential to divert significant amount of fermentable sugars towards the biosynthesis of functional exopolysaccharides. In the food Industry, EPS produced by lactic acid bacteria are mainly used as food additives and act as viscosifiers, stabilizers, emulsifiers or gelling agents to modify the rheological properties and texture of food products (Bajpai et al. 2016). In the dairy Industry EPS are widely used to improve the body and texture of fermented milk products like dahi and yoghurt. Exopolysaccharides also confer health benefits like antioxidant, cholesterol regulating, antitumor, antiviral and immunomodulatory activities. (Madhuri and Prabhakar 2014)

The health risks associated with the consumption of high fat diet lead to the increased demand for low fat dairy products. However, the role of milk fat in the development of flavour, body and texture of the dairy products is undeniable (Güven et al. 2005). Exopolysaccharides which are naturally produced by some lactic acid bacteria during fermentation process can act as natural bio thickener to improve texture, decrease syneresis and improve the sensory properties of low fat dairy products (De vuyst et al. 2003). Hence, the present study aims at isolation of exopolysaccharide producing lactic acid bacteria from curd samples and utilization of EPS producing isolates for the preparation of low fat curd.

Department of Dairy Science, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

K Radha(✉)

Department of Dairy Science, College of Veterinary and Animal Sciences, Mannuthy-680 651, Thrissur, Kerala, India

Email: radhavet@gmail.com; Mobile:9744481598

Materials and Methods

The main objective of the study was to develop low fat curd using exopolysaccharide (EPS) producing isolates. The study was carried out at Department of Dairy Science, College of Veterinary and Animal Sciences, Mannuthy.

Isolation and Identification of lactic acid bacteria from curd samples

Twenty-five curd samples were collected from different households and student hostels. Samples were thoroughly stirred, and one gram of each sample was taken and serially diluted with sterile peptone water (one per cent). Then one ml of diluted sample taken from appropriate dilution was plated on De Man Rogosa and Sharpe (MRS) agar and incubated at 37°C for 48 hours. The individual colonies with different morphology were picked and grown in MRS and M17 broth (Patil et al. 2015). The lactic acid bacteria were identified by morphological and biochemical tests comprising catalase activity, oxidase test, gram staining, Hugh leifson test, arginine hydrolysis, IMViC and sugar utilisation tests according to the procedure described by Collins and Lyne (2004).

Screening for EPS production

The isolates were streaked on MRS and M17 with 4 per cent sucrose medium for examination of exopolysaccharide production. The mucoid colonies with a slimy appearance were considered to possess the ability to produce EPS.

Capsular and ropy polysaccharide formation

The ability of cultures to form a capsule was evaluated by capsule staining method (Anthony, 1931). Screening for ropy EPS production was performed according to Dabour and Lapointe (2005) on MRS or M17 agar containing 0.08 g/L ruthenium red. The inoculum was streaked and the plates were incubated at 37°C. After incubation, the presence of white coloured mucoid slimy colonies indicates the presence of ropy strains.

Purification and quantification of exopolysaccharides

Isolation of exopolysaccharide from lactic acid bacteria was done by the method suggested by Bajpai et al. (2016). Quantification of EPS was done by the Phenol sulphuric acid method as per Dubois et al. (1956) with some modifications and using glucose as standard.

Preparation of low fat curd

Low fat curd was prepared as per the method of Behare et al. (2009). Whole milk was standardized to below 0.5 per cent fat and 8.7 per cent SNF. Then it was heated at 90°C for 10 minutes and promptly cooled to 30°C. Two selected high EPS producing

isolates and non-EPS producing culture (Mesophilic mixed dahi culture NCDC 352 obtained from NCDC, Karnal) were used as starter culture for preparation of low fat curd. Two per cent of starter culture was inoculated and incubated at 37°C.

Analysis of physico – chemical properties of low fat curd with EPS

Curd Samples were examined for fat, total solids, acidity, setting time, whey separation and sensory quality. The titratable acidity of curd sample was determined according to the procedure described by Bureau of Indian Standards (1981). Fat content of curd was determined by the Rose gottlieb method outlined by A.O.A.C (2005). Total solids content of curd was determined by the procedure prescribed by IS (1997). Time required for setting of the curd was noted in hours. Whey separation was determined by the method of Wachter-Rodarte et al. (1993). Thirty gram of curd was centrifuged at 1535rpm for 20min and the expelled whey was measured. Whey separation (per cent) was expressed as weight (g) of expelled whey per 100g curd. Sensory evaluation of curd samples was carried out with a 11 member panel including teachers and post graduate students of Department of Dairy Science, CV&AS, Mannuthy. The panel of judges assessed the curd samples according to the methodology described by Nelson and Trout (1965).

Statistical analysis

The data obtained were subjected to statistical analysis using the software SPSS version 24. Means of parameters like fat, total solids, whey separation, titratable acidity, curd setting time were compared by Repeated measure Analysis of variance (RMANOVA). In case of sensory evaluation data were analysed by Friedman test.

Results and Discussion

Screening for EPS production

32 EPS producing cultures were isolated from 25 curd samples. In a study conducted by Van et al. (1998) 182 *Lactobacillus* strains were screened and sixty EPS producing strains were identified. They also found that sucrose is the excellent source for abundant EPS production. Similar findings were reported by De Vuyst et al. (2003). In the present study also EPS producing cultures showed mucoid colonies on MRS agar or M17 agar with 4 per cent sucrose.

Capsular polysaccharide production

Out of the 32 EPS producing isolates, only three of the isolates showed capsules. Capsules were observed by capsule staining. The present results are in agreement with Behare et al. (2009) who had identified two isolates of *Lactococcus lactis* which were able to produce capsular polysaccharides. (Fig 1)

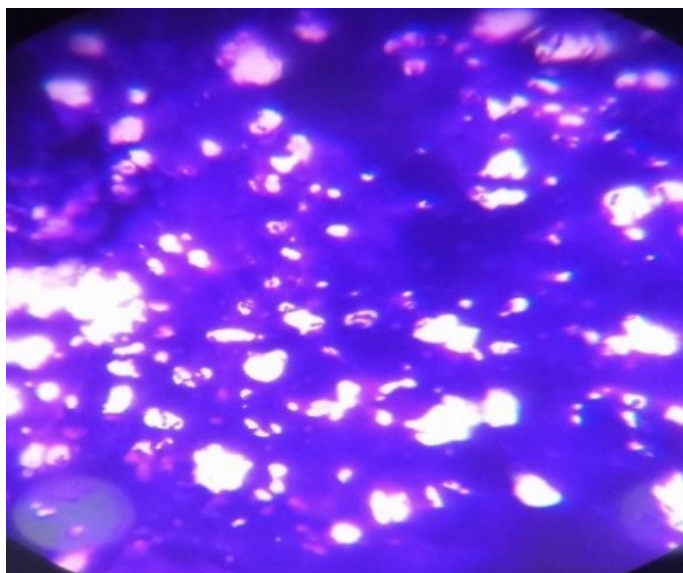


Fig. 1 Capsular polysaccharide

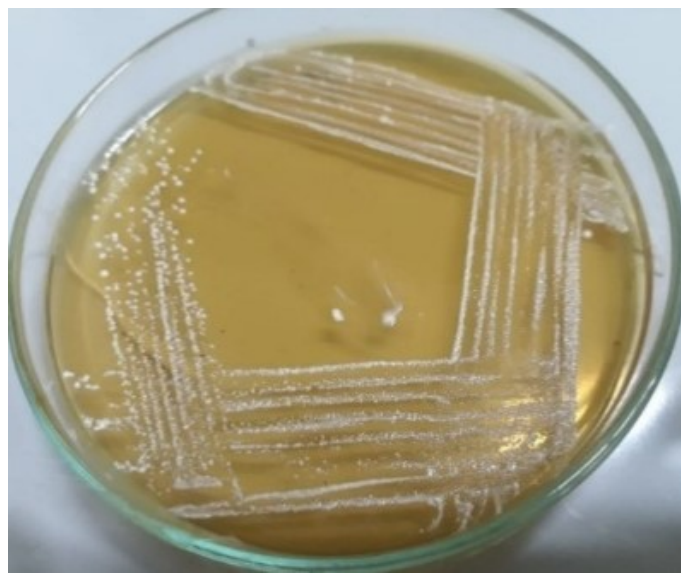


Fig. 2 Ropy polysaccharide

Ropy polysaccharide production

29 isolates were able to show white colonies on media with ruthenium red which indicates the presence of ropy polysaccharide producing strains. Dabour and Lapointe (2005) reported that ruthenium red staining method is the ideal method for detection of ropy polysaccharides. He also observed that the production of EPS prevents the ruthenium red stain and colonies appear as white. The non-ropy strains appear as pink colonies. Kersani et al. (2017) had also reported similar findings for the detection of ropy polysaccharide produced by the EPS producing strains. Hence the results of the present study agrees with previous observations of different researchers. (Fig 2)

Identification of EPS producing lactic acid bacteria

A total number of 32 EPS producing cultures were isolated from 25 curd samples collected from different sources. Based on morphological, biochemical and sugar utilisation tests the isolates were grouped into three genera namely *Lactococcus*, *Lactobacillus* and *Streptococcus*. Among the 32 isolates, two isolates were gram positive cocci in chains and showed arginine negative test hence they were grouped as *Streptococcus thermophilus*. Eight of the isolates were *Lactobacillus*. Remaining 22 isolates were identified as *Lactococcus*. Among 22 isolates, seven isolates tested were Arginine negative and were identified as *Lactococcus lactis* ssp. *cremoris* and fifteen isolates tested were arginine positive and they are identified as *Lactococcus lactis* ssp. *lactis*. All the 32 EPS producing isolates were catalase and oxidase negative.

Behare et al. (2009) had isolated 47 EPS producing lactic acid bacteria from different dahi and milk samples. *Streptococcus*, *Lactococcus*, *Lactobacillus* and *Enterococcus* species were

identified as EPS producer based on biochemical and morphological characteristics. Badel et al. (2010) had isolated EPS producing lactic acid bacteria from dairy products and obtained *Streptococcus*, *Lactococcus*, *Lactobacillus*, *Leuconostoc* and *Bifidobacteria* as EPS producers.

Quantification of EPS by phenol sulphuric acid method

The exopolysaccharides produced by 32 isolates were quantified by phenol sulphuric acid method. Concentration of EPS was determined from the glucose standard curve. The EPS produced by the isolates varied from 23 to 109 mg/L (Table 2). The maximum EPS production was observed in two isolates of *Lactococcus lactis* ssp. *lactis* with 109 mg/L and 107.4 mg/L respectively. Cerning (1995) observed that the yield of EPS from lactic acid bacteria like *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris* and *Lactobacillus casei* varied from 30 to 600mg/L. Behare et al. (2009) reported that two isolates of *Lactococcus lactis* namely B6 and KT24 were able to produce EPS. These strains produced 193 mg/L and 184 mg/L of EPS respectively in deproteinized whey medium. Seo et al. (2015) had also purified EPS from *Lactobacillus plantarum* YML009 using ethanol precipitation with a yield of 260mg/L. In the present study, the wide variations in EPS yield among different cultures can be attributed to the difference in strains.

Analysis of low fat curd

Fat

The fat content of control, T1 and T2 samples were 0.1 per cent. Guzel et al. (2005) reported that yoghurt had the same fat as that of the milk from which it was prepared. Ayana and Ibrahim (2015)

Table 1 Physico-chemical and sensory properties of low fat curd made by EPS producing cultures

Parameters	Control	T1 (EPS)	T2 (EPS)
A. Physicochemical parameters			
Fat (%)	0.1	0.1	0.1
Total solids (%)	9.11±0.00 ^a	9.13±0.01 ^b	9.12±0.00 ^c
Titrateable acidity(% LA)	0.63±0.00 ^a	0.61±0.00 ^b	0.61±0.00 ^b
Curd setting time(hours)	8.91±0.09 ^a	7.9±0.14 ^b	8.12±0.06 ^b
Whey separation (%)	66.32±0.14 ^a	41.38±0.16 ^b	43.42±0.20 ^c
B. Sensory parameters			
Flavour	39.78±0.34 ^a	45.53±0.26 ^b	42.31±0.29 ^c
Body and texture	27.11±0.30 ^a	29.06±0.16 ^b	28.61±0.17 ^b
Colour and appearance	8.79±0.13 ^a	9.25±0.13 ^a	9.17±0.12 ^b
Acidity	7.97±0.13 ^a	8.69±0.13 ^b	8.69±0.16 ^b
Container	4.86±0.06 ^a	4.86±0.05 ^a	4.86±0.05 ^a
Total Score	88.51±0.71 ^a	95.33±0.54 ^b	93.67±0.56 ^c

Means are average of six replications.

Means with different superscript within the same column differ significantly ($p < 0.05$)

Means within a column bearing same letter as superscript are homogenous

Table 2 Yield of EPS (mg/L)

Isolates	Yield (mg/L)
1	61.5
2	103.6
3	23.5
4	51.2
5	45.4
6	107.4
7	48.4
8	41
9	58
10	46.5
11	74.9
12	29.7
13	109.2
14	69.3
15	73.5
16	35.3
17	20.9
18	35.5
19	52.1
20	48.6
21	42.3
22	82.1
23	70.6
24	52.2
25	57.4
26	35.7
27	31.6
28	101.4
29	29.3
30	65.1
31	45.6
32	33.9

observed that there was no change in fat content of the low fat yoghurt prepared with EPS and non EPS producing cultures. In the present study, there was no significant difference in fat per cent between control and treatment groups of low fat curd samples. The results are in accordance with the earlier findings.

Total solids

The mean total solids content of control, T1 and T2 were 9.11±0.00, 9.13±0.01 and 9.12±0.00 per cent respectively. The mean total solid content of treatment groups of low fat curd was significantly higher than control. Significant difference was also found between treatment groups. Similar results were also reported by Ayana and Ibrahim (2015). Karasu et al. (2015) also reported that addition of EPS resulted in higher total soluble solids contents in curd. In the present study also the mean total solids content of curd samples prepared with EPS producing cultures was significantly higher than the control curd prepared with non EPS producing culture. The increase in total solids content could be due to the ability of EPS to bind with milk constituents such as proteins which could have increased the density.

Titrateable acidity

The mean titrateable acidity of control and treatment groups of low fat curd samples were 0.63±0.00, 0.61±0.00 and 0.62±0.01 per cent lactic acid. The mean titrateable acidity of control curd was significantly higher than treatment groups. There was no significant difference in titrateable acidity between treatment groups. The majority of EPS producing cultures utilize carbohydrates as their energy source as well as their carbon source for EPS formation, but the non EPS producing cultures utilize carbohydrates as their energy source and for lactic acid production (Lo et al. 2007). Kanika and Nivedita (2019) also

reported that the titratable acidity of curd incorporated with EPS producing culture was 0.67 and that of control curd was 0.78 per cent lactic acid. In the present study titratable acidity of low fat curd prepared with EPS producing cultures and non EPS producing culture were within the standards prescribed by FSSAI. The difference in acidity between control and treatment groups could be attributed to the difference in strains.

Whey separation

The mean whey separation of low fat curd samples of control, T1 and T2 were 66.32 ± 0.14 , 41.38 ± 0.16 and 43.42 ± 0.20 per cent respectively. Whey separation of treatment groups of low fat curd were significantly lower than the control. Doleyres et al. (2005) reported that the whey separation was reduced in dahi incorporated with EPS producing cultures due to the ability of EPS to bind with water. Higher EPS producing cultures showed maximum effect on control of syneresis. Kanika and Nivedita (2018) had reported that the water holding capacity was good (63.4 per cent) for curd incorporated with EPS producing culture compared to control (48.5 per cent). In the present study also the whey separation of low fat curd incorporated with EPS producing cultures were lower than the control. It can be attributed to the ability of EPS to bind with water.

Curd setting time

Time required for setting of control, T1 and T2 curd samples were 8.9 ± 0.09 , 7.9 ± 0.14 and 8.12 ± 0.06 hours respectively. Behare et al. (2009) observed that the time required for the setting of the curd prepared by EPS producing cultures was six hours whereas, setting time of control curd with non EPS producing culture was eight hours. Patil et al. (2015) reported that the time required for setting of curd incorporated with EPS producing culture was seven hours and control curd prepared with non EPS producing cultures had taken nine hours for setting. In the present study, the mean curd setting time values of control was significantly higher than treatment groups. There was no significant variation in the curd setting time between treatment groups. The faster setting of curd prepared by EPS producing cultures could be attributed to the increase in total solids content caused by the production of EPS. The results obtained in the present study are in accordance with the previous reports.

Sensory evaluation

The mean overall scores of control, T1 and T2 were 88.51 ± 0.71 , 95.33 ± 0.54 and 93.67 ± 0.56 . The overall scores of treatment curd samples were significantly higher than control samples. The mean flavour scores, Body and texture scores of treatment groups of curd samples were significantly higher than control. There was no significant difference in body and texture scores between the treatments groups of curd samples. The colour and appearance scores of treatment groups of curd samples prepared with EPS producing cultures were significantly higher than control. There

was no significant difference between treatments groups.

In a study conducted by Behare et al. (2009) the overall acceptability score was 92.5 ± 0.06 for dahi prepared with EPS producing cultures and it was 87.3 ± 0.09 for curd prepared with non EPS producing culture. Qin et al. (2011) had also found higher sensory scores for fermented milk prepared with EPS producing *Streptococcus thermophilus*. Yoghurt made with non EPS producing cultures had significantly lower ratings for overall acceptability when compared to yoghurt prepared with EPS producing cultures (Ibrahim 2015).

In the present study, the overall scores of curd samples prepared by EPS producing cultures were significantly higher than control samples. This could be due to the improved mouth feel caused by the increase in total solids content and improved appearance due to the prevention of syneresis by EPS.

Conclusions

From the above findings, it can be concluded that most of the lactic acid bacteria are capable of producing exopolysaccharides. Ropy polysaccharides are produced by majority of lactic acid bacteria. Capsular polysaccharides are produced by only a few lactic acid bacteria. The bacterial cultures capable of producing EPS can be used to improve the sensory and rheological properties of low fat curd. The use of EPS producing cultures can reduce the whey separation and increase the total solids content in low fat curd. Time required for setting of curd can also be reduced by the use of EPS producing cultures.

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The compositional and biochemical characteristics of traditional Diyarbakir Örgü cheese during the ripening period

Abdulkerim Hatipoğlu¹ and Serafettin Çelik²

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Abstract: This study was conducted to determine the changes in compositional and various biochemical parameters of traditional Diyarbakir Örgü cheese during storage period. For this purpose, cheese samples were collected from eight different small dairy plants. In this study, it was observed that the composition of the cheese samples during the storage period changed as follows: dry matter decreased from 55.87% to 52.29%, fat from 26.56% to 23.47%, fat in dry matter from 38.84% to 37.07%, protein from 26.56% to 23.47%; ash increased from 7.31% to 9.32%, salt from 6.31% to 8.16%, salt in dry matter from 11.40% to 15.48%, while the parameters investigated in relation to the biochemical properties of the cheese samples ranged as follows: 18.38-22.96 for SH, 5.47-5.64 for pH, 0.17-0.21% for soluble nitrogen in pH 4.6, 0.03-0.05% for soluble nitrogen in 12% TCA, 0.25-0.46% for lipolysis level and 4.19-5.61% for ripening index. In this study, the proteolysis level of the cheese during the ripening was determined electrophoretic analysis. According to the Turkish Food Codex Communique on Cheese (Notice No: 2015/6), DÖC is a semi-fat (25 ≤ milk fat < 45) and hard cheese with the desired moisture content (up to 50%) and salt (>7.5%).

Keywords: Biochemical properties, Composition, Diyarbakir Örgü cheese, Pasta-filata cheeses

Introduction

The industrial production and marketing of traditional and regional cheeses are of a great contribution to national economies. Such cheeses are valuable export products for countries such as Germany (3.67 billion dollars), France (3.28 billion dollars) and Italy (2.67 billion dollars) (WTO, 2019). The vast majority of regional cheeses are sold in the region or country they are produced in, for which there are two main reasons. Firstly, traditional cheeses are usually produced in accordance with regional taste and secondly, most of the cheeses produced don't have sufficient marketing opportunities in other countries.

In Turkey, most regional cheeses are produced from raw milk. This increases the risk of infection and intoxication from fresh cheeses caused by pathogenic microorganisms, especially *Brucella spp.* (Kaynar, 2011; Elmali and Uylaser, 2012; Hatipoğlu and Çelik, 2012; Kesencas et al. 2012). Therefore, the production process of regional cheeses should be reevaluated in the context of the pasteurization of the milk and starter culture applications. In addition, cheeses of high quality and economic value should be produced in accordance with the demands of the global market. For this purpose, many studies have been carried out on traditional cheeses (Drake et al. 2001; Hort and Le Grys, 2001; Lawlor et al. 2001; Ritvanen et al. 2001; Singh et al. 2003; Caspia et al. 2006; Kucukoner and Haque, 2006; Uysal, 2008).

Diyarbakir Örgü cheese (DÖC) should be produced industrially while preserving its conventional characteristics (taste, aroma, composition etc.). DÖC is widely consumed in Diyarbakir and surrounding provinces, and is sold just above the cost in markets in cities such as Istanbul, Ankara and Izmir. The production process of DÖC is similar to that of pasta-filata cheeses such as Mozzarella, Caciocavallo and Povola dei Nebrodi. In the production of traditional DÖP, usually raw ewe milk is used. In order to produce the cheese, the milk is coagulated with liquid rennet at 29-30 °C for 54-70 minutes in a way that it should coagulate. After the coagulation, the curd which is put in the cheesecloth is pressed and fermented. Cord pulling method is used in order to test whether or not the curd is suitable for scalding. Fermented curd is submerged into the boiler (water, 78-87 °C) by placing it in perforated stainless steel pails and scalded

¹ Department of Nutrition and Dietetics, Faculty of Health Sciences, Mardin Artuklu University, Mardin, Turkey

² Department of Food Engineering, Faculty of Engineering, Harran University, Sanliurfa, Turkey

Abdulkerim Hatipoğlu (✉)
Department of Nutrition and Dietetics, Faculty of Health Sciences,
Mardin Artuklu University, Mardin, Turkey
E-mail: abdulkerimhatipoglu@artuklu.edu.tr; Tel: (+90) 482 212 1395

for 2-7 minutes. The scalded curd dough is placed on the bench, stretched threadlike by cutting in portions of 200 g on average and shaped as typical braid by weaving. Fresh cheese is left for approximately 20-30 minutes in brine (usually 12 Bé). Then, the cheese is placed into lacquered tin plates or plastic jerry cans and stored at 6 ± 1 °C for approximately 4-6 months by adding 17-21 Bé brine. As it matures, the humidity increases and become softer. DÖC is a kind of semi fat and hard cheese (Hatipoglu, 2014, Anonymous, 2015).

DÖC, which is a traditional Anatolian cheese, is produced mostly in the Karacadag Basin situated in the South of the province of Diyarbakir. Due to the small arable areas of the basin and the presence of large meadow-pasture areas, animal husbandry, especially sheep farming, is carried out in general (Hatipoglu and Celik 2012). There were 1164.521 sheep, 157.144 goats and 128.376 cattle (domestic cows, crossbred cows, culture (non-domestic) cows and buffalo) that were being milked in the basin. Approximately 296.000 tons of cattle milk, 97.976 tons of sheep milk and 16.566 tons of goat milk are produced in the basin (Anonymous, 2019).

DÖC production is seasonally performed in portable facilities in the production area (Hatipoglu, 2014). This study is especially important as it is the first study conducted on the ripening characteristics of DÖC. In the present study, the composition and various biochemical properties of DÖC produced in small dairy farms in the Karacadag Basin were investigated.

The composition and maturity of the cheese affects the consumer's nutritional preferences. This study is the first study conducted in the production region of traditional DÖC. The findings of the research may help to standardize and preserve the composition and biochemical properties of the cheese in the industry. Thus, it may provide basic data for the cheese to reach world markets in the near future. In addition, for researchers, this study will provide an insight into the definition and characterization of the composition and biochemical parameters of DÖC as a fieldwork.

Materials and Methods

The study was conducted in eight small-scale dairy plants, selected upon the suggestion of the Diyarbakir Provincial Directorate of Agriculture and Forestry and local cheese producers. Fresh cheese samples (4 kg) were collected four times at a week intervals from each dairy plant. The collected cheese samples were stored and ripened at 6 ± 1 °C for 6 months.

Composition

The ratios of dry matter (DM) (gravimetric method) (Anonymous, 1989), fat (butyrometric method) (Anonymous, 1989), ash (gravimetric method) (Anonymous, 1989), salt (Mohr method) (Anonymous, 1989) and protein (Dumas method, by Leco FP

528) (Anonymous, 1989) of the cheese samples were determined on the 1st, 30th, 60th, 90th and 120th days of the ripening period.

Biochemical parameters

Titration acidity (TA) (Dagdemir et al. 2003), pH (WTW 330i, Germany) (Yazici and Dervisoglu, 2003), soluble nitrogen (SN) at pH 4.6 (Dumas method, by Leco FP 528) (Gripon et al. 1975), SN in Trichloroacetic acid (TCA) (Dumas method, by Leco FP 528) (Tavaria et al. 2003) and lipolysis level (Ocak et al. 2015) analyzes were performed on the 1st, 30th, 45th, 60th, 90th and 120th days of the storage period.

Electrophoresis

The degradation level of the proteins was determined by the urea-PAGE method in accordance with the density of the protein bands obtained on the 1st, 30th, 60th, 90th, 120th, 150th and 180th days of the storage period by using vertical slab-gel electrophoresis (BioRad Laboratories Ltd. Watford, UK). The gels obtained were stained with Comassie Brilliant Blue G250 (Blakesley and Boezi 1977; Andrews, 1983; Hynes et al. 1989).

Statistical analysis

The data obtained were analyzed by the one-way ANOVA and the differences between the significant averages were determined by the Tukey multiple comparison test (Yıldız & Bircan, 1994).

Results and Discussion

Composition

The ANOVA revealed that there were statistically highly significant ($P < 0.01$) differences between all compositional parameters (DM, protein, fat, fat in DM, ash, salt and salt in DM) of the cheese samples during storage period (Table 1). The DM, fat and protein ratios of DÖC decreased during the period. In contrast, salt, salt in DM and ash rates increased. The fat in DM rate of the cheese followed a fluctuating course throughout the period and it was determined that this ratio in ripened cheese decreased slightly compared to fresh cheese.

The average DM and protein ratios of commercial Örgü cheese were reported by Ozdemir et al. (1998) as 44.84% and 21.69%, and as 50.88% and 21.75% according to Celik and Turkoglu (2007). Additionally, the average DM and protein ratios of traditional Lavash cheese were reported by Celik et al. (2001) as 45.80% and 17.52%. The average values of the same components of Caciocotta cheese were reported by Albenzio et al. (2006) as 46.43% and 16.8%. Finally, the average DM and protein values of Dil cheese were reported by Uysal (2008) as 53.78% and 23.95%.

On the other hand, the fat ratios of the samples determined in the study (Table 1) were found to be higher than those reported for

Örgü cheese by Ozdemir et al. (1998) (% 14.7) while lower than Örgü (% 20.6) reported by Celik and Turkoglu (2007) and Dil cheeses (% 23.64) reported by Uysal (2008) in the literature. As a result of the laboratory analysis, the mean ratio of fat in DM of DÖC was higher than the value (% 32.23) reported by Ozdemir et al. (1998) for traditional DÖC and lower than those (% 40.46) reported by Celik and Turkoglu (2007) commercial Örgü cheese and Uysal (2008) (% 43.96) for Dil cheese.

According to the data obtained from the present study, the average ash and salt ratios of DÖC were determined to be higher than those reported by Ozdemir et al. (1998), Celik and Turkoglu (2007), and Uysal (2008). Similarly, the mean ratio of salt in DM of DÖC obtained in the study were also determined to be higher than that reported for Örgü (% 9.02 and % 13.68), Lavash (% 13.95) and Dil (% 13.23) cheeses (Ozdemir et al. 1998; Celik et al. 2001; Celik and Turkoglu, 2007; Uysal, 2008).

As seen from the above comparisons, the fact that all of the composition parameters of DÖC (especially DM and fat in DM) were determined to be different from those of other studies may be due to the difference in the race/breed/diet of the dairy animals and the fat content of the raw milk used in the production of the cheeses. Small-scale dairy plants do not only produce DÖC from sheep's milk, they also use cow's and goat's milk (approximately 20%). Therefore, they mix the sheep, goat and cow's milk without a fixed rate and produce DÖC from the milk of this mixture. Additionally, it was observed in the local area study that the brines used in the cheese production had different amounts of salt (17-21 Bé) (Hatipoglu, 2014).

Based on the storage period, the average ratio of fat in the DM of DÖC (37.73%) was determined to be between full-fat cheese (45%) and fatty cheese (30%) (Anonymous, 2015; Anonymous, 2006a; Anonymous, 2006b). DÖC is similar to Dil and Kashar cheeses in terms of its production technique and composition properties. Therefore, DÖC can be defined as a fatty cheese based on the cheese standards mentioned above. However, according to the Turkish Food Codex Communiqué on Cheese, DÖC is considered as a semi-fatty cheese (Anonymous, 2015). DÖC is expected to be a full fat cheese type. However, DÖC cheese and butter fat are also produced at the same time in the Karacadag region. According

to the information received in the region, milkfat obtained from milk in the form of cream is used in the production of butterfat, while the remaining partially skimmed milk is used in the cheese production.

As can be seen from Table 1, the ratios of fat, protein and DM of DÖC gradually decreased during the storage period. This may be due to the degradation of proteins and lipids in the cheese matrix by enzymes and microorganisms during the storage period, and may also be due to the low molecular weight compounds formed as a result of the degradation products passing from the cheese into the brine (Grappin and Beuvier, 1997). In addition, it is thought that the amount of salt diffusing from the brine into the cheese matrix is less than the protein and lipid degradation products passing from cheese to brine.

Celik and Turkoglu (2007) reported that the DM ratio of Örgü cheese produced from raw and pasteurized milk decreased significantly during the ripening period. Moreover, the DM ratio of Dil cheese which was produced from raw, thermised, and pasteurized milk and ripened for 90 days in brine, decreased during the 90 day storage period (Uysal, 2008). In addition, Arboatti et al. (2014) reported that the DM ratio of Mozzarella cheese decreased during a 43 days of storage period.

According to the results of the present study (Table 1), the average DM ratio of DÖC (54.43%) corresponds to the DM ratio (at least 50%) specified in the legislation for Dil cheese.

During the scalding process, there was a positive relationship between the pH of the curd and the DM level of the DÖC. In contrast, it has been reported that while the pH of the curd decreased, the DM ratio of the final product increased in a study conducted on Cheddar cheese (Walstra and Jennees, 1984). This can be explained by the decrease in the water binding ability of the curd as the scalding pH approaches the isoelectric point of casein (pH 4.6).

The protein ratio of DÖC decreased significantly ($P<0.01$) during the storage period (Table 1). This may be due to the fact that low molecular weight nitrogenous compounds migrated from the cheese matrix into the brine. These values obtained from the

Table 1 The mean values of the composition parameters (%) of the samples and the groups formed during the storage period (n=30)

SP (day)	DM	Protein	Fat	Fat in DM	Ash	Salt	Salt in DM
1	55.44±1.13 ^a	26.56±0.64 ^a	21.56±0.80 ^a	38.84±0.99 ^a	7.31±0.33 ^c	6.31±0.27 ^c	11.40±0.52 ^c
30	55.32±1.13 ^a	25.84±0.64 ^b	20.71±0.80 ^b	37.38±0.99 ^{bc}	8.77±0.33 ^b	7.70±0.27 ^b	13.95±0.52 ^d
60	54.75±1.13 ^a	25.23±0.64 ^c	20.33±0.80 ^{bc}	37.07±0.99 ^c	9.19±0.33 ^a	8.04±0.27 ^a	14.72±0.52 ^c
90	53.78±1.13 ^b	24.36±0.64 ^d	20.19±0.80 ^{bc}	37.47±0.99 ^{bc}	9.22±0.33 ^a	8.11±0.27 ^a	15.09±0.52 ^b
120	52.84±1.13 ^c	23.47±0.64 ^e	20.06±0.80 ^c	37.88±0.99 ^b	9.32±0.33 ^a	8.16±0.27 ^a	15.48±0.52 ^a
Mean	54.43±1.13	25.20±0.64	20.57±0.80	37.73±0.99	8.77±0.33	7.67±0.27	14.13±0.52

Means with different letters in the same column are significantly different ($P<0.01$). SP: Storage period; DM: Dry matter

study were higher than the protein ratios reported for Örgü (Akyuz et al. 1998; Ozdemir et al. 1998; Turkoglu et al. 2003; Celik et al. 2006 Celik and Turkoglu, 2007), Lavash (Celik et al. 2001) and Dil cheeses (Uysal, 2008). Furthermore, in another study in the literature, it was reported that the protein content in Kaskaval cheese was reduced during a storage period of 56 days (Andronoiu et al. 2015).

The average fat content of DÖC decreased significantly ($P < 0.01$) during the ripening period (Table 1). This may be due to the breakdown of the products resulting from the hydrolysis of the lipids passing into the brine (Grappin and Beuvoir, 1997).

The average fat ratio of DÖC was determined as 21.56% and 20.06% on the 1st and 120th days of the ripening period, respectively. These values are similar to the fat ratio determined in a study on Örgü cheese which matured for 90 days in brine (Celik and Turkoglu, 2007). This study reported that the amount of fat in Örgü cheese produced from raw and pasteurized milk decreased significantly ($P < 0.05$) during the ripening period. In the survey studies, different researchers reported that the average fat ratios of Örgü cheese were between 14.72-17.86% (Ozdemir et al. 1998; Akyuz et al. 1998; Turkoglu et al. 2003). Similarly, it was reported that fat content in Kaskaval cheese decreased during the storage period (Andronoiu et al. 2015).

Although the ratio of fat in DM changed irregularly, decreases in the matured (120 days) cheese compared to fresh (1 day) cheese were observed during the ripening period of DÖC (Table 1). On the 120th day of the ripening period (in mature cheese), the fat ratio in DM was determined as 37.88%. This value is lower than the value reported by Celik and Turkoglu (2007) for Örgü cheese while it is higher than the values reported by Ozdemir et al. (1998) and Aksu et al. (1999). This difference may be due to the fat content of the milk used in the cheese production, the temperature and duration of the scalding process of the fermented curd.

The ash ratio of DÖC increased until the 60th day of the ripening period and then reached equilibrium (Table 1). This was parallel to the salt passing to DÖC and caused by the increase in the salt ratio. The ash ratio was determined as 7.31% on the first day of the period and as 9.32% at the end of the period (120th day). Various studies reported that the ash ratio of Örgü cheese ranged from 6.43% to 8.01% (Akyuz et al. 1998; Ozdemir et al. 1998; Aksu et al. 1999; Turkoglu et al. 2003;). The ash ratio of Dil cheese matured for 90 days was reported as 5.18% on the first day and as 5.98% on the last day (Uysal, 2008). It was reported in another study that the ash ratio of the Caciocavallo cheese ripened in brine for 150 days was 3.84% in fresh cheese and it continuously increased to 5.50% in the mature (150th day) cheese (Perna et al. 2014).

At the beginning of the storage period, due to the very high difference between the salt content of fresh cheese and the brine,

the salt proportion of the DÖC samples increased rapidly until the 30th day of the period which continued until the 60th day. On the 60th day of the storage period, the salt's transition to cheese was balanced (Table 1). Similarly in a study by Arboatti et al. (2014), it was reported that the increase in the salt content of the Mozzarella cheese matured in brine during the storage period. On the other hand, the salt ratio of Örgü cheese were reported as 5.32-6.03% in another studies (Ozdemir et al. 1998; Akyuz et al. 1998; Turkoglu et al. 2003). In addition, the salt ratio of the Maras finger cheese matured in brine was reported as 5.3% (Tekinsen, 1996). In a study, the average salt ratios of 90-day old Dil cheeses produced from raw, thermised and pasteurized milk were reported as 5.02, 4.80 and 5.12, respectively (Uysal, 2008). In the present study, the average salt ratio of the cheese was 8.16% on the 120th day of the storage period. This could be caused by the high salt content in the brines (17-21 Bé) used by the plants that were the subject of the study. The ratio of salt in DM increased gradually during the ripening period (Table 1). At the end of this period, the average ratio of the salt in the DM of DÖC was determined as 15.48%. It was reported in another study that this ratio was between 13.77-15.66% at the end of a 90-day storage period of Örgü cheese produced from raw and pasteurized milk (Celik et al. 2006). Various studies reported that the ratio of the salt in the DM of Örgü cheese was between 10.02-14.12% (Ozdemir et al. 1998; Akyuz et al. 1998; Aksu et al. 1999). The ratios of salt in DM were measured as 9.49%, 9.12% and 9.72% for mature (90th days) Dil cheese made from raw, thermized and pasteurized milk, respectively (Uysal, 2008).

According to the Turkish Food Codex Communique on Cheese, this value should be 7.5% at the most. Therefore, based on reference, it can be said that the average ratio of salt in DM of the DÖC was high in the present study (Table 1). However, this is due to the fact that the Dil cheese specified in Turkish standards, namely TS 3002, applies to cheese made from pasteurized milk, and because it is considered as a fresh cheese that is freshly consumed without being ripened in brine. Nowadays, the tendency to present cheeses such as Kashar, Örgü, Dil and Lavash, the fermented curd of which is scalded at high temperatures, for fresh consumption after packaging under vacuum has increased. At the same time, it is known that all of the mentioned cheese varieties (except Kashar) are mostly offered for consumption after being ripened in brine. These cheeses, which are semi-hard, have a high salt content due to their maturation in the brine that contains high amounts of salt.

Biochemical parameters

The difference between biochemical parameters of DÖC were found to be highly significant ($P < 0.01$) in terms of storage period. The TA value of the DÖC fluctuated during the storage period (Table 2).

This was also reported in similar studies in the literature (Uysal, 2008; Celik and Turkoglu, 2007; Tarakci and Kucukoner, 2006). The mean TA value decreased up to the 45th day of the storage period and then increased (Table 2). On the 45th day of the period, the average TA value of the DÖC was the lowest (18.38 SH) and the highest mean TA value (22.96 SH) was seen on the 120th day of the period. It was reported that alkaline and neutral compounds caused by proteolysis and free acids formed as a result of lipolysis can lead to a decrease in TA (Uysal, 2008; Guven and Konar, 1997). In addition, it was reported that the acidity of the cheese is caused by lactic acid, acetic acid, butyric acid, formic acid, free fatty acids resulting from lipolysis and free amino acids formed as a result of proteolysis (Yilmaztekin, 2001).

In the present study, the mean TA value was determined as 20.35 SH (Table 2). Celik and Turkoglu (2007) reported the average TA value of Örgü cheese made from raw milk and pasteurized milk which was followed by a fluctuating course during a 90 day ripening period was determined as 22.28 SH and 21.14 SH, respectively. This study revealed that TA increased until the 30th day and decreased after the 60th day. Furthermore, Uysal (2008) reported the TA of Dil cheese made from raw milk decreased until the 30th day of the ripening period and then started to increase. According to the results of studies conducted by various researchers on Dil, Örgü and Lavash cheeses, the mean TA values were found to be 22.89; 22.86, 15.11, 49.33; 22.74, respectively (Ozdemir et al. 1998; Celik et al. 2001; Turkoglu et al. 2003; Aksu et al. 1999; Kocak et al. 1997).

According to the relevant standard, the highest value of TA of the Dil cheese is 1.0% LA (44.44 SH). During the ripening period in the present study, the DÖC samples were determined to be in compliance with this standard (Anonymous, 2006a).

The average pH values of the DÖC samples increased until the 30th day of the storage period and started to decrease after the 60th day. This increase in the pH of the samples may be due to the formation of alkaline compounds as a result of proteolysis, and the decrease starting from the 60th day may be caused by the

rapid increase in the lipolysis level. It was reported that the pH values of the Örgü cheese increased up to the 15th day of the ripening period and then decreased until the end of the period (Celik and Turkoglu, 2007). In another study, it was reported that the average pH values of Dil cheese increased up to the 60th day of the ripening period and decreased relatively on the 90th day of the period (Uysal, 2008). "In addition, it was stated that the pH of Mozzarella fluctuated during 43 days of storage (Arboatti et al. 2014). Similarly, it was reported that the pH values of Kashkaval cheese which was stored for 180 days fluctuated between 5.0-5.3 (Pappa et al. 2019). A similar result was stated for the Caciocavallo cheese which was matured for 150 days (Perna et al. 2014). Celik and Turkoglu (2007) reported the average pH of Örgü cheese made from raw milk and pasteurized milk was 5.07 and 5.08, respectively. In their study, Anar et al. (2000) reported that the average pH of Örgü cheese was 5.30. Uysal (2008) reported that the average pH values of Dil cheese made from raw, thermised and pasteurized milk were 5.07, 5.14 and 5.19, respectively. In a survey study, the average acidity of Dil cheese was expressed as 5.20 pH (Kocak et al. 1997). According to the results of the present study, the average pH of DÖC was higher than the values reported in the studies mentioned above.

The average lipolysis values of the DÖC samples (Table 2) were lower than the values of Örgü cheese made from raw and pasteurized milk (1.49 and 1.27 mg KOH/g-fat) reported by Celik and Turkoglu (2007), and higher than the values of the Dil cheese made from raw, thermised and pasteurized milk (0.25, 0.22 and 0.34 mg KOH/g-fat) determined by Uysal (2008).

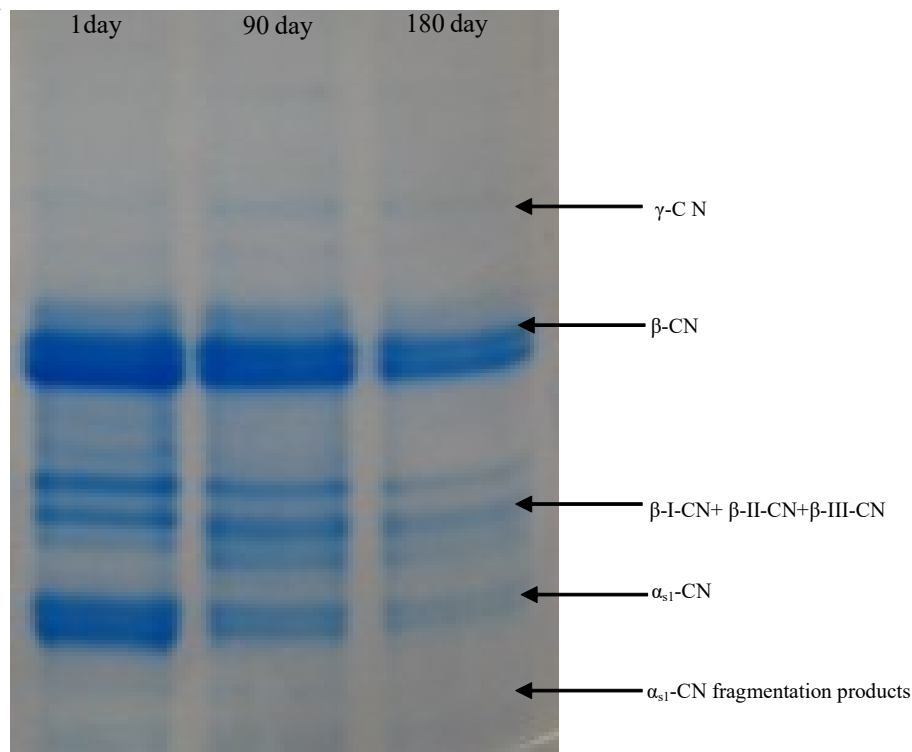
In the present study, the lipolysis value of DÖC increased significantly ($P<0.01$) during the storage period (Table 2). The average lipolysis value of DÖC was 0.25 mg KOH/g-fat on the first day of the ripening period, while it was 0.46 mg KOH/g-fat on the 120th day of the period. Similarly, various studies reported that the lipolysis values of Örgü and Dil cheeses increased significantly ($P<0.05$) during the 90-day storage period (Uysal, 2008; Celik and Turkoglu, 2007). It has been stated that the level of lipolysis in the cheese matured in brine was affected by the

Table 2 The mean values of various biochemical parameters of DOC and the groups formed during the storage period (n=30)

Storage Period (day)	Acidity		Lipolysis Value (mg KOH/g fat)	SN in pH 4.6 (%)	SN in TCA (%)	RI (%)
	TA (SH)	pH				
1	20.26±2.03 ^c	5.59±0.05 ^b	0.25±0.02 ^f	0.17±0.01 ^f	0.03 ^f	4.19±0.20 ^f
30	18.50±2.03 ^d	5.64±0.05 ^a	0.29±0.02 ^e	0.18±0.01 ^e	0.04 ^e	4.47±0.20 ^e
45	18.38±2.03 ^d	5.62±0.05 ^a	0.33±0.02 ^d	0.19±0.01 ^d	0.04 ^d	4.66±0.20 ^d
60	20.41±2.03 ^c	5.58±0.05 ^b	0.37±0.02 ^c	0.19±0.01 ^c	0.04 ^c	4.86±0.20 ^c
90	21.61±2.03 ^b	5.54±0.05 ^c	0.41±0.02 ^b	0.20±0.01 ^b	0.05 ^b	5.18±0.20 ^b
120	22.96±2.03 ^a	5.47±0.05 ^d	0.46±0.02 ^a	0.21±0.01 ^a	0.05 ^a	5.61±0.20 ^a
Mean	20.35±2.03	5.57±0.05	0.35±0.02	0.19±0.01	0.04	4.83±0.20

Means with different letters in the same column are significantly different ($P<0.01$). TA: Titration acidity (SH), SN: Soluble nitrogen, TCA: 12% Trichloroacetic acid, RI: Ripening index

Fig. 1 Urea-PAGE electrophoretogram of DÖC during the storage period



origin of the milk used in production, milk pasteurization, milk lipase, bacterial lipase, brine concentration and maturation temperature (Abd El-Salam, 1987). Moreover, Fox et al. (2000) reported that most of the proteinase enzymes synthesized by psychrotrophic bacteria dissolved in water was removed by whey while most of the psychrotrophic lipase enzymes were temperature-resistant and caused rancidity during the storage period. As a result of the activities of these enzymes, an increase in the lipolysis value of the cheeses was observed in the present study (Table 2).

According to the results of this study, the average ratio of SN in 4.6 pH of DÖC was higher than the values reported for Dil cheese (Uysal, 2008). The difference between SN in pH 4.6 for different cheeses may be caused by the different microflora of the milk used by each plant.

The average values of SN in pH 4.6 of the DÖC samples showed an increase during the 120 days of ripening period (Table 2). Similarly, it was reported that the average ratio for SN in pH 4.6 of Dil cheese made from raw milk was 0.13% in fresh cheese (day 1) and 0.14% in mature cheese (day 90) (Uysal, 2008). There is a positive correlation between SN in pH 4.6 and SN in TCA values. It has been determined that monitoring the changes in the ratio of these two parameters in cheese gives information about the degree of maturation (Uysal, 2008; Fox et al. 2000).

As can be seen from Table 2, there was a continuous increase in the rate of the average SN in TCA of the DÖC during the storage

period. Celik and Turkoglu (2007) reported that the average ratio of SN in TCA in Örgü cheese made from raw milk and pasteurized milk increased during the 90-day storage period. On the other hand, Uysal (2008) stated that SN in TCA of Dil cheese made from raw milk did not change until the 30th day of the ripening period and that SN in TCA values of Dil cheeses which were made from thermised milk and pasteurized milk remained constant until the 60th day of the storage period while these values increased slightly on the following days. Studies have reported that the ratio of SN in TCA of Kasserli, Caciocavallo and Kashkaval cheeses produced from raw milk were higher than the values of DÖC. However, Kashar cheese produced from raw milk and pasteurized milk showed no difference in terms of this parameter (Sert et al. 2007; Moatsou et al. 2011; Perna et al. 2014; Pappa et al. 2019).

The RI value increased parallel to the protein degradation in DÖC matrix during the storage period (Fig 1). According to the data obtained from this study, the average RI of DÖC was calculated as 4.83%. Tarakci and Kucukoner (2006) reported that the RI of Kashar cheese was between 10.50-10.59% in fresh cheese (5 days) and between 20.95-18.70% in mature cheese (90 days). In addition, the RI of fresh (2 days) herby cheese was reported as 5.75% and as 13.57% for the mature (90 days) cheese (Tarakci et al. 2005). Arboatti et al. (2014) calculated the RI of 43-day (mature) Mozzarella cheese as 3.87%. In the survey studies, the RI of Dil, Örgü and Lavash cheeses were reported as 12.45, 3.09 and 2.30%, respectively (Kocak et al. 1997; Ozdemir et al. 1998; Celik et al. 2001).

Electrophoresis

The density of the bands representing the α_{s1} -Cn was relatively low compared to the bands representing the β -Cn on the electrophoretogram of the DÖC samples (Fig 1). This can be explained by the chymosin activity which was higher than the plasmin activity in the cheese curd (Mcsweeney and Sousa, 2000). Similarly, chymosin is also known to hydrolyse α_{s1} -Cn (Creamer and Mills, 1971; Creamer, 1976). And this situation confirms resistance of β -CN to chymosin hydrolysis as reported by Fox et al. (1993). Due to the high salt concentration (17-21 Bé) of the brine, it was estimated that the activity of plasmin decreased and β -Cn degradation slowed down (Fallico et al. 2005). Similar results were also reported for different types of cheeses (Milanovic et al. 1998; Saldamli and Kaytanli, 1998; Katsiari et al. 2000; Pavia et al. 2000; Gobetti et al. 2002; Kongo et al. 2009).

On the other hand, it has also been reported that high scalding temperature, high pH and low moisture level decrease the chymosin activity in the cheese matrix, while they increase the enhancing effect of plasmin activity on β -Cn (Singh et al. 2003; Kelly et al. 1996; Sousa et al. 2001). The electrophoretogram of the DÖC samples showed that γ -Cn was formed from β -Cn (Figure 1). This revealed that plasmin activity existed in the cheese (Di Cagno et al. 2003). Plasmin activity is also known to be an important parameter in the differentiation of cheese varieties (Sousa et al. 2001).

Furthermore, Carretero et al. (1994) reported that these breakdown products emerged in cheese made from goat's milk. In addition, the effect of bacteria on the level of proteolysis in cheese was expressed (Fox, 1989). Although starter culture was not used in the production of DÖC, non-starter lactic acid bacteria in natural flora of the milk can also increase the level of proteolysis of the cheese.

Conclusions

Since DÖC is not produced within a certain standard, the fat in DM, salt in DM and DM contents of the cheese should be standardized. In addition, in order to reduce the salt content of the cheese mass, keeping DÖC in vacuum packages instead of preserving it in brine with high salt content is a more suitable option. According to the Turkish Food Codex Communiqué on Cheese (Notice No: 2015/6), DÖC is a semi-fat ($25 \leq$ milk fat < 45) and hard cheese with the desired moisture content (up to 50%) and salt ($> 7.5\%$). Until now, industrially produced cheeses under the name of "Örgü cheese" did not represent the traditional characteristics of DÖC. For this reason, the demand for this cheese could not be met sufficiently. In conclusion, it is thought that adopting the cheese to the dairy industry without losing its natural characteristic properties will gain more importance in the near future.

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Moisture sorption characteristics of heat desiccated milk sweet ‘*Khoa-peda*’ prepared from buffalo milk

Somnath G Pagire¹, Aswin S Warriar² and IK Sawhney³

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Abstract: Water activity (a_w) is a valuable criterion in food processing affecting food product design and in ensuring food safety during storage. *Khoa-peda* is an indigenous khoa based dairy sweet prepared by mixing khoa and sugar and is popular all over India. It has a maximum shelf life of 15 days at refrigerated temperatures. The moisture sorption parameters of *Khoa-peda* prepared from buffalo milk were determined at three different temperatures 15, 25 and 35°C using a gravimetric-static method. The moisture sorption isotherms were found to be of sigmoid shape reflecting a Type II isotherm. At all water activities, the equilibrium moisture content was lower at higher temperatures. From amongst the GAB, Modified Mizrahi, Oswin, Caurie and Halsey models applied to the experimental sorption data of *khoa-peda*, the GAB model best described the sorption isotherm throughout the entire range of a_w . Temperature dependence of GAB parameters was determined by using Arrhenius type equation to correlate the GAB constants with temperature. The hysteresis effect was observed at all the temperatures over the entire range of water activities. The hysteresis loop was of Type ‘C’ according to Everett classification. The effect of temperature was found to reduce the hysteresis. The hysteresis amplitude reduced from 0.303 at 15°C to 0.253 at 35°C, total hysteresis units decreased from 1.55 units at 15°C to 1.25 units at 35°C and the hysteresis energy reduced from 67.72 kJ/kg of water at 15°C to 32.25 kJ/kg of water at 35°C.

Keywords: Hysteresis, *Khoa-peda*, Moisture sorption isotherm, Sorption models

Introduction

Khoa-peda, a heat desiccated milk sweet popular in the Indian subcontinent, has been a part of Indian culture since ancient times. As compared with *burfi*, *khoa-peda* is granular in texture having dry body because of comparatively lower moisture content. It has been reported that the quantity of *khoa-peda* produced in India far exceeds any other indigenous milk based sweets (Aneja et al. 2002). Milk is concentrated in an open pan to a dough-like consistency, called ‘*Khoa*’, which forms the base material for the preparation of *khoa-peda*. *Khoa* can be made from cow milk or buffalo milk, however, *khoa* made from buffalo milk is found to have increased yield, better sensory quality and better textural attributes (Gupta and Gupta 2013; Jha et al. 2014; Choudhary et al. 2017). To make *khoa-peda*, *khoa* is mixed with sugar while heating and some optional ingredients, such as, flavoring and / or coloring substances and nuts may be added. The stirring and heating process during the production of *khoa-peda* dries out a part of the moisture, while sugar acts as a preservative and both of these attributes contribute towards the increased shelf life of *khoa-peda* in comparison to *khoa*. A number of different variants of *khoa-peda* are made with regional based preference of consumers. Various researchers have studied and reported shelf life for *khoa-peda*. Yadav and Beniwal (2009) and Londhe et. al. (2012) have reported that the shelf life of *khoa-peda* at ambient temperature is hardly more than two days. The shelf life of *khoa-peda* has been found to be 8 days of storage at 25°C (Banjare et. al. 2015) and a maximum of 15 days at refrigerated temperatures (Indiramma et al. 2002)

Water activity and sorption behavior are two most powerful concepts available for understanding and controlling shelf life of foods (Labuza 1968). Equilibrium relationship between moisture content of food and the water activity at any temperature is quantified in the form of moisture sorption isotherms. Knowledge of sorption isotherm of a food product is essential for product and process development, besides food engineering applications (Delgado and Sun 2002). Moisture sorption properties affect physicochemical and biological phenomena in

¹Tetra Pak India, Pune, Maharashtra, India

²University Dairy Plant, Kerala Veterinary and Animal Sciences University, Thrissur, Kerala, India

³Dairy Engineering Division, National Dairy Research Institute, Karnal, Haryana, India

Aswin S Warriar (✉)

²University Dairy Plant, Kerala Veterinary and Animal Sciences University, Thrissur, Kerala, India

Email: aswinwarriar@kvasu.ac.in

foods, such as, enzymatic degradation, microbial activity, food microstructure, sensory quality deterioration, nutrient losses, and other changes limiting the shelf life of food products (Caballero-Ceron et. al. 2015). Information on moisture sorption isotherms of various similar traditional dairy products, viz., *khoa*, *burfi*, dietetic *rabri* and *kalakand* . have been reported in literature by Sawhney et. al. (1991); Chetana et. al. (2005); Ghayal et. al. (2013) and Deshmukh et. al. (2017), respectively.

The objective of the present study was therefore to obtain the experimental data on moisture sorption characteristics *khoa-peda* from buffalo milk at different temperatures, to analyze it with different recommended models and generate information to quantify moisture sorption parameters, which could help in developing *khoa-peda* as intermediate moisture milk sweet.

Materials and Methods

Preparation of khoa-peda

Khoa-peda samples used in this study were prepared from fresh buffalo milk collected from Institute's Experimental Dairy as per the standard procedure described by Aneja et. al. (2002). Khoa was prepared by boiling milk in a steam jacketed kettle with continuous stirring till desired texture was attained. Sugar was then added at the rate of 30% of khoa (w/w). Peda was made in to balls of about 20 to 25 g size by rolling between the palms and then flattened into cylindrical shapes using moulds. The average % composition of khoa-peda test sample was: moisture: 15.12; fat: 29.00; protein: 16.10; lactose: 15.06; sucrose: 21.34; ash: 2.67.

Measurement of sorption equilibrium

Adsorption and desorption isotherms of khoa-peda prepared from buffalo milk were determined at 15, 25 and 35 °C over a water activity range of 0.11 to 0.97 using static moisture gain / loss from test samples. The standard sorption apparatus recommended by COST 90 Project (Wolf et. al. 1985) was used in determining the sorption equilibrium of khoa-peda. The sorption device consists of sorption containers and support for weighing dishes in which the samples were exposed to humid atmospheres in the containers. Seven reagent grade salts, namely, lithium chloride, magnesium chloride, magnesium nitrate, ammonium sulphate, potassium bromide, potassium chloride, barium chloride and potassium sulphate were chosen to cover the water activity range of 0.11 to 0.97 (Greenspan 1977). The salts were dissolved in distilled water at 100 °C and cooled to test temperatures of 15, 25 and 35 °C for crystallization to form saturated solutions. The sorption apparatus were placed in constant temperature cabinets maintained at the temperatures of 15, 25 and 35 °C and allowed to equilibrate at respective temperatures for 3 days before the samples were placed in them.

Weighed quantity (1.00 ± 0.05 g) of each of the sample was taken in the tared dishes which were then placed in the respective

sorption containers. Samples were weighed at a regular interval of 24 h and the equilibrium was adjudged to be reached when three consecutive weight measurements showed no significant difference i.e. less than 0.001 g. Moisture content of each sample was measured by oven method as described in IS 2785 (1979). Measurements for each experiment were done in triplicate, for each water activity and for each temperature. The average value of the equilibrium moisture content (EMC) was taken for further analysis.

Sorption models

Due to complex nature of foods, no single model is general enough to predict the sorption isotherms of foods. In this study five models were used to fit the experimental data on moisture sorption and the relevant isotherm equations are given below:

(i) Halsey Equation (Halsey 1948)

$$a_w = e^{-a/W^b} \quad (\text{Eq.1})$$

(ii) Oswin Equation (Oswin 1946)

$$W = a \left\{ \frac{a_w}{1 - a_w} \right\}^b \quad (\text{Eq.2})$$

(iii) Caurie Equation (Caurie 1981)

$$\ln \frac{1}{W} = \ln \frac{1}{CW_o} + \frac{2C}{W_o} \ln \frac{1 - a_w}{a_w} \quad (\text{Eq.3})$$

(iv) Modified Mizrahi Equation (Mizrahi and Karel 1977)

$$W = \frac{a + a_w(ca_w + b)}{a_w - 1} \quad (\text{Eq.4})$$

(v) GAB Equation (Van den Berg and Bruin 1981)

$$W = W_m \frac{Cka_w}{(1 - ka_w)[1 - ka_w + Cka_w]} \quad (\text{Eq.5})$$

Where, W, W_m and a_w are equilibrium moisture content, monolayer moisture content and water activity respectively. The other symbols are the model constants.

Linear regression analysis was conducted to analyze the fit of two parameter sorption models, viz., Halsey, Oswin, Caurie and 2nd order polynomial regression analysis was conducted for the

three parameter polynomial regression fit to the experimental data to the Modified Mizrahi and GAB

The accuracy of fit was evaluated by calculating the root mean square percent error (RMS %).

$$RMS\% = \sqrt{\frac{1}{n} \left[\sum_1^n \left(\frac{W_{EXP} - W_{PRE}}{W_{EXP}} \right)^2 \right]} \times 100 \quad (Eq.6)$$

Where, W_{EXP} and W_{PRE} are EMC values, experimentally obtained and predicted by the model, respectively and n is the number of data points.

Temperature dependence of GAB isotherm parameters

Weisser (1985) proposed using the exponential relationship between GAB parameters (W_m , C , and k) and temperature to extend the GAB model to incorporate the temperature effect. The temperature dependence of isotherm parameters was thus determined by correlating the GAB (T) constants with temperature by using the following form of Arrhenius-type of equations:

$$C(T) = C' \exp(H_i - H_m / RT) \quad (Eq.7)$$

$$k(T) = k' \exp(H_L - H_m / RT) \quad (Eq.8)$$

$$W_m(T) = W'_m \exp(\Delta H' / RT) \quad (Eq.9)$$

Where H_i is the total heat of sorption of the first layer on primary sites, H_m the total heat of sorption of the multilayer, which differs from the heat of condensation of pure liquid water, H_L is the heat of condensation of pure water vapor and $\Delta H'$ the partial molar enthalpy of sorption, R is the universal gas constant, J/(mol-K).

Moisture sorption hysteresis

Moisture sorption hysteresis has been related to the nature and state of components of foods and temperature (Zhengyong et al. 2008). Hysteresis in *khoa-peda* was observed in this study by comparing the adsorption and desorption isotherms at 15, 25 and 35°C. The magnitude of hysteresis was determined in terms of area between adsorption and desorption isotherm as relative hysteresis units, hysteresis amplitude and the total hysteresis energy as described by Wolf et al. (1972). For relative hysteresis units, the adsorption EMC values were subtracted from desorption EMC values at a regular water activity intervals of 0.05 and the resulting difference was plotted versus the corresponding water activity. Graphical integration of the area under the resulting curve was carried out to obtain the hysteresis units. The hysteresis amplitude for a given water activity was characterized by a ratio of difference in desorption (D) and adsorption (A) to

desorption ((D-A)/D). The total hysteresis energy was evaluated from the sorption data using the following generalized equation described by Johnston and Duckworth (1985):

$$\Delta G = RT \int_o^u \ln p dw \quad (Eq.10)$$

Where ΔG is free energy change per sorption cycle (J), T is absolute temperature, p is the ratio of adsorption to desorption vapor pressure for w moles of sorbed water, o is the lower closure point and u is the upper closure point of hysteresis loop. Graphical integration of above was obtained by plotting ‘ $RT \ln p$ ’ versus ‘ w ’. The resultant curve is known as the Everett and Whitton plot. The area under this curve represents the total hysteresis energy and the shape of the curve reflects the relative variation in extent of the hysteresis effect with moisture content.

Statistical analysis

A One-way ANOVA followed by paired t-test and correlation analysis were performed using SYSTAT-12 to compare moisture content sorbed by the samples at different temperatures during the adsorption and desorption. The level of confidence required for significance was selected at $P 0.05$. Linear regression analysis was conducted to analyze the fit of two parameter sorption models and second order polynomial regression analysis was conducted for the three parameter polynomial regression fit to the experimental data. The accuracy of fit of different models was evaluated by calculating the root mean square percent errors (% RMS) between the experimental and predicted values.

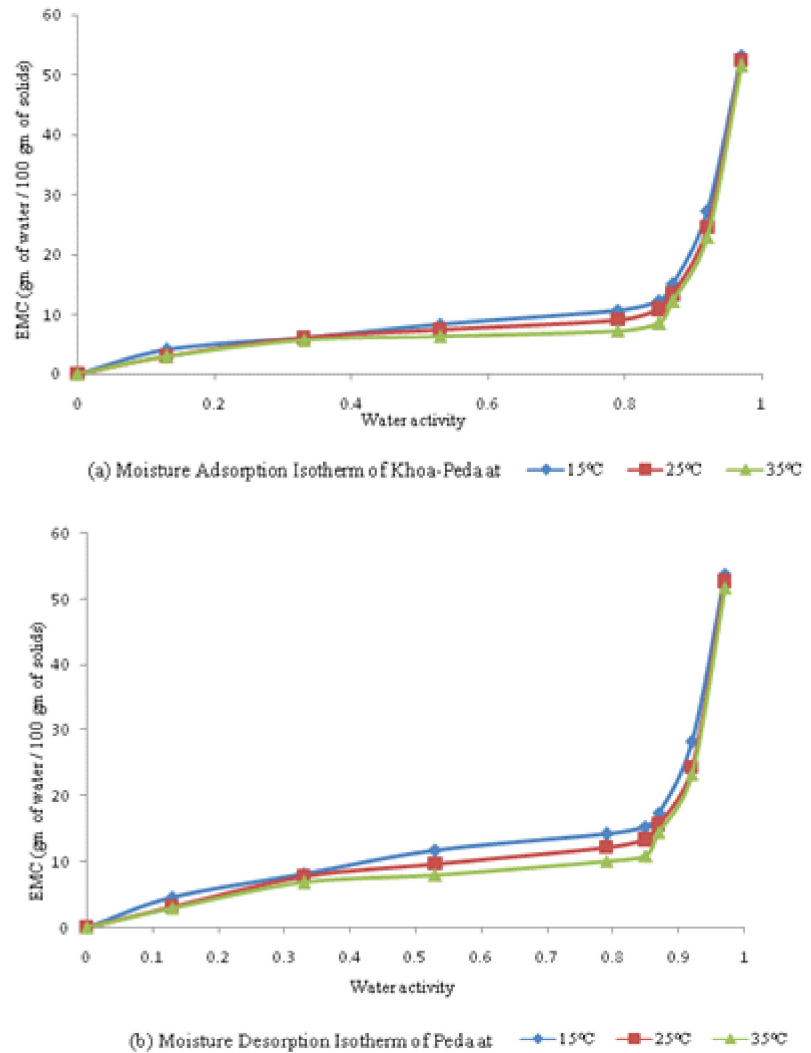
Results and Discussion

Sorption Isotherm of *khoa-peda*

The moisture adsorption behavior of *khoa-peda* measured at 15, 25 and 35°C as observed in Fig.1(a) is manifested in the form of sigmoid shaped curves reflecting a Type II isotherm, which is typical to the most of the foods. No discontinuity was observed in the graphical data. The isotherms demonstrate an increase in equilibrium moisture content with increasing water activity. In all isotherms the moisture uptake was slow up to a water activity of 0.35 a_w , it was moderate between 0.35 and 0.85 a_w , and the equilibrium moisture content increased sharply beyond 0.85 a_w . Similar behavior was observed for desorption isotherms in Fig. 1(b). The isotherms showed that the product adsorbed proportionately more water towards the later part of the curve. Similar types of observations were found by Lin et al. (2005) for milk powders, Sawhney et al. (2011) for dried acid casein from buffalo whole milk, Sawhney et al. (1991) for *khoa* and Sharma et. al. (2009) in *basundi*.

The experimental data points of isotherm showed clear temperature dependence. At water activities 0.33 a_w and above

Fig. 1 Moisture Adsorption and Desorption Isotherms of Khoa-Peda at different temperatures



an increase in temperature resulted in decrease in equilibrium moisture content of *khoa-peda*. The difference decreased with increasing water activities beyond 0.85 a_w and almost vanished at water activities greater than 0.90. The negative temperature effect on equilibrium moisture content has been observed in many other food systems (Iglesias and Chirife 1976), and in *khoa*, the heat desiccated milk product from buffalo milk (Sawhney et al. 1991). In nearly all the dairy products, water sorption is an exothermic process; hence, for given moisture content their water activity increases with temperature (Van den Berg and Bruin 1981). This trend may be due to a reduction in the total number of active sites for water binding as a result of physico-chemical changes in the product induced by temperature (Al-Muhtaseb et al. 2004). At increased temperatures, water molecules get activated to higher energy levels, become less stable and break away from the water binding sites of the material, thus decreasing the equilibrium moisture content.

The analysis of variance (one-way ANOVA) was performed using SYSTAT-12 to compare moisture content sorbed by the samples

at different temperatures during the adsorption and desorption to study the effect of temperature on moisture content. The results revealed that the effect of temperature on moisture content was statistically significant ($P < 0.05$) over the temperature range 15–35°C.

Isotherm modeling

The experimental data on moisture sorption was fitted to five different two and three parameter models using Eq. (1) to (5). Estimated parameters and root mean square per cent error (% RMS) for selected models of isotherm in the water activity and temperature ranges studied are presented in Table 1 for adsorption isotherm and Table 2 for desorption isotherm. The lower is the value of % RMS for predicted and experimental values; the better would be the goodness of fit. A good description of the isotherm is considered, on average, to be smaller than % RMS of 7.0 when a model is applied (Palou et al. 1997). Examination of the results in above tables indicated that the GAB models best described the experimental adsorption data for *khoa-peda* throughout the entire range of water activity. In the present study, at 15, 25 and 35°C

the GAB model predicted the adsorption and desorption isotherm between 4.87 and 6.18% RMS. This was followed by Modified Mizrahi with % RMS between 6.52 and 14.86. Since Modified Mizrahi equation was not defined for the whole range of water activity (0.11-0.97), this study resulted in the GAB as best fitted equation for both adsorption and desorption isotherms. GAB equation was fitted precisely up to 0.90 a_w as well as it provide the better evaluation of amount of water tightly bound by primary adsorption sites.

The values of monolayer moisture content 'Wm' and GAB constants C and k calculated for *khoa-peda* at three temperatures 15, 25 and 35°C for adsorption and desorption are presented in

Table 3. The results show that the adsorbed monolayer moisture content of *khoa-peda* decreased from 5.79 at 15°C to 5.48 g/100g solids at 35°C. Similarly, the desorption monolayer decreased from 6.51 at 15°C to 5.90 g/100g solids at 35°C. The monolayer moisture was higher in desorption than the adsorption and decreased with increase in temperature. The temperature dependence of monolayer moisture has been linked to a reduction in sorption active sites as a result of physicochemical changes induced by temperature. This behavior has also been reported for many other food systems (Iglesias and Chirife 1976).

Temperature Dependence of GAB Parameters

Table 1 Estimated parameters and root mean square percent error for selected models of adsorption isotherm at different temperatures for *khoa-peda*

Equation	Temperature	Constants			% RMS
		a	b	c	
Halsey	15°C	1.8529	2.3013	-	9.0118
Oswin		7.6219	0.3187	-	6.8457
Caurie		-3.6891	8.4444	-	11.453
Modified Mizrahi		-2.6436	-7.9712	10.3548	6.5291
GAB		-0.11	0.1541	0.0139	6.2838
Halsey	25°C	0.9982	2.11	-	15.269
Oswin		6.6475	0.348	-	11.713
Caurie		-4.1827	13.0939	-	15.8986
Modified Mizrahi		-1.5886	-10.6296	12.3867	10.8604
GAB		-0.1034	0.1667	0.0116	4.8712
Halsey	35°C	1.1595	2.2987	-	16.4489
Oswin		6.0545	0.3084	-	14.4137
Caurie		-3.9692	11.9738	-	18.0382
Modified Mizrahi		-2.05	-7.2971	9.4453	14.8618
GAB		-0.0416	0.1117	0.0256	5.483

Table 2 Estimated parameters and root mean square percent error for selected models of desorption isotherm at different temperatures for *khoa-peda*

Equation	Temperature	Constants			% RMS
		a	b	c	
Halsey	15°C	1.9692	2.8311	-	7.6187
Oswin		9.3363	0.2574	-	7.6252
Caurie		-1.8853	0.6846	-	15.606
Modified Mizrahi		-2.9489	-11.187	14.4402	7.5953
GAB		-0.099	0.1477	0.0045	6.1829
Halsey	25°C	1.4661	2.5764	-	7.9267
Oswin		8.7744	0.2975	-	7.4042
Caurie		-1.9606	0.6778	-	14.6618
Modified Mizrahi		-3.6528	-6.8809	10.3712	7.1698
GAB		-0.1078	0.1569	0.0064	6.7896
Halsey	35°C	1.5435	2.9232	-	10.5508
Oswin		6.9879	0.2441	-	7.6726
Caurie		-2.2503	0.8646	-	10.6023
Modified Mizrahi		-2.9105	-7.1538	10.4613	8.1363
GAB		-0.0854	0.1585	0.0104	6.3082

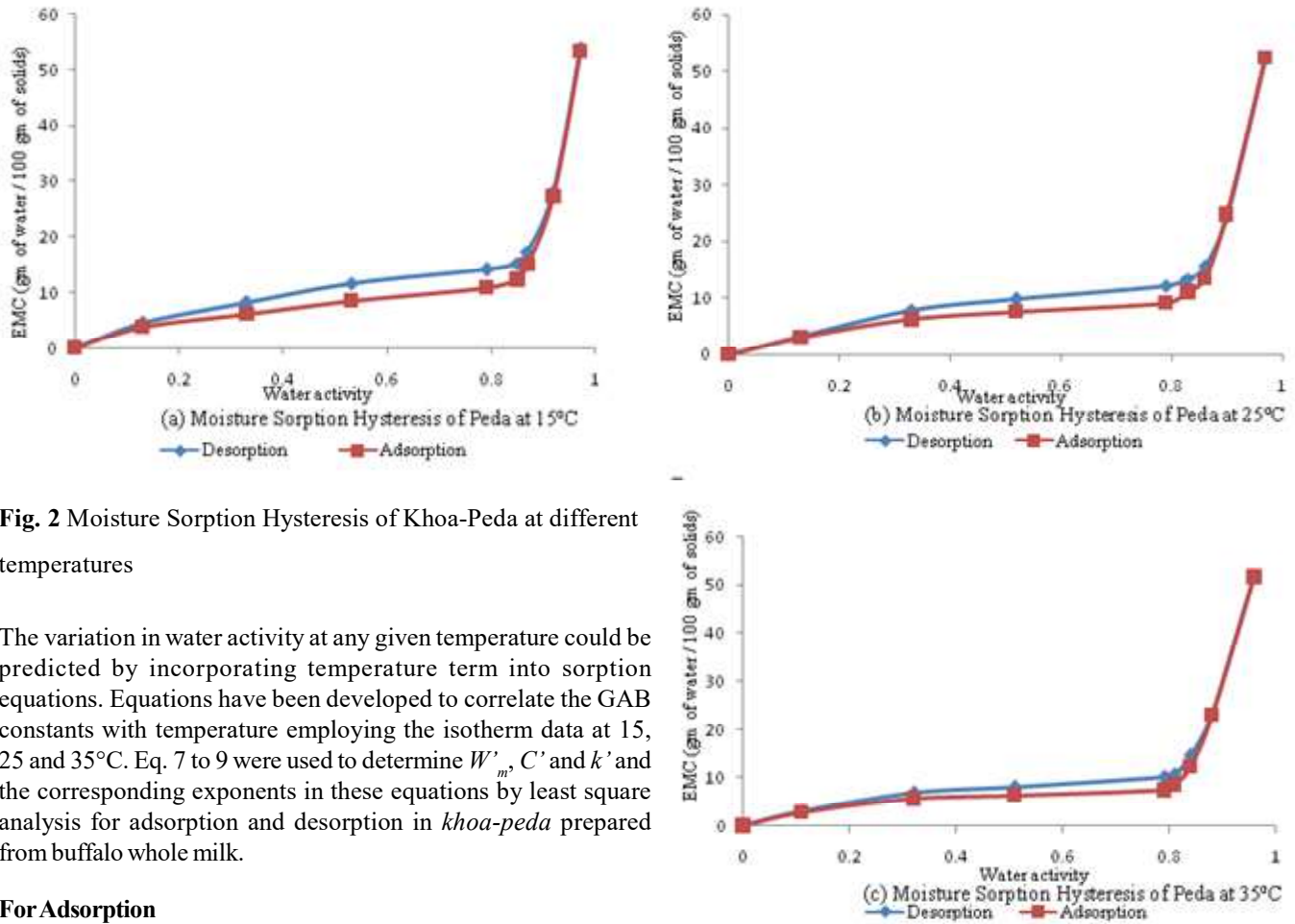


Fig. 2 Moisture Sorption Hysteresis of Khoa-Peda at different temperatures

The variation in water activity at any given temperature could be predicted by incorporating temperature term into sorption equations. Equations have been developed to correlate the GAB constants with temperature employing the isotherm data at 15, 25 and 35°C. Eq. 7 to 9 were used to determine W'_m , C' and k' and the corresponding exponents in these equations by least square analysis for adsorption and desorption in *khoa-peda* prepared from buffalo whole milk.

For Adsorption

$$W'_m(T)a = 1.0213.exp(2.8614 \times 103 / RT) \dots\dots (Eq.11)$$

$$C(T)a = 0.0173.exp(17.918 \times 103 / RT) \dots\dots (Eq.12)$$

$$K(T)a = 0.0123.exp(9.2611 \times 103 / RT) \dots\dots (Eq.13)$$

For Desorption

$$W'_m(T)d = 1.4261.exp(3.6170 \times 103 / RT) \dots\dots (Eq.14)$$

$$C(T)d = 0.0215.exp(18.618 \times 103 / RT) \dots\dots (Eq.15)$$

$$K(T)d = 0.0201.exp(8.4551 \times 103 / RT) \dots\dots (Eq.16)$$

The Equations 11 to 16 together with equation of GAB model (Eq. 5) can now be used to calculate the equilibrium water content of the *khoa-peda* at any given water activity and temperature for adsorption as well as desorption isotherm. These equations could gainfully be used in shelf life simulation and storage and dehydration of the *khoa-peda*.

Moisture Sorption Hysteresis

The phenomenon of moisture sorption hysteresis is of importance in drying and rewetting of dried powder. The practical implications of hysteresis are of significance because of its effect on chemical and microbiological deterioration of the product. The hysteresis effect was observed at all the temperatures almost over the entire range of water activities in *khoa-peda* prepared from buffalo whole milk. The moisture sorption hysteresis of *khoa-peda* at 15, 25 and 35°C are shown in figure 2. The hysteresis effect exhibited by *khoa-peda* shows that the adsorption and desorption isotherms were distinctly apart from each other. The hysteresis loop was classified as Type - C according to Everett and Whitton classification (Kapsalis 1981), which begins about 0.9 a_w (upper closing point) and extends over the rest of the isotherm up to 0.12 a_w (lower closing point). The distribution of hysteresis loop relative to water activity showed a marked change at various water activities. The hysteresis effect became minimum beyond 0.85 a_w and the adsorption and desorption isotherm of *khoa-peda* coincides with each other at about 0.91 a_w at 15°C, 0.9 a_w at

Fig. 3 Everett and Whitton Plot for Moisture Sorption Data on Khoa-Peda at different temperatures

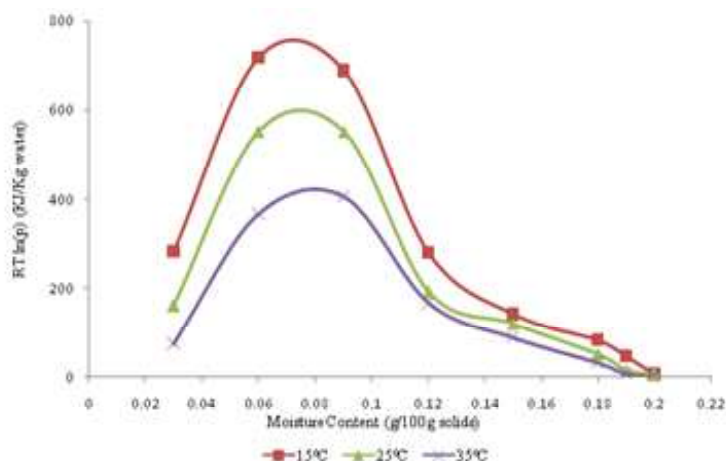


Table 3 Parameter values for the GAB model to describe the adsorption (A) and desorption (D) isotherms of *khoa-peda* at different temperatures

Parameter	Temperature, °C					
	15		25		35	
	A	D	A	D	A	D
W_m	5.79	6.51	5.54	6.04	5.48	5.90
k_m	0.67	0.66	0.65	0.67	0.64	0.69
C	26.08	51.72	18.45	38.45	14.67	31.24
% RMS	6.2838	6.1829	4.8712	6.7896	5.483	6.3082

25°C and 0.88 a_w at 35°C. Maximum hysteresis was observed between 0.30 and 0.85 a_w .

Bell and Labuza (2000) reported that foods with high sugar content frequently exhibit this phenomenon and explained that when the water moves out from capillaries of the product, during moisture desorption, the narrow ends of surface pores trapped and held water internally below the water activity where the water should have been released, thus there was greater moisture content at a low range of water activity. During adsorption, the pure water would dissolve solutes, that is lactose, sucrose and salts present in *khoa-peda* and dissolution of solutes increased the surface tension resulting in lower water activity at given moisture.

A paired t-test and correlation analysis were performed using SYSTAT-12 to compare moisture content sorbed by the samples at different temperatures during the adsorption and desorption. Paired t-test revealed that the adsorption and desorption differ significantly at all the three temperatures with $t_{0.05} = 4.66$ at 15°C, $t_{0.05} = 3.29$ at 25°C, and $t_{0.05} = 3.44$ at 35°C. Further, high correlation ($\gamma = 0.998$) between adsorption and desorption data revealed significant association between them.

The hysteresis loop was evaluated in terms of relative hysteresis units. The total hysteresis in *khoa-peda* was 1.55 units at 15°C. The magnitude of hysteresis was smaller at higher temperatures. Yan et al. (2008) attributed this phenomenon to the increased elasticity of capillary walls and greater capability of forming hydrogen bond between protein/carbohydrate and water. In

khoa-peda from buffalo whole milk, the effect of increasing the isotherm temperature was found decreasing the total hysteresis from 1.55 units at 15°C to 1.35 at 25°C and 1.21 at 35°C. It also resulted in limiting the span of the loop along the isotherm.

The hysteresis amplitude ratio varied throughout the water activity range of 0.12 to 0.9 at all the temperatures. However, the maximum value for this ratio in *khoa-peda* was 0.303 at 15°C. It reduced with increase in temperature to 0.284 at 25°C and 0.253 at 35°C. In most foods, the sorption capacity decreases with increasing temperatures because of negative excess heat of sorption (Bizot et al. 1985). The results obtained on *khoa-peda* are in agreement to this.

Total hysteresis energy was determined employing the Eq.10. The Everett and Whitton plots of sorption data on *khoa-peda* at 15, 25 and 35°C are shown in the Figure 3. In this figure the x-axis has been expressed, for the convenience in interpretations, on a moisture content basis, where as for calculating the total energy, the number of mole of water has been used. The Everett and Whitton plot indicate that the greater contribution to hysteresis energy is made in the moisture content range 6 to 10% (d.b.). The energy of hysteresis was not uniform over the whole water activity range, but varied with the moisture content. The hysteresis energy increased in the beginning with the moisture content and there after decreased abruptly and remained almost uniform. The total energy of hysteresis decreased with increase in temperature; it was 60.61 kJ/kg of water at 15°C, 45.63 kJ/kg of water at 25°C and

32.25 kJ/kg of water at 35°C. The effect of temperature was thus found to reduce hysteresis energy.

Conclusions

Moisture sorption isotherm of *khoa-peda* prepared from buffalo milk obtained in the temperature range of 15 to 35°C were sigmoid type II, common to most foods. Sorption data was best described by GAB model. Equations have been developed correlating the G.A.B. constants with temperature to calculate the equilibrium water content $W(T)$ of *khoa-peda* at any given water activity and temperature by means of GAB(T) constants. The hysteresis effect classified as 'Type-C' extends from water activity range from 0.1 to 0.9. The effect of increasing the isotherm temperature was found decreasing the total hysteresis from 1.55 units at 15°C to 1.35 at 25°C and 1.21 at 35°C. It also resulted in limiting the span of the loop along the isotherm. The hysteresis amplitude ratio reduced from 0.303 at 15°C to 0.253 at 35°C. The total energy of hysteresis decreased with increase in temperature; it was 60.61 kJ/kg of water at 15°C, 45.63 kJ/kg of water at 25°C and 32.25 kJ/kg of water at 35°C. The effect of temperature was thus found to reduce hysteresis energy. The results would be valuable in appraising the shelf life of *khoa-peda* under varying storage conditions and design of suitable packaging systems. It may also be used to optimize the processing conditions and to evaluate the quality of ingredients used in the manufacture of multi-component intermediate moisture food with *khoa* as a base material

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RESEARCH ARTICLE

A comparative study of food products developed from standard dairy milk and lactose hydrolysed milk on their organoleptic qualities

Debanjana Bhattacharyya, Mini Sheth and Vijayata Sengar

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Abstract: In Lactose intolerance, human body is unable to digest lactose present in milk and milk products due to the absence of lactase enzyme and leads to gastrointestinal symptoms. Such individuals are deprived of consuming normal dairy milk and milk products which is an important source of various nutrients. The objective of the study is to develop food products from lactose hydrolysed milk (LHM) and evaluate its organoleptic properties in comparison to regular milk products. Standard methods were used to develop six food products namely *cold cocoa, rose milk, white sauce pasta, vegetable au gratin, kheer and Sandesh* using standard dairy milk and lactose-free milk. Organoleptic evaluation was performed in duplicates using nine point hedonic scale by 50 semi-trained panel members who qualified the threshold test. No significant difference was observed in the organoleptic qualities of all the products except mouth feel and overall acceptability of white sauce pasta prepared from LHM which increased significantly by 6.8% ($p < 0.001$) and 4.12% ($p < 0.05$) respectively. However, after taste and taste of rose milk prepared from LHM decreased significantly by 3.8% ($p < 0.05$) and 5.1% ($p < 0.01$) respectively. All the six food products namely

cold cocoa, rose milk, white sauce pasta, vegetable au gratin, kheer and Sandesh prepared from lactose hydrolysed milk were accepted by the sensory panelist for most organoleptic qualities. Thus, products prepared from lactose hydrolysed milk can be recommended to lactose intolerant individuals which will serve as an important food group full of vital nutrients in their daily diet.

Keywords: Food products, Lactose intolerance, Lactose hydrolysed milk, lactase, Organoleptic evaluation

Introduction

Milk and dairy products are nutrient-dense foods, supplying energy and high-quality protein with a range of essential micronutrients (especially calcium, magnesium, potassium, zinc, and phosphorus) in an easily absorbed form. (Rizzoli, 2014; Black et al. 2002; Bailey et al. 2010; Bechthold et al. 2019; Kardinaal et al. 1999; Pfeuffer and Watzl, 2017; Burgess, 2014). Globally the prevalence of lactose intolerance is observed to be 5-15% among British population, whereas almost 100% is observed in Asia. In India, it is reported to be 27.4% in north India and 66.6% in southern India (Tandon et al. 1981). Lactose intolerance is an inability to digest lactose present in milk and milk products due to the absence or decreased production of lactase enzyme and leads to gastrointestinal symptoms such as bloating, stomach pain, vomiting, diarrhoea etc. Lactose intolerance are of various types depending upon its cause. Primary lactose intolerance- genetically predetermined reduction of enzyme activity during childhood and adolescence, Secondary lactose intolerance- reduction in enzymic activity in response to intestinal injury and surgery or diseases like inflammatory bowel diseases, giardiasis, rotavirus, celiac disease, tropical sprue etc. Congenital lactose intolerance- enzyme activity absent from birth. Lactose intolerance is one of the factors that may influence milk consumption. Studies suggest that lactose maldigesters consume less milk than digesters, possibly as a result of experiences of unpleasant symptoms after ingestion of lactose-containing dairy products. Such individuals are deprived of consuming standard dairy milk and milk products (Bayless et al. 2017; Rosado et al. 1987).

Department of Foods and Nutrition
Faculty of Family and Community Sciences
The Maharaja University of Baroda, Vadodara 390 002, Gujarat, India

Debanjana Bhattacharyya (✉)
Department of Foods and Nutrition
Faculty of Family and Community Sciences
The Maharaja University of Baroda, Vadodara 390 002, Gujarat, India.
Email: debanjanabhattacharyya18@gmail.com

Materials and Methods

Procurement of milk and other raw ingredients

Lactose hydrolysed milk was procured from Gujarat Cooperative Milk Marketing Federation (GCMMF), Anand, Gujarat, India and standard milk of the same brand was procured from the local market. The nutritive value of LHM is similar to standard dairy milk except for the value of lactose present in both.

All other ingredients of standard brands that were needed to make the food products were purchased from the local market.

Standardisation of the recipes

A total of six products were standardised two in each category of beverages, main course and dessert respectively namely *cold cocoa*, *rose milk*, *white sauce pasta*, *vegetable au gratin*, *kheer* and *Sandesh*.

Cold cocoa was made from cocoa powder (1 tbsp), milk (500ml), sugar (4 tbsp) and corn flour (2 and 1/2 tsp). Milk was boiled with sugar, cocoa powder was added to it and was boiled further. When the sugar and cocoa powder dissolved completely in the milk then we took corn flour dissolved in cold milk (1/4 cup) and added it to the above mixture. Once it thickens as our desired consistency then we allow it to cool. It was garnished with chopped dry fruits and grated chocolate. The entire process of making cold cocoa required 15 minutes.

Rose milk is another beverage wherein milk (500 ml) was boiled with sugar (2tbsp), rose syrup (2 tbsp) was added to it, stirred and garnished with chopped dry fruits.

White sauce pasta is an Italian cuisine. In a cooking pan, onion and garlic was sautéed till they were softened, we added 3-4 tbsp of refined flour into the above sautéed onions and cooked it for more 5 mins, now we poured milk (500ml) and allowed it to boil. Then we added the boiled pasta (250gm) in to it and boil it further for more 10 mins. It was flavoured using oregano, Italian herbs and chili flakes.

In Vegetable au gratin onion and garlic were sautéed till they softened. Vegetables (1 cup) such as carrots, onions, tomatoes, beans were added into it and sautéed further. Now 3-4 tbsp of refined flour was added to it and sautéed for more 5 mins, now we poured milk (500ml) into it and allow the vegetables to cook further and flavouring was incorporated using oregano, Italian herbs and chilli flakes.

Kheer is an Indian porridge. In this milk (1 litre) was boiled with sugar (120 gm) and cardamom (5-6 pieces), vermicelli (240 gm) was added into the milk and boiled further for more 10 minutes. Once the vermicelli were cooked and desired consistency was achieved then it was ready to be served. (vermicelli used in this

recipe was roasted from before, if not then one need to roast the vermicelli and then add it into the milk).

Sandesh is a popular dessert from West Bengal in India. Firstly we added sugar (320 gms) and cardamom powder (1 tbsp) into the milk (1.5 l) and stirred it continuously for a period of 20 mins in an open vessel on low flame and allowed it to cook till it became thick in consistency thereafter it was given desired shape. 1.5 litre of milk condensed to give *Sandesh* of around 250 grams.

Threshold test

Threshold test is a test conducted to select panelist who will perform organoleptic evaluation of all the developed food products. In this, sweet, salt, sour and bitter solutions were developed and tasted by the panelists. Panelists who were able to identify the solutions correctly were selected for conducting organoleptic evaluation. Thus the selected panelist performed organoleptic evaluation of the various food products developed from both standard milk and lactose hydrolysed milk.

Organoleptic evaluation

Sensory evaluation of the food products was performed using 9 point hedonic scale by 25 panelists in duplicates wherein panelists were asked score the food products for the various sensory attributes such as colour and appearances, texture, aroma, mouth feel, after taste etc.

Statistical analysis

Statistical analysis was performed using statistical package for social sciences (SPSS) software (version 25). Results were expressed in terms of mean and standard deviation for all the six food products. Students t test was used to determine the significance difference between both the two food products prepared from LHM and standard milk at a p value of 0.5.

Results and Discussion

Organoleptic qualities of Beverages (cold cocoa and rose milk)

As seen in table 1 below, no statistical differences were seen any of the organoleptic properties for cold cocoa. However there was statistically significant reduction in after taste and taste by 3.8% (p value < 0.05) and 5.1% (p value < 0.01) respectively.

Organoleptic qualities of white sauce pasta and veg au gratin with and without lactose hydrolysed milk

As mentioned in Table 2 no significant difference was observed in the organoleptic qualities between pasta made from both the standard milk and LHM, except mouth feel and overall

acceptability of white sauce pasta prepared from LHM which was significantly higher by 6.8% ($p < 0.001$) and 4.12% ($p < 0.05$) respectively. Similarly no difference were observed in veg au gratin prepared from both standard milk and lactose hydrolysed milk. However, score of mouth feel of standard milk was higher than score of mouth feel of veg au gratin prepared from lactose hydrolysed milk.

Organoleptic qualities of *kheer* and *Sandesh* with and without lactose hydrolysed milk

As seen in Table 3, No differences were observed in *kheer* and *Sandesh* prepared from standard milk and LHM. However, taste,

after taste and over all acceptability of standard milk were reported to be higher in score for *kheer* whereas texture and mouth feel received higher score for *kheer* made up of lactose hydrolysed milk. Visually *Sandesh* prepared from lactose hydrolysed milk was much darker (brownish) in colour. Overall acceptability of *Sandesh* prepared from standard milk received higher score compared to *Sandesh* made from LHM.

Milk is an important source of nutrients. In one of the studies, when lactose consumption was equivalent to 240 ml of milk or less a day, symptoms are likely to be negligible. (Suarez et al. 1995).

Table 1 Organoleptic evaluation of beverages made out of standard milk and LHM

Beverages	Organoleptic properties	Mean \pm S.D (Standard milk)	Mean \pm S.D (LHM)	t value	P value	%diff
Cold cocoa	Colour and appearance	7.48 \pm 0.974	7.56 \pm 0.884	0.496	0.622 ^{NS}	1.06
	Aroma	7.44 \pm 0.951	7.48 \pm 0.909	0.265	0.792 ^{NS}	0.53
	Texture	7.36 \pm 0.964	7.4 \pm 1.01	0.260	0.796 ^{NS}	0.54
	After taste	7.52 \pm 1.182	7.42 \pm 0.95	0.532	0.597 ^{NS}	1.32
	Taste	7.52 \pm 1.126	7.66 \pm 0.96	0.312	0.757 ^{NS}	1.86
	Mouthfeel	7.6 \pm 0.969	7.42 \pm 1.032	1.055	0.297 ^{NS}	2.36
	Over all acceptability	7.58 \pm 0.992	7.58 \pm 0.81	0.000	1.000 ^{NS}	0
	Rose milk	Colour and appearance	7.82 \pm 1.119	7.5 \pm 1.093	1.719	0.092 ^{NS}
Aroma		7.58 \pm 1.108	7.26 \pm 1.084	1.908	0.062 ^{NS}	4.22
Texture		7.72 \pm 0.991	7.48 \pm 1.092	1.288	0.204 ^{NS}	3.10
After taste		7.74 \pm 0.986	7.44 \pm 0.951	1.976	0.054*	3.87
Taste		7.82 \pm 0.919	7.42 \pm 0.928	2.694	0.010**	5.11
Mouthfeel		7.72 \pm 1.011	7.44 \pm 0.951	1.705	0.095 ^{NS}	3.62
Over all acceptability		7.8 \pm 0.969	7.56 \pm 0.884	1.731	0.090 ^{NS}	3.07

Note: NS=non-significant; *= <0.05 , **= <0.01 , ***= <0.001

Table 2 Organoleptic evaluation of white sauce pasta and veg au gratin made out of standard milk and LHM

Main course		Mean \pm S.D (Standard milk)	Mean \pm S.D (LHM)	t value	P value	%diff
White sauce pasta	Colour and appearance	6.98 \pm 1.204	6.78 \pm 1.016	1.167	0.249 ^{NS}	2.86
	Aroma	6.68 \pm 1.115	6.78 \pm 1.093	0.573	0.569 ^{NS}	1.49
	Texture	6.98 \pm 1.04	7.08 \pm 1.007	0.598	0.553 ^{NS}	1.43
	After taste	6.72 \pm 1.196	6.96 \pm 1.009	1.205	0.234 ^{NS}	2.98
	Taste	6.7 \pm 1.074	6.9 \pm 0.995	0.953	0.345 ^{NS}	2.98
	Mouth feel	6.76 \pm 1.17	7.22 \pm .996	2.837	0.007**	6.80
	Over all acceptability	6.78 \pm 1.055	7.06 \pm 0.998	1.705	0.095*	4.12
	Vegetable au gratin	Colour and appearance	7.46 \pm 1.147	7.28 \pm 1.179	1.219	0.229 ^{NS}
Aroma		7.28 \pm 1.246	7.5 \pm 1.111	1.355	0.182 ^{NS}	3.02
Texture		7.42 \pm 1.162	7.52 \pm 1.015	0.626	0.534 ^{NS}	1.34
After taste		7.42 \pm 1.197	7.52 \pm 1.035	0.670	0.506 ^{NS}	1.34
Taste		7.4 \pm 1.143	7.5 \pm 1.035	0.590	0.558 ^{NS}	1.35
Mouth feel		7.38 \pm 1.93	7.42 \pm 1.052	0.252	0.802 ^{NS}	0.54
Over all acceptability		7.48 \pm 1.216	7.54 \pm 1.054	0.387	0.700 ^{NS}	0.80

Note: NS=non-significant; *= <0.05 , **= <0.01 , ***= <0.001

Table 3 Organoleptic evaluation of desserts (*kheer* and *Sandesh*) made out of standard milk and Lactose hydrolysed milk

Desserts		Mean \pm S.D (Standard milk)	Mean \pm S.D (LHM)	t value	p value	%diff
Kheer	Colour and appearance	7.9 \pm 0.909	7.76 \pm 0.894	0.943	0.35 ^{NS}	1.77
	Aroma	7.8 \pm 0.99	7.68 \pm 1.039	0.759	0.452 ^{NS}	1.53
	Texture	7.8 \pm 0.756	7.9 \pm 0.863	0.670	0.506 ^{NS}	1.28
	After taste	7.84 \pm 0.912	7.76 \pm 1.001	0.496	0.622 ^{NS}	1.02
	Taste	7.96 \pm 0.781	7.9 \pm 0.931	0.417	0.679 ^{NS}	0.75
	Mouthfeel	7.8 \pm 0.782	7.92 \pm 0.877	0.759	0.452 ^{NS}	1.53
	Over all acceptability	8.02 \pm 0.685	7.92 \pm 0.752	0.962	0.341 ^{NS}	1.24
Sandesh	Colour and appearance	7.64 \pm 1.174	7.62 \pm 0.987	0.110	0.913 ^{NS}	0.261
	Aroma	7.62 \pm 1.008	7.52 \pm 0.931	0.538	0.593 ^{NS}	1.31
	Texture	7.5 \pm 1.055	7.62 \pm 0.945	0.651	0.518 ^{NS}	1.6
	After taste	7.58 \pm 1.247	7.64 \pm 0.875	0.335	0.739 ^{NS}	0.79
	Taste	7.54 \pm 1.216	7.64 \pm 0.985	0.509	0.613 ^{NS}	1.32
	Mouthfeel	7.44 \pm 1.198	7.66 \pm 0.798	1.244	0.219 ^{NS}	2.95
	Over all acceptability	7.56 \pm 1.181	7.74 \pm 0.853	1.055	0.297 ^{NS}	2.38

NS= non-significant; * = <0.05, ** = <0.01, *** = <0.001

Lactose intolerance problems can be controlled by using β -galactosidase which hydrolyses lactose into glucose and galactose and provides a sweet flavour. Glucose is a source of energy and galactose plays a vital function in brain development (Adam et al. 2004; Aggarwal et al. 2019).

Lactose hydrolysed milk can be further utilized in preparation of fermented dairy products such as cheese, yoghurt, *dahi* and heat desiccated products such as *khoa*. Lactose hydrolysis in milk imparts better digestibility and sweet taste, leading to a recent rise in the demand for lactose hydrolyzed dairy products (Aggarwal et al. 2019).

In a study in India, sensory evaluation of low lactose *khoa* prepared from hydrolysed buffalo milk was performed on the basis of 100 point composite card. Statistical analysis revealed no significant change ($p > 0.05$) in flavour and body and texture in *Khoa* prepared from lactose hydrolyzed milk compared with fresh milk *Khoa* samples until the 21st day of storage but a significant change ($p < 0.05$) was observed on the 28th day. However, the flavour scores of *Khoa* prepared from fresh milk had slightly higher scores than *Khoa* prepared from lactose hydrolyzed milk. The variation can be attributed to the development of a sweet flavour owing to the release of monosaccharides i.e. glucose and galactose released upon lactose hydrolysis in milk used for the preparation of *Khoa* (Aggarwal et al. 2019) unlike the present study. Similar results were obtained where higher sweetness in lactose hydrolysed *gulab jamun* (a deep-fried *khoa* Indian dessert) compared with control was observed (Harini & Rao, 2011). In the present study also, *Sandesh* prepared from lactose hydrolysed milk had higher sweetness compared to *Sandesh* prepared from standard dairy milk. While heating, during *Sandesh* preparation a slight brown colour appeared due to Maillard reaction in lactose hydrolysed milk compared to *Sandesh* prepared

from standard dairy milk. Well known, that lactose hydrolyzed milk has a sweeter taste and more prone to strong Maillard reaction. (Csanádi et al. 2010). In one of the study, organoleptic properties of yogurt made up of lactose hydrolysed milk and standard revealed no significant changes between control and the test samples upon evaluation by triangle test, however the addition of fruit and sugars increased sweetness when detected by triangle test (Ismail et al. 1983).

In another study, the use of low lactose milk with varying degree of hydrolysis of lactose may have an impact on the textural properties of yogurt. In a study, yogurt prepared using 50%, 70% and 90% hydrolysed lactose revealed significantly higher scores for body and texture, flavour and overall acceptability than control ($P < 0.05$). whereas lower scores were observed for 90% hydrolysis. (Nagaraj et al. 2009). From the above studies we can say that a wide variety of food products can be developed from lactose hydrolysed milk without compromising on its organoleptic properties at both household and commercial level.

Conclusions

On the basis of the results obtained the sensory panelist accepted all the six food products prepared from lactose hydrolysed milk based upon organoleptic qualities. Milk and milk products are highly nutritious food especially in calcium and protein. In a predominant vegetarian population milk is the only good quality protein consumed by the people. Thus, food products derived from lactose hydrolysed milk can be replaced by those prepared from conventional standard milk as avoidance of milk and milk products have been the most common method of treating lactose intolerance. Lactose present in lactose hydrolysed milk is broken down into glucose and galactose thus resulting into sweeter

taste compared to standard milk. Therefore, milk based recipes which requires added sugar can be developed using LHM with lesser amount of sugar and may be recommended to lactose intolerant individuals. Also, development of various recipes can help in adding variety to the diet as many children and elderly do not like plain milk.

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Significance of storage study on α -amylase inhibitory activity, α -glucosidase inhibitory activity and pancreatic lipase inhibitory activity of fermented milk-based beverage

Dhvany Kinariwala¹ and Subrota Hati²

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Abstract: In the study, 3 potent *Lactobacillus* cultures were considered to prepare fermented milk-based beverage. Three products were prepared by using the three cultures namely *L. fermentum* (M2), *L. fermentum* (M7) and *L. paracasei* (M11) in 1:1 ratio. Based on sensory evaluation, combination of M2:M7 (1:1) product was selected during storage study (upto 9 days) determining pH, acidity (% lactic acid), α -amylase, α -glucosidase and pancreatic lipase inhibitory activities. Self-life study was also done for this selected final product. Decrease in pH and increase in acidity of product were also observed during storage. Slight reduction of *Lactobacillus* count was found (from 8.64 to 8.32 log cfu/mL) for both flavored and without flavored products (8.71 to 8.38 log cfu/mL). However, at 0 day, α -amylase, α -glucosidase and pancreatic lipase inhibitory activities of fresh products (fermented milk without flavor) were 51.49%, 38.10% and 98.71% respectively while product with added flavor also produced 41.28%, 69.55% and 96.12% inhibitions respectively. Best results were observed for lipase inhibitory activity throughout the storage periods for both the products.

Keywords: Fermented milk beverage, Lipase inhibitory activity, *Lactobacillus*, α -Amylase inhibitory activity, α -Glucosidase inhibitory activity, Self-life study

Introduction

The metabolic disorder is firmly identified with way of life and central obesity, filling in as a hazard factor for metabolic sicknesses, for example, type 2 diabetes and cardiovascular ailment (Naydenov et al. 2012). The quantity of individuals with diabetes has ascended from 108 million out of 1980 to 422 million of every 2014. The worldwide commonness of diabetes among adults more than 18 years old has ascended from 4.7% in 1980 to 8.5% in 2014. Diabetes prevalence has been rising more quickly in center and low-pay nations (Emerging Risk Factors, 2010). Diabetes is a noteworthy reason for visual impairment, kidney failure, heart attacks, stroke and lower appendage removal. In 2016, an expected 1.6 million passing were legitimately brought about by diabetes. Another 2.2 million passing were inferable from high blood glucose in 2012. Practically, 50% of all deaths inferable from high blood glucose happen before the age of 70 years. WHO states that diabetes was the seventh driving reason for death in 2016. A large number of individuals around the globe live with diabetes. Cho et al. (2018) assessed that in 2017 there are 451 million (age 18-99/ years) individuals with diabetes around the world. These figures were relied upon to increment to 693 million by 2045. It was assessed that practically 50% surprisingly (49.7%) living with diabetes are undiscovered.

Diabetes mellitus (DM) is arriving at conceivably plague extents in India. DM keeps on expanding because of fast cultural and social changes, which incorporate ageing populations, expanding urbanization, dietary changes, diminished physical activity and unfortunate behavior (Tripathy et al. 2017). As per International Diabetes Federation estimates, around 415 million individuals had DM in 2015 and this number is relied upon to ascend to 642 million by 2040 (<http://www.idf.org/idf-diabetes-atlas-seventh-edition>).

There are different treatments available for preventing or managing diabetes, but many researches have been done to prove that probiotics have antidiabetic properties (Zhang et al. 2016). The term probiotic is a moderately new word signifying “for life” and it is as of now used to name microbes (friendly bacteria) related with valuable impacts for people and creatures (Metchnikoff, 1907). Fermentation is an old development and

¹Dept. of Biochemistry, St. Xavier’s College (Autonomous), Ahmedabad-380 009, Gujarat, India

²Dept. of Dairy Microbiology, Anand Agricultural University, Anand-388 110, Gujarat, India

Subrota Hati (✉)
Dairy Microbiology Department, SMC College of Dairy Science,
Anand Agricultural University, Anand-388 110, Gujarat, India
Email: subrota_dt@yahoo.com, Mobile: +91-9409669561

various civic establishments in different pieces of the world have utilized it to improve the capacity characteristics and nutritive estimation of numerous transitory sustenance, for example, milk, vegetables, meat, fish and grains. Lactic acid bacteria (LAB) is the major group of organisms that play key role in such kind of fermentation. Their utilization as dairy starter societies have turned into an industry where they are principally connected with assembling of matured dairy items, for example, cheddar, yogurt, buttermilk, sour cream, and so forth. LAB establish an enormous heterogeneous gathering of microorganisms of extensive significance for industrial applications, for example, the production of healthy sustenance and feeds. LAB share the property of changing over fermentable starches essentially to lactic acid (Ljungh and Wadstrom, 2006). Phenolic rich compounds from fruits generally shows the antioxidative and antidiabetic activity (Gajera et al. 2017). It has been proven through research that Jamun has antidiabetic properties. Jamun seed and pulp invigorated the release of insulin from the cultured Langerhans cells from both normal and diabetic rats (Baliga et al. 2013). The aim of the study is to evaluate the potentiality of α -Amylase inhibitory activity, α -Glucosidase inhibitory activity, Lipase inhibitory activity of milk-based beverage prepared with lactobacillus cultures.

Materials and Methods

Collection of LAB isolates and their maintenance

The LAB cultures used in the present study were obtained from the Culture Collection of Dairy Microbiology Department, SMC College of Dairy Science, Anand (Table 1). The LAB cultures were propagated in sterilized reconstituted skim milk. The transfer was given every week during the study. Before use in the study, they were activated by propagation in MRS broth by daily transfers.

Product preparation

The pasteurized and standardized milk were purchased from Amul shop, Anand, India for making curd/dahi. Batches for preparation of the products using combination of selected cultures.

Combinations of cultures were taken as follows:

Heat treatment was given to milk at 90°C for 10 min
↓
Allowed to cool down (37°C)
↓
Transfer the milk to six sterile clean and dry beakers in equal volume (500 mL)
↓
Inoculation of selected cultures in combination to each beaker at the rate of 2% (v/v)
↓
Mix all the beakers thoroughly
↓
Incubation at 37°C for 9 h
↓
Curd formation
↓
Transfer all beakers to refrigerator at 6±2°C for 2 h
↓
Flavor [2% salt and cumin, 12% Jamun (*Syzygium cumini* L.) pulp] was added after blending curd

Combination-1: *L. fermentum* (M2) and *L. fermentum* (M7)

Combination-2: *L. fermentum* (M2) and *L. paracasei* (M11)

Combination-3: *L. fermentum* (M7) and *L. paracasei* (M11)

Milk without inoculation of cultures was taken as control.

Sensory evaluation

The products were subjected to the sensory evaluation by an expert trained panel of judges using nine-point hedonic scale (Rahman et al. 2009). The product was served at 10°C. The score for colour and appearance, flavour, body and texture and overall acceptability were recorded as nine-point hedonic scale (Rahman et al. 2009).

Evaluation of final product during storage

Product made by combination of M2 and M7 cultures were selected based on its acidity and palatability. This product was

Table 1 *Lactobacillus* cultures used in the study

Sr.No.	Culture name	Source of isolation	Selective Media	Growth conditions	Gene bank accession no.
1	<i>Lb. fermentum</i> (M2)	Fermented rice beverage	MRS Agar	37°C for 24h	MF951094
2	<i>Lb. fermentum</i> (M7)	Fermented rice beverage	MRS Agar	37°C for 24h	MF951099
3	<i>Lb. paracasei</i> (M11)	Fermented rice beverage	MRS Agar	37°C for 24h	MG027695

also stored for 9 days and evaluated on 0th day, 3rd day, 6th day and 9th day by performing *in vitro* tests as follows.

Determination of pH

pH of control (milk), product without flavour (fermented milk) and product with flavour (beverage) were measured by using digital pH meter (Cole Parmer, India).

Determination of titratable acidity

The titratable acidity of products was estimated by the procedure described in (IS: 1479, Part I, 1960). Ten mL of sample was taken into a porcelain dish and an equal volume of distilled water was added to it. Then 1 mL of phenolphthalein indicator was added, and it was titrated against 0.1 [N] NaOH till the appearance of light pink colour, which persisted for 30 seconds in the solution. Titratable acidity was calculated by the following formula:

$$\text{Acidity (\% Lactic acid)} = \frac{9 \times V \times N}{X} \times 100$$

Where,

V = Volume (mL) of 0.1 [N] NaOH required for the titration

N = Normality of NaOH solution

X = Volume of milk (mL) taken for titration

Determination of *Lactobacillus* counts

Lactobacilli counts of bacterial cultures were determined as per the method described by IDF standards (117A:1989: doi: 10.1016/0168-1605(93)90043-G). One mL sample was taken out from the tubes and added to 9 mL phosphate buffer tubes (1:10 dilution). Similarly, required numbers of serial dilutions were prepared. One mL diluted sample from appropriate tubes was transferred to labelled petri plates (performed in triplicates), then 15-20 mL of melted and cooled (45°C) MRS agar was poured to respective petri plates. The content was mixed thoroughly by tilting and rotating the plates and allowed it to solidify and then additional layer (5-7 mL) of the same agar was poured completely over the solidified medium. Again, allow it to solidify, then incubated at 37°C for 24-48 h in inverted position. Typical colonies were calculated, and the counts were expressed as cfu/mL.

α -Amylase inhibitory activity

Reaction mixture containing 200 μ L phosphate buffer (100 mM, pH = 6.8), 10 μ L α -amylase (2 U/mL), and 100 μ L supernatant of different cultures was preincubated at 37°C for 5 min. Then, the 100 μ L 1% soluble starch (100 mM phosphate buffer, pH 6.8) was added as a substrate and incubated further at 37°C for 20 min; 1 mL of the DNSA colour reagent was then added and boiled for 10

min. The absorbance of the resulting mixture was measured at 540 nm using Systronic PC based double beam Spectrophotometer, 2206 (Telagari and Hullatti, 2015). The results are expressed as percentage inhibition, which was calculated using the formula,

$$\text{Inhibitory activity (\%)} = (1 - A_s/A_c) \times 100$$

Where,

A_s is the absorbance in the presence of test substance and A_c is the absorbance of control.

This test was performed for control (milk), product without flavour (fermented milk) and product with flavour (beverage) on different days of storage period.

α -Glucosidase inhibitory activity

Reaction mixture containing 1.5 mL phosphate buffer (100 mM, pH = 6.8), 2.5 μ L α glucosidase (1 U/mL), and 100 μ L supernatant of different cultures was preincubated at 37°C for 5 min. Then, 500 μ L P-NPG (5 mM) as a substrate was added and incubated further at 37°C for 10 min. The reaction was stopped by adding 100 μ L Na_2CO_3 (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using Systronic PC based double beam Spectrophotometer, 2206 following the method Shai et al. (2011). The results are expressed as percentage inhibition, which was calculated using the formula,

$$\text{Inhibitory activity (\%)} = (1 - A_s/A_c) \times 100$$

Where, A_s is the absorbance in the presence of test substance and A_c is the absorbance of control.

This test was performed for control (milk), product without flavour (fermented milk) and product with flavour (beverage) on different days of storage period.

Pancreatic lipase inhibitory activity

Pancreatic lipase inhibitory activity was carried out by measuring the release of 4-methylumbelliferone (4MU) from the substrate 4-methylumbelliferyl oleate (4MUO) (Sergent et al. 2012) with some modifications. Reaction mixture containing 1.7 mL phosphate buffer (100 mM, pH = 6.8), 100 μ L supernatant of different cultures, 100 μ L 0.25 mM 4MUO, and 2 μ L pancreatic lipase (1U/mL) were mixed and incubated at 37°C for 30 min. Then, 100 μ L 0.1 M sodium citrate was added to stop the reaction. Fluorescence from the release of 4MU was measured at 260nm using Systronic PC based double beam Spectrophotometer, 2206 (Add reference). The percentage of pancreatic lipase inhibitory activity was calculated as follows:

$$\text{Pancreatic lipase inhibitory activity (\%)} = ((A_c - A_s) \times A_c) \times 100$$

Where, A_c is the absorbance of the control and A_s is the absorbance of the sample.

This test was performed for control (milk), product without flavour (fermented milk) and product with flavour (beverage) on different days of storage period.

Statistical Analysis

All the data were subjected to statistical analysis using one factor and two factor Completely Randomized Design (CRD) as per the requirement. The significance was tested on basis of comparison between calculated value and Table F-value. Standard error of mean value, co-efficient of variance (C.V.) and critical difference (C.D.) were determined. The values for microbial counts were log transformed before analysis.

Results and Discussion

Formulation of product

The fermented milk-based beverage appeared most appropriate for use as a vehicle for M2, M7 and M11 cultures. Therefore, three variants of the product were made using combinations of selected *Lactobacillus* cultures. To improve the palatability of the product, Jamun pulp, salt and cumin were added.

Sensory evaluation of products prepared using selected cultures

Pasteurized and standardized milk (4.5% Fat, 8.5% SNF) were used to prepare products using different combinations of selected cultures. Milks were inoculated with 2% rate of cultures and incubated for 9 h. When the curd was formed and transferred to refrigerator for 2 h and then the product was evaluated by an expert panel of judges for evaluating the flavour, body and texture, acidity, colour and appearance and overall acceptability using nine-point hedonic scale (Table 2).

The flavour score, body and texture score, acidity score, colour and appearance score and overall acceptability score of the variants of product were obtained in the range of 5.87 to 9, 7.62 to 8.62, 6 to 8.75, 7.5 to 8.25 and 6.12 to 8.5 respectively. The product prepared with M2 and M7 in combination showed distinctly higher scores. Hence these cultures (M2 and M7) were selected based on the sensory attributes and considered for further use.

Table 2 Sensory Score (9-point Hedonic scale) of three products

Variants of product	Flavour score	Body & Texture score	Acidity score	Colour & Appearance score	Overall acceptability score
M2-M7	8.81±0.11	8.27±0.19	8.58±0.09	8.10±0.11	8.44±0.06
M2-M11	6.04±0.13	7.75±0.07	6.15±0.08	7.79±0.18	6.17±0.05
M7-M11	7.00±0.10	7.83±0.11	6.60±0.22	7.96±0.15	7.19±0.11

Each observation is mean of six replicates (n=6)

Analysis of the selected product during storage

Product was prepared with M2 and M7 in combination, stored and studied for 9 days. Different testing parameters were determined during storage i.e. pH, acidity, *Lactobacillus* count, sensory quality, α -amylase inhibitory activity, α -glucosidase inhibitory activity and pancreatic lipase inhibitory activity of the product. Milk was used as control for all the experiments.

pH of the product

pH of milk (control), fermented milk (product without flavour) and beverage (product with flavour) were checked at 0th day (very first day of product made), 3rd day, 6th day and 9th day of storage. Product was stored under refrigeration condition at $7\pm 2^\circ\text{C}$. Changes were observed and is depicted in Fig. 1.

Based on statistical analysis, it was observed that there is no significant ($P>0.05$) difference between flavoured and without flavoured product in terms of their pH. In fact, difference in pH of the product from 0th day to 9th day was also not significant ($P>0.05$).

pH of stored buttermilk prepared by using five strains of *L. acidophilus* and one strain of *L. casei* were studied by

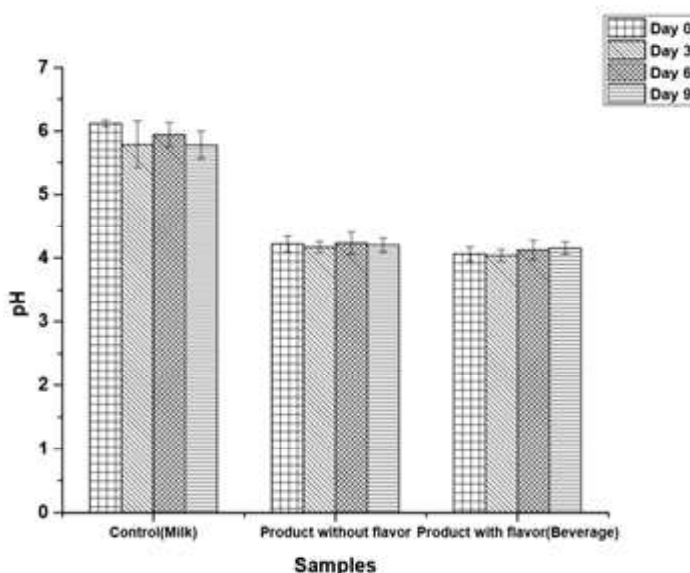


Fig. 1 Changes in pH during storage of product

Nighswonger et al. (1996). On the 0th day, they observed pH of cultured buttermilk samples (n=3) were in the range of 4.5 to 4.6 and did not change during storage period (28 days) at 5°C. They also checked pH of stored yogurt in which initial pH of three batches of yogurt (made with culture CM-2) were 4.5 to 5 which decreased to 4.2 to 4.4 during storage period upto 28 days at 7°C. In our study, pH was dropped from 6.1 to 5.7 during storage period (upto 9 days) and similarly, pH of fermented milk did not change during storage and pH of the stored products which did not vary significantly during storage periods was also supported by Irigoyen et al. (2005).

Acidity of the product

Acidity of milk (control), fermented milk (product without flavour) and beverage (product with flavour) were measured at 0th day (very first day of product made), 3rd day, 6th day and 9th day of storage period of product. Product was stored at 7±2°C. Changes was observed and the trend is presented in Fig.2.

It was observed that there is no significant (P>0.05) difference between flavoured and without flavoured product in terms of their acidity. In fact, difference in acidity of the product from 0th day to 9th day was also not significant. But slight variation in acidity of the product was observed.

Mani-Lopez et al. (2014) had studied the titratable acidity of fermented products during storage of 35 days. They recorded the highest acidity values of yogurt containing *L. delbrueckii* ssp. *bulgaricus*. Lactic acid (%) ranged from 0.72 to 0.74% at initial stage and increased during storage period from 0.82 to 1.04%. Acidity in fermented milks without *L. delbrueckii* ssp. *bulgaricus* varied from 0.67 to 0.79% and from 0.74 to 0.83% at the end of the storage. Dave and Shah (1997) reported initial acidity in its probiotic yogurts and fermented milk of 0.68 and 0.77%, separately; they likewise found after 5 days at 4°C to estimations of 0.82 to 0.84%, and steadily qualities were observed (30 days) from the day 6 of storage. Another study by Donkor et al. (2007) evaluated the generation of acetic and lactic acids in milk fermented by *L. acidophilus* and *L. casei* related with yogurt bacteria during 28 days of cold storage. Korbekandi et al. (2009) announced comparative outcomes in yogurts with *L. casei*. In our study, acidity of fermented milk without flavor was ranged from 0.87 to 0.97% and acidity of fermented milk with Jamun flavor was ranged from 0.79 to 1.15%. These values are approximately similar with Mani-Lopez et al. (2014).

Analysis of product based on viable count of *Lactobacillus*

Lactobacillus count of fermented milk (product without flavour) and beverage (product with flavour) were taken at 0th day (very first day of product made), 3rd day, 6th day and 9th day of storage period of product. Product was stored at 7±2°C. Changes was observed and that trend is exhibited in Fig.3.

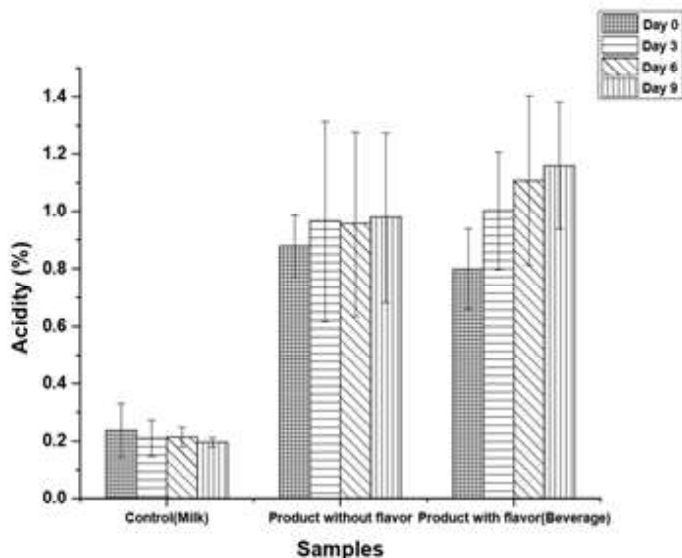


Fig. 2 Changes in acidity during storage

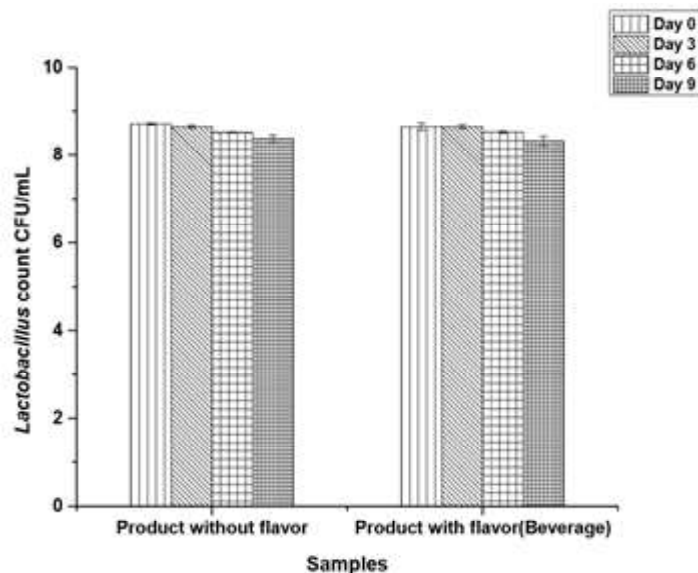


Fig. 3 Total viable count (cfu/ml) of *Lactobacillus* during storage

From the Fig. 3, *Lactobacillus* count in fermented milk and beverage was same at 3rd day and almost similar at 6th day of storage. Thus, Jamun did not show any inhibitory effect on viable counts of *Lactobacillus*. *Lactobacillus* count were higher in fresh product and then decreased gradually during its storage.

Viability of *L. acidophilus* and *L. casei* in fermented milk products (yogurt and buttermilk) were investigated by Nighswonger et al. (1996) during refrigerated storage at 5 to 7°C. They observed that total number of lactobacilli in cultured buttermilk containing different strains of *L. acidophilus* declined significantly during storage (28 days) at 5°C and increased slightly (P>0.05) beyond

7 days. They concluded that all strains retained viable population at 28th day of storage above 10⁶ CFU/g in the cultured buttermilk stored at 5°C. Significant (P<0.05) decrease of viable counts were also observed in stored yogurt at 7°C except for *L. acidophilus* L-1. They did this experiment on two different agar medium and observed different results. In our study, viable counts of *Lactobacillus* ranged from 8.71 to 8.38 log cfu/ml and 8.64 to 8.32 log cfu/ml were observed in case of fermented milk product without Jamun flavour as well as fermented milk with Jamun flavour (beverage) respectively.

α--Amylase inhibitory activity of the product

α-Amylase inhibition ability of milk (control), fermented milk (product without flavour) and beverage (product with flavour) were measured at 0th day (very first day of product made), 3rd day, 6th day and 9th day of storage period of product. Product was stored at 7±2°C. Changes in inhibition rate was observed and the trend is presented in Table 3.

Significant difference (P<0.05) was observed between flavoured and without flavoured product in terms of their inhibition capacity. Results showed reduction in inhibition of α-amylase activity from 0th day to 9th day which suggest that diabetic patient needs to consume product when it is fresh for more benefits and inhibition was ranged from 51.49% to 30.48% for fermented milk (without flavoured product) and 41.28% to 3.27% for beverage (flavoured product). Vankudre et al. (2015) had performed comparative analysis of whey from cow and buffalo milk fermented with *Lactobacillus* species. They observed the highest α-amylase inhibition in product fermented by *L. delbeurkii* and noted that cow milk whey (46.59%) has shown higher α-amylase inhibitory activity than buffalo milk whey (32.29%).

α-Glucosidase inhibitory activity of the product

α-glucosidase inhibition ability of milk (control), fermented milk (product without flavour) and beverage (product with flavour) were measured at 0th day (very first day of product made), 3rd day, 6th day and 9th day of storage period of product. Product was stored at 7±2°C. Changes in inhibition rate was observed and trend is depicted in Table 3.

Significant difference (P<0.05) was observed between flavoured and without flavoured product in terms of their inhibition capacity. Reduction in inhibition of α-glucosidase activity was observed from 0th day to 6th day for fermented milk product and after that product lost the capacity to inhibit activity and therefore table shows negative activity on 9th day which suggest that diabetic patient can consume product till 6th day of the storage. On the other hand, flavoured product (beverage) showed better ability to inhibit α-glucosidase activity and difference in inhibition rate was not significant (P>0.05) during storage of it. In fact, beverage has capacity to inhibit α-glucosidase activity on 9th day was almost similar with capacity of without flavoured product to inhibit α-glucosidase activity on 0th day. Inhibition ranged from 38.10% to 17.89% for fermented milk (without flavoured product) and 69.55% to 34.73% for beverage (flavoured product).

Shori and Baba (2014) had checked α-glucosidase inhibitory activity of cow milk yogurt and camel milk yogurt during 21 days of storage period. They observed increased (P<0.05) inhibition of α-glucosidase for plain-camel milk yogurt (8.4% to 13.7%) and *Allium sativum*-camel milk yogurt (11.7% to 18.8%). Inhibition reduction was observed for plain-cow milk yogurt from 11.3% to 5.5%. α-Glucosidase inhibition by *Allium sativum* cow milk yogurt was reduced (15.2% to 12.8%) after 7 days of storage but it was still higher than inhibition by plain-cow milk yogurt during storage study. Ramchandran and Shah (2008) had also stated that fermented milk products have α-glucosidase inhibitory activities.

Pancreatic lipase inhibitory activity of the product

Pancreatic lipase inhibition ability of milk (control), fermented milk (product without flavour) and beverage (product with flavour) were measured at 0th day (very first day of product made), 3rd day, 6th day and 9th day of storage period of product at 7±2°C (Table 3).

Based on statistical analysis, there was no significant (P>0.05) difference observed between flavoured and without flavoured product. Fermented milk and beverage, both showed excellent ability to inhibit lipase activity till last storage day of the product about 85.93% and 81.88%, respectively. Inhibition ranged from 98.71% to 85.93% for fermented milk (without flavoured product)

Table 3 Evaluation of selected product for three enzyme inhibition activity during storage

Storage period (days)	Inhibition (%)					
	α-Amylase activity		α-Glucosidase activity		Lipase activity	
	Fermented milk	Beverage	Fermented milk	Beverage	Fermented milk	Beverage
0	51.49±0.23	41.28±0.18	38.10±0.01	69.55±0.00	98.71±0.04	96.12±0.06
3	29.71±0.22	20.50±0.31	19.97±0.02	63.20±0.01	95.63±0.01	91.99±0.00
6	26.85±0.07	07.97±0.14	17.89±0.02	59.65±0.00	87.10±0.21	84.13±0.24
9	30.48±0.05	03.27±0.12	-41.80±0.02	34.73±0.00	85.93±0.06	81.88±0.06

Each observation is mean of three replicates (n=3)

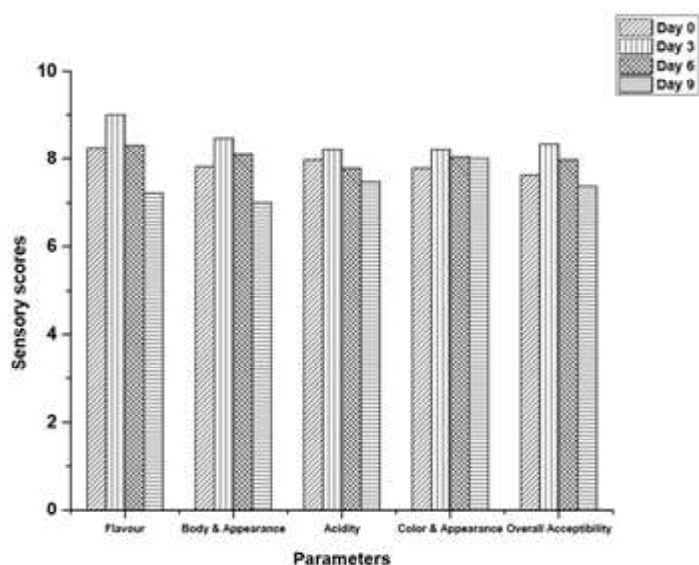


Fig. 4 Sensory evaluation of products during storage period

and 96.12% to 81.88% for beverage (flavoured product). Skim milk was fermented by using different strains of *L. helveticus*, *L. acidophilus*, *L. lactis*, *L. brevis*, *L. plantarum*, *L. delbrueskiissp bulgaricus* and *Pediococcus pentosaceus* for checking its lipase inhibitory activity at different temperatures (30°C, 33°C, 37°C and 42°C). Graph indicated that SC45 strain of *L. helveticus* has the highest ability to inhibit lipase at 42°C in compare to other strains (49.75 ± 2.13%). At 42°C, more than 30% lipase inhibition was reported for different strains such as SC77 and SC40 of *L. delbrueskiissp bulgaricus*, SC1 and SC63 of *L. acidophilus*, SC18 and SC34 of *L. lactis*, SC57 of *Pediococcus pentosaceus* and SC80 of *L. plantarum*. It was concluded that all different strains of LAB species have less or more capability to inhibit pancreatic lipase at different temperatures (Gil-Rodríguez and Beresford, 2019). α -Amylase and α -glucosidase play key role in diabetes, but obesity is the strongest risk factor for diabetes and therefore fermented milk-based product should have ability to inhibit pancreatic lipase which is responsible for fat metabolism.

Sensory evaluation of the product

The pasteurized and standardized milk was used to prepare product using M2 and M7 in combination. They were inoculated at 2% rate and incubated for 9 h. The curd was transferred to refrigerator for 2 h and then the product was judged by an expert panel of judges using nine-point hedonic scale. Sensory evaluation of final product (n=4) for different attributes was done during storage period using nine-point hedonic scale as mentioned above. It is depicted in Fig.4.

The flavour score, body and appearance score, acidity score, colour & appearance score and overall acceptability score of the products were obtained in the range of 7.22 to 9, 7 to 8.47, 7.47 to

8.22, 7.78 to 8.22 and 7.38 to 8.34 respectively. Highest score for all the sensory attributes was observed at the 3rd day of storage. Sensory scores indicated that beverage was acceptable to consume upto 9th day.

Conclusions

Product shows the highest α -amylase inhibitory activity, α -glucosidase inhibitory activity and pancreatic lipase inhibitory activity at 0th day for both flavoured (51.49%, 38.10% and 98.71% respectively) and without flavoured products (41.28%, 69.55% and 96.12% respectively). The results showed that microorganisms differed in growth, acid production and antidiabetic activities. Fermented milk without flavor (Jamun) showed slightly higher *Lactobacillus* growth in compare to flavored product (beverage). Jamun boost antidiabetic activity in α -glucosidase activity. Sensory results also indicated that beverage produced by using combination of cultures was acceptable upto 9th day. Further study is required to validate the health claim of antidiabetic activity of the fermented products.

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Milk from healthy or infected cattle as a source of multi-drug resistant, AmpC β -lactamase-producing *Escherichia coli*

A Mahanti¹, SN Joardar¹, S Bandyopadhyay², S Ghosh³, K Batabyal¹ and I Samanta¹

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Abstract: Drinking raw unpasteurized milk or products made from raw milk is a recent trend observed among the people due to their belief in enhancing nutritional properties and better taste. It poses a great risk of microbial transmission along with antibiotic resistance gene pool (extended spectrum beta-lactamase or ampC) among the consumers. The study was conducted to detect the occurrence of AmpC producing *E. coli* in raw milk samples collected from healthy and mastitic cattle in West Bengal. In total, 450 cow milk samples were collected from all the six agro-climatic zones of West Bengal. The samples were collected from healthy as well as infected cattle suffering with clinical or sub-clinical mastitis, irrespective of age and breed. The collected milk samples were tested with MAST INDEX reagent for the detection of sub-clinical mastitis. *E. coli* isolates obtained from cattle milk samples (n=450) were subjected to cefoxitin-cloxacillin double disc synergy test and PCR based confirmation of *bla*_{AmpC} class I integron and CITM genes. All the ACBL producing *E. coli* isolates were subjected for their resistance to other antimicrobials. All the levofloxacin and Co-Trimoxazole resistant ACBL-producing isolates were studied for PMQR, *sul1*, *sul2*, *sul3* genes.

E. coli was isolated from 205 collected milk samples (205/450, 45.6%). The phenotypical test detected 231/383 isolates (60.3%) were AMPC producers which were also found to possess *bla*_{AmpC}. Comparison of ACBL-producing *E. coli* occurrence in cattle with mastitis, sub-clinical mastitis and healthy cattle revealed significantly higher occurrence rate in infected cattle than the healthy ones (p<0.05). Phenotypical antibiotic resistance pattern of the isolates showed resistance against penicillin-G (100%), cefoxitin (100%), ampicillin (42%), co-trimoxazole (13.5%) and levofloxacin (5.2%). Out of 30 co-trimoxazole resistant isolates identified, 25 (83%) and seven (30.4%) isolates possessed *sul1* and *sul2* gene, respectively, whereas, four (13.3%) isolates possessed both *sul1* and *sul2* genes. Three levofloxacin resistant isolates possessed *qnrS* gene. This is the first systemic study with substantial numbers of milk samples from healthy and infected cattle depicting occurrence of ACBL-producing *E. coli* and linkage present in the isolates between the genes encoding ACBL and quinolone or sulphonamide resistance.

Keywords: AmpC β -lactamase, *E. coli*, Mastitis, Milk, West Bengal

Introduction

Drinking raw unpasteurized milk or products made from raw milk is a recent trend observed among the health-conscious people in the United States, European Union and other countries due to their suppose in enhanced nutritional properties and better taste (EFSA panel on biological hazards, 2011). It poses a great risk of microbial transmission along with antibiotic resistance gene pool (extended spectrum beta-lactamase or ampC) among the consumers.

Production of extended spectrum β -lactamase (ESBL) and ampC β -lactamase is associated with resistance to life saving antimicrobials such as third-generation cephalosporins, penicillin and monobactams in *Enterobacteriaceae*. Further, ampC β -lactamase-producing organisms can also resist β -lactamase inhibitors for example clavulanic acid (EFSA panel on biological hazards, 2011). Presence of AmpC- β -lactamases is confirmed earlier in milk borne *Enterobacteriaceae* group of pathogens such as *E. coli* and *Klebsiella pneumoniae* (Bauernfeind et al. 1996). Our

¹ Department of Veterinary Microbiology, West Bengal University of Animal and Fishery Sciences, 37, K.B. Sarani, Belgachia, Kolkata-700037, West Bengal, India

² Eastern Regional Station, ICAR-Indian Veterinary Research Institute, Belgachia, Kolkata, West Bengal, India

³Sasya Shyamala Krishi Vigyan Kendra, Ramakrishna Mission Vivekananda Educational and Research Institute, Arapanch, Sonarpur, West Bengal, India

Indranil Samanta (✉)
Department of Veterinary Microbiology, West Bengal University of Animal and Fishery Sciences, 37, K.B. Sarani, Belgachia, Kolkata-700 037, West Bengal, India
Email: isamanta76@gmail.com; Phone: +91-9062918679

prior study in India established the occurrence of ESBL-producing *E. coli* in raw milk samples collected from mastitic cattle (Bandyopadhyay et al. 2015). Hitherto no systemic study of ampC producing organisms with substantial numbers of milk samples collected from healthy and /or mastitic cattle is documented from any part of the globe. The present study was conducted to detect the occurrence of ampC producing *E. coli* in raw milk samples collected from healthy as well as infected cattle suffering with clinical or sub-clinical mastitis in West Bengal, the state with one of the largest cattle population in the country with an increasing trend (Government of India, 2019). The study also aimed to establish possible linkage between *ampC* and class I integron, PMQR and sulphonamide resistance genes in *E. coli* isolates from collected raw milk samples.

Materials and Methods

In total, 450 cattle milk samples were collected randomly from all the six agro-climatic zones of West Bengal, India. The samples were collected from healthy as well as infected cattle suffering from clinical or sub-clinical mastitis, irrespective of age and breed (Table 1).

The collected milk samples were tested with MAST INDEX (Tulip Diagnostics Limited, India) reagent for the detection of sub-clinical mastitis. Milk samples from the cattle with clinical mastitis were collected following standard guidelines. All the four quarter milk samples were mixed and brought into the laboratory maintaining cold chain.

Isolation, identification and PCR based confirmation of *Escherichia coli* from milk samples

Escherichia coli were isolated and identified from collected milk samples following standard guidelines (Quinn et al. 1994). The isolates were subjected to 16S rRNA gene specific PCR for confirmation of *E. coli* (Wang et al. 1996).

Phenotypical and PCR based detection of AmpC beta-lactamase (ACBL) in *E. coli* isolates

All the *E. coli* isolates were subjected to cefoxitin-cloxacillin double disc synergy (CC-DDS) test for phenotypic confirmation of ACBL production (Tan et al. 2009). PCR based detection of *bla*_{AmpC} genes in *E. coli* isolates was performed by previously described method (Féria et al. 2002).

PCR based detection of class I integron and plasmid-mediated AmpC β-lactamase gene (CITM) in ACBL producing-*Escherichia coli* isolates

All the ACBL producing- *E. coli* isolates were studied for the presence of class I integron and plasmid-mediated AmpC β-lactamase gene (CITM) genes following the protocols described earlier (Mazel et al. 2000; Van et al. 2008).

Antimicrobial sensitivity of ACBL producing-*E. coli* isolates

All the ACBL producing *E. coli* isolates were subjected for their sensitivity and resistance to different antimicrobials by disc diffusion method (CLSI 2014). The antibiotics used were cotrimoxazole (25 µg), cefoperazone (75 µg), chloramphenicol (30 µg), ampicillin (10 µg), cefoxitin (30 µg), gentamicin (10 µg), tetracycline (30 µg), amikacin (30 µg), levofloxacin (5 µg), penicillin-G (10 U), tobramycin (30 µg), ceftriaxone (30 µg), ceftizoxime (30 µg), ceftazidime (30 µg) and cefotaxime (30 µg) (HiMedia, India).

Detection of PMQR (*qnrA*, *qnrB*, *qnrS*) and sulfonamide resistance genes (*sul1*, *sul2*, *sul3*)

All the levofloxacin and co-trimoxazole resistant ACBL-producing *E. coli* isolates were studied for detection of plasmid mediated quinolone resistance genes and *sul1*, *sul2*, and *sul3* genes, respectively (Frank et al. 2007; Kar et al. 2015).

Statistical analysis

Occurrence of ACBL-producing *E. coli* in different types of cattle according to their health status (clinical mastitis, sub-clinical mastitis and healthy) was compared by chi-square test using SPSS software version 17.0 (SPSS Inc.).

Results and Discussion

ACBL-producing *Enterobacteriaceae* group of organisms produce resistance against cephalosporins and their higher versions either due to overexpression of chromosomal AmpC coding gene (*bla*_{AmpC}) or plasmid-mediated genes (CITM or *bla*_{CMY-2}) (Schmid et al. 2013). The double disc synergy test using cefoxitin-cloxacillin (CC-DDS) discs for phenotypic confirmation of ACBL production showed 231/383 *E. coli* isolates (60.3%) in the present study were AMPC producers which were also found to possess *bla*_{AmpC} (Table 1, Figure 1, Figure 2). Although there is paucity of reports from livestock sector to corroborate with the present findings, previous studies have documented lower occurrence of *bla*_{AmpC} gene in *E. coli* isolates from mastitic milk of cattle in India and Taiwan (Kar et al. 2015; Su et al. 2016). The plasmid mediated AmpC gene (CITM) was present in 51 (51/231, 22.08%) ACBL producing *E. coli* isolates. Evaluation with other studies may not describe similar occurrence rate of ACBL/ESBL associated genes in the isolated organisms as the occurrence rate varies with breed of cattle, management practices, nutrition, veterinary treatments, use of disinfectants and slurry management (Ibrahim et al. 2016).

Comparison of ACBL-producing *E. coli* occurrence in cattle with mastitis, sub-clinical mastitis and healthy cattle revealed significantly higher occurrence rate ($p < 0.05$, Table 1) in infected cattle than healthy ones. Similarly, earlier studies also detected more prevalence of ESBL-producing isolates in mastitic cattle

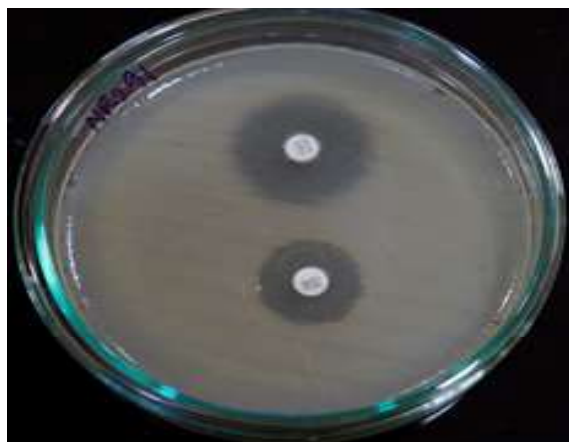


Fig. 1 Cefoxitin-cloxacillin double disc synergy (CC-DDS) test for phenotypic detection of ACBL producing-*E. coli*

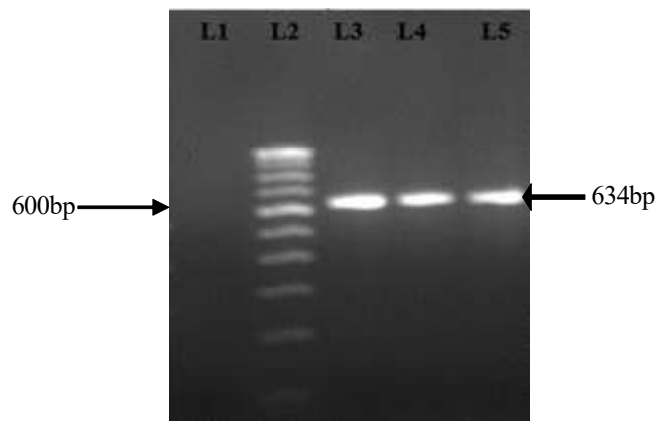


Fig. 2 PCR gel image showing amplification *blaAmpC* (634 bp) in *E. coli* isolated from milk samples. L1, negative control; Lane 2, 100 bp DNA ladder; Lane 3-4, representative positive samples; Lane 5, positive control

Table 1 ACBL producing *E. coli* isolated from cattle of diverse health status in different agro-climatic zones of West Bengal, India

Sl. No.	Agroclimatic zone	Name of the District	Total no of Isolate (ACBL positive)			
			CM	SCM	H	Total
1	Hill Zone	Darjeeling	21(18)	34(21)	9(1)	64(40)
2	Tarai Zone	Darjeeling	0	11(10)	15(2)	26(12)
		Coochbehar	0	17(14)	12(1)	29(15)
3	Old Alluvial Zone	Malda	8(7)	12(5)	5(0)	25(12)
		Burdwan	12(12)	8(7)	2(0)	22(19)
4	New Alluvial Zone	Murshidabad	14(11)	12(7)	2(0)	28(18)
		Hooghly	2(2)	0	2(0)	4(2)
5	Red and Laterite zone	Paschim Medinipur	12(11)	12(9)	7(1)	31(21)
		Purulia	12(8)	6(4)	6(1)	24(13)
6	Coastal and Saline zones	Purba Medinipur	0	9(7)	6(1)	15(8)
		North 24 Parganas	24(16)	48(26)	5(1)	77(43)
		South 24 Parganas	9(8)	26(20)	3(0)	38(28)
Total			114(93) ^a	195(130) [*]	74(8) ^{*a}	383(231)

CM: Clinical Mastitis, SCM: Sub-clinical Mastitis, H: Healthy

^{*a} differs significantly (p<0.05)

than the healthy ones probably due to additional exposure to higher generation cephalosporins during treatment (Su et al. 2016).

Transmission of ACBL/ESBL producing organisms is regulated by mobile genetic elements specially class 1 integrons. The present study revealed lower occurrence of class 1 integron (18.6%) in ACBL producing *E. coli* isolates depicting their low transmission possibility to consumers.

Phenotypical antibiotic resistance pattern of the isolates showed resistance against Penicillin-G (100%), Cefoxitin (100%), Cefotaxime (66.2%), Ampicillin (42%), Ceftazidime (32.9%), Ceftizoxime (22.5%), Co-Trimoxazole (13.5%), Cefoperazone (8.2%) and Levofloxacin (5.2%) (Table 2). All the ACBL-producing *E. coli* isolates were sensitive to Gentamicin and Amikacin in the

present study. Resistance to penicillin, ampicillin and higher generation cephalosporins might be due to hyperproduction (by gene amplification or mutation) of AmpC enzyme (Srinivasan et al. 2007).

Out of 30 co-trimoxazole resistant isolates identified in present study, 25 (83%) and seven (30.4%) isolates possessed *sull* and *sul2* gene, respectively, whereas, four (13.3%) isolates possessed both *sull* and *sul2* genes. Similarly, *sull* was obtained in higher frequency than *sullI* in sulfisoxazole-resistant *E. coli* isolated from mastitic cattle (Lanz et al. 2003). Three levofloxacin resistant isolates possessed *qnrS* gene in PCR and *qnrA* or *qnrB* genes were not detected in any of the studied isolates. Moreover, all *qnrS* positive and majority of *sul*-positive isolates possessed class-1 integron. Similarly, none of the *E. coli* or *Klebsiella* isolates from mastitic milk samples in United Kingdom possessed

Table 2 Multi-drug resistance pattern of ACBL producing *E. coli* isolates (n=231) from bovine milk

Resistance profile detected	No of isolate
CX, P, CTX, CAZ, CZX, CPZ, COT, C, LE, TE, CTR, AMP, TOB	4
CX, P, CTX, CAZ, CZX, CPZ, COT, C, LE, CTR, AMP, TOB	1
CX, P, CTX, CAZ, CZX, CPZ, COT, LE, CTR, AMP, TOB	1
CX, P, CTX, CAZ, CZX, COT, LE, TE, CTR, AMP	5
CX, P, CTX, CAZ, CZX, COT, C, TE, AMP	1
CX, P, CTX, CAZ, CZX, CPZ, CTR, AMP	8
CX, P, CTX, CAZ, CZX, CPZ, AMP	1
CX, P, CTX, CAZ, CPZ, COT, AMP	3
CX, P, CTX, CAZ, COT, CPZ, AMP	1
CX, P, CTX, CAZ, COT, TE, AMP	6
CX, P, CTX, CAZ, CZX, LE	1
CX, P, CTX, CAZ, CZX, COT	1
CX, P, CTX, CAZ, CZX, AMP	7
CX, P, CTX, CZX, COT, AMP	2
CX, P, CTX, CAZ, COT, AMP	1
CX, P, CTX, CAZ, COT, TE	2
CX, P, CTX, CAZ, CZX	20
CX, P, CTX, CAZ, AMP	11
CX, P, CTX, CAZ, COT	2
CX, P, CTX, TE, AMP	4
CX, P, CTX, AMP	41
CX, P, CTX	30
CX, P	78

any of the *qnrA* or *qnrB* genes, but the latter were positive for *qnrS* (Timofte et al. 2014). The present study confirmed the occurrence of multi drug resistant ACBL-producing *E. coli* in collected raw milk samples and a possible linkage between ACBL determinants and the resistance genes for sulphonamides and quinolones. Earlier study also indicated about linkage between ACBL/ESBL determinants with sulphonamides, aminoglycosides and tetracycline (Wichmann et al. 2014).

Conclusion

The present study makes the public awareness to avoid consumption of raw milk which might be the source of antimicrobial resistant bacteria.

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Exopolysaccharide production potential of different strains of *Lactobacillus plantarum*

Riya KB, K Radha, CT Sathian and MV Chinnu

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Abstract: In the present study, the exopolysaccharide production potential of different strains of *Lb. plantarum* was investigated. Six different strains of *Lb. plantarum* were screened for their ability to produce EPS and the growth conditions were optimized for maximum EPS production. For this different strains of *Lactobacillus plantarum* were grown on MRS broth supplemented with carbon sources (glucose, sucrose) and nitrogen sources (yeast extract and beef extract) at three different levels for maximum EPS production. Sucrose (15g/L) was found to be the best source of carbon for maximum EPS production in strains NCDC 373 (50.87±7.67 mg/L) and NCDC 377 (54.50±1.27 mg/L). In NCDC 382 and UBLP-40, maximum EPS yield (88.48±3.55 and 84.41±3.24 mg/L) were obtained with 25g/L of sucrose. Beef extract acted as the best source of nitrogen for maximum EPS production in five strains (NCDC 378, NCDC 377, NCDC 382, UBLP-40, NCDC 20). FT-IR spectral analysis and prebiotic activity score of the purified EPS of *Lb. plantarum* strains NCDC 382 and UBLP-40 revealed the presence of prominent polysaccharide functional groups and their prebiotic potential respectively.

Keywords: Carbon and nitrogen sources, Exopolysaccharide, *Lb. plantarum*, Functional groups, Prebiotic activity

Introduction

Exopolysaccharides (EPS) are long chain polysaccharides biosynthesized extracellularly by bacteria. EPS produced by food grade lactic acid bacteria (LAB) with GRAS (Generally Regarded as Safe) status is receiving a renewed interest as these polymers are capable of improving the mouthfeel, texture and stability of fermented dairy products. *Lactobacillus plantarum* - an EPS producing LAB is a versatile, Gram positive facultative anaerobe that can be found in a wide range of habitats such as dairy, meat etc. It can grow to high cell densities which makes it desirable for industrial application. EPS producing *Lb. plantarum* strains also act as a textural agent and as a new potentially probiotic strain. More than 50 percent of the *Lactobacillus plantarum* species isolated from dairy and non-dairy products were found to be active producers of EPS (Adebayo-Tayo and Onilude 2008).

Carbon sources such as glucose, sucrose, lactose, galactose and nitrogen sources like yeast extract, beef extract and tryptone are utilized by LAB strains for EPS production. Thus, supplementation with carbon and nitrogen sources in culture medium influences the production and concentration of EPS (Imran et al. 2016). Recent studies have shown that optimization of growth conditions is an important criteria that can maximize the EPS production by LAB. *Lb. plantarum* also has the potential to produce EPS which can serve as prebiotics. These polysaccharides exhibit high resistance to gastric juices and will be metabolized more slowly than oligosaccharides, thereby exert prebiotic effects in more distal colonic regions. The presence of EPS also prolongs the survival of probiotic microorganisms like Bifidobacteria in the gastrointestinal tract of the host (Hongpattarakere et al. 2012). The aim of the present study was to determine the optimum carbon and nitrogen source for maximum production of EPS by different strains of *Lb. plantarum* which could help to maximize the EPS production commercially. In addition, the chemical characterization and the prebiotic potential of the EPS were also determined.

Department of Dairy Science, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

K Radha(✉)

Department of Dairy Science, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Email: radhavet@gmail.com

Materials and Methods

Collection of bacterial cultures and media

The *Lb. plantarum* cultures NCDC 20, NCDC 373, NCDC 377, NCDC 378, NCDC 382 and *Escherichia coli* NCDC 247 were procured from National Collection of Dairy Cultures (NCDC), National Dairy Research Institute, Karnal. Probiotic *Lactobacillus plantarum* UBPL-40 (DVS) culture was procured from Unique Biotech, Hyderabad. Lactobacillus MRS agar (Himedia Laboratories Ltd. Mumbai) was used for the enumeration of *Lactobacillus plantarum*. The addition of ruthenium red (Sisco Research Laboratories Pvt Ltd) improved the specificity of this medium for the screening of exopolysaccharide producing strains. Lactobacillus MRS broth was used as a medium for the growth of exopolysaccharide producing cultures. Tryptone yeast extract broth was purchased from Himedia Laboratories Ltd., Mumbai for testing the prebiotic activity of exopolysaccharide.

Screening of LAB for EPS production

Ropy polysaccharide production

The ability of *Lb. plantarum* cultures to produce ropy polysaccharide was examined in MRS agar containing 0.08 g/L ruthenium red. 20 mL of autoclaved molten culture medium maintained at 45°C was poured into sterile petri plates. The inoculum was quadrant streaked over the agar surface to obtain isolated colonies. The plates were incubated at 37°C in an anaerobic jar for 48 hours. After incubation the strains that produced white coloured mucoid slimy colonies were recorded as strains capable of producing EPS as per Mostefaoui *et al* (2014).

Capsule formation

The mucoid slimy colony obtained was subjected to capsule staining method (Anthony, 1931). The smear prepared was air dried without heat fixing followed by the addition of crystal violet (primary stain). Slide was then saturated with 20 per cent copper sulphate for one minute and gently washed with distilled water and observed under oil immersion objective of the microscope. Cells containing capsule appear as purple coloured surrounded by clear or faint blue halo.

Optimization for enhanced EPS production

Optimization studies were carried out by using different levels of carbon sources like glucose, sucrose (15, 20, 25g/L) and nitrogen sources such as yeast extract and beef extract (20, 25, 30 g/L) for all the six strains of *Lb. plantarum*. They were supplemented individually into the MRS broth followed by inoculation of each flask with 18 hour old inoculum, to determine the best source of carbon and nitrogen respectively. The

combined effect of carbon and nitrogen supplement on EPS production was also investigated.

Isolation of exopolysaccharide

Isolation of EPS was performed by the method suggested by Bajapi *et al.* (2016). *Lb. plantarum* was cultured in MRS broth and incubated at 37°C for 24 hours. Eighty per cent trichloroacetic acid (Himedia Laboratories Pvt. Ltd.) was added to denature the protein content (Goh *et al.* 2005). The MRS broth was further left for homogenization in a shaker (90 rpm) for 30-40 minutes. Broth was transferred to centrifuge tubes and centrifuged at 8000 x g for 20 minutes at 4°C. The supernatant collected was added to absolute ethanol (double volume of supernatant) and kept at 4°C. The upper layer of ethanol was discarded and the precipitate obtained was again centrifuged at 8000 g for 20 minutes at 4°C to obtain the crude EPS (Adebayo-tayo and Onilude 2008). The EPS obtained was added with equal volume of de-mineralized water and mixed well with the help of vortex. It was then transferred into dialysis membrane of average flat width-37.7 mm and average diameter-25.4 mm with molecular weight cut off 12,000 to 14,000 Da (Himedia Laboratories Pvt Ltd) and kept at 4°C for 48 hours to remove simple carbohydrates like residual lactose present in EPS (Rimada and Abraham 2003). Thus, purified EPS was isolated.

Quantification of exopolysaccharide

Determination of EPS yield was done by phenol sulphuric acid method as described by Dubois *et al.* (1956). Sample (50 µl) was taken in sugar tube and made to a final volume of one millilitre with distilled water. Blank was set up with one millilitre of distilled water. Mixed with one millilitre of five per cent phenol solution (Himedia Laboratories Pvt Ltd) followed by addition of five millilitre of sulphuric acid (Himedia Laboratories Pvt Ltd). Sample and blank tube were shaken well and kept in water bath for 10 minutes and then cooled to room temperature. Absorbance of sample was noted at 490 nm. Blank was used as the reference sample. Concentration of EPS was determined from the glucose standard curve as shown in Figure 1, plotted by measuring the concentration of known set of glucose standards generated through serial dilution of glucose stock solution (Goh *et al.* 2005).

Chemical characterization of exopolysaccharide

Fourier Transform Infrared Spectrophotometer- Attenuated total reflectance (FTIR-ATR) analysis

Infrared spectroscopy helps to detect the charged groups such as sulphate, carboxyl, phosphate or pyruvate present in EPS (Shao *et al.* 2014). FTIR-ATR analysis was done using Perkin Elmer (Singapore). By FTIR-ATR technique, the functional groups present in the purified EPS of top two EPS producing strains of *Lb. plantarum* were recorded within the spectrum range from 4000 to 600 cm⁻¹.

Prebiotic activity of EPS

Prebiotic activity of lyophilized EPS was tested for the top two EPS producing strains of *Lb. plantarum*, according to the method of Tsuda et al. (2009). Probiotic *Lb. plantarum* and *E. coli* strains were inoculated into tryptone yeast extract broth (TY broth) containing 0.2 per cent glucose and EPS respectively and incubated for 18 hour at 37°C. Optical density (OD) of the culture at 660 nm was measured at zero hour and 18 hour.

$$\text{Prebiotic activity} = (\text{Growth rate of LAB} - \text{Growth rate of } E. coli)$$

$$\text{Growth rate} = (\text{Log OD 660 of TYP at 18 hour} - \text{Log OD 660 of TYP at zero hour}) / (\text{Log OD 660 of TYG at 18 hour} - \text{Log OD 660 of TYG at zero hour})$$

{TYP:- TY broth containing EPS , TYG:- TY broth containing glucose}

Results and Discussion

Screening of *Lb. plantarum* cultures for EPS production

All the tested *Lactobacillus plantarum* strains were able to produce translucent, creamy white coloured colonies on MRS agar plate incorporated with 0.8 g/L of ruthenium red (Fig 2). The presence of EPS prevented the absorption of ruthenium red present in the medium into the bacterial cell, distinguishing the

EPS producing strains with white colonies from the non-EPS producing strains. Similar results were reported by Imran et al. (2016). Visualization of capsular EPS was performed by Anthony’s counterstaining method. None of the strains tested produced capsular polysaccharides.

Optimization studies for maximal EPS production

In order to improve the EPS production by *Lb. plantarum*, the influence of carbon sources (glucose, sucrose) and nitrogen sources (yeast extract, beef extract) were studied. In the present study wide variations in EPS yield was noticed depending upon the carbon and nitrogen supplements. EPS production was found to be strain dependent.

Effect of carbon sources on EPS production

The mean EPS yield of different *Lb. plantarum* strains supplemented with different levels (15, 20, 25 g/L) of glucose and sucrose are presented in Table 1. The mean EPS yield of different strains ranged from 9.73±1.93 to 88.48±3.55 mg/L. Sucrose, at the rate of 15g/L was found to be the best source of carbon for maximum EPS production in strains NCDC 373, NCDC 377 where, EPS yields of 50.87±7.67 mg/L and 54.50±1.27mg/L were obtained respectively. In NCDC 382 and UBLP-40, maximum EPS yield of 88.48±3.55 and 84.41±3.24 mg/L were obtained with sucrose at the rate of 25 g/L. Tallon et al. (2003) found that when sucrose

Fig 1. Standard curve of glucose

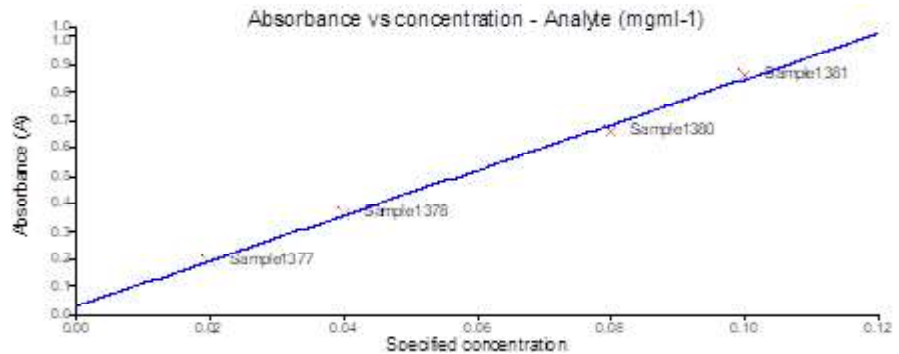


Table 1. EPS yield of *Lb. plantarum* cultures at different levels of glucose and sucrose supplementation

<i>Lb. plantarum</i> strains	EPS YIELD (mg/L)					
	Glucose			Sucrose		
	15g/L	20g/L	25g/L	15g/L	20g/L	25g/L
NCDC 378	19.35±0.35 ^{ab}	37.85±7.17 ^b	34.50±7.63 ^{ab}	33.58±4.60 ^{ab}	9.73±1.93 ^a	29.23±13.67 ^{ab}
NCDC 373	25.08±1.09 ^{bc}	20.53±0.24 ^{abc}	29.48±2.03 ^c	50.87±7.67 ^d	13.6±1.37 ^a	18.31±1.16 ^{ab}
NCDC 377	28.47±2.92 ^b	13.57±1.66 ^a	42.33±3.21 ^d	54.50±1.27 ^e	37.33±.79 ^{cd}	35.90±1.66 ^c
NCDC 382	42.07±1.77 ^b	18.61±2.15 ^a	64.77±1.10 ^d	64.65±2.33 ^d	14.48±.31 ^a	88.48±3.55 ^e
UBLP-40	32.87±2.25 ^b	32.4±1.95 ^b	25.7±1.49 ^a	42.68±2.67 ^c	46.40±.20 ^c	84.41±3.24 ^a
NCDC 20	60.27±3.16 ^c	51.17±6.16 ^{bc}	29.85±1.32 ^{ab}	48.32±11.95 ^{abc}	34.99±8.65 ^{ab}	26.97±1.57 ^a

Means bearing different superscript within the same row differ significantly.

Means are averages of six replications

was added as carbon supplement, EPS yield of 25.3 mg/L was obtained whereas, when glucose was used the yield was 22.9 mg/L in *Lb. plantarum* EP56. Among the six strains tested, only two strains (NDDC 378, NCDC 20) had showed better EPS production when glucose was used as a carbon source. Fan et al. (2007) observed that due to the relative easing in polymerization mainly disaccharides were found to be a better carbon source than monosaccharides for EPS production.

Effect of nitrogen sources on EPS production

The mean EPS yield of different *Lb. plantarum* strains supplemented with different levels (20, 25, 30 g/L) of yeast extract and beef extract are presented in Table 2. The mean EPS yield of different strains ranged from 5.33±1.62 to 69.03±6.43 mg/L. Beef extract at 30g/L was the best source of nitrogen for maximum EPS production in three of the strains studied (NCDC 378 (67.68±8.10), UBLP-40 (66.60±3.65) and NCDC 20(69.03±6.43mg/L). Beef extract at 20g/L and 25g/L were found to be the best source of nitrogen for maximum EPS production in NCDC 377(56.98±6.60) and NCDC 382 (57.81±1.29) respectively. In the presence of yeast extract supplement only strain NCDC 373, showed greater EPS production.

Combined effect of carbon and nitrogen sources on EPS production

The EPS production level was found to be higher in strains NCDC 382 and UBLP-40 when the optimized levels of their carbon and nitrogen source were added (Table 3). By the synergistic effect of sucrose and beef extract at 25 g/L, the EPS yield was 146.81±10.03 mg/L in NCDC 382. In strain UBLP-40, the EPS yield was 138.21±8.10 mg/L EPS by the combined effect of sucrose at

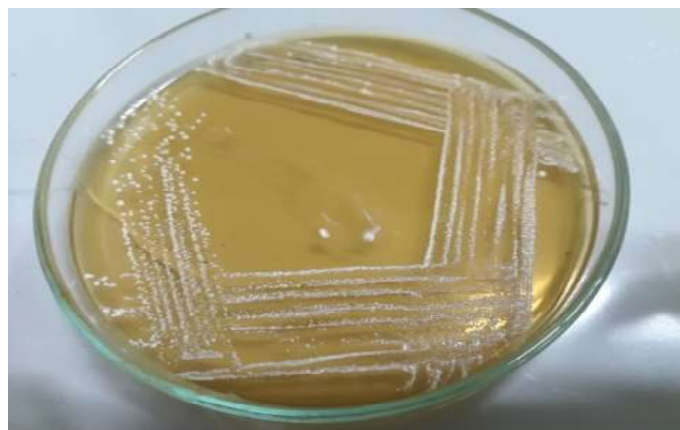


Fig.2. Appearance of slimy colonies on MRS agar

25 g/L and beef extract at 30 g/L. Thus improvement in EPS production can be achieved by the combined effect of carbon and nitrogen at their optimized levels. Ismail and Nampoothiri (2010) had also reported that highest EPS production (108 mg/L) in probiotic *Lb. plantarum* was obtained when carbon and nitrogen sources were added at their optimized levels.

FTIR-ATR characterization of EPS

FTIR-ATR spectra of purified EPS of top two EPS producing *Lb. plantarum* strains NCDC 382 and UBLP-40 were evaluated. FTIR-ATR spectrum of EPS produced by *Lb. plantarum* NCDC 382 is shown in Fig 3. For the strain NCDC 382, characteristic absorption bands appeared at 3330.96 cm⁻¹, 2840 cm⁻¹, 1645.77 cm⁻¹, 1410.49 cm⁻¹ and 1014 cm⁻¹. A broad stretching in the region 3330.96 cm⁻¹ represents the stretching vibration of the hydroxyl groups of carbohydrate. A sharp absorption band at 2840 cm⁻¹ represents

Table 2. EPS yield of *Lb. plantarum* cultures with different levels of supplementation of yeast extract and beef extract

<i>Lb. plantarum</i> strains	EPS YIELD (mg/L)					
	Yeast extract			Beef extract		
	20g/L	25g/L	30g/L	20g/L	25g/L	30g/L
NCDC 378	5.33±1.62 ^a	24.88±2.07 ^b	41.08±2.13 ^{bc}	48.83±10.04 ^{cd}	60.55±1.84 ^d	67.68±8.10 ^d
NCDC 373	62.72±5.08 ^d	39.73±5.67 ^c	17.65±2.81 ^a	35.55±2.95 ^{bc}	53.97±1.49 ^d	26.43±2.85 ^{ab}
NCDC 377	41.63±2.16 ^{ab}	40.80±1.21 ^{ab}	33.00±7.09 ^a	56.98±6.60 ^c	52.51±1.98 ^{bc}	34.63±2.11 ^a
NCDC 382	42.07±3.41 ^b	43.08±4.78 ^b	36.00±1.70 ^b	17.33±1.78 ^a	57.81±1.29 ^c	41.23±0.99 ^b
UBLP-40	30.65±2.94 ^b	42.98±2.62 ^c	15.42±1.70 ^a	28.08±2.68 ^b	32.33±1.33 ^b	66.60±3.65 ^d
NCDC 20	29.13±1.45 ^a	32.85±4.01 ^a	42.93±3.05 ^{ab}	37.97±5.86 ^{ab}	49.98±6.67 ^{bc}	69.03±6.43 ^d

Means bearing different superscript within the same row differ significantly.

Means are averages of six replications.

Table 3 Mean EPS yield of *Lb. plantarum* cultures supplemented with carbon and nitrogen source and Mean prebiotic activity score of EPS (Mean± SE)

<i>Lb. plantarum</i>	EPS yield (mg/L)	Prebiotic activity
NCDC 382	146.81±10.03	0.21±0.11
UBLP-40	138.21±8.10	0.41±0.61

Means are averages of six replications

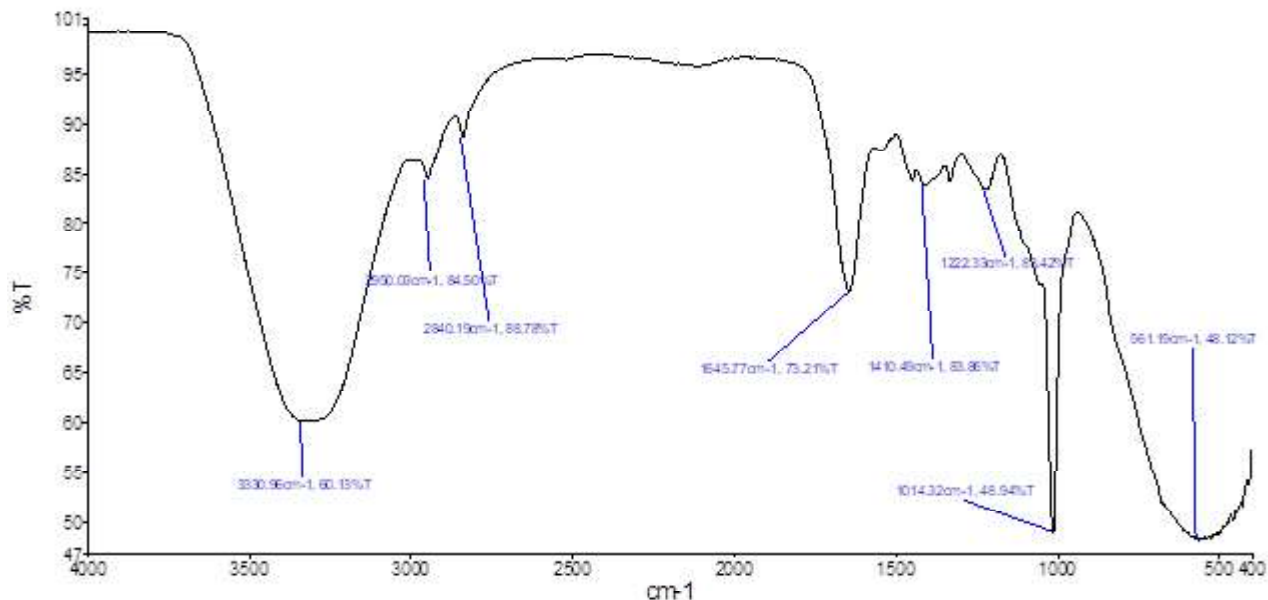


Fig.3. Absorption peaks of the EPS produced by *Lb. plantarum* NCDC 382

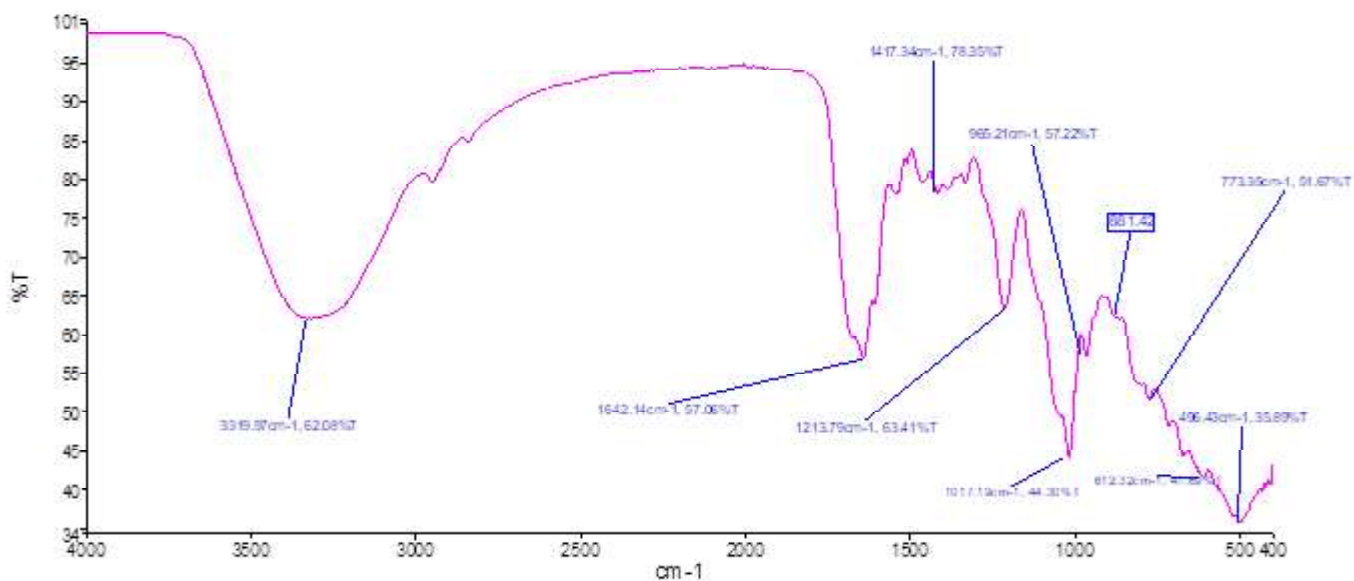


Fig.4. Absorption peaks of the EPS produced by *Lb. plantarum* UBLP-40

methyl group. The absorption peaks at 1645.77 cm⁻¹ and 1014 cm⁻¹ represent single carbon bond and carbonyl group respectively which are the typical absorption peaks of a polysaccharide (Imran et al. 2016).

Intense absorption peaks at around 3319.97 cm⁻¹, 1642.14 cm⁻¹, 1213.79 cm⁻¹, 1017.19 cm⁻¹ and 881.42 cm⁻¹ were observed in *Lb. plantarum* UBLP-40 strain (Fig 4). A broad stretching in the region of 3319.97 cm⁻¹ represents the stretching vibration of the hydroxyl groups of carbohydrate (Wang et al. 2010). The absorption band at 1213.79 cm⁻¹ usually represents the stretching vibrations of carboxylic acids, esters and ethers. Braissant et al. (2009) reported that peak at 898 cm⁻¹ could be attributed to the B-glycoside

linkage between sugar monomers. The absorption peaks at 1642.14 cm⁻¹ and 1017.19 cm⁻¹ represent single carbon bond and carbonyl group respectively. Thus FTIR-ATR spectrum revealed that the EPS of strain NCDC 382 and UBLP-40 are complex polysaccharides containing different functional groups. The presence of β -linkages in strain UBLP-40 also had an influence on the prebiotic potential of substrate.

Prebiotic activity of EPS

Prebiotic activity was found out by determining the growth rate of LAB and growth rate of *E. coli*, by Ultraviolet Visible (UV-Vis)

Spectrophotometer. The mean prebiotic activity score of NCDC strain 382 and UBLP-40 were 0.21 ± 0.11 and 0.41 ± 0.16 respectively (Table 3). Prebiotic activity score of both the strains tested were high as they showed high growth rate compared to the growth rate of *E. coli*. This suggests that the EPS produced by both strains of *Lb. plantarum* can be used as prebiotics. The strain UBLP-40 showed higher prebiotic activity score when compared to strain NCDC 382. This may be due to the presence of certain β -linkages in EPS of UBLP-40 strain and their accessibility to glycolytic enzymes that could favour the growth of Bifidobacterium in the gastrointestinal tract (Grosu-Tudor et al. 2013).

Conclusions

The presented results provide important insight on the production of EPS from different strains of *Lb. plantarum* and the culture conditions affecting their production. Optimization of growth conditions resulted in an impressive increase in EPS production in the strains tested. Chemical characterization of EPS by FTIR-ATR analysis revealed prominent functional groups corresponding to polysaccharide. The isolated EPS also showed significant prebiotic activity.

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RESEARCH ARTICLE

Association of genetic variants of forebrain embryonic zinc finger-like (*FEZL*) gene exon 3 with clinical mastitis in Murrah buffaloes (*Bubalus bubalis*)

Bharat Kumar, Archana Verma and Ishwar Dayal Gupta

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Abstract: Forebrain embryonic zinc finger-like (*FEZL*) gene is an important candidate gene, which affects the host disease resistance. The gene has been mapped on BTA22 and consists of 6 exons and 5 introns spanning over 4685 bp. Present investigation was carried out with an aim to identify genetic polymorphism within exon 3 of *FEZL* gene and its association with incidence of clinical mastitis in Murrah buffaloes. Genomic DNA extracted from 110 Murrah buffaloes were amplified using two sets of primers and respective PCR products of 554 bp and 474 bp were obtained. A total of 4 nucleotide sequence variations in exon 3 were observed. Two SNPs g.1556C>T and g.1707G>A were non synonymous, which resulted in amino acid substitution of isoleucine to threonine and methionine to valine at protein position 31 and 83, respectively. PCR-RFLP using *MspI* for primer 3.1 and *DraI* for primer 3.2 revealed monomorphic patterns, while *TaqI* for primer 3.1 exhibited polymorphism with CC, CT and TT genotypes with respective frequencies of 0.35, 0.21 and 0.44. Association of identified genotypes with clinical mastitis has been found to be significant ($p < 0.01$). Odds Ratio (OR) analysis confirmed significant association (OR=6.8531; 95% CI) and TT genotype of SNP g.1556C>T of *FEZL* gene emerged as the most favoured and may be used as a potential marker for selecting

Murrah buffaloes less susceptible to mastitis, after validation in a larger herd.

Keywords: *FEZL*, Genetic variants, Mastitis, Murrah buffalo, SNPs

Introduction

Mastitis, an inflammatory disease of the mammary gland, is generally caused by invading pathogens resulting in intramammary infections. It is the most devastating disease of dairy animals as well as dairy industry, due to treatment cost, reduction in milk production and milk quality, discarding milk and culling of the affected animals (Aitken et al. 2011). Generally, genetic variation in complex traits is controlled by a large number of loci with small to moderate effects (Kemper and Goddard, 2012). Moreover, breeders' focus has always been towards selection of animals for more milk production, which results in an increase in the mastitis incidences. Considering animal to animal variation in the occurrence of mastitis under same management system, role of genetics towards resistance/susceptibility to this disease is imperative (Rupp and Boichard, 2003). Consequently, search for potential genetic markers associated with udder health was attempted. Therefore, to decrease the incidence of mastitis and increase milk production of dairy animals, genetic variants in the candidate genes may be identified and included in marker-assisted selection (MAS) breeding programs (Loor et al. 2011; Pighetti and Elliott, 2011).

Mastitis causing bacteria invade the mammary gland, and produce harmful substances resulting in inflammation. In response to pathogen infiltration, the vertebrate immune system has evolved multiple defense systems, which can be broadly classified into innate and adaptive immunity, to repel and kill the invasive microbe. The innate immune response exists in all multicellular organisms and it is mediated by the physical barrier of the teat end, immunocytes such as macrophages, neutrophils, natural killer (NK) cells, and certain soluble factors (Sordillo and Streicher, 2002). The immunocytes have receptors called Pattern Recognition Receptors (PRRs), which recognize specific Pathogen Associated Molecular Patterns (PAMPs). The interaction between PRR and PAMP stimulates extra cellular

Animal Genetics and Breeding Division
ICAR-National Dairy Research Institute, Karnal-132 001, India

Archana Verma (✉)
Animal Genetics and Breeding Division
ICAR-National Dairy Research Institute
Karnal-132001, India
Email: archana.ndri@gmail.com

Tel: (O): 91-184-2259105; (M): 09416079659

complement pathway as well as intracellular signaling pathways culminating in inflammatory responses. The major PRRs involved in intracellular signaling pathways are Toll-like receptors (TLRs) and Nucleotide Oligomerization Domains (NODs). NODs are a family of cytosolic proteins that has been implicated in innate recognition of bacteria and host defense (Matteo et al. 2017). Forebrain embryonic zinc finger-like (FEZL) protein is an important member of this domain, which is involved in transcriptional regulation of neuronal development. The structure of C2H2 type zinc-finger proteins is maintained and coordinated by zinc ions and contain two cysteines in one chain and two histidine in the other chain. Crystallographic studies revealed that classical zinc-finger domains have two β -sheets and one α -helix. It has been established that there exists crosstalk between neuronal development and immunity via downstream cytokine expression. Zinc fingers act as regulators of T helper cell differentiation, cytokines, cytokine receptors, and other components of cytokine signaling pathways (Powell et al. 2019), inhibit the replication and expression of certain viruses, promote viral degradation (Ishida, 2020) and exhibit immunomodulatory function through activation of T lymphocyte differentiation, which provide defense against invading pathogens (Zhu et al. 2020).

Mastitis induces FEZL expression in mammary glands resulting in increased expression of the axon-attracting molecule semaphorin 5A (SEMA5A) which in turn induces expression of TNF- α and IL-8 (Sugimoto et al. 2006; Dettileux, 2009; Fonseca et al. 2011). Susceptibility to mastitis results from an impaired immune response due to the lower transcriptional activity of the gene. Thus, FEZL has been identified as one of the QTLs influencing mastitis in dairy animals (Ogorevc et al. 2009). Bovine FEZL gene has been mapped on BTA 22, spans over 4685 bp comprising of six exons, only four of which are coding with 459 amino acids long polypeptide (Sugimoto et al. 2006). Present study aimed to identify genetic variants in exon 3 of FEZL gene and their association with clinical mastitis in Murrah buffaloes.

Materials and Methods

Selection of animals, blood collection and genomic DNA isolation

A total of 110 Murrah buffaloes maintained at Livestock Research Center, ICAR-National Dairy Research Institute, Karnal were included in present study. Blood samples were collected from each animal by jugular vein puncture in a sterile vacutainer (Beckton-Dickinson vacutainer) containing 0.5% EDTA solution (10 μ l/ml of blood), which was duly approved by Institute Animal Ethics Committee (IAEC). Genomic DNA was isolated by phenol-chloroform method described by Sambrook and Russell (2001). Data regarding incidence of clinical mastitis were recorded from the treatment register of Animal Health Complex and depending on the incidence of clinical mastitis, animals were categorized as healthy and mastitis affected. Cows which never suffered from mastitis up to first four lactations were classified as healthy, while

those animals who suffered from mastitis were included in the affected category.

PCR and sequencing

Two sets of primers targeting partial intron 2 and exon 3 of FEZL gene were designed using Primer 3 software and synthesized from Eurofins. Polymerase chain reaction (PCR) was carried out in 25 μ l of reaction mixture containing 20 pmole of each primer, 200 μ M dNTP, 1 unit of *Taq* polymerase, 10X PCR buffer and 50 ng of genomic DNA. PCR amplification was performed in a programmable thermal cycler (MJ Research, PTC 200) under the following conditions: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 55°C and 53°C respectively for both the primers for 30s, extension at 72°C for 40s and final extension at 72°C for 10 min and cooling at 4°C. The PCR products were loaded on 1.0% agarose gel in 1xTBE along with 100 bp DNA marker and ethidium bromide (1 μ l/100 ml) staining and visualized, size estimated and documented under Gel Documentation system (Minibis). A single sharp band confirmed proper amplification of the target DNA. PCR products were custom sequenced with both forward and reverse primers through 1st BASE sequencing INT, Malaysia. The edited DNA sequences were aligned using ClustalW software and the SNPs were identified. Nucleotide sequences were translated using ExpASY translate tool and amino acid substitutions were analysed for their effect on protein function using SIFT software.

PCR-RFLP

Restriction enzymes were selected using NEBcutter V2.0. and the amplicons of 554bp and 474bp were subjected to restriction digestion to identify polymorphism. The RE digestion was set up with 6 units of enzyme, 6 μ l PCR product, 1.5 μ l buffer and milliQ water to make up the reaction volume to 10 μ l and the mixture was incubated for 6 hours at 37°C for *Msp*I and *Dra*I; 65°C for *Taq*I. The restriction fragments were resolved on 2.5% agarose gel with 100 bp DNA ladder, visualized in UV transilluminator to screen for respective restriction bands. Genotype patterns were assessed and photographed with gel documentation system.

Statistical Analysis

The genotype and allele frequencies were calculated by simple scoring of homozygotes and heterozygotes. Population genetic indices *viz.* effective allele number (N_e) to estimate reciprocal of homozygosity, Shannon Index (I) as a measure of gene diversity and PIC (Polymorphism Information Content) were estimated using POPGENE v 1.3.2 (population genetic analysis tool). The Odds Ratio (OR) between mastitis affected and non-affected genotypes were calculated.

Results and Discussion

SNP genotypes and population genetic indices

Complete exon 3 of bubaline FEZL gene was amplified using two sets of forward and reverse primers. Amplicons of 554bp covering nt.1387..1940 and 474bp covering nt.1843..2316 of FEZL gene were obtained (Table 1). Nucleotide sequence alignment resulted in 4 SNPs in complete exon 3. Two SNPs: g.1556C>T and g.1707A>G are non-synonymous types of mutations resulted in amino acids substitution of isoleucine to threonine and methionine to valine at p.31 and p.83 respectively, while g.1940C>T and g.1997G>T were synonymous type of mutations. SIFT software indicated p.Met83Val substitution was tolerated, while AA substitution p.Ile31Thr affect the protein function (deleterious) (Table 2). This amino acid replacement potentially influences neutrophil function through ligand response modification and disease resistance (Lee et al. 2006).

PCR-RFLP was carried out using MspI and TaqI for 554 bp amplified fragment and DraI for 474 bp amplicon. Exon 3.1-TaqI at locus g.1556C>T exhibited polymorphism with CC, AT and TT genotypes, while monomorphism was revealed by other two amplicon-RE combinations. Frequencies of observed genotypes for the g.1556C>T polymorphisms in the mastitis affected and healthy buffaloes CC, TT, and CT were 0.51, 0.24, 0.25, and 0.20, 0.64, 0.16 respectively. The restriction fragment sizes, overall allele and genotype frequencies, population genetic indices are shown in Table 3. Genotypic and allelic frequencies were greater than 0.1, thus qualifying for association analysis. PIC, He and Ne are the indices of evaluating the genetic variation in population since higher the values of PIC and He, greater is the genetic variation. Effective Allele Number (Ne) which estimates the reciprocal of homozygosity and Shannon index (I), estimates gene diversity. Since Ne value of detected alleles is closer to the absolute number, population distribution of the allele is more uniform and moderately polymorphic as evident by PIC (0.3785). There is no similar report

Table 1 Primer sequences, their annealing temperatures and PCR products of FEZL gene in Murrah buffaloes

Gene Region	Targeted Region	Primer Sequence (5'...3')	Annealing temperature (°C)	Product size (bp)
Exon 3-1	1387..1940	F:CTT TTC CGG AAT GCAGAC TT	55	554
	Partial intron 2..partial exon 3	R: CACAGC CTG GTT GAT GAC C		
Exon 3-2	1843..2316	F:AAACCAACT GTG GCG TGT G	53	474
	Partial exon 3	R: TCACCT TTC CGCACACCT		

Table 2 Nucleotide changes and amino acid substitution in exon 3 of FEZL gene in Murrah buffaloes

S.No.	Nucleotide position	Gene Region	Type of Mutation	Amino Acid Substitution	SIFT* Prediction
1	g.1556C>T	Exon 3	Non-Synonymous	p.Ile31Thr	Affects protein function
2	g.1707G>A		Non-Synonymous	p.Met83Val	Tolerated
3	g.1940C>T		Synonymous	p.Ala160Ala	-
4	g.1997G>T		Synonymous	p.Thr179Thr	-

Table 3 Overall genotypes, allele frequencies and genetic indices of FEZL loci in Murrah buffaloes

Exon-RE combination	Genotype/ allele size (bp)	Frequency	Allele Frequency	Ne	I	PIC	χ ²
Exon 3.1- <i>MspI</i>	CC: —	0.00	C	0.00	-	-	-
	CG:—	0.00	G	1.00			
	GG: 554	1.00 (110)					
Exon 3.1- <i>TaqI</i>	CC: 554	0.35 (39)	C	0.459	1.9963	0.6913	0.3785
	CT: 170, 384, 554	0.21 (23)	T	0.541			0.003*
	TT: 170, 384	0.44 (48)					
Exon 3.2- <i>DraI</i>	AA: —	0.00	A	0.00	-	-	-
	AT: —	0.00	T	1.00			
	TT: 474	1.00 (110)					

Number of animals exhibiting a particular genotype has been indicated in parenthesis

*Significant (P ≤ 0.01)

Ne =Effective number of alleles; I = Shannon’s information index;

PIC = Polymorphic Information Content

Table 4 Association of identified genotypes with incidence of clinical mastitis in Murrah buffaloes

Locus	Genotype frequency		P value	Odds ratio (CI=95%)
	Genotype	Mastitic(n=55)		
Exon 3.1- <i>TaqI</i>	CC	28	11	6.8531 [2.665-17.619]
	CT	14	09	4.1880 [1.463-11.991]
	TT	13	35	1

available in literature to compare and contrast the findings of present research on buffaloes. However, Dubey et al. (2014) observed monomorphic RFLP patterns using *EaeI* and *MspI* restriction enzymes for 978 bp fragment covering intron 3 to intron 5 including exons 4 and 5 regions of FEZL gene in 20 healthy and 20 mastitis affected Murrah buffaloes. Ali et al. (2019) reported three SSCP patterns in 229bp amplicon with highest frequency: 0.8625 and 0.923 of A allele in mastitic and healthy cows respectively, while frequency of allele B was least (0.0125) in mastitic and allele C (0.014) in healthy animals. They also reported that the population was not in HW equilibrium, suggesting selective advantage of G locus of FEZL gene. Sugimoto et al. (2006) also observed three genotypes with the highest (0.894) frequency of homozygotes in Holstein cattle.

Association of genotypes with incidence of clinical mastitis

Statistical analysis was carried out to find out association between genotypes of identified SNPs. Among the identified genotypes, animals with TT genotypes for SNP g.1556C>T showed significant association with mastitis. Odds Ratio analysis revealed strong association (OR=6.851, 95% CI=2.665-17.619) indicating that animals with CC genotype are at higher risk for mastitis, while those with TT genotype exhibit least susceptibility to the disease (Table 4). There are no reports regarding association with FEZL genotypes with mastitis in buffaloes, however, Sugimoto et al. (2006; 2013) reported significant association of 12G/13G polymorphism with SCC and mastitis in Japanese cattle. Ali et al. (2019) reported significant association of CC genotype having highest mean somatic cell counts (674.92±48.1) compared to AB genotypes (226.16±4.9) with susceptibility to mastitis in Egyptian Holstein cattle, allele B was least frequent in mastitic animals, and allele C had the least frequency in normal animals. Moreover, the presence of allele (B) is associated with lower SCC while the presence of allele (C) is associated with higher SCC indicative of higher susceptibility to mastitis. Fonseca et al. (2011) studied expression of mastitis-related genes, however, they did not find significant expression of FEZL gene in mastitis affected Holstein crossbred animals. Sugimoto et al. (2006) and Ali et al. (2019) reported increased expression of FEZL and SEMA5A genes in mastitic than healthy cows, due to enhanced antimicrobial/immunomodulatory activity of FEZL, facilitating migration of neutrophils through induced production of interleukin8 by TNF- α , which is effective for clearing infecting pathogens from host

tissue (Sugimoto et al. 2006; Zhu et al. 2020). This further supports the role of FEZL as a potential genetic marker for mastitis resistance/susceptibility.

Conclusions

This is the first report on identification of SNPs/genetic variants of FEZL gene associated with incidence of clinical mastitis in buffaloes. It is evident from the findings of the present research that TT genotype of SNP g.1556C> in FEZL gene is a potential genetic marker for mastitis resistance, which could be exploited for marker-assisted selection (MAS) program aimed to improve mastitis resistance in buffaloes, after validation in larger population.

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Development of a mobile application to control Brucellosis and its effect in Knowledge gain among the commercial dairy farmers of Northern India

Arjun Prasad Verma¹, Hans Ram Meena², Diksha Patel³, Manish Sawant⁴ and BS Meena⁵

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Abstract: Quick and timely dissemination of technological information from the agricultural research system to grassroots level and farmers' reaction in the form of feedback to the research system is one of the most critical inputs in technology transfer. Information and Communication Technology plays an important and crucial role where its potential can be exploited to strengthen the linkage amongst research and extension system. In this context, mobile application was developed on brucellosis prevention in dairy animals and it was tested for its effectiveness in terms of knowledge gain by the commercial dairy farmers (n=60) from six district of Haryana and Punjab states. The study revealed that, the overall mean knowledge gain on Brucellosis in dairy animals through mobile application was effective ($p < 0.01$) in terms of knowledge gain among the commercial dairy farmers (17.65). Pearson's correlation analysis of socio-demographic profile with knowledge gain revealed that knowledge gain through Mobile application on brucellosis was positively and significantly correlated with education, landholding, income, herd size, milk production and experience in dairy farming at 5 % level of significance. It is evident from this study that Information Communication Technologies plays significant role in knowledge gain and empowerment of dairy farmers and hence still more institutional intervention, support and innovation is required in

developing such technology transfer mechanisms in developing countries like India.

Keywords: Dairy farmer Effectiveness, Empowerment, Knowledge, Mobile Apps

Introduction

Animal husbandry is an integral component of Indian agriculture supporting livelihood of more than two-thirds of the rural population. India possesses rapid growth in animal husbandry sector to attain self-sufficiency in the production of livestock products. Livestock has considerable potential for generating additional income as well as employment through various sectors. Dairy farmers' rarely apply scientific technology for management of their farm animals. Brucellosis being one of the highly contagious ubiquitous reproductive diseases harms not only dairy animals but also pertains risk to public health and highly prevalent among bovine population of the country (Patel et al. 2014). Serological study indicates the high rate of Brucellosis on dairy farms in Haryana and Punjab with an overall herd prevalence of 65.54 per cent. It's state-wise herd prevalence was 62.79 per cent in Haryana and 72.72 per cent in Punjab (Chand and Chhabra, 2013). The livelihood security of the dairy farmers can be increased further by enhancing their access to information on brucellosis. Quick and timely dissemination of technological information on brucellosis from the livestock research system to end users and feedback to the research system are utmost important for the transfer of animal husbandry technology (Sasidhar and Sharma, 2006). Innovative information and communication technologies when applied to conditions in rural areas can help to improve communication, increases participation, and disseminate information and share knowledge and skills. The use of such technologies makes extension process faster, efficient and more effective. Improved communication and information access is directly related to social and economic development (Meena and Singh, 2013). The growth and spread of new information and communication technology in rural India in recent years provide a viable alternative to overcome the physical barriers of face to face inter-personal communication. Dissemination of knowledge on improved practice through appropriate delivery methods plays an important role and it can

¹ Krishi Vigyan Kendra, Jhansi; Banda University of Agriculture & Technology, Banda-210 001 (Uttar Pradesh), India

^{2& 5} Dairy Extension Division, ICAR-NDRI, Karnal-132001 (Haryana), India

³ Krishi Vigyan Kendra, Banda University of Agriculture & Technology, Banda-210 001 (Uttar Pradesh), India

⁴ Department of Veterinary and Animal Husbandry Extension, Bombay Veterinary College, (MAFSU) Parel, Mumbai -400 012, India

Arjun Prasad Verma (✉)
Krishi Vigyan Kendra, Jhansi; Banda University of Agriculture & Technology, Banda-210 001 (Uttar Pradesh), India
Email: arjunverma45753@gmail.com

be multiplied manifold by modern ICT applications such as mobile application. Information provided through modern information technology gadgets should be client oriented, needbased, demand-driven, site-specific, user-friendly and should be in local language (Tiwari et al. 2010). In this context, mobile application (Brucellosis Advisor Apps) on brucellosis prevention in dairy animals was developed and its effectiveness in enhancing knowledge among the commercial dairy farmers was analysed.

Materials and Methods

The present study was carried out in six districts of Haryana and Punjab state of India. From Haryana, three different districts viz. Hisar, Sirsa and Karnal and from Punjab states, three districts viz. Ferozpur, Gurdaspur and Ludhiana were selected based on highest cattle population. From each district 20 commercial dairy farmers were selected on the basis of snowball sampling method. Total of 120 commercial dairy farmers were selected for the present study. Out of 120 respondents, 60 commercial dairy farmers were selected to assess the effectiveness of Mobile Application (Bilingual i.e. Hindi & English) in knowledge gain on Brucellosis prevention in dairy animals. The study had followed the experimental research design i.e. Before-and-After without control design. That is the commercial dairy farmers were subjected to well-developed knowledge test in order to ascertain the pre exposure knowledge score and subsequently exposed to the mobile application on brucellosis prevention in dairy animals and then reassessed them for the gain on knowledge regarding the brucellosis in dairy animals.

Statistical Analysis

The knowledge test was developed on brucellosis and was assessed among the commercial dairy farmers through exposure to the developed mobile application. The knowledge levels of the subjects before and immediately after exposure were assessed to find out the knowledge gain on brucellosis in dairy animals. The gain in knowledge on brucellosis was taken as an indicator for the effectiveness of the mobile application in knowledge gain. The mean gain in knowledge also expressed in terms of percentage of knowledge gain. In order to test effectiveness of the developed mobile application statistically, 'z' test was applied to find out, whether there existed any significant difference between the pre-exposure and immediate post-exposure knowledge due to the treatment i.e. exposure to the mobile application.

Results and Discussion

Development of mobile application on brucellosis prevention in dairy animals

Mobile application was operationalized as "the act or process by which application software is developed for mobile devices which delivered as mobile application using server-side or client-side

processing to provide an "application-like" experience for a mobile user. Through mobile apps subject matter content (Prevention of Brucellosis) through text and images transferred to the commercial dairy farmers. It helps to educate the user about a technology and can be stored and operated from any type of android smart phone and can be downloaded through Google play store or by sending through Shareit, Xender apps or by sending APK link through SMS which can be installed in smart phone. Once downloaded there is no need of internet to run the application. The components of mobile apps have been broadly classified in to two major parts viz. technical component and subject matter component.

Technical part

Technical component was done by Outline systems, a private firm from Chandigarh who developed the mobile application.

Subject matter

Information on Brucellosis had been taken as the subject matter (Table 1) to develop the Mobile application. In apps there were 7 components and were presented in the following table.

Validation of mobile application on brucellosis prevention in dairy animals

Validation of Mobile Application is the extent to which it reflects a specific domain of content. Expert in the concerned field were asked to rate the relevance and appropriateness of the Mobile Application on Brucellosis prevention in Dairy animals. The findings revealed (Table 3) that the experts were highly satisfied (100%) with the characteristics of Mobile Application like relevancy of Application, accuracy and appropriateness of the health information, simplicity of content in enhancing knowledge on brucellosis, suitability of Mobile Application to field condition and easy in obtaining desired information on brucellosis. The experts also indicated that there was scope of improvement in certain features of the Mobile Application i.e. navigation in getting information and gestural design of application, quality of photograph used in Application (Apps), engagement of the users and aesthetic value.

Socio-demographic profile of respondents

The findings revealed (Table 4) that 45.83 percent of the respondents belonged to young age category that ranges up to 35 year of age. About one third (34.16%) of the respondents had higher secondary education, an equal number of the respondents (39.17%) were having small and large family size, majority of the respondents (54.17%) had medium experience (8 to 15 years) in commercial dairy farming. Almost half of the respondents (44.17%) have medium land holdings followed semi-medium, large and small land-holding i.e. 27.50, 20.00 and 5.83 percent, respectively. Majority (72.50%) of the respondents were engaged in dairy with

crop farming. The average annual income of commercial dairy farmers was Rs. 1060000 per annum. About 41.67 percent of the respondents were rearing medium herd size (33 to 59 animals), and possess 54.74, 34.74 and 10.52 percent crossbred cow, buffalo and indigenous cow as major composition of herd size in the study area, respectively. A perusal of Table 4 revealed that 38.33 percent of the respondent's comes under low production category (<123.92 litres), and cent percent of the possessed and used mobile and television for obtaining information in dairying. The findings

of the study are in line with the study of Obonyo and Gufu (2015) and Rathod et al. (2016).

Effectiveness of mobile application in knowledge gain of commercial dairy farmers on brucellosis

The results revealed (Table 5) that before exposure to Mobile application, mean knowledge scores was 5.38. The mean knowledge gain scores after fifteen days exposure was 23.03.

Table 1 Subject matter component of mobile application

Sl. No	Components	Content
1	About brucellosis	It includes information about brucellosis, alternative name, its causal organism, host species and losses due to brucellosis in dairy animals.
2	Mode of transmission	It covers the information through which brucellosis transmitted in other animals.
3	Sign and symptoms	It includes different types of clinical syndromes occur due to brucellosis to make dairy farmers aware.
4	Prevention and control	Preventive and control measures of brucellosis to be followed by dairy farmer in his or her farm for earning more profit.
5	Vaccination	It includes information on right stage of vaccination, name of vaccine, source of vaccine and precaution in storage of vaccine etc.
6	Ask an expert	It includes contact details of different institution for detailed information on brucellosis.
7	FAQs	Commonly asked questions along with their answers in simple language were added in FAQ component.

Table 2 Supporting components of mobile application

S. No	Components	Description and Uses
1	Home	7 components in which text in particular language along with images are present.
2	Change language	In this the farmer can change language at ease. Two languages are present in apps viz., Hindi and English
3	About app	This page reveals the importance of mobile apps to different stakeholder involved in dairying
4	Team	Names of the advisory committee member who guided the researcher in development of the apps.
5	Acknowledgement	Acknowledgement is given to ICAR-NDRI, Hon. Director & VC for providing grants and guidance for development of mobile apps on Brucellosis prevention in Dairy animals.
6	Website link	It the address of institution for further enquiry about Brucellosis.
7	Log out	After getting knowledge regarding Brucellosis, farmer can exit the app by logging out.

Table 3 Validation of mobile application on brucellosis prevention in dairy animals (n=30)

S. No.	Characteristics for validation	Weighted mean score (%)
1	Relevancy of Mobile Application on Brucellosis prevention in dairy animals	100
2	Accuracy and appropriateness of the health information in Mobile application	100
3	Simplicity of content in enhancing knowledge on brucellosis	100
4	Navigation in getting information and gestural design of application	98.33
5	Quality of photograph used in Application (APK)	98.00
6	Suitability of Mobile Application to field condition	100
7	Easy in obtaining desired information on brucellosis	100
8	Mobile application provide good engagement to the users	97.22
9	Mobile application having good aesthetic value	96.33

The mean gain in knowledge scores was 17.65. In term of percentage, before exposure of Mobile application knowledge of commercial dairy farmers was 13.13 percent and after exposure of Mobile application knowledge was 56.18 percent. Overall knowledge gain among commercial dairy farmers in term of percentage was 43.05. It was also evident (Table 5) that the difference in means of pre and post-test is highly significant in term of knowledge gain on brucellosis at 0.01 levels of significance with z value 19.54 and standard error of mean 0.886. This shows that Mobile application has played a vital role in disseminating knowledge among the commercial dairy farmers. These findings were in line with Jayakumar and Manoharan (2002), Vidya et al.

Table 4 Distribution of respondents on the basis of Socio-demographic profile (n=120)

Sl. No.	Variables	Category	Frequency	Percentage
1	Age	Young (up to 35 years)	55	45.83
		Middle (36 to 50 years)	39	32.50
		Old (more than 50 years)	26	21.67
2	Education	Primary	12	10.00
		Middle	12	10.00
		Secondary	26	21.67
		Higher secondary	41	34.16
		Graduate and above	29	24.17
3	Family Size	Small (<5)	47	39.17
		Medium (5 to 7)	26	21.66
		Large (>7)	47	39.17
4	Land Holding	Marginal (<1 ha)	3	2.50
		Small (1 ha to 2 ha)	7	5.83
		Semi medium (2 ha to 4 ha)	33	27.50
		Medium (4 ha to 10 ha)	53	44.17
		Large (> 10 ha)	24	20.00
5	Occupation	Dairy Farming	11	9.17
		Dairy + Crop farming	87	72.50
		Dairy + Crop farming+ Service	13	10.83
		Dairy + Crop farming+ Business	9	7.50
6	Annual Income	Low (<8.69 lakhs)	57	47.50
		Medium (8.69 to 15.36 lakhs)	43	35.83
		High (>15.36 lakhs)	20	16.67
7	Herd Size	Small (<33 animals)	47	39.17
		Medium (33 to 59 animals)	50	41.67
		Large (>59 animals)	23	19.16
8	Experience in commercial Dairy Farming	Low experience (<8 years)	23	19.17
		Medium experience (8 to 15 years)	65	54.17
		High experience (>15 years)	32	26.66
9	Milk Production (Litre/day)	Low (<123.92)	46	38.33
		Medium (123.92 to 232.45)	41	34.17
		High (>232.45)	33	27.50
10	ICT Tools possession and utilization	Radio	76	63.33
		Mobile	120	100
		Television	120	100
		Computer	47	39.17

Table 5 Knowledge Gain among respondents due to exposure of Mobile application (n=60)

Treatment	Mean		Mean		Z value	Standard Error Mean		
	Before Exposure	%	After Exposure	%				
Mobile Application	5.38	13.13	23.03	56.18	17.65	43.05	19.54**	0.886

**Significant at 0.01 level of significance

Table 6 Correlation analysis between various factors and knowledge gain through Mobile Application (n=60)

S.No.	Independent variables	'r' value
1.	Age	0.101 ^{NS}
2.	Education	0.231*
3.	Family Size	-0.076 ^{NS}
4.	Land holding	0.213*
5.	Occupation	0.051 ^{NS}
6.	Annual Income	0.273*
7.	Herd size	0.278*
8.	Milk production	0.079 ^{NS}
9.	Experience in commercial dairy farming	0.254*
10.	ICT tool possession	-0.087 ^{NS}

(2010), and Roy and Tiwari (2014) where their respective expert system namely, Instructor Controlled Interactive Video, Educational Video-DVD on Dairy Health Management Practices, Goat Health Management Information System (GHMIS) were resulted in improvement in knowledge level after exposure. Sasikala et al. (2012) revealed significant difference with respect to mean knowledge scores and the farmers gained overall knowledge of pig farming practices about 32.56 percent. The results are in conformity with the results of Anandaraja et al. (2006), Mahmoodabad et al. (2008), Mooventhan and Philip(2012), Vanetha (2013) and Meena et al. (2014).

Pearson's correlation analysis with knowledge gain due to exposure of mobile application

The results (Table 6) revealed that knowledge gain through Mobile application on brucellosis was positively and significantly correlated with education, landholding, income, herd size, milk production and experience in dairy farming at 5 % level of significance. It was not significantly correlated with variables like age, family size, occupation, milk production and ICT tools possession. The findings of Ahmed et al. (2020) contrary to study and reveals that variables age, experience, and socioeconomic status hold an insignificant relationship with the knowledge level of dairy farmers. In the present study, since dairy farmers had higher education level this could have made to gain in knowledge about Mobile Application on brucellosis prevention as well as size of the landholding and knowledge gain on brucellosis had positive effect due to the fact that it provides the economic base for dairy farmers to practice new technologies for achieving maximum profit. The annual income of the respondents directly influences the economic viability, stability and rational behaviour of an individual. Hence, increase in the income levels related increase access to ICT tools; this might be the reason behind the gain in knowledge regarding brucellosis through Mobile Application. Dairy farmers with large herd size are more likely to obtain information from a variety of sources. With increase in experience in dairy farming, farmers got more chance to know about different disease in their animals and it might be the reason

behind the knowledge gain of the respondents on brucellosis through Mobile Application.

Conclusions

Mobile based technology dissemination was found effective among the commercial dairy farmers and it would emerge as a new paradigm in agricultural technology transfer and development of livelihood opportunities for people in the developing countries. Hence, efforts may be taken up by the extension personnel to disseminate knowledge on such aspects by using ICT tools especially mobile application to educate dairy farmers and thereby maximizing their profit. Thus, it can be concluded that mobile application not only facilitate the increased knowledge of the respondents, but also assists in the retention of the knowledge gained.

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Impact analysis of women centric technological interventions in rural dairy farming

Kuppusamy Ponnusamy¹, Parvinder Singh Oberoi¹ and Anil Kumar²

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Abstract: Women continue to lack adequate access to extension, technology, market and credit despite their active involvement in major activities of crop and dairy farming. ICAR-National Dairy Research Institute (NDRI), Karnal implemented a research project titled, “Empowerment of farm women through livestock technologies” during 2015-2017. Two women groups were formed in Pingli and Jundla villages of Karnal district, Haryana, India with 20 members in each group to facilitate the technology diffusion in dairy farming based on their identified needs, interests, preferences and level of knowledge and skill through participatory approach. The impact of interventions was assessed based on pre and post interventions by developing a set of indicators for each intervention in 2018. The findings revealed that the farm women not only gained economic returns due to introduced technologies, but also motivated fellow farm women to adopt the use of cream separator, supplementing yeast in cattle feed, mechanical lassi maker/butter churn, pouch making machine, preparation of value added dairy products for home consumption and marketing, cultivation of bajra napier for throughout the year fodder supply, cultivation of berseem fodder variety BL-42, head

load manager for carrying fodder and dung and chaff cutter with safety net. These technologies were found to significantly increase their work efficiency, reduce drudgery and contribute to additional income among the adopted families. A participatory approach and convergence of development agencies would facilitate farm women empowerment and serve as role model for fellow women in dairy farming.

Keywords: Dairying, Impact assessment, Technology interventions, Women empowerment

Introduction

Women continue to lack adequate access to technologies, market, credit, extension services and resources which are often reported to hamper the farm productivity significantly (Ponnusamy et al. 2017). The inherent knowledge and skill of women in crop and dairy farming always exist. However, they do not get proper motivation and opportunity to acquaint themselves with innovations being promoted by various development agencies. If proper avenues are created for women in accessing technology, facilities and equipments, there are tremendous chances for them to excel in scientific endeavours (IFAD, 2009) and resultant productivity enhancement would provide rich dividends in their hands which they can use for making better livelihood in their families. Government of India has given due importance to women empowerment in the country (Borkar et al. 2017). Patel et al. (2016) listed key gender women issues such as limited ownership of land and livestock, less access to capital and knowledge, lack of knowledge in processing of livestock products limited role in marketing of livestock, lack of women specific livestock related training and inadequate efforts for the capacity building of women. The role of women in dairying gains importance in view of 70 per cent of activities being handled by them. A well-organized mechanism to facilitate proper technology access to women is expected to help in better technology penetration and dissemination of innovations in dairying (Ponnusamy and Pachaiyappan, 2018). Considering these facts in view, a project titled, “Empowerment of farm women through livestock technologies” was implemented by ICAR-National Dairy Research Institute (NDRI), Karnal in two villages with an objective of capacity building of farm women through technological

¹Dairy Extension Division, ICAR-National Dairy Research Institute, Karnal, Haryana, Pin 132 001, India

² ICAR-Central Institute for Women in Agriculture, Bhubaneswar, Odisha, Pin 751 003, India

Kuppusamy Ponnusamy (✉)
Dairy Extension Division, ICAR-National Dairy Research Institute,
Karnal, Haryana, Pin 132 001, India
Email: ponnusamyk@hotmail.com

interventions. This collaborative project was funded by Central Institute for Women in Agriculture (CIWA), Bhubaneswar.

Materials and Methods

The project titled “Empowerment of farm women through livestock technologies” was implemented in Pingli and Jundla villages of Karnal district in Haryana during 2015-17. The initial rapport was built with village elders and key resource persons to understand the prevailing farming and socio-economic scenario in both the villages. Based on their inputs, two farm women groups with 20 members each were formed in both the villages. The age of group members ranged between 25 to 50 in both the villages. By deploying Participatory Rural Appraisal (PRA) techniques such as transect walk, social map, technology adoption matrix and problem rankings; various issues being faced by women in dairying were documented. The major identified issues were lack of knowledge on preparation of farm made feeds, landless farmers not having their own fodder production, gap in adoption of important scientific management practices, lack of knowledge in scientific and hygienic production of milk and milk products and lack of awareness on government schemes. A set of technologies was identified to address the issues which affect the dairying especially the farm women who were involved in dairy farming based on the base-line information collected. These technologies include complete feed preparation for dairy animals; use of minerals mixture for improving reproductive performance of dairy animals & milk production; early mastitis detection techniques using California Mastitis Kit; fodder crop (Bajra napier grass) for scarcity months; long duration Berseem variety (BL42) for more cuts; chaff cutter with safety lock (Blade Guard, Flywheel Lock, Warning Roller); urea molasses mineral block making by hand operated machine; silage making in bags for fodder conservation at a smaller scale; introducing stool while milking to improve milker’s comfort, Serrated Sickle with comfortable handle (CIAE Bhopal tool) for fodder harvesting, Head load manager, Dung scraper, Straw Hoe, Animal washing brush for increasing dairy workers comfort; scientific techniques for traditional Milk product preparation, dairy product making and packing using simple machines “electric butter churner, cream separator, lassi making machines and pouch sealing machines.

Later, farm women members were given training and demonstration on various aspects of dairy farming technologies as well as exposure visit to NDRI. Two veterinary camps were organized to identify and treat major ailments in dairy animals. The critical inputs were supplied to women group members who in turn were motivated to share their experiences of technological interventions with fellow women in the villages. The impact of interventions which were carried out in the adopted villages during 2015-2017 was documented in 2018 with participation of 40 women through PRA techniques in both the villages. The opinion on both qualitative and quantitative parameters was

gathered on the basis of demonstration of proven technologies and their subsequent adoption by farm women.

Results and Discussion

The results pertaining to nine technological interventions which were implemented through participatory mode are furnished here under:

Cream separator

Cream separator is used for preparing cream as well as skim milk. Farm women were manually preparing as well as purchasing cream and skim milk from the market before the introduction of cream separator machine by NDRI among them.

The difference between manually prepared/ purchased cream and skim milk from market and prepared cream and skim milk using mechanical cream separator supplied by NDRI is presented in Table 1. The cream separator supplied by NDRI was found to be relatively more lucrative for the farm women as they could prepare cream and skim milk at a low cost by using the same and got more quantity of skim milk and cream. The quality, colour appearance, consistency, flavour and fat & SNF percentage of both cream and skim milk prepared using cream separator supplied by NDRI were better than the purchased and manual products (cream and skim milk). A team of dairy science experts while visiting the village for evaluation of dairy products prepared by women expressed satisfaction about the quality of products. History of market purchased products is difficult to assess from quality and freshness point of view. The milk products which were prepared at household using sanitary and hygienic protocols were perceived to be free from adulterants, fresh and good for health. They also could save time and money by using this NDRI intervention. This indicates that when women are exposed to improved appliances, the acceptability and adoption could be enhanced due to simplicity in usage and cost affordability.

Yeast supplementation to dairy animals

Farm women were not using yeast for addressing heat stress in their dairy animals as they were earlier not aware of this technique. They were exposed to the use of yeast through demonstration and training.

Heat stress in dairy animal during summer months can cause substantial drop in milk production due to lower dry matter intake and feeding of poor quality of roughages having more lignified fibres and higher heat of fermentation (Ponnusamy *et al*, 2019). By assessing visible indicators such as rumination, locomotion or undigested grains in dung, producers can better anticipate heat stress issues and make the necessary adjustments before dairy production starts falling.

Among the nutritional measures shown to alleviate heat stress, the use of the rumen-specific live yeast *Saccharomyces cerevisiae* shows positive effects on the rumen environment and consequently on milk production. The rumen-specific live yeast acts as a rumen modifier. It works by improving overall rumen environment and function through the control of rumen pH. The inclusion of yeast in ration of dairy animals also helps to improve animal performance through enhanced fiber degradation. The recommended dose for large animals was 5-10 gms / day or 25-50 gms per 100 kg of feed.

Benefits of supplementation of yeast to dairy animals by the farm women are highlighted in Table 2. On an average, the milk production increased by around half liter/day. Yeast use was found to be beneficial for dairy animals (cow/buffalo) in terms of palatability, digestibility and resistant to heat stress though it had no effect on quality of milk. Farm women reported that use of yeast was also good for their animal’s physical condition i.e. body score. Further, they were motivated to maintain this practice in future as they found it very advantageous. A liaison was established with local traders for supplying yeast in times of

Table 1 Assessment of cream separator as perceived by women on its utility

Parameters	Earlier practice of preparing cream and skim milk by women in the villages by manual method	Cream and skim milk separated through mechanical cream separator supplied by NDRI
Cost of cream / kg in Rs	220-240	100-110 (because obtained skim milk Sale Rs. 30/ Liter)
Use the cream / month / kg	1	3 (also used in Ghee making)
Cost of skim milk / kg in Rs	32-34 (Purchased packed milk)	25-30 (Sales)
Quantity of skim milk kg / month	4-5	12-15
Fat percentage in cream	High	Low
SNF percentage in cream	Low	High
Fat & SNF percentage in skim milk	Variable	Adjustable
Beneficial for health	Reasonable	Good
Colour & appearance	Good	V.Good
Flavour & taste	Fair	V.Good
Consistency of cream & skim milk	Fair	V.Good
Time saving	No	Yes
Microorganism quality in both	Fair	Good
Churned butter quality and ghee quality prepared from cream	Fair	Good

Table 2 Perceived assessment of parameters on use of yeast

Parameters	Ration without yeast	Ration with supplementation of yeast
Increase in milk production (Liter)	5.4- 5.6	5.8 – 6.0
Effect on organoleptic quality of milk	Acceptable	No change
Body condition of animal	Normal	Improved
Palatability and intake of fodder	Normal	Improved
Digestive problems (Anorexia, loose faeces / diarrhea)	Frequent occurrence	Less frequent occurrence
Level of heat stress observed (panting rate)	More	Lesser

Table 3 Assessment of lassi making machine as per the perception of farm women

Parameters	Manual lassi maker/ butter churn	Purchased from market	Electronic lassi making machine/ butter churn
Cost of equipment (Rs.)	400-1000	2500	4500- 5000
Cost of lassi/ liter (Rs.)	70	100-120	125
Time taken (in minute)	30	20	10
Purity (%)	80	40-50	80
Colour & appearance of lassi	Good	Good	Very good
Consistency of lassi	Fair	Fair	Good
Flavour & taste of lassi	Good	Good	Very good

need for women group members. Results of the present study demonstrate that there was an increase in production performance in 20 lactating animals by using easily transferable simple nutritional interventions.

Mechanical lassi maker/ butter churn

Lassi making machine is useful both for making lassi and churning butter. Farm women were found to use the manual *lassi* maker or wooden churner in both the villages. Table 3 indicates the difference between the manual lassi maker and the electronic *lassi* maker provided by NDRI. With respect to the cost of equipment, electronic lassi maker (Rs. 4500-5000) was costlier than the manual one (Rs. 400-1000). However, the electronic *lassi* maker supplied by NDRI was perceived to be much better than the manual one. It could save twenty minutes time for preparing *lassi*. The texture, appearance, consistency, flavour of lassi, fat & SNF quality and butter prepared by the electronic *lassi* maker were given better weightage as perceived by women than the

lassi and butter prepared with manual churn as well as the one purchased from market. It was found to save labour. Hence, farm women could be able to make improved quality products. Awareness was the issue as women were found to follow the traditional methods although availability is not a problem in nearby urban areas.

Pouch packing machine

Pouch packing machine is used for sealing the pouches and packing milk and milk products. This was a new intervention for the farm women. The machine performed better than the manual way of packaging. It saved time and cost as well as farm women were found no ripping, transportation, spacing and handling problem in pouch packing, which led to increase in the shelf life of milk and milk products (Table 4).

Preparation of value-added milk products

Table 4 Perceived assessment of pouch packing machine by farm women

Parameters	Impact
Time saving, traditional rubber band packed polythene packets vs pouch making	Less time consuming
Operational cost of packaging milk with machine(Rs/litre)	Reduced
Effect on Shelf life of packed milk	Improved
Pouch ripping issues (number)	Reduced
Incidences of leakage of milk from pouches during transportation	Reduced
Ease of handling milk pouches	Easier

Table 5 Perceived assessment of preparation of value-added milk products by women

Parameters	Type of products purchased before training	Type of products prepared after training
Preparation of milk products	Ghee, Chhach and Curd	<i>Masala Paneer</i> , Flavoured <i>lassi</i> & <i>milk</i> , Whey drink, <i>Kalakand</i> , <i>Burfi</i> , <i>Gulabjamun</i> and <i>Rasagulla</i>
	Average quantities of different milk products purchased per household (per month) and their purchasing cost/kg	Average quantities of different milk products prepared after training per household (per month) their cost benefit
	Quantity kg. Cost (Rs)	Quantity kg. Cost (Rs.)/ kg. Benefits Rs. / kg
<i>Paneer</i>	2-2.5 270-280	3-3.5 200 70-80
Flavoured <i>lassi</i>	1 120	2 40 80
Flavoured milk	2 100	4-5 40 60
Whey drink	No 50	12 8-10 40-42
<i>Kalakand</i>	1 400	1 200 200
<i>Burfi</i>	1 350-400	2 180-200 170-200
<i>Gulab jamun</i>	1.5 300	2 140-150 150-160
<i>Rasagulla</i>	1 300	2 140-150 150-160
	Comparison of quality of purchased vs prepared dairy products by the households	
	Purchased products	Prepared products
Flavour& taste	Good	Very good
Chance of adulteration in products	Possible	No

Selling raw milk fetches only Rs. 25 to 40/ litre depending upon the fat content either from cow or buffalo. When the milk is converted into various products, it is possible to enhance the profit significantly. Therefore, value addition becomes important. As depicted in Table 5, farm women learned the preparation of value-added products from milk like *paneer*, flavoured milk and *lassi*, whey drink, *Kalakand*, *Burfi*, *Gulabjamun* and *Rasagulla* after undergoing training and demonstration. It helped them to prepare more quality dairy products at home rather than buying from market. They also saved Rs.70-80 in *paneer*, Rs. 80 in flavoured *lassi*, Rs. 60 in flavoured milk, Rs. 40-42 in whey drink and more than Rs.150 in *Kalakand*, *Burfi*, *Gulabjamun* and *Rasagulla*, by preparing the dairy product at their home. The home-made milk products were perceived to be pure and had a better taste and quality as compared to the dairy products purchased from market. During interaction, the farm women revealed that sometimes they could not get the flavour for making flavoured milk/ *lassi*. If women are educated about availability of flavours either through local vendors/traders or through online purchase, they could easily prepare the marketable quality dairy products and earn appreciable returns (Sharma et al. 2015). Linking women groups with potential buyers of milk products including established sweet shops, hostels of school and college, hotels, restaurants and canteens with approach like public private partnership (PPP) can create better opportunities for earning more profit (Ponnusamy et al. 2017). Affirmative policies should be implemented to strengthen women’s capabilities, thus enabling them to articulate their needs and interests for health, technology, education and social, economic well-being (Kelkar et al. 2017).

Hybrid bajra napier cultivation as animal green fodder

Since awareness of farmers on improved fodder varieties from national institutes was limited, rooted slips of hybrid bajra napier was procured from Indian Grassland and Fodder Research

Institute (IGFRI), Jhansi and distributed to farm women in both the villages. Women were motivated to plant the rooted slips even in vacant piece of land, bunds, borders and backyards apart from cultivation in the mainland. Table 6 showed that hybrid bajra napier introduced by NDRI had better performance than conventional local sorghum fodder crop. Within 30- 35 days after planting of napier, the fodder was ready for first cut, number of cuts (10-12) were more per year, yield per acre herbage (600 q/ acre) was also more than local sorghum fodder crop. It was available for feeding the animals throughout the year and had ability to withstand moisture stress. The adoption of each innovation is site specific and depended on local human and material resources (Misra et al. 2009).

Berseem fodder variety- BL 42

The berseem variety BL- 42 introduced by NDRI had exhibited better performance than the local berseem variety (Table 7). Within 40- 45 days after sowing of BL- 42, the fodder was ready for first cut, number of cuts (7-8) were more per year, per acre herbage yield (500 q/ acre) was also more than the local variety. It was available for a longer period i.e. 7 months in a year.

Head load manager

Drudgery in certain operations among farm women is a vital issue which needs to be addressed with systematic approach (Ponnusamy et al. 2015). Farm operations such as fodder collection and dung cake collection are done by farm women. Head load manager was essential equipment for reducing the drudgery of farm women. Specifications for head load manager include over head frame height (25.6 cm), frame length (20 cm), frame width (36 cm), width of the over head frame (ear to ear distance) with 20.3 cm and length of the back frame (40.8 cm). Designs are available in steel sheet tube / stainless steel sheet and tin sheet for making

Table 6 Assessment of Hybrid Bajra Napier as perceived by farm women

Parameters	Hybrid Napier	Local sorghum fodder crop
First cut after planting (days)	30-35	50-60
Subsequent cuts with intervals(days)	20-22	40-45
Number of cuts per year	9-10	2-3 (only berseem 6-7 cuts)
Herbage yield in quintal/ Acre	600	350
Perceived palatability	Good	Good
Fodder availability in round the year	Yes	No
Ability to withstand moisture stress	Yes	No

Table 7 Comparison of berseem variety- BL 42 with local berseem variety as perceived by farm women

Parameters	Local variety	BL 42 variety
First cut after sowing (days)	55-60	40-45
Subsequent cuts with intervals (days)	35	25
Number of cuts per year	5	7-8
Herbage yield in quintal/ acre	400	500
Round the year fodder availability	5 Months	7 Months
Perceived palatability	Good	Good

Table 8 Perceived assessment of head load manager by farm women

Parameters	Manual	Use of head load manager
Maximum load lifting (kg)	10-12	18-20
Drudgery	Yes	No
Comfort while carrying the load	Uncomfortable	Comfortable
Pressure and pain on head & neck and body	Observed	Less observed

Table 9 Assessment of chaff cutter by farm women as perceived by farm women

Parameters	Local chaff cutter	Chaff cutter supplied by NDRI
Time taken in chaffing the fodder (Minutes)	15	10
Easiness to operate	Less easy	More easy
Quantum of energy or effort used in cutting the fodder through chaff cutter	More	Less
Extent of reduction of the accident cases with supplied chaff cutter	-	40% less
Utility of flywheel Safety Lock	Not available	More useful
Utility of blade Guard	Not available	Yes
Utility of warning roller	Not available	Yes
Complaint of blood clotting and lump in hand	Yes	No
Space for operation (sq.ft)	70	60
Quantity of fodder chaffed per 5 minute (Kg.)	12-15	15-18

its frame. After using this equipment, farm women could lift around 6-8 kg more fodder and did not feel any pain on the head & neck and body while carrying the load. They considered head load manager as very useful equipment (Table 8).

Chaff cutter

Farm women were using local chaff cutter for cutting of chaff for their dairy animals. After using the chaff cutter given by NDRI, which was procured from Division of Agricultural Engineering in IARI, New Delhi, they found it very useful than the local chaff cutter. They could cut more quantity of chaff in lesser time by using chaff cutter. The advanced features of the chaff cutter such as flywheel safety lock, blade guard and warning roller helped in reduction of accidental cases by around 40 per cent. This chaff cutter also helped farm women in reducing their drudgery to some extent in terms of time, energy and easiness to operate (Table 9).

Conclusions

Technology access to women is a long-neglected issue in agricultural progress in spite of their significant involvement in farming activities. Isolated attempts in technological empowerment of farm women did not percolate as a nation-wide movement although government makes enormous efforts in this direction. The experiences as documented in Pingli and Jundla villages of Karnal district by NDRI, Karnal would serve as a learning platform for fellow farm women in other parts of the country for faster dissemination of simple technologies. Besides the availability of critical inputs for technology adoption, there is urgent need to assess and validate simple technologies

considering local needs and resources for profitable and sustainable dairy farming and these issues may be addressed by the planners and policy makers.

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Interventions to sustain dairy production and productivity in changing climate of Namakkal district, Tamil Nadu

N Akila¹ and M Jothi Lakshmi²

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Abstract: Dairy animals play a major role in livelihood of farming community in Namakkal district of Tamil Nadu. But continuous reduction in average rainfall of 854.95 mm between 1995-2005, 777.47mm between 2006-2014 and 708.04mm between 2015-2019 due to climate change causes severe drought and drought results in scarcity of available pasture and fodder and decline in dairy animal population from 2.85lakhs in 18th livestock census, 2009 to 2.49 in 19th livestock census, 2014 in Namakkal district. Also, dairy animals become susceptible for heat stress, prone to vector borne diseases and affect the reproductive performances. Hence, suitable interventions to improve the performance of dairy animals in summer and hot humid seasons were introduced. It was found that though the temperature humidity index persisted almost similar over the years (2012 – 2018) and induced moderate heat stress ranged between 80 – 83, the productivity of animal increased from 4.1lt to 6.15lt in summer and 4.15lt to 6.59lt in hot humid season by adopting the climate resilient interventions. The productive and reproductive performance of the dairy animals indicated that housing, feeding and health management interventions improved the lactation yield from 945 lts to 2103 lts with 7.41 to 9.85 months lactation length though the average maximum temperature in summer from 2012 – 2018 was 39.17°C. Even in drought and crop failure times, farmers could get stable income of Rs.2270/month/animal through milk and manure of cow by adopting scientific dairy housing and feeding management practices like thatched roof sheds, trees around the sheds for good ventilation, green fodder cultivation and feeding (15-20kg/

day/animal), balanced low cost concentrate feed, mineral mixture (40gms/day/animal) along with Sodium bicarbonate (50gms/day/animal) feeding and ad libitum good quality drinking water (70lt – 90lt/day/animal).

Keywords: Dairying, Drought, Productivity, Summer season

Introduction

Dairying is not only an important source of food and income, but also the sign of assets in rural India for poor people. Dairy farming is an important livelihood option for the vulnerable sections namely landless, small and marginal farmers who form major stakeholders in the smallholder dairy (Rao and Birthal 2002; Thirunavukarasu et al. 2014). India's milk production is 187.7 million tons during 2018-19 compared to 17 million tons in 1950-51 (BAHS 2019). Dairy animals play a major role in livelihood of farming community in Namakkal district of Tamil Nadu. Most of the farmers were of small holders and unaware of proper housing for their animals and the animals were tied in open space under tree shades / other shades. They rear dairy animals by grazing and feeding crop residues. But continuous reduction in rainfall of 854.95 mm average rainfall between 1995-2005, 777.47mm between 2006-2014 and 708.04mm between 2015-2019 (Gramin Krishi Mausam Sewa, Agromet Field Unit, Indian Meteorological Department, Namakkal, 2019) and changing climate causes severe drought and drought results in scarcity of feed and fodder and decline in dairy animal population from 2.85lakhs in 18th livestock census, 2009 to 2.49 in 19th livestock census, 2014 in Namakkal district (18th & 19th Livestock Census 2009 & 2014, State Department of Animal Husbandry, Tamil Nadu). Climate change is one of the major threats for the sustainability of livestock production systems in tropical countries. Sere et al. (2008) reported that heat stress has adverse effects on the productive, reproductive and health performances of dairy animals. Dairy animals become susceptible for heat stress, prone to vector borne diseases and affect the reproductive performance. This further cause reduction in income and would probably force the family to abandon dairy farming and dispose the dairy animals.

In India, the upper temperature limit of comfort zone for optimum milk production is 27°C, about two degrees higher than the same

¹Krishi Vigyan Kendra, Veterinary College and Research Institute campus, Namakkal 637 002, Tamil Nadu, India

²Veterinary University Training and Research Centre, Perambalur- 621 220, India

N Akila (✉)
Krishi Vigyan Kendra, Veterinary College and Research Institute campus, Namakkal 637 002, Tamil Nadu, India
Email: akila2528@gmail.com

reported in temperate countries Dutt et al. (1992). This is perhaps because the crosses of exotic breed with native Indian breeds have adapted to climatic conditions in the country. However, the average annual temperature is higher than this upper limit in several parts of the country, particularly in South Eastern region comprising of the states of Andhra Pradesh and Tamil Nadu. Even though daily maximum temperature exceeds 40°C for a few hours each day, cool nights help to lose the stored heat which allows the dairy cattle to produce at near optimal levels Bouraoui et al. (2002). Hence a study was conducted to introduce suitable interventions to overcome heat stress in animals and improve the productive and reproductive performance of the animals.

Materials and Methods

The study was conducted in Vadavathurpanchayat, the NICRA village of Erumapatti block, Namakkal district, Tamil Nadu. Crossbred and non-descript dairy cattle were mostly reared by the farmers. A meagre percent of farmers (6%) owned buffaloes and indigenous cattle. The climate resilient interventions under animal science module such as physical modification of sheds, better feeding, health and summer management practices were taken up between 2012 and 2019. Under physical modification of environment, suitable modifications in dairy sheds such as applying foggers and wet the animals two times / day, tree plantation around the sheds, keeping thatched roof over the asbestos sheet to minimise the heat were taken up in nine sheds and these sheds kept as model instructional sheds to create awareness among other farmers during the training programmes. Under the management practices, green fodder production with drought tolerant varieties and feeding (20kg/animal/day), preparation of low cost concentrate feed and feeding the animal in correct ration (400gms/lit of milk/day), mineral mixture (40gms/day/animal) along with sodium bicarbonate (50gms/day/animal) and common salt feeding (30gms/day/animal), providing ad libitum good quality drinking water (70-90lt/day/animal), regular deworming and vaccination against Foot and Mouth disease and silage preparation with crop residues were carried out. The temperature and relative humidity available in the manual weather station installed in the village were recorded daily.

The Temperature Humidity Index (THI) of dairy animals was measured as

$$THI = 0.8 * T + RH * (T - 14.4) + 46.4$$

where T = ambient or dry-bulb temperature in °C and RH = relative humidity. The levels of stress were separated into:

- Mild (72 to 79 THI)
- Moderate (80 to 89 THI)
- Severe (90 THI or greater)

The THI measured to assess the risk of heat stress based on the recorded data. The total milk production and productivity were calculated in summer and hot humid season from 2012 – 2018 and related to THI. Also 100 dairy farmers were selected randomly out of 317 beneficiary farmers to study the before and after effect of implementation of interventions with the secondary data related to productive and reproductive parameters. All the selected farmers were attended skill training programmes on dairy farming and demonstrations on concentrate feed mixture preparation and received critical inputs like fodder seeds, feed ration, mineral mixture, deworming and vaccination in different years from the inception of the scheme.

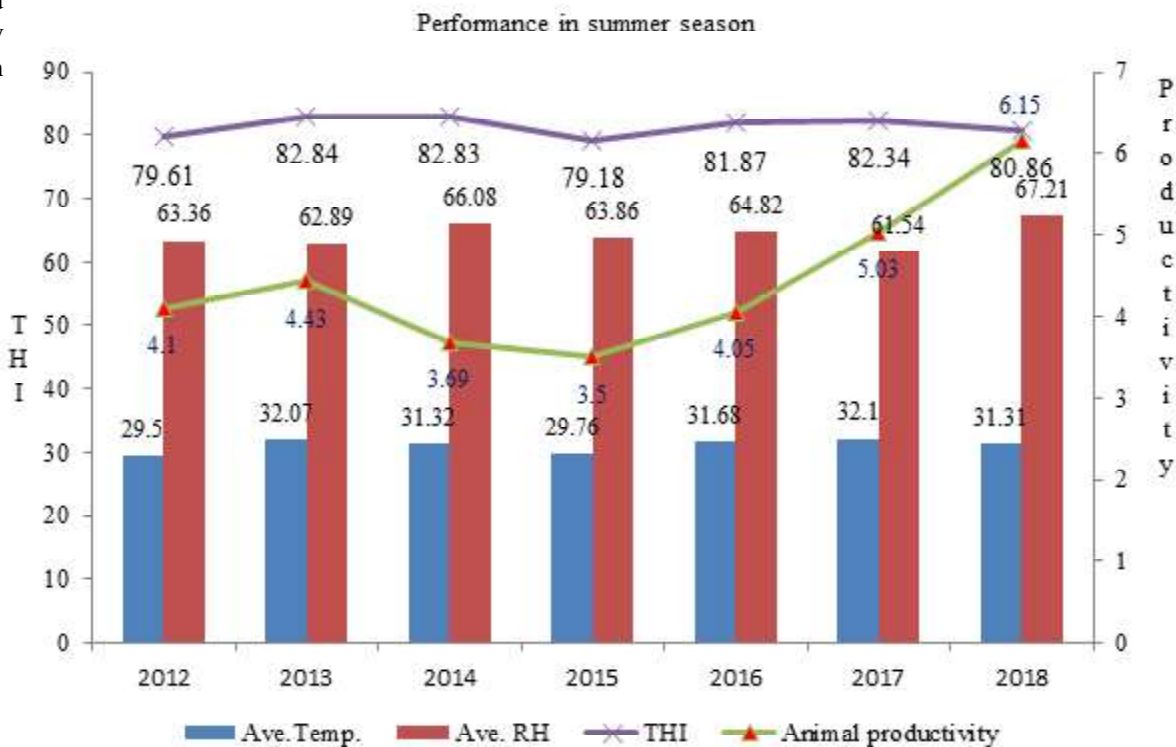
Results and Discussion

The performance of dairy cattle in summer and hot humid season is presented in fig 1 and 2. It is found that though the temperature humidity index persisted almost similar over the years (2012 to 2018) and induced moderate heat stress ranged between 80 to 83, the productivity of animal increased from 4.1lt to 6.15lt in summer and 4.15 lt to 6.59lt in hot humid season by adopting the climate resilient interventions such as proper sheds, trees around the sheds, water splashing on animals in hot days, mineral mixture (40gms/day/animal), sodium bicarbonate (50gms/day/animal) and common salt (20gms/day/animal) feeding with balanced concentrate feed @ 400gms/lit of milk production by 52% of the farmers. Whereas the similar interventions were adopted partially by 48% of the farmers as per convenience. 37% of the farmers

Table 1 Productive and reproductive performance of dairy animals (n=100)

Parameter	Before intervention	After intervention
Lactation length (Months)	7.4	9.85
Lactation yield (lt)	945	2103
Fat & SNF (%)	3.7 & 7.2	4.5 & 7.8
Incidence of Repeat breeding (%)	48	10
Calving interval (Months)	16	12
Feed cost /animal /day (Rs.)	107.00	115.50
Gross Income	30420	65800
Gross Expenditure	29980	38550
BC Ratio	1:1.01	1:1.71

Fig. 1 THI and Milk productivity in summer season



followed green fodder production and feeding to dairy cattle and 6% of them produced silage. The animal productivity reached to 7.18lt in winter and 7.03 lt in rainy season which was higher than the productivity obtained in summer and hot humid seasons. This might be due to the heat stress during the summer and hot humid seasons and dairy breeds are more susceptible to heat stress than meat breeds, and higher milk producing animals had increased metabolic heat production and this causes more susceptibility to heat stress as compared to low milk producing animals (Das et al. 2016). Change in climatic condition directly affects the production and reproduction level of animal about 58.3% and 63.3%, respectively (Singh et al. 2012). Johnson et al. (1963), Shinde et al. (1990); Mandal et al. (2002) and Zewdu et al. (2014) also reported that milk yield and dry matter intake exhibited significant declines when temperature humidity index (THI) reached 77. Average daily milk yield/cow was reduced by 0.886 kg per unit increase of THI (Das, S.K., 2012).

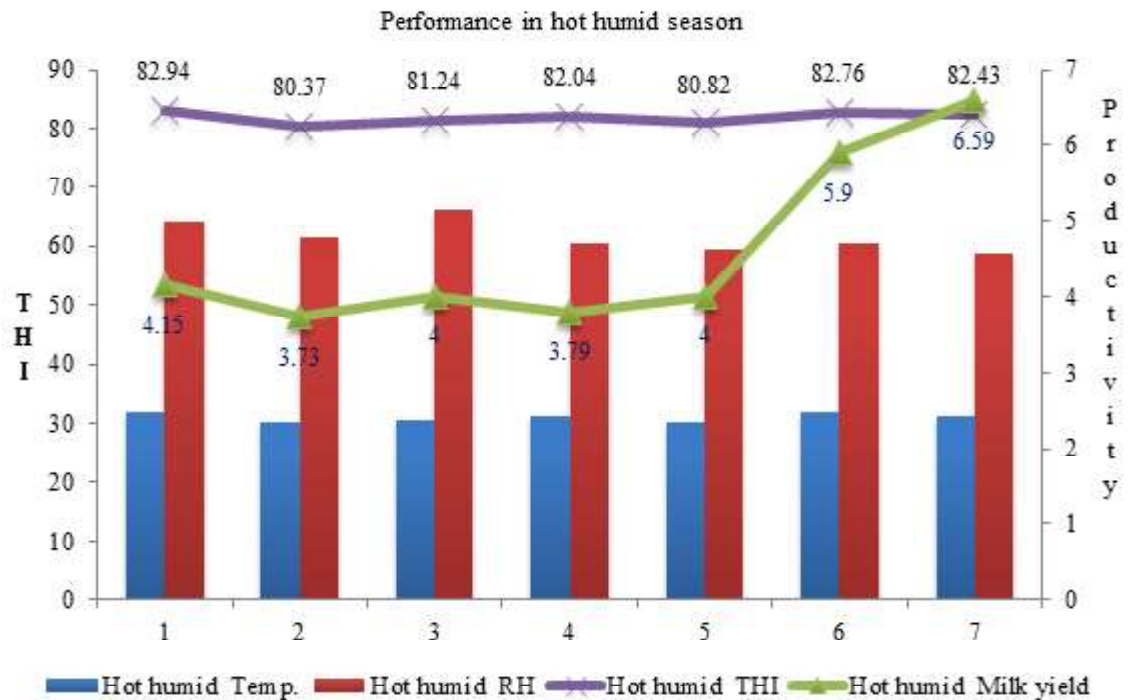
Though animals were under moderate heat stress in summer and hot humid season 91001 lts of milk production was achieved as against high production in winter (110558lts) by 167 cattle of that village with 4.5 fat and 7.8 SNF due to the adoption of summer management practices. Regular mineral mixture(40gms), green fodder (15to20kg), crop residue silage, low cost concentrate fed @ 400gms/lt of milk production, good quality drinking water (70lt) and altered time of grazing improved the animal performance (Ponnusamy et al. 2019).

The animal health and productivity depends on climatic, social and economic factors and mostly on environment in which they live (Sivakumar et al. 2017 and Rajadurai et al. 2018). The improved shelters helped the animal to dissipate the heat, increased the comfort of the animal and sustained the production. The foggers helped to maintain the correct body temperature. The farmers perceived that the animal shelter with standard spacing (60-70sq.ft) helped to increase the production performance i.e. enhanced daily milk production by 300-500 ml in dairy animals during hot weather.

The farmers were motivated to adopt planting trees nearby the sheds and wetting of gunny bags and applied it over the roof and sides during hot summer, to protect the animal from direct sunlight with the advantage of alleviating heat stress in animals and increase the feed conversion ratio. Almost all the dairy farmers adopted feeding mineral mixture which is exhibited well by improved health status of the animal by no symptoms of panting and salivation, increase in milk production, fat and SNF % and increase in calf birth weight. The green fodder and silage, mineral mixture assisted to maintain the milk production in dairy animals. The farmers regularly followed vaccination and deworming of their animal which helped to prevent the disease outbreak, decreased the treatment cost and increased the profitability of the small holder farmers.

The productive and reproductive performance of the dairy animals is presented in Table 1 and it was found that housing, feeding and health management interventions improved the

Fig. 2 THI and Milk productivity in hot humid season



lactation yield from 945 lts to 2103 lts with 7.41 to 9.85 months lactation length though the average maximum temperature in summer from 2012–2018 was 39.17°C.

The additional cost incurred by the farmers in adopting the interventions was Rs.12000/- in installing foggers in dairy sheds, fodder cultivation (Rs.6000/50cents), and mineral mixture feeding (Rs.55/month/animal). Foggers are onetime expenditure alleviate heat stress to animals and provides comfort that enhances milk production. Also green fodder feeding and mineral mixture increased the production performance of the animals. Hence the additional cost incurred by the farmers in adopting the interventions is not actually a burden to the farmers. Infact balanced concentrate feed ration helped the farmers to reduce the feed cost what they spent before learning the correct feed ration. Deworming and vaccination was the cost free intervention to the farmers either from the scheme or from the animal husbandry department. Hence, a slight modification in the shed by installing foggers, planting trees around the sheds, water splashing on the animals where foggers are not available are the interventions really helped to improve the animal performance. Like that correct concentrate feed ration with mineral mixture, sodium bicarbonate and common salt feeding, green fodder feeding and ad libitum good quality drinking water are the successful interventions in feeding management assisted to maintain the performance of the dairy cattle

Conclusions

The productive and reproductive performance of dairy animals is based on the environment provided to them with suitable

management practices in feeding. Due to the successful interventions implemented in the study village the dairy population increased from 317 to 878 in seven years (2012 to 2018) and annual production increased from 1,77,702 to 7,81,430 lit. Four Government (*aavin*) and private milk outlets opened to collect milk from farmers that motivated the farmers to continue dairying. Even in drought and crop failure times, famers could get stable income of Rs.2270/month/animal through milk and manure of cow by adopting scientific dairy management practices. Since majority of the dairy farmers possess small herd size, efforts should be taken to propagate season based management practices on large scale to sustain the productivity and enhance the income to farmers.

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Microbial profiling and adulteration patterns among street dairy food sold around the Ramnagar, Varanasi, Uttar Pradesh

Kumari Pooja, Arvind, Dinesh Chandra Rai, Shikha Pandhi and Akansha Gupta

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Abstract: The study was performed to assess the microbiological quality of street food sold around the Ramnagar, Varanasi, Uttar Pradesh. Eighty samples of different street foods including Lassi, Rabri, Kulfi, Rasmalai were collected from different areas of the Ramnagar region and the microbial quality and adulteration pattern of each sample was assessed. Apart from this, a survey was also conducted to assess the awareness pattern of food safety among the population. A very high level of coliform was observed in all samples of dairy products. The adulterants were not present but have a wide variation in acidity and moisture content. Further, the consumer survey indicated that only 35 % of people know about FSSAI and 74.16% of people known about the fatal diseases associated with the consumption of unclean street food.

Keywords: Kulfi, Lassi, Microbiological safety, Survey

Street food vending is an ubiquitous phenomenon that is essentially urban and forms an essential part of the unorganized food sector. It shows varied patterns amongst different regions and cultures concerning the scale and pattern of street food vending (Malhotra, 2017). As per FAO 1989, "Street food is defined as ready-to-eat foods and beverage prepared and/or sold by vendors especially in streets or other similar public places". Street food is mostly cherished for its exceptional flavors,

convenience and the position which they acquire in the cultural and communal legacy of societies. It has become an important part of the majority of people belonging to low-income groups as a source of reasonable nutrients and governs the nutritional status of such populations (Muzaffar et al. 2009). Even though street food offers various sensorial as well as economic benefits, there is a huge need to think about the safety aspect associated with consumption of these street foods as they may serve as a transmission means for various foodborne illnesses within a population (Tambekar et al. 2011).

Maintenance of hygiene in street food vicinity is imperative to ensure food safety and nutritional security. Mishandling and ignorance towards hygiene procedures by the food vendor may permit the pathogenic microorganism to get in touch with the food. These microorganisms may survive in some cases and multiply sufficiently to a number that may cause illness in the consumer. Lack of proper infrastructure facilities may provide the opportunity for the microorganism to infest food at any time during the whole preparation phase (Gadi et al. 2013). Hence, assuring the safety of food is important for the welfare of a human being, a society, and a nation. Dairy-based street food has been widely consumed in different parts of the country such as Lassi, Rasmalai, Kulfi, etc and acts as a sole source of nutrients and energy. They may serve as a potential source of various microbial contaminants due to its good nutritional profile that acts as an efficient substrate for microbial growth. Also, prevalent adulteration practices of dairy-based products may have put a question on the safety of these dairy-based street foods. The present study was performed to evaluate the microbial quality and adulteration pattern of street food sold in the vicinity of the Ramnagar area in Varanasi, Uttar Pradesh. Dairy products such as Lassi, Rabri, Kulfi, and Rasmalai were evaluated for the above-mentioned criteria. Also, a consumer survey was conducted to evaluate consumer's perceptions of hygiene and the safety of street foods.

The present study was conducted in Ramnagar, Varanasi in Uttar Pradesh. Ramnagar was selected purposely based on several street dairy products that are easily available and consumed by a large population.

Department of Dairy Science and Food Technology, Institute of Agricultural Sciences,
Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India

Arvind (✉)
Department of Dairy Science and Food Technology
Institute of Agricultural Sciences
Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India
Email- arvind1@bhu.ac.in

Eighty samples of different street foods including lassi, Rabri, Kulfi, Rasmalai were collected from the different vendors and shops who are directly or indirectly engaged in the street food preparation in the different areas of Ramnagar, Varanasi district, U.P. Two hundred grams of each sample was collected in a separate sterile polythene bag and stored at 4°C in a refrigerator for further use. The microbial parameters such as Total plate count, Yeast and Mold count and coliform count were analyzed in samples. Different adulteration detection tests were performed to check the presence of starch, cane sugar, vanaspati and metanil yellow in the procured samples.

Isolations and enumerations of microbes were performed using serial dilutions and spread plate technique. One gram of sample was properly mixed with distilled water one ml of resultant homogenate was added to 9ml of sterile saline water in a test tube and diluted serially to obtain a series of dilutions up to 10^{-7} . For bacterial isolations 1ml or 0.5ml of the appropriate dilutions from each tube was aseptically pipette out and plated on to different selective and differential media for coliform count (Violet Red Blue Agar and MacConkey Agar) using the streak plate technique or plate pouring technique. All the bacterial plates were incubated in an inverted position under aerobic conditions 37°C for 24-48 hr. The fungal isolations were performed in Potato Dextrose Agar (PDA) and Total Plate Count. The plates were incubated at 25-27°C for 2-3 days (Kumbhar et al. 2009). The number of microbial counts was calculated using the following formula as counted as colony forming units (c. f. u.) per gram/ml:

$$\text{Colony forming units/g} = \frac{\text{No. of colonies} \times \text{dilution factor}}{\text{Weight of Aliquot taken(ml)}}$$

Acidity of various samples were determined using the method as recommended by Ranganna (2001). Take one gram of milk product sample and dissolve it in 10ml of distilled water. This was then titrated against 0.1N NaOH using few drops of phenolphthalein solution as indicator. The end point was judged by the appearance of pink color that persists for 10 to 15 seconds. The titred value was noted and results were calculated as percent total acids using the following equation:

$$\% \text{ Acidity} = \frac{\text{Titre reading} \times \text{Normality of NaOH} \times \text{volume made up to} \times 90}{\text{Weight of sample} \times \text{volume of aliquot taken} \times 1000} \times 100$$

The moisture content of sample was determined according to the Association of Official Analytical Chemists method (AOAC, 1995). Each sample (10 g) was placed in an oven at 105°C for 3 h. Reading was taken at a constant weight. The moisture content was then expressed as the percentage (%) of the dry weight of sample.

$$\% \text{ Moisture} = \frac{W_1 - W_2}{W} \times 100$$

Where, W_1 = Weight of plate & sample before drying, W_2 = Weight of plate & sample after drying & W = weight of sample.

One gram of sample was added in test tube followed by addition of 0.1ml of resorcinol and 1ml of hydrochloric acid. Boiled for 2-3 minutes in waterbath. Appearance rose red color indicates the presence of canesugar (Navale and Gupta, 2016).

1gram of sample was measured in test tube and kept for incubation in boiling water bath (80°C) for 5 minutes. After Incubations, the test tube was cooled and adds 3-4 iodine crystal balls then mixed the content well. Appearance of blue black color indicates the presence of starch in product (Patel, 2019).

One gram of sample was taken in a test tube followed by addition of 10 drops of hydrochloric acid. Mixed up with gentle shaking and examined after 5 minutes for any color change. Appearance of red color indicates the presence of vanaspati in the sample (Patel, 2019).

One gram of sample was added in a test tube. Few drops of concentrated hydrochloric acid were added and observed for any color change. Appearance of magenta red color indicate the presence of metanil yellow color (Attrey, 2017).

Twenty questions were prepared for the survey questionnaire. The questionnaires were designed to obtain information about the consumer perspective about the safety and hygiene followed during the food handling procedures by street food vendors. It took approximately 20-30 minutes to complete answering the self-assessment questionnaire. The questionnaire encloses personal information of consumers, and their knowledge regarding street food and food adulteration.

All the samples procured from the Ramnagar vicinity in Varanasi, Uttar Pradesh were analyzed for both microbial assessments as well as for quality assessment based on various adulteration detection quick tests. The microbial profiling reveals the presence of bacteria, coliforms and yeast and mold. Presence of high coliform count poses direct food safety concerns and may serve as a source for occurrence of food borne infections. The possible reasons for this poor bacteriological quality of the product could be the use of contaminated water or water with poor bacteriological quality, lack of adequate storage facility or the unhygienic surrounding conditions may serve as a source of bacterial contamination to the food (Noor, 2019). Also, the use of earthen pot, leafy *donna* without washing for serving the food could be a major cause of bacterial load. Poor personal hygiene of the street food vendor could also be one of the reasons as most of the vendors did not adopt good personal hygiene and aprons were not in use (Mali et al. 2019). All the samples have shown negative results for the presence of adulterants like starch, cane sugar, vanaspati and artificial colors which indicate that fair practice has been employed by the vendor in that aspect. The

Table 1 Microbial and adulteration profiling of street dairy products sold around Ramnagar Varanasi (N=20)

Sample	Assessment Parameters	
Lassi	Colony count in Log ₁₀ (CFU/ml)	Adulterations test
	Coliform Count- 2.6-3.1	Acidity- 0.95-2.3
	Total Plate Count - 6.1-7.0	Starch Test- Negative
Rabri	Coliform Count- 4.71-7.09	Cane Sugar- Added
	Total Plate Count- 8.30-9.30	Vanaspati- Negative
	Yeast and mold count- 6.69-7.20	% Moisture- 44.72-46.02
Kulfi	Coliform Count- 1.90-3.26	Starch Test- Negative
	Total Plate Count- 4.05-4.76	Vanaspati- Negative
	Yeast and mold count- 2.30-3.26	% Moisture- 20.95-41.30
Rasmalai	Coliform Count- 4.62-5.23	Starch Test- Negative
	Total Plate Count- 6.07-6.81	Vanaspati- Negative
	Yeast and mold count- 5.69-6.14	Cane sugar - Present
		Starch Test- Negative
		Metanil yellow Test- Negative

Table 2 Consciousness for hygiene & food safety among consumers

Parameter	Frequency (N=120)	Percentage%
1-Aware about FSSAI and its laws or rules?		
(a)Yes	42	35
(b)No	78	65
2-Awareness about causing fatal disease?		
(a)Yes	89	74.16
(b)No	31	25.83
3-Aware about adulterations and its bad effect on health?		
(a)Yes	50	41.66
(b)No	70	58.33
4-Aware about vending surrounding?		
(a)clean	15	12.5
(b)not clean	37	30.83
(c)sometime clean or not clean	48	40
(d)rarely clean	20	16.66

probable reason for addition of these ingredients to the food product by the vendor is to improve or preserve the appearance of the product (Alimi and Workneh, 2016). From the results given in Table 1, it can be concluded that there is a need for creating more awareness regarding the adoption of hygienic practices during food practices that must be put across to ensure the food safety of street food products. The factors such as level of education, income, knowledge of food safety and age generally influence the awareness of the consumers regarding the food safety product (Alimi and Workneh, 2016). Further, the consumer survey (Table 2) revealed that only 35 % of people know about FSSAI and only 74.16% of people know about the fatal diseases that are associated with the consumption of unsafe street food. This scenario necessitates the requirement to increase awareness amongst the consumers regarding the concept of food safety and hygiene and their effect on public health. They must also need to be made aware of various adulteration practices followed by the food vendors and their associated impacts on human health as only 41% of consumers are aware of the adulterants

present in dairy foods. It can be concluded that consumer gives more preference to taste over hygiene.

Conclusions

Street food sector is a booming sector in the developing world but necessitates the need to ensure the adoption of good hygienic practices by the food vendor to protect the health of consumers. The present study indicates that all the samples procured from the street vendors of Ramnagar were found to be poor in terms of bacteriological quality but have shown the absence of adulterants. Lack of knowledge about hygiene and infrastructural facilities like potable water, storage facilities, and unhygienic surroundings could have been the potential contributor. Hence, there is a need to bring various improvements in the street food scenario, especially regarding the hygienic surroundings and food safety aspect. The present study findings show the need for more attention to good manufacturer practices and good hygiene practices to reduce street food

contaminations. Primarily, development and adoption of suitable public policies that deals with all the aspects of street food vending starting from the raw material to the hygienic preparation and selling should be encouraged to ensure food safety.

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SHORT COMMUNICATION

Effect of azolla as feed supplement on milk production of lactating buffaloes at Neemuch district Madhya Pradesh

Shilpi Kerketta¹, SS Sarangdevot¹, PS Naruka¹, Shilpi Verma¹, CP Pachauri¹, AK Singh², JP Singh¹ and SS Bhadauria³

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Abstract: Azolla holds the promise of providing a sustainable feed for livestock. Azolla, a floating fern reported to be a very good source of protein, essential mineral elements and vitamins for livestock. The present study was undertaken to know effect of feeding azolla on milk production performance (milk yield, fat%, SNF%) of lactating buffaloes under field conditions. A total of 40 lactating buffaloes were selected and categorized into two groups each having 20 buffaloes. The animals in control group (T0) were fed on conventional ration of paddy straw, green fodder and concentrate mixture as per recommendation of NRC. In the treatment group (T1) 2 kg of fresh azolla was supplemented over conventional ration. The effect of fresh Azolla supplementation in lactating buffaloes was studied in an early lactation period of 90 days. The average milk yield (kg/d) was found significantly higher ($P<0.05$) in T1 (7.4 ± 0.08) than T0 (6.5 ± 0.13). On an average milk yield increase by 0.9 L/day over control group animals. These results showed that supplementing of azolla caused an increase in milk yield by 13.8%. The average percentage of milk fat, milk protein, SNF showed no significant difference between two groups but the fat% was found higher in T1 as compared to T0. Peak milk yield was also found significantly ($P<0.05$) higher in azolla fed group than the control group. Thus, it can be concluded that azolla can serve as a potential alternative nutrient supplement for the lactating buffaloes for the improvement of productivity in

terms of milk specially where/when green fodder availability is scanty. However, further studies are needed for determining the replacement amount of azolla for concentrate to make it cost effective for farmers.

Keywords: Azolla, Buffalo, Milk, Milk fat, Milk SNF

India has huge and rich livestock population which play significant role in national economy. At present India is a leading country in milk production out of this majority of production i.e., 56% is being contributed by buffaloes. The milk production is not dependent on only one factors rather than several factors, one among them is decreasing feed and fodder for the supporting the growing livestock's population. There is acute shortage of feed and fodder for dairy animals due to lack of land availability for growing fodder for animals and high population density. Today shortage of dry fodder, green fodder and concentrate has been estimated to be 21%, 35.6% and 44%, respectively (Kumar, 2018). To maintain continuous milk production it is essential to be provided with adequate feed and fodder, along with other supplement. Primary consideration on feed resources must be to identify the feed resources in ample supply to provide the bulk of a ration for the local herd and the supplements needed to balance the animal's nutrition. One among them is Azolla as a feed supplement which could be a good alternative for green fodder and concentrates to fulfill the nutrient requirement of animal.

Azolla is a free floating, rapidly growing aquatic fern on water surface forming symbiotic relationship with the cyanobacterium *Anabaena azollae*, which fixes atmospheric nitrogen. Azolla is rich in crude protein content which varies between 15.4 to 27.93% i.e. 4 to 5 times more protein of excellent quality in comparison to hybrid napier and lucerne, respectively (Pillai et al. 2002). Along with this it is also good source of minerals (calcium, phosphorous, potassium, ferrous, copper, magnesium) and vitamins (vitamin A, vitamin B12, Beta Carotene). Azolla meal contains 25.78% crude protein, 15.71% crude fiber, 3.47% ether extract, 15.76% ash and 30.08% nitrogen free extract on the air-dry basis (Basak et al. 2002). Azolla pinnata had high crude protein content and better mineral profiling (Calcium, phosphorus and Potassium are 1.67%, 0.31% and 2.68%) respectively (Bhatte et

¹KVK Neemuch, Madhya Pradesh, India

²Department of Animal Nutrition, FVAS, RGSC, BHU, India

³PS to DES

A K Singh (✉)
Department of Animal Nutrition, FVAS, RGSC, BHU, Varanasi, Uttar Pradesh 221 005, India
Email: vetabhi04@gmail.com

al. 2020). Azolla can act as a valuable green feed supplement for dairy cattle to improve productivity (Chatterjee et al. 2013). Due to easy cultivation, good nutritive value and high productivity it may be used as a beneficial fodder supplement as reported by various scholars (Prabina and Kumar, 2010). One hectare of azolla can produce 540-720 kg of protein per month. Various work on economics and nutritive value of azolla rich in protein and low in lignin content indicates that it has been one of the most economic and efficient feed substitutes for livestock which can be easily digested. Due to its beneficial characteristics Azolla is called "Green Gold" (Wagner, 1997). Keeping in view the above features of the supplement the objective of the present study was to evaluate the effect of Azolla as feed supplement on milk production performance of lactating buffaloes.

The present study was carried out at farmers field of various villages of Neemuch district (Madhya Pradesh, India). For trial a total of 20 locally available buffalo breeds having 2nd to 5th parity of early lactation stage were selected. The lactating buffaloes were divided into two groups of 10 buffaloes each. Treatment 1 (T0) (n=10): Control group (Farmers' practices), no supplementation of Azolla as feed supplement. Treatment 2 (T1) (n=10): Supplementation of Azolla as feed supplement at rate of 2 kg/day/animal for 90 days. In control group (T0) animals were fed with wheat straw with market purchased concentrate mixture, whereas the treatment group (T1) was fed with same quantity of roughages and concentrate with supplementation of fresh green Azolla pinnata. The buffaloes were hand-milked twice daily morning and evening. Before commencement of the trial the milk yield of individual buffalo was noted as well as after commencement of the experiment the milk yield was recorded fortnightly for a period of 3 months. Along with this on each milk recording day the milk sample was subjected for the analysis of Fat % and SNF %. The data was recorded for 90 days and the comparison was carried out for milk yield, fat percentage in

Table 1 Effect of azolla supplementation on milk yield and fat% in lactating buffaloes

	T0	T1
Milk yield (L/day)	6.5±0.13 ^a	7.4±0.08 ^b
Fat%	5.81±0.20	6.01±0.25
Average increase in milk yield	-	0.9
% increase in milk yield	-	13.8
% increase in Milk fat	-	0.2
Total Milk Yield for 90 days (L)	585±0.53 ^a	666±0.58 ^b

Means bearing different superscripts in a row differ significantly (P<0.05)

between groups. The data were compiled and analyzed using T-test (Snedecor and Cochran, 1994) with the help of SPSS package programme (SPSS 9.00 software for Windows, SPSS Inc., Chicago, IL).

For demonstration of the azolla unit a kuccha trench was made at farmers land. Before this a plain area was selected and then cleaned and leveled. A trench of area 3m X 1.5 m X 0.2 m dug, further covered with silpaulin sheet to prevent draining of water into the earth. The size of the sheet was 0.5m longer and wider than the pit and was secured by a layer of mud at the edges. Filtered fertile soil 10-15 kg uniformly spread over the silpaulin sheet. Cow dung slurry 2kg along with super phosphate 20 gms mixed in 10 litre of water and poured into the pit. Pour water into the pit until a 10 cm space left from top. Finally add about 1 kg of fresh azolla culture on the surface of water, so that in 10-15 days azolla will multiply rapidly to fill the whole pond. As per requirement azolla is fed 1.5-2.0 kg/animal/day and the investment in milk production can be minimized

Economics of Azolla production (Kuccha azolla pit)

Cost of silpaulin sheet	Rs. 340.00
Nylon Shady net (5 x 2 mt). @ Rs. 30/- mt	Rs. 300.00
Azolla 2 kg @ Rs. 55/-kg	Rs. 110.00
Super phosphate 240 gms@ 20g/month	Rs. 6.00
Total cost	Rs. 756.00

From the above data it is clear that the cost of construction a kuccha azolla pit is very easy and cheap. Here the cost of labour, and dung is excluded, as the farmer himself dug the pit without hiring the labour, using dung excreted by his own cow/buffalo. Giving due consideration to the one-time cost of permanent structure and recurring expenses, the cost of Azolla production would be less than 65 paisa per Kg.

The average milk yield (kg/d) over 6 fortnights was significantly higher (P<0.05) in T1 (7.4±0.08) than T0 (6.5±0.13). Similar trend was also found for fat % however the result was non significant i.e, 5.81±0.20 and 6.01±0.25 for T0 and T1 respectively. The result was in concordance with Chaterjee et al. (2013), performed trial in crossbred cattle and found significant increase in milk yield in control and azolla fed group cattle (7.14±0.08 vs 6.42±0.13L/day). Here the fat corrected milk followed increasing trend with 7.04±0.15 (Control) and 7.92±0.09 (Azolla fed) groups. Further the milk yield showed increasing trend and it increased to 10.5 L/day from 8.8 L/day after 90 days of azolla feeding per day with conventional feed Mustard oilseed cake in lactating buffaloes (Singh et al. 2017; Meena et al. 2017). Hence the total milk yield for 90 days also have significant difference between control and treatment group i.e, 585±0.53 L and 666±0.58 L respectively.

Average increase in milk yield recorded to be 0.9 L/day. Percent increase in milk yield found to be high (13.8%), however percent increase in fat recorded to be very low (0.2), indicating the azolla rich in protein and mineral content is helpful in enhancing milk yield but have little effect on fat%. Bhatt et al (2020) concluded that *Azolla pinnata* feeding improves the feeding and resting behavior without affecting the overall performance of calves. Similarly, an average milk yield increase of 1.70 L/day was reported by Singh et al. (2017) in lactating buffaloes and tremendous increase in milk yield (19.32 %) was found. It was also supported by Mathur et al. (2013), with 20.96 % and 16.90 % increase in milk yield in cattle and buffalo respectively and by Rawat et al. (2015) and Gowda et al. (2015) in cross bred cattle. Fat % when compared was also reported to be slightly increased i.e 4.3 to 4.7 in cattle and 6.2 to 6.7 in buffaloes. Due to increase in milk yield per day the overall milk production for observation period (90 days) was also found to be significantly ($P < 0.05$) higher in azolla fed group as compared to control group (666 ± 0.58 versus 585 ± 0.53 L).

Conclusion

From above study it can be concluded that azolla can serve as a potential alternative nutrient supplement for lactating buffaloes for the improvement of productivity in terms of milk as this is a good source of protein especially where/when green fodder availability is scanty. It enhances milk yield along with fat % in lactating buffaloes improving economic status of farmers. However, further studies are needed for determining the replacement amount of Azolla for concentrate to make it cost effective for farmers.

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