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Process Optimization for the Development of Lactose Hydrolyzed *Kulfi*

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Abstract:- Milk sugar (lactose) was hydrolyzed to 70, 75 and 80 per cent using enzyme Lactozym (3000 LAU/ml) @ 1.5 ml/L at 40 °C and incubating for 90, 120 and 150 minutes, respectively. The lactose hydrolyzed milk of all 3 levels was used for *kulfi* preparation. It was evident that melting rate and penetration values increased as hydrolysis increased. Lactose hydrolysis contributes to the development of caramel colour and flavour which is suitable to plain *kulfi*. It also contributes softness to the product. The decline in flavour scores was observed in lactose hydrolyzed *kulfi* as a result of excessive sweetness. To overcome this lactose hydrolyzed *kulfi* was prepared by reducing the added sugar (40, 50 and 60 per cent). The *kulfi* prepared from 70 per cent lactose hydrolyzed milk with 40 per cent sugar reduction was more acceptable and had appreciable sweetness, caramel colour and flavour with uniform body and texture.

Keywords:- *Kulfi, Lactozym, Lactose Hydrolysis.*

I. INTRODUCTION

Kulfi, also known as Malaikulfi/Malai-ka-burf is an indigenous frozen dairy product, which closely resembles ice cream in composition. Traditionally *kulfi* is prepared by evaporating sweetened flavoured milk by slow heating with almost continuous stirring to keep milk from sticking to the bottom of the vessel until its volume is reduced by a half thus concentrating the milk. It has a distinctive taste due to caramelization of lactose and sugar during the lengthy heating process (David, 2014). *Kulfi* contains no air; it is solid dense frozen milk. A typical composition range for the components used in the *kulfi* mix is milk fat 10-16 per cent, milk solids not fat 9-12 per cent, sucrose 9-12 per cent, corn syrup solids 4-6 per cent, stabilizers/ emulsifiers 0-0.5 per cent, total solids 36-45 per cent and water 55-64 per cent (Singh *et al*., 2017).

Lactose, a disaccharide is the primary carbohydrate found exclusively in mammalian milk. Lactose is the principal solid constituent in bovine milk, representing about 35 per cent of the totals solids in normal milk, and is the principal constituent in many dairy products. Therefore, the properties of several dairy products, especially concentrated and dehydrated products, are dominated by certain properties of lactose, especially its solubility, crystallization behavior, mutarotation properties and its propensity to maillard browning (Paul *et al*., 2009). In food industry it is used in confectionary, which helps in improving texture without excessive sweetness. Lactose derivatives such as lactulose, lactitol and lactobionic acid has various functional and technical benefits (Seki *et al*., 2012).

Cow's milk contains 3.0 - 4.6 per cent lactose, whereas the buffalo milk and goat milk contains 3.28 - 4.8 and 4.0 - 5.5 per cent of lactose, respectively (Kanwal *et al*., 2004). Lactose cannot be absorbed as such, but it must be hydrolyzed into glucose and galactose by the action of intestinal β-galactosidase (Nivetha and Mohanasrinivasan, 2017). A significant number of the adult population (almost 75 per cent) is unable to digest lactose due to a genetic deficiency of this enzyme – lactase deficiency (Vesa *et al*., 2000).

Crystallization is a distinctive feature of lactose. Lactose can be present in dairy products in two crystalline forms, α-hydrate and β-anhydride, as well as an amorphous 'glass' mixture of α and β- forms. The crystals are hard and not very soluble. If their size in food products is over 10-16 μm they can be detected in mouth and create a defect called 'sandiness' (Ganzle *et al*, 2008). Sandiness due to lactose crystallization is one of the most objectionable texture defects in frozen dairy products. The limitations of lactose can be greatly minimized by hydrolyzing the milk sugar (Ganzle *et al*, 2008). Acid hydrolysis and enzymatic hydrolysis are two methods of lactose hydrolysis. The first method is characterized by very severe pH (1-2) and temperature (100-150 °C) conditions, thus rendering the end product unsuitable for use as food ingredients. This method is suitable only for hydrolysis of lactose in absence of protein whereas enzymatic hydrolysis is suitable both in presence and absence of protein. Enzymatic hydrolysis is preferable than acidic hydrolysis as the former process allows milder conditions of pH and temperature, and does not cause bad flavours, odors and colours (Das *et al*., 2015).

Lactose hydrolyzed products are better tolerated by lactose intolerant people and because of inhibition of lactose crystallization results in soft, smooth and fairly uniform body and texture of the product (Ganzle *et al*, 2008). Use of lactose hydrolyzed milk also have other advantages such as increased sweetening power favoring reduction in quantity of sugar and the calorie content of the final product, increased

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browning due to the release of monosaccharides which interact with proteins during processing favoring the colour and distinctive caramelized taste of products (Harini and Ramachandra, 2012). So the objective of this study was to optimize the level of lactose hydrolysis in milk for *kulfi* preparation.

II. MATERIALS AND METHODS

Ingredients Fresh whole milk was procured from Students Experimental Dairy Plant (SEDP) of Dairy Science College, Hebbal, Bengaluru. Fresh cream (40% fat and 6% SNF) which was obtained after separating the fresh whole milk and 'Sagar' brand skim milk powder were used for standardization of milk (fat 5%, SNF 8.5%). Good quality cane sugar was purchased from the local market. Carboxy Methyl Cellulose was used as the stabilizer and emulsifier in this study.

Enzyme lactase (β-galactosidase), commercially available as 'LACTOZYM', manufactured by Novo Nordisk A/S, Denmark, 3000 LAU/ml activity, type HP-G was used for hydrolysing lactose.

Degree of lactose hydrolysis (DH per cent) Lactose contents of milk and the residual lactose after hydrolysis were determined colourimetrically by the method of Nickerson *et al*. (1976). The level of lactose hydrolysis was calculated by:

Per cent lactose hydrolysis = (X-Y)/X

Where, 'X' is the amount of lactose in milk prior to hydrolysis and 'Y' is the amount of lactose in milk after hydrolysis.

Preparation of lactose hydrolyzed milk 4 L of fresh milk was standardised to 5.0 per cent fat and 8.5 per cent SNF. The standardized milk was pasteurized and cooled to 40 ºC and milk was equally divided into 4 batches of 1 L each. The milk was incubated at 20, 30 and 40 ºC with varying enzyme concentration of 0.5, 1 and 1.5 ml/l to obtain 60, 70 and 80 per cent lactose hydrolysis.

Production of lactose hydrolysed kulfi Lactose hydrolysis of standardized milk was carried out and further, lactose hydrolysed milk was used in the preparation of *kulfi* and served to panel of judges along with control.

Production of reduced sugar kulfi The optimised lactose hydrolysed *kulfi* was tested for different levels of sugar like 40, 50 and 60 per cent sugar reduction. The product thus prepared was subjected to different physical and sensory attributes in comparison with control *kulfi*.

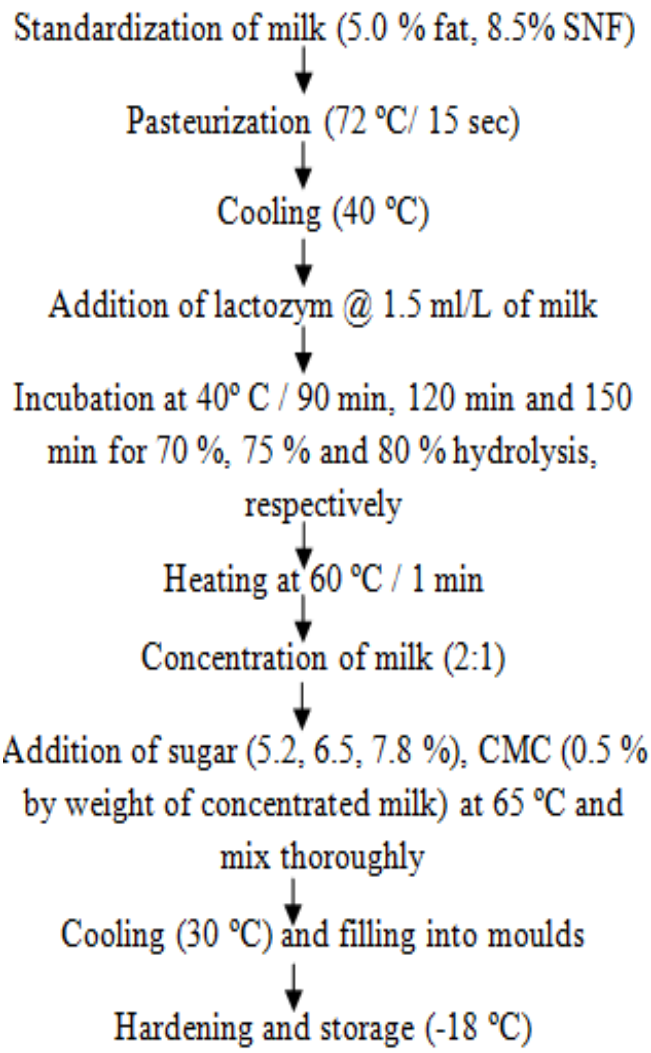

Fig 1:- Flow Diagram for Production of Lactose Hydrolyzed *Kulfi*

Analytical methods Standard of ISI: SP 18 (Part XI) 1981 was adopted for carrying out chemical analysis such as fat, moisture, protein, lactose and ash contents.

Specific gravity of *kulfi* mix was estimated at 30 ºC by using a standard specific gravity bottle of 50 ml capacity, taking distilled water as the standard liquid. The melting rate of the *kulfi* was observed by drawing 10g of the sample on to a wire net placed on a funnel over a beaker immediately after removal from the *kulfi* moulds. The time taken by the sample for complete melt down and dripping into the beaker at room temperature was noted. The melting rate was expressed as ml/15 min. Using a cone penetrometer, penetration value was determined as soon as *kulfi* were drawn from the molds after hardening. The distance in millimeter by which the cone travels in 5s of the sample was noted. For each sample reading were recorded at 3 different spots and the mean value was noted.

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Sensory evaluation Kulfi samples were given to a panel of five judges for sensory evaluation. Each judge was supplied with standard score card of a total of 9 Point Hedonic Scale for colour and appearance, body and texture, flavor and overall acceptability. The scores given by panel of judges were then statistically analyzed. The samples were code numbered to avoid identification and bias.

Statistical analysis The results which are the average of three replications will be statistically analyzed by subjecting to statistical analysis (R Programme, R- Version 3.1.3) using ANOVA technique for one way analysis with independent samples that helps in interpretation (Zar, 2003).

III. RESULTS AND DISCUSSION

Effect of enzyme concentration on the degree of lactose hydrolysis in milk The effect of enzyme concentration on the degree of lactose hydrolysis in milk is shown in Table 1 and Figure 1.The degree of lactose hydrolysis is known to be affected by many parameters such as preheat treatment, temperature of incubation, period of incubation and concentration of enzyme etc. In the investigation pasteurized cooled milk was used. It was recommended by earlier workers that pasteurization should precede lactase treatment for its optimum activity. They reported that milk contained a thermo labile lactase inhibitor and such a compound would be of considerable biological interest because of its possible effect on lactose digestion in mammals. Increased lactase activity in heated milk could be due to exposure of sulfydryl groups of whey protein upon heating (El-Neshawy *et al*., 1988).

With the increase in level of enzyme concentration from 0.5 to 1.5 ml/l, there was an increase in the extent of hydrolysis at all durations of incubation. Lactose hydrolysis of 70, 75 and 80 per cent was attained with enzyme concentration of 1.5 ml/l at 90,120 and 150 min of incubation, respectively. On the contrary, the enzyme concentration of 0.5 ml/l had taken more than 180 min of incubation for achieving 70 per cent of lactose hydrolysis. In case of 1 ml/l of enzyme, the duration of incubation was 150, 180 and 210 min, respectively for attaining 70, 75 and 80 per cent of lactose hydrolysis. However, in both the levels of enzyme concentration (0.5 and 1 m/l), 100 per cent hydrolysis could not be achieved even after incubating for 210 min. The maximum hydrolysis attained was 72.50 and 82.50 at 0.5 and 1 ml/l enzyme concentration. Harini and Ramachandra (2012) reported that 1 ml/L of enzyme ('Lactozym' from *Kluveromyces fragilis*) incubated at 40° C for 30, 60, 180, and 300 min resulted in 25, 50, 75 and 100 per cent hydrolysis, respectively in cow milk containing 3.5 per cent fat and 8.5 per cent SNF. The hydrolysis of 80 per cent was obtained at 37°C after 1 h incubation when 'Lactozyme' was added at the rate of 1.0 ml/L at pH 6.5 (Suresha and Jayaprakasha, 2004).

Duration of incubation (min)	Enzyme concentration (ml/l)		
	0.5	1.0	1.5
	Extent of hydrolysis (per cent)		
30	20.00[a]	32.50[a]	40.00[a]
60	30.00[b]	50.00[b]	52.50[b]
90	42.50[c]	55.00[c]	69.95[c]
120	50.00[d]	65.00[d]	75.00[d]
150	57.50[e]	72.50[e]	80.70[e]
180	67.50[f]	77.50[f]	89.00[f]
210	72.50[g]	82.50[g]	100.00[g]
CD(*P*=0.05)	2.0	3.4	3.55

Table 1:- Effect of Enzyme Concentration on the Degree of Lactose Hydrolysis in Milk

Note:

- Each value is mean of three trials
- Figures in a column with different alphabets differ significantly
- Temperature of incubation - 40 °C
- Enzyme – LACTOZYM with 3000 Lactase Activity Units / ml

It is observed from the result that at constant temperature of 40 °C there was significant increase in the degree of hydrolysis with the increase in enzyme concentration from 0.5 to 1.5 ml/l. Abbasi and Saeedabadian (2013) observed extent of lactose hydrolysis as 25, 50, 75 and >75 per cent when 1.5 ml/L Lactozym from Kluyveromyces fragilis with 3,000 LAU/ml activity is added to pasteurized milk and then incubated for 15, 35, 75 and 240 min at 40 °C.

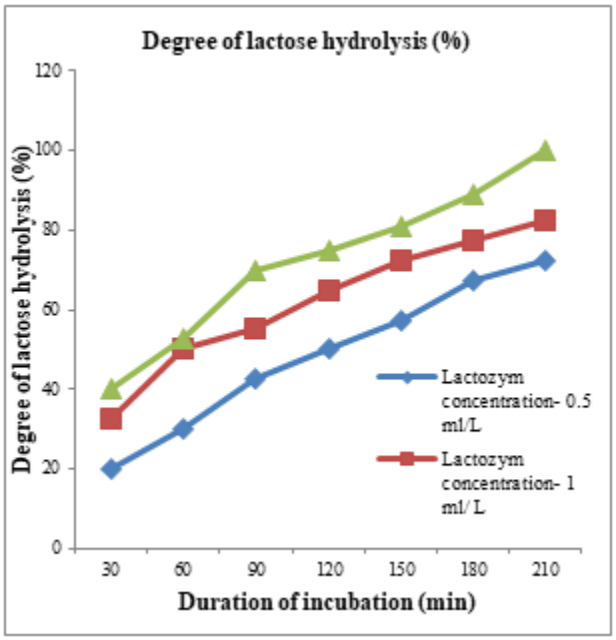

Fig 2:- Effect of Lactozym Concentration on the Degree of Lactose Hydrolysis in Milk

Effect of lactose hydrolysis on chemical composition of kulfi Effect of lactose hydrolysis on chemical composition of *kulfi* is presented in Table 2. The chemical composition of lactose hydrolyzed *kulfi* indicates that the degree lactose hydrolysis has no significant effect on any of the components of *kulfi* except the lactose content. The lactose hydrolysis resulted in a rapid decrease in the lactose content as a result of conversion of lactose to glucose and galactose. The lactose content in the control sample without hydrolysis was 10.14 per cent whereas it decreased to 4.03, 3.35 and 2.48 per cent at 70, 75 and 80 per cent lactose hydrolysis. Similar observation was made by Harini and Ramachandra (2012), who observed lower lactose and ash content in lactose hydrolyzed khoa compared to unhydrolyzed khoa.

Effect of lactose hydrolysis on physical properties of kulfi Effect of lactose hydrolysis on some of the physical characteristics of *kulfi* is presented in Table 3. Extent of lactose hydrolysis has no significant effect on the specific gravity concerned. However there was significant increase in melting rate and penetration value at 80 per cent level of hydrolysis compared to control, but 70 and 75 per cent levels of hydrolysis does not show significant difference as compared to control. The melting rate of control and *kulfi* prepared from the lactose hydrolyzed milk at 70, 75 and 85 per cent hydrolysis was noted to be 17.76, 18.57, 20.83 and 22.62 ml/15 min, respectively.

The results also demonstrated that as level of hydrolysis increases the penetration value also increases. The penetration value at 0, 70, 75 and 80 per cent level of hydrolysis was observed to 33.18, 34.16, 36.21 and 38.80 mm/5s, respectively. The increase in the melting rate and the penetration values was due to increase in the softness of the product. As hydrolysis increases, the hardness decreases. This is because the hydrolytic products, both glucose and galactose, sufficiently inhibit the formation of lactose crystals resulting in soft, smooth and fairly uniform body and texture of the hydrolyzed product.

Abbasi and Saeedabadian (2013) reported that in vanilla ice cream with lactose hydrolyzed milk the melting rate in ml/30 min increased from 35 to 46 when the degree of hydrolysis increased from 0 to >75 per cent. A similar increase in the penetration value was observed in khoa prepared from lactose hydrolyzed milk. The penetration value in mm/5s in khoa prepared from 0, 25, 50, 75 and 100 per cent lactose hydrolyzed milk were 22.58, 28.51, 42.52, 59.82 and 79.39, respectively (Harini and Ramachandra,2012).

Degree of hydrolysis (per cent)	Chemical constituents (per cent)				
	Moisture	Fat	Protein	Lactose	Ash
0(control)	61.33[a]	10.12[a]	6.65[a]	10.14[a]	1.27[a]
70	61.35[a]	10.12[a]	6.67[a]	4.03[b]	1.27[a]
75	61.29[a]	10.13[a]	6.68[a]	3.35[c]	1.28[a]
80	61.34[a]	10.13[a]	6.73[a]	2.48[d]	1.27[a]
CD (*P*=0.05)	0.04	0.005	0.05	0.27	0.01

Table 2:- Effect of Lactose Hydrolysis on Chemical Composition of *Kulfi*

Note:-
- Each value is mean of three trials
- Figures in a column with different alphabets differ significantly
- Control- Standard plain *kulfi* with 13 per cent sugar

Degree of hydrolysis (per cent)	Physical properties		
	Specific gravity	Melting rate (ml/15min)	Penetration value (mm/5s)
0 (control)	1.096	17.76[a]	33.18[a]
70	1.096	18.57[a]	34.16[a]
75	1.097	20.83[ab]	36.21[ab]
80	1.098[a]	22.62[b]	38.80[b]
CD (*P*=0.05)	0.01	1.48	1.72

Table 3:- Effect of Lactose Hydrolysis on Physical Properties of *Kulfi*

Note:
- Each value is mean of three trials
- Figures in a column with different alphabets differ significantly
- Control- Standard plain *kulfi* with 13 per cent sugar

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Effect of lactose hydrolysis on sensory qualities of kulfi It can be observed from the Table 4 that hydrolysis has significant effects on all sensory attributes of the *kulfi*. As the degree of lactose hydrolysis increased from 0 to 80 per cent, the scores for *kulfi* samples with respect to colour and appearance attribute increased from initial 7.55 to 8.55, indicating that the lactose hydrolysis favour the development of caramelized colour which is suitable for *kulfi*. The scores for colour and appearance of *kulfi* with 0, 70, 75 and 80 per cent lactose hydrolyzed milk were 7.55, 8.04, 8.44 and 8.55, respectively.

In case of body and texture it could be observed that there was a significant increase in the score for *kulfi* with 70 per cent hydrolyzed milk when compared to other samples. Highest score was obtained at 75 per cent hydrolysis. Further increased hydrolysis decreased the sensory scores in respect of body and texture attribute. However in both cases there was no significant difference as compared to control. The scores awarded for body and texture were 7.58, 8.18, 7.60 and 7.23 for *kulfi* prepared with 0, 70, 75 and 80 per cent lactose hydrolyzed milk, respectively.

Increase in body and texture score in case of *kulfi* samples at 70 and 75 per cent hydrolysis was due to increase in the softness of the product. As hydrolysis increases, the hardness decreases. This is because the hydrolytic products, both glucose and galactose, sufficiently inhibit the formation of lactose crystals resulting in soft, smooth and fairly uniform body and texture of the hydrolyzed product. Similar increase in the body and texture score was observed by El-Neshawy *et al*. (1988) in ice cream made from hydrolyzed lactose reconstituted milk. They observed that out of 30, control without lactose hydrolyzed reconstituted milk secured 18 while the ice cream with 50 % and 75 % lactose hydrolyzed reconstituted milk secured 28 and 29, respectively.

The lactose hydrolysis affects the flavour of the *kulfi* significantly. The *kulfi* samples with different levels of lactose hydrolyzed milk show significant difference in their flavour from that of control, but there was no significant difference among them. As the level of lactose hydrolysis increased from 0 to 80 per cent, the flavour scores decreased from initial 8.10 to 7.21. The scores awarded on nine point hedonic scale for flavor attribute were 8.10, 7.58, 7.42 and 7.21 for *kulfi* prepared with 0, 70, 75 and 80 per cent lactose hydrolyzed milk, respectively. Although lactose hydrolysis favors the development of caramelized nutty flavour in the developed *kulfi*, the flavour score decreased as hydrolysis proceeds as compared to control. This was due to the increased sweetness of the hydrolyzed product.

Similar observations were made by Harini and Ramachandra (2012) in khoa prepared from lactose hydrolyzed milk. They observed a decrease in the flavor score from 8.06 to 7.90 and increase in the sweetness scores from 7.99 to 8.92 when the degree of lactose hydrolysis increased from 0 to 100 per cent. They reported that the reduction in the flavour score is due to defects such as increased sweetness, saltiness, caramel like or syrup like and lacking vanilla flavour.

It could be observed from the table that the *kulfi* with 70 per cent lactose hydrolyzed milk was rated higher for the overall acceptability than other samples. The *kulfi* with 75 per cent lactose hydrolysis scored higher than that of control, whereas the *kulfi* with 80 per cent lactose hydrolyzed milk scored less as compared to control. The scores awarded for overall acceptability attribute was 7.75, 8.23, 7.83 and 7.55, respectively for *kulfi* prepared with 0, 70, 75 and 80 per cent lactose hydrolyzed milk.

Effect of sugar reduction on sensory qualities of lactose hydrolyzed kulfi (70 per cent) Effect of sugar reduction on the sensory attributes of 70 per cent lactose hydrolyzed *kulfi* is depicted in Table 5. The data shows that, the reduction of sugar in 70 per cent lactose hydrolyzed *kulfi* has no significant effect on any of the sensory attributes except flavour. It is observed from the table that lactose hydrolyzed *kulfi* with 40 per cent sugar reduction rated higher for all the sensory attributes than the control and other sugar reduced *kulfi* samples.

Level of hydrolysis (per cent)	Colour & appearance	Body & texture	Flavour	Overall acceptability
	Scores on 9 point hedonic scale			
0	7.55^{a}	7.58^{a}	8.10^{a}	7.75^{ab}
70	8.40^{b}	8.18^{b}	7.58^{b}	8.23^{a}
75	8.44^{b}	7.60^{a}	7.42^{b}	7.83^{ab}
80	8.55^{b}	7.23^{a}	7.21^{b}	7.55^{b}
CD(*P*=.05)	0.12	0.13	0.10	0.13

Table 4:- Effect of Lactose Hydrolysis on Sensory Qualities of *Kulfi*

Note:
- Each value is mean of three trials
- Figures in a column with different alphabets differ significantly
- Control- Standard plain *kulfi* with 13 per cent sugar

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Level of sugar reduction (per cent)	Colour & appearance	Body & texture	Flavour	Overall acceptability
	Scores on nine point hedonic scale			
0(control)	7.55	7.58	8.10[a]	7.75
40	8.00	7.90	8.50[a]	8.08
50	7.78	7.70	8.03[ab]	7.68
60	7.63	7.33	7.40[b]	7.37
CD (*P*=0.05)	0.18	0.41	0.16	0.23

Table 5:- Effect of Sugar Reduction on Sensory Qualities of Lactose Hydrolyzed *Kulfi* (70 percent)

Note:

- Each value is mean of three trials
- Figures in a column with different alphabets differ significantly
- Control- Standard plain *kulfi* with 13 per cent sugar
- 40 per cent sugar reduced *kulfi* - 7.8 per cent sugar
- 50 per cent sugar reduced *kulfi* – 6.5 per cent sugar
- 60 per cent sugar reduced *kulfi* – 5.2 per cent sugar

The lactose hydrolysis results in the breakdown of lactose to glucose and galactose. The relative sweetness factor of lactose is 16, whereas that of glucose and galactose is 74 and 60. Thus the hydrolysis of lactose in milk and milk products to its monosaccharides results in increased sweetness. Similar observations were made by Lindamood *et al*. (1989) in ice cream mixes treated with lactase. They reported that the hydrolysis of lactose increased the sweetness and the percentage sweetness of control, 25, 50 and 100 per cent lactose hydrolyzed ice cream mixes were 16, 16.7, 17.2 and 18.5, respectively.

In case of the developed lactose hydrolyzed *kulfi* a similar trend was noted. The hydrolysis of lactose resulted in increased sweetness, thus reducing the flavour score as discussed earlier. So an attempt was made to decrease the level of added cane sugar in the optimized 70 per cent lactose hydrolyzed *kulfi*. It can be observed from the table that lactose hydrolyzed *kulfi* with 40 per cent sugar reduction was rated higher for all the sensory attributes than the control and other sugar reduced *kulfi* samples. The 50 per cent and 60 per cent sugar reduction was rejected due to low sweetness.

Abbai and Saeedabadian (2013) reported 25-50 per cent sugar reduction in case of vanilla ice cream prepared with 50 – 75 per cent lactose hydrolyzed milk.

IV. CONCLUSION

The present investigation was carried out to develop *kulfi* from the lactose hydrolyzed milk. The addition of 1.5 ml/L of Lactozym (3000 LAU/ml) resulting in 70 % lactose hydrolysis was found superior in its physical properties such as melting rate and penetration value and also with respect to its sensory properties. The hydrolysis of lactose in milk results in elimination of lactose intolerance and increase softness and creaminess by preventing the sandiness in *kulfi* due to lactose crystallization. The 70 % lactose hydrolysis reduced the sugar percentage by 40 %.

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