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Effect of Storage (at – 18 °C) on Physicochemical, Sensory and Microbiological Properties of Lactose Hydrolyzed Functional *Kulfi*

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Abstract:- In *kulfi* prepared with 70 % lactose hydrolyzed milk added with 7.8 % sugar, 2 % oat flour, 1 % flaxseed oil and 4 % Whey Protein Concentrate were separately incorporated in order to enrich functional β-glucan, ω3 fatty acid (Alpla Linolenic Acid) and whey proteins to *kulfi*. The developed Lactose Hydrolyzed Functional *Kulfi* was subjected to the evaluation of storage stability for 50 days at -18 °C. As the storage days increased the melting rate and penetration value decreased whereas peroxide value and free fatty acids increased in both control and the developed *kulfi*, but both were stable for 50 days in terms of oxidative stability. The sensory attributes of control as well as experimental sample of *kulfi* were decreased slightly at each subsequent analysis interval. The control as well as product was found acceptable on the basis of overall acceptability score. From microbiological point of view the TBC, coliform and yeast and mold count was within the standards prescribed by BIS up to 50 days in both control and experimental *kulfi*.

Keywords:- *Kulfi, Lactose Hydrolysis, β- Glucan, ω3 Fatty Acid, Whey Proteins, Storage Stability, Functional and Sensory Properties.*

I. INTRODUCTION

Kulfi, also known as Malaikulfi/Malai-ka-burf is an indigenous frozen dairy product, which closely resembles ice cream in composition. 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. Lactose, a disaccharide is the primary carbohydrate found exclusively in mammalian milk. Cow's milk contains 4.5–5 per cent lactose. Lactose is responsible for the sandiness defect in ice cream and frozen desserts, also it leads to lactose intolerance in certain individuals. These limitations of lactose can be greatly minimized by hydrolyzing the lactose using β- galactosidase (lactase) enzyme (Thomas *et al*., 2019). 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 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).

Oat (*Avena sativa*) is a cereal grain rich in dietary fiber as β-glucan and protein. Oats are a good source of beta glucan and as such a good source of dietary fiber (Weightman *et al*., 2004). Beta Glucan is a polymer of D-glucose linked with glycosidic bonds at β (1 →3), β (1 →4), β (1 →6) and is typically found in the endosperm cell wall in oats, barley. Commercially β-glucan is obtained from oats, barley, mushrooms and some microorganisms. Beta glucan constitutes 1 per cent of wheat grains, 3-7 per cent of oats and 5-11 per cent of barley (Skendi *et al*., 2003). Studies have indicated the hypocholesterolaemic effect of this compound, leading to 20–30 per cent reduction of LDL-cholesterol, and to an expected overall effect of reduced cardiovascular disease risk (Gallaher, 2000). In 1997, the FDA of US has formally acknowledged as functional the products made of whole-grain oats or oat fiber with a minimum of 0.75 g β-glucan/ serving size (Angelov *et al*., 2006).

Flaxseed (*Linum usitatissimum*) commonly known as linseed, is a member of the genus Linum in the family Linaceae. Commercially, flax is grown both for its seeds and for its fibres and its seed is one of the richest plant sources of the α-linolenic acid (ALA), an ω-3 fatty acid (Gebauer *et al*., 2006). Besides ALA, soluble and insoluble fibres, phytoestrogenic lignans, and an array of antioxidants (tocopherols) are also present in flax seed oil significantly. "The potential health benefits of ω-3 fatty acids have been widely reported for several conditions including cardiovascular disease, hypertension, atherosclerosis, brain development, diabetes, cancer and neurological disorders (Gogus and Smith, 2010).

Whey proteins are by-products of the cheese making process and were once considered a waste product, but now are considered as a valuable by-product (Marshall, 2004). Whey protein structure is rich in branched chained amino acid such as leucine, valine, and isoleucine (Berber *et al*., 2015). Whey protein concentrates possess excellent functional properties which make them compatible with any type of products (Berber *et al*., 2015). Whey's amino acid profile makes it ideal for body composition and to support

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protein synthesis and muscle growth. Other bioactive components found in whey might benefit additional aspects of health in active people and trained athletes by improving immune function and gastrointestinal health and exhibiting anti-inflammatory activity. Whey components, such as IgA, glutamine, and lactoferrin, can rectify common complaints among athletes, including repeated infections and gastrointestinal disturbances (Marshall, 2004).

Kulfi, a frozen dessert which is enjoyed by consumers of all ages, is a fair means of offering good nutrition to the consumer. If lactose hydrolyzed milk is used along with addition of oat flour powder, flaxseed oil and whey protein concentrate to milk for preparation of *kulfi*, it becomes a functional *kulfi* and can extend therapeutic benefits to consumers. The objective of this study was to study the shelf stability of developed lactose hydrolyzed functional *kulfi*.

II. MATERIALS AND METHODS

Ingredients Fresh whole milk was procured from Students Experimental Dairy Plant (SEDP) of Dairy Science College, Hebbal, Bengaluru. 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. Oat groats were obtained from Satwik foods, Goa. The groats were roasted at 60- 70 ºC for 10 to 15 min and powdered to obtain oat flour. Virgin single press processed flaxseed oil manufactured by 'UNIFINE', Ahmedabad was obtained from local market. Whey Protein Concentrate - Lactoprot, Lactomin 80 was procured from Lactoport Deutschland, Germany.

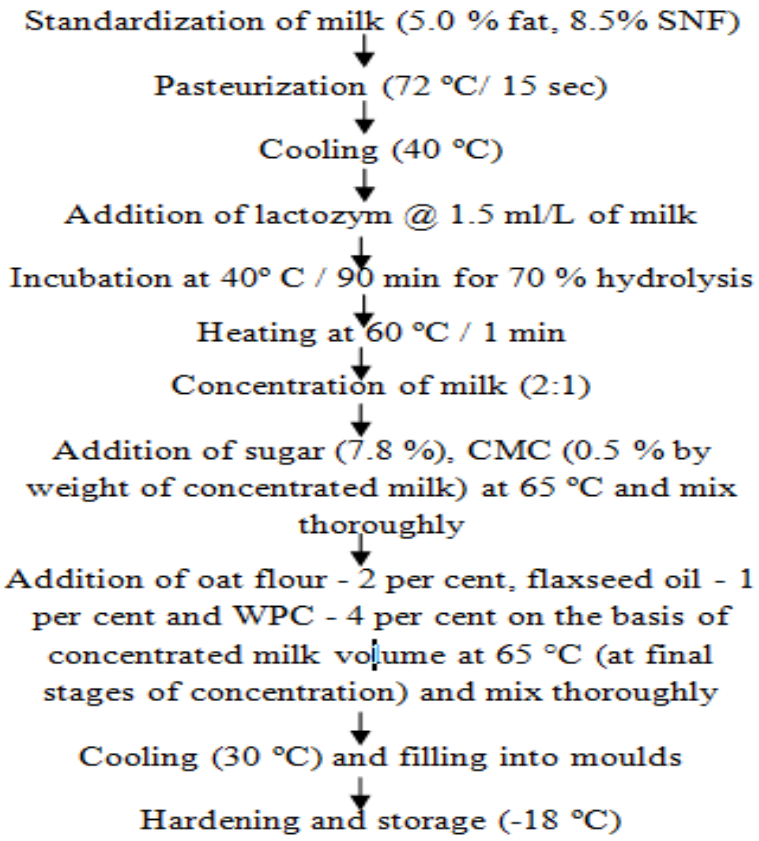

Fig 1:- Flow chart for the preparation of lactose hydrolyzed functional *kulfi*

Storage Studies The *kulfi* samples prepared by lactose hydrolysis of milk and with incorporation of oat flour, flaxseed oil and whey protein concentrate which showed maximum sensory score were subjected for the evaluation of storage stability for 50 days at -18 °C. The chemical (peroxide value, Free Fatty Acid value), physical (melting rate and penetration value), sensory and microbiological parameters (Total bacterial count, coliform count, yeast and mold count) of the formulated *kulfi* were evaluated from the day of preparation at every 10 days interval by employing standard methods.

Standard of ISI: SP 18 (Part XI) 1981 was adopted for carrying out chemical analysis. The melting rate of the *kulfi* was observed by drawing 10 g 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

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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 5 s of the sample was noted. For each sample reading were recorded at 3 different spots and the mean value was noted.

Microbiological analysis Total bacterial count, coliform count and yeast and mold count and of *kulfi* samples were determined as per the standard methods given in IS SP 18 Part (XI) 1981. Counts obtained were expressed as $\log_{10}$ cfu/g.

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 storage (at -18 °C) on melting rate and penetration value of Kulfi The effect of storage on melting rate and penetration value of *kulfi* is presented in table 1, fig 1 and 2, respectively. The result shows that as the storage days increases the melting rate and penetration value keeps on decreasing in both control and the experimental *kulfi*, indicating that hardness is increased during storage. The melting rate in case of control decreased from 17.76 to 17.10 whereas in case of experimental *kulfi* the melting rate was decreased from 17.48 to 17.05 ml/15min as the period of storage increased from 0 to 50. Similar trend was also observed in penetration value in both control and experimental *kulfi*. The penetration value in case of control decreased from 33.18 to 30.30 whereas in case of experimental *kulfi* the penetration value was decreased from 34.32 to 32.55 mm/5s as the period of storage increased from 0 to 50.

It was observed that compared to control the rate of decrease of penetration value and melting rate was less in the developed *kulfi*. This may be due to the presence of the added ingredients such as oat flour and WPC which may prevent the formation of ice crystals in the *kulfi* because of their water binding ability.

The results are in agreement with Jha (2005) who conducted shelf life studies of low fat and low calorie kulfi at -15 °C for 60 days. He observed that hardness in 10 kg/50 g increased from 3.67 on 0th day to 4.2 on 60th day of storage in case of control whereas in the experimental sample hardness increased from 3.3 to 5.3. Meltdown rate in ml/min increased from 14 to 19 in experimental sample compared to 14 to 16 in case of control.

Storage period (Days)	Control *kulfi*		Experimental *kulfi*	
	Melting rate (ml/15min)	Penetration value (mm/5s)	Melting rate (ml/15min)	Penetration value (mm/5s)
0	17.76^{a}	33.18^{a}	17.48^{a}	34.32^{a}
10	17.71^{ab}	33.05^{a}	17.44^{ab}	34.25^{a}
20	17.60^{b}	33.00^{a}	17.42^{ab}	34.10^{a}
30	17.35^{c}	31.00^{b}	17.35^{ab}	33.25^{b}
40	17.20^{d}	30.50^{b}	17.30^{b}	32.90^{bc}
50	17.10^{d}	30.30^{b}	17.05^{c}	32.55^{c}
CD(*P*=0.05)	**0.05**	**0.47**	**0.06**	**0.19**

Table 1:- Effect of storage (at -18 °C) on melting rate and penetration value of *Kulfi*

Note:

- Each value is mean of three trials
- Figures in a column with different alphabets differ significantly
- Control *kulfi* - Standard plain *kulfi* with 13 per cent sugar
- Experimental *kulfi* – 70 per cent lactose hydrolyzed *kulfi* with 7.8 per cent sugar, 2.0 per cent oat flour, 1.0 per cent flaxseed oil and 4.0 per cent WPC

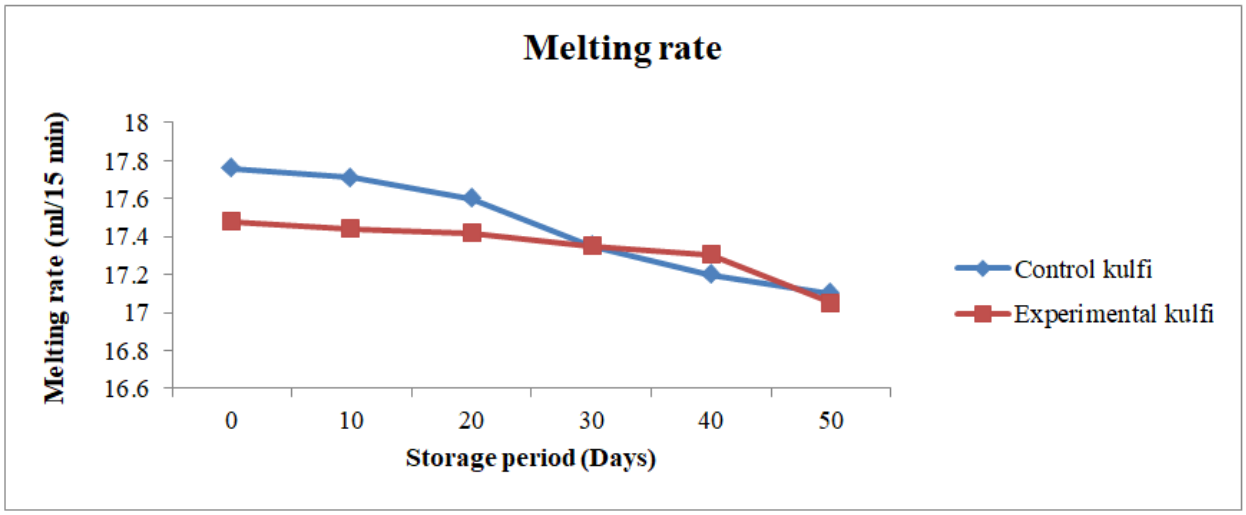

Fig 1: Effect of storage (at -18 °C) on melting rate of *kulfi*

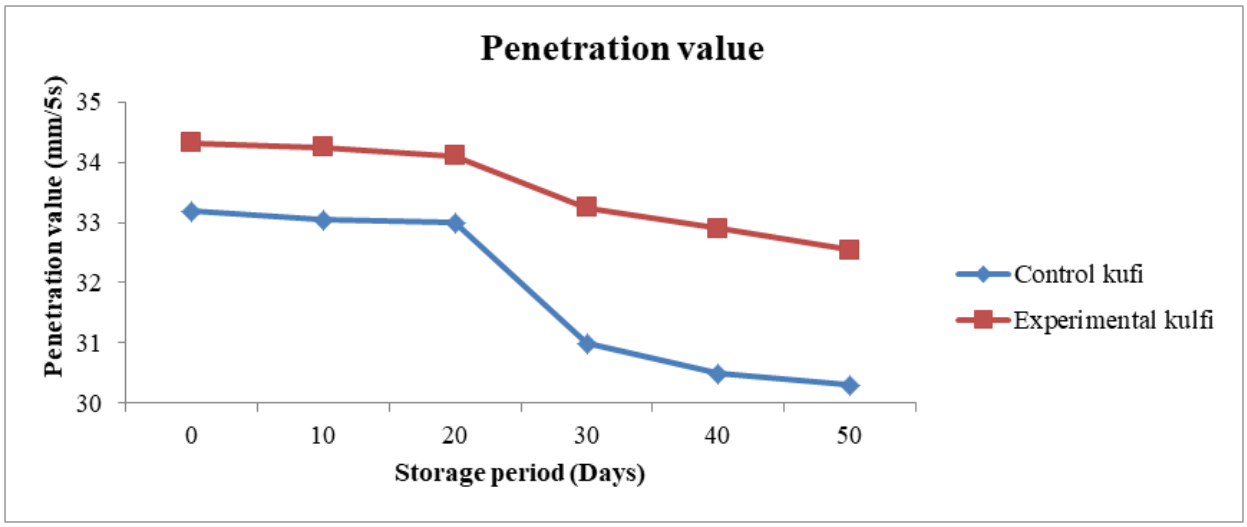

Fig 2:- Effect of storage (at -18 °C) on penetration value of *kulfi*

Effect of storage (at -18 °C) on peroxide value and free fatty acids of Kulfi The effect of storage on peroxide value and free fatty acids of *kulfi* is presented in table 2, figure 3 and 4, respectively. Result shows that both peroxide value and free fatty acid values keep on increasing in both control and developed *kulfi* samples during storage. Peroxide value and free fatty acids indicates the extent of oxidation and formation of primary oxidation products. Flaxseed oil is highly polyunsaturated (76 per cent PUFA) and thus, highly susceptible to atmospheric oxygen, high temperature and metal ions. It is reported that α- linolenic acid is 20 times more susceptible to oxidation as compared to oleic acid (Decker *et al*., 2012).

It is evident from the table that as the storage period progressed, the peroxide value and free fatty acids increased. In case of control the peroxide value increased from 0.25 on 0^{th} day to 1.63 on 50^{th} day while the free fatty acid value increased from 0.74 on 0^{th} day to 1.28 on 50^{th} day. Whereas in the case of developed *kulfi* the peroxide value increased from 0.27 on 0^{th} day to 2.18 on 50^{th} day while the free fatty acid value increased from 0.92 on 0^{th} day to 1.60 on 50^{th} day. Both control and the developed product were stable for 50 days in terms of oxidative stability.

Similar results have been reported by Avinash (2014) in stability of microencapsulated omega-3 fatty acids rich flaxseed oil powder in ice-cream. He reported that as the storage period progressed, the PV increased and reached to the maximum on 30th day of storage indicating the formation of hydroperoxides to the maximum level of 3.03 meq peroxides/kg oil and FFAs content increased continuously during the storage of 120 days in the ice-cream samples to 1.67 ml N NaOH/100 g fat. This increase in FFAs could be due to the hydrolysis of unprotected/ free oil present in the MFOP used in the preparation of omega- 3 fortified ice cream.

It is evident from the results that the rate of increase in the peroxide value and free fatty acid value was higher in case of developed kulfi compared to control. This may be attributed to the presence of flaxseed oil in the free form without any encapsulation techniques.

Storage period (Days)	Control *kulfi*		Experimental *kulfi*	
	Peroxide value (meq peroxides /kg of oil)	Free fatty acids (per cent oleic acid)	Peroxide value (meq peroxides /kg of oil)	Free fatty acids (per cent oleic acid)
0	0.25[a]	0.74[a]	0.27[a]	0.92[a]
10	0.83[ab]	1.15[b]	1.13[b]	1.43[b]
20	1.45[bc]	1.18[b]	1.89[bc]	1.48[b]
30	1.50[c]	1.21[b]	1.99[c]	1.55[b]
40	1.58[c]	1.24[b]	2.09[c]	1.58[b]
50	1.63[c]	1.28[b]	2.18[c]	1.60[b]
CD(*P*=0.05)	**0.24**	**0.11**	**0.32**	**0.14**

Table 2:- Effect of storage (at -18 °C) on peroxide value and free fatty acids of *kulfi*

Note:

- Each value is mean of three trials
- Figures in a column with different alphabets differ significantly
- Control *kulfi* - Standard plain *kulfi* with 13 per cent sugar
- Experimental *kulfi* – 70 per cent lactose hydrolyzed *kulfi* with 7.8 per cent sugar, 2.0 per cent oat flour, 1.0 per cent flaxseed oil and 4.0 per cent WPC

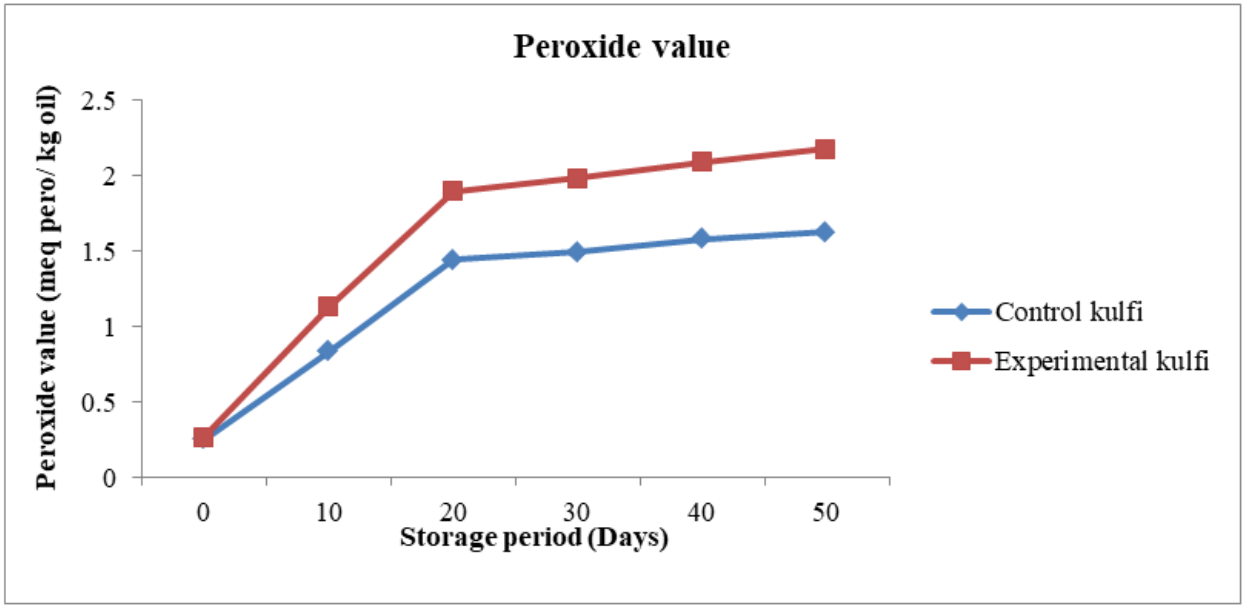

Fig 3: Effect of storage (at -18 °C) on peroxide value of *Kulfi*

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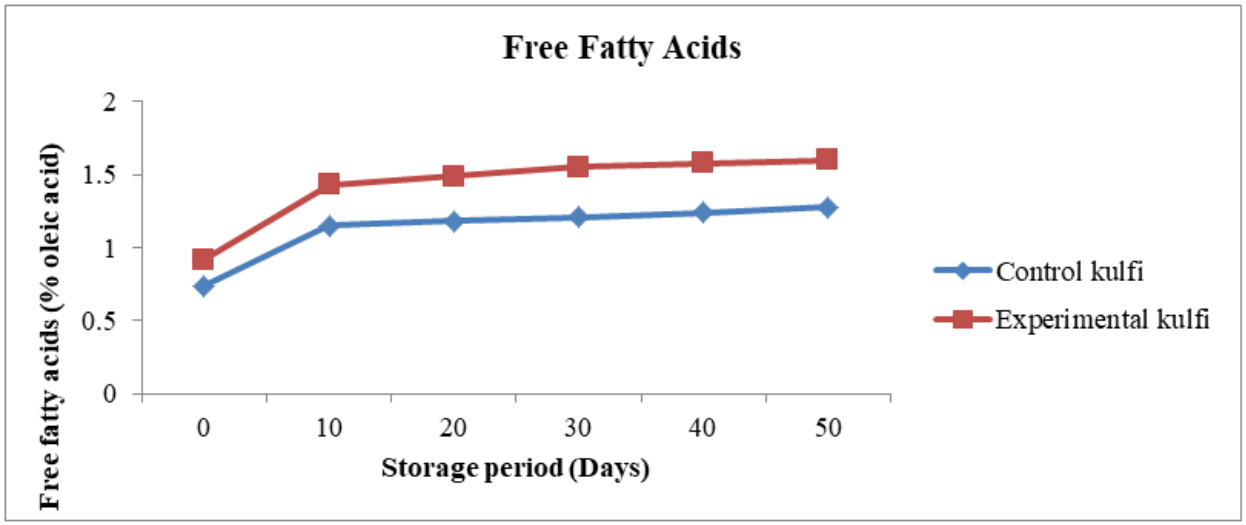

Effect of storage (at -18 °C) on sensory attributes of Kulfi The effect of storage on the sensory attributes of *Kulfi* is presented in table 3. From the results pertaining to the effect of storage on sensory attributes of *kulfi* it can be observed that score for all the sensory attributes of control as well as experimental sample of *kulfi* were decreased slightly at each subsequent analysis interval. At the end of storage the product was found acceptable on the basis of overall acceptability score in both the cases i.e. control as well as experimental. It was concluded from the results that lactose hydrolyzed functional *kulfi* can be stored up to 50 days without affecting its sensory attributes.

Similar observations were reported by Jha (2005) who conducted shelf life studies of low fat and low calorie *kulfi* at -15 °C for 60 days. Sensory scores for all the attributes of control as well as experimental sample of *kulfi* were decreased slightly at each subsequent analysis interval. At the end of storage the experimental sample was scored 7.50, 7.40, 7.21 and 7.20 for colour and appearance, body and texture, flavour and overall acceptability, respectively while the control *kulfi* scored 7.68, 7.88, 7.70 and 7.60, respectively.

Effect of storage (at -18 °C) on microbiological quality of Kulfi The microbiological analysis shown on table 4 indicates that the total bacterial count, coliform count and yeast and mold count of both control and experimental *kulfi* was less than maximum limit as prescribed in BIS standards of *kulfi*.

From microbiological point of view the experimental *kulfi* maintains quality as that of control *kulfi* and the product is safe for consumption up to 50 days of storage at -18 °C.

Storage period (Days)	**Control *kulfi***				**Experimental *kulfi***			
	Colour & appearance	**Body & texture**	**Flavour**	**Overall acceptability**	**Colour & appearance**	**Body & texture**	**Flavour**	**Overall acceptability**
0	7.55^{a}	7.58^{a}	8.10^{a}	7.75^{a}	7.82^{a}	8.13^{a}	7.86^{a}	7.95^{a}
10	7.53^{a}	7.57^{a}	8.09^{a}	7.75^{a}	7.80^{ab}	8.12^{a}	7.77^{a}	7.93^{a}
20	7.54^{a}	7.56^{a}	8.09^{a}	7.80^{a}	7.79^{b}	8.12^{a}	7.73^{a}	7.85^{ab}
30	7.52^{a}	7.51^{ab}	8.04^{a}	7.70^{a}	7.78^{bc}	8.01^{b}	7.64^{ab}	7.68^{bc}
40	7.22^{b}	7.41^{bc}	7.64^{b}	7.55^{ab}	7.76^{c}	7.98^{c}	7.52^{b}	7.50^{c}
50	7.22^{b}	7.36^{c}	7.64^{b}	7.40^{b}	7.76^{c}	7.95^{d}	7.20^{c}	7.27^{d}
CD(*P*=0.05)	**0.13**	**0.05**	**0.18**	**0.16**	**0.01**	**0.01**	**0.13**	**0.07**

Table 3:- Effect of storage (at -18 °C) on sensory attributes of *kulfi*

Note:
- Each value is mean of three trials
- Figures in a column with different alphabets differ significantly
- Control *kulfi* - Standard plain *kulfi* with 13 per cent sugar
- Expt *kulfi* – 70 per cent lactose hydrolyzed *kulfi* with 7.8 per cent sugar, 2 per cent oat flour, 1 per cent flaxseed oil and 4 per cent WPC

Storage period (days)	Processes	Total bacterial count	Coliform count	Yeast and mold count
		log_{10} cfu/g		
0	**Control**	4.52^{a}	Nil	Nil
	Product	4.70^{a}	Nil	Nil
10	**Control**	4.59^{b}	Nil	Nil
	Product	4.79^{b}	Nil	Nil
20	**Control**	4.65^{c}	Nil	Nil
	Product	4.89^{c}	Nil	Nil
30	**Control**	4.76^{d}	Nil	Nil
	Product	4.93^{cd}	Nil	Nil
40	**Control**	4.83^{e}	Nil	1.0^{b}
	Product	4.97^{d}	1.0^{b}	1.0^{b}
50	**Control**	4.87^{e}	1.0^{b}	1.0^{b}
	Product	5.00^{d}	1.3^{b}	1.3^{b}
CD(P=0.05)	**Control**	**0.02**	**0.30**	**0.28**
	Product	**0.03**	**0.31**	**0.31**

Table 4:- Effect of storage (at -18 °C) on microbiological quality of *kulfi*

Note:
- Each value is mean of three trials
- Figures in a column with different alphabets differ significantly
- Control *kulfi* - Standard plain *kulfi* with 13 per cent sugar
- Experimental *kulfi* – 70 per cent lactose hydrolyzed *kulfi* with 7.8 per cent sugar, 2.0 per cent oat flour, 1.0 per cent flaxseed oil and 4.0 per cent WPC

CONCLUSION

The present investigation was carried out to determine the storage stability of developed lactose hydrolyzed *kulfi* supplemented with oat flour, flaxseed oil and WPC. The effect of supplementation of these functional ingredients on chemical composition, physical properties, sensory attributes and microbiological analysis shows that, the control as well as product was acceptable up to 50 days at –18 °C.

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