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Effect of Supplementation of Whey Protein Concentrate on Physicochemical and Sensory Properties of Lactose Hydrolyzed *Kulfi*

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Abstract:- In *kulfi* prepared with 70 % lactose hydrolyzed milk added with 7.8 % sugar 2 % oat flour and 1 % flaxseed oil, Whey Protein Concentrate (2, 4 and 6 per cent) was separately incorporated in order to enrich whey proteins to *kulfi*. The effect of this functional ingredient on chemical composition, sensory attributes and physical properties such as melting rate and penetration value were studied separately. As the level of supplementation of WPC increased from 0 to 6 per cent, moisture, fat, ash and the melting rate decreased whereas the protein content, specific gravity and penetration value increased significantly. 70 per cent lactose hydrolyzed *kulfi* with 4 per cent WPC was found better in physico-chemical and sensory attributes.

Keywords:- *Kulfi, WPC, Whey Proteins, Functional and Sensory Properties.*

I. INTRODUCTION

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). Three groups of WPC have come to dominate in world trade: low protein WPC containing 25 to 45 per cent protein, medium protein WPC containing 45 to 60 per cent protein and high protein WPC containing 60 to 80 per cent protein (Nielsen, 1988). In United States the requirements for the composition of WPC are protein (min) 25 per cent, fat 0.2- 10 per cent, ash 2-15 per cent, lactose (max) 60 per cent and moisture 1-6 per cent (Giri, 2007).

Whey protein concentrates possess excellent functional properties which make them compatible with any type of products (Berber *et al.*, 2015). The functional properties of WPC that are exploited in meat products include emulsification, gelling, water and fat absorption and binding whereas in beverages the solubility of WPC at different pH's is of great advantage. Binding, cohesion and adhesion properties are widely used in food coatings (Berber *et al.*, 2015). WPCs have also been used as a base material for the manufacture of coffee whiteners, whipped toppings, imitation sour creams and instant breakfast foods.

Whey has potent antioxidant activity, likely by contributing cysteine-rich proteins that aid in the synthesis of glutathione (GSH), a potent intracellular antioxidant (Walzem *et al*., 2002). As a result of the glutathione/ antioxidant component of whey, it is being investigated as an anti-aging agent (Marz, 2002).

Whey protein concentrates have been researched extensively in the prevention and treatment of cancer. Glutathione stimulation is thought to be the primary immune-modulating mechanism (Bouous, 2000). Whey also have anticancer potential due to iron binding capacity, as iron may act as a mutagenic agent causing oxidative damage to tissues (Weinberg, 1996).

Whey's amino acid profile makes it ideal for body composition and to support 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 WPC it becomes a functional *kulfi* and can extend therapeutic benefits to consumers. The objective of this study was to optimize the level of supplementation of WPC to *kulfi* prepared from 70 % lactose hydrolyzed milk.

Whey Components	% of Whey Protein	Benefits
β-Lactoglobulin	50-55	Source of essential and branched chain amino acids
α-Lactalbumin	20-25	Primary protein found in human breast milk; Source of essential and branched chain amino acids
Immunoglobulins	10-15	Primary protein found in colostrums; Immune modulating benefits
Lactoferrin	1-2	Antioxidant, antibacterial, antiviral and antifungal; Promotes growth of beneficial bacteria
Lactoperoxidase	0.50	Inhibits growth of bacteria
Bovine Serum Albumin	5-10	Source of essential amino acids; Large protein
Glycomacropeptide	10-15	Source of branched chain amino acids

Table 1:- Composition of whey proteins (Marshall, 2004)

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. Whey Protein Concentrate - Lactoprot, Lactomin 80 was procured from Lactoport Deutschland, Germany.

Preparation of lactose hydrolyzed kulfi 4 L of fresh milk was standardised to 5.0 % fat and 8.5 % SNF. The standardized milk was pasteurized and cooled to 40 ºC. 1.5 ml/L of Lactozym was added and incubated at 40 ºC for 90 min to obtain 70 % lactose hydrolysis. This milk was further used to prepare lactose hydrolyzed *kulfi* as per the procedure outlined by Thomas *et al* (2019). To this oat flour @ 2 % and flaxseed oil @ 1 % on the basis of concentrated milk volume is added (Thomas *et al*., 2019).

Preparation of lactose hydrolyzed kulfi supplemented with WPC To the *kulfi* prepared WPC was added @ 2, 4 and 6 % on the basis of concentrated milk volume at 65 ºC (at the final stage of condensation) and mixed thoroughly. This mix was cooled to 30 ºC, filled into moulds and hardened at -18 ºC.

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 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 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.

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 WPC on chemical composition of lactose hydrolyzed kulfi The effect of supplementation of WPC on the chemical composition of *kulfi* is presented in Table 1. Result shows that the addition of WPC at 2, 4 and 6 per cent level has significant effect on all the constituents except lactose content.

Due to addition of WPC, net weight of kulfi increased, which decreased the concentrations of fat, ash and moisture

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content in the product. Increased levels of WPC from 0 to 6 per cent, decreased the moisture content significantly from 60.61 in control to 58.49 in 6 per cent WPC supplemented kulfi, fat content from 11.25 to 10.64 and ash content from 1.28 to 1.26, respectively. As the levels of WPC increased, the protein percentage increased significantly in the kulfi from 7.02 in the control to 11.83 per cent in the kulfi supplemented with 6 per cent WPC, which might be due to the presence of high protein content (80 per cent) in WPC. Similar observations are made by Giri et al. (2013) in stevia sweetened kulfi enriched with 3 per cent WPC. They reported that compared to control kulfi without any addedWPC the *kulfi* prepared with 3 per cent WPC contained fat per cent (control- 10.7, product-10.4) protein per cent (control- 6.9, product - 8.7), ash content (control- 1.1, product -1.0) and moisture content in per cent (control- 64.9, product - 63.0).

Effect of WPC on physical properties of lactose hydrolyzed kulfi The effect of supplementation of WPC on the specific gravity, melting rate and penetration value of *kulfi* is presented in Table 2 and figures 1, 2 and 3, respectively. From the results it is evident the supplementation of WPC in *kulfi* has significant effect on the physical properties. It was noticed that increased levels of WPC increased the specific gravity of *kulfi* mix. It may be attributed to the water binding ability of WPC. Similar findings have been reported by Giri *et al*. (2013) in stevia sweetened *kulfi* enriched with 3 per cent WPC (Control-1.086, product- 1.095).

The melting rate decreased significantly as the level of WPC addition increased. The decreased melting rate of treated *kulfi* samples may be attributed to the improved water-binding ability and stability of *kulfi* samples as a result of WPC incorporation. The interaction and association of whey proteins with casein micelles probably resulted in improved melting resistance in *kulfi* samples. Giri *et al*. (2013) reported that increase in the level of WPC in stevia sweetened *kulfi* (0, 2, 3 and 4 per cent) resulted in a gradual decrease in melting rate (14.81, 13.27, 12.58 and 11.93 ml/15 min).

Level of WPC (per cent of kulfi mix)	**Chemical constituents (per cent)**				
	Moisture	**Fat**	**Protein**	**Lactose**	**Ash**
0(control)	60.61[a]	11.25[a]	7.02[a]	4.04	1.28[a]
2	60.00[b]	11.05[b]	8.63[b]	4.04	1.28[a]
4	59.35[c]	10.85[c]	10.22[c]	4.04	1.27[b]
6	58.49[d]	10.64[d]	11.83[d]	4.03	1.26[c]
CD(*P*=0.05)	**0.17**	**0.002**	**0.23**	**0.01**	**0.003**

Table 1:- Effect of supplementation of WPC on chemical composition of lactose hydrolyzed *Kulfi*

Level of WPC (per centof kulfi mix)	**Physical properties**		
	Specific gravity	**Melting rate (ml/15min)**	**Penetration value (mm/5s)**
0 (control)	1.112[a]	17.48[a]	34.32[a]
2	1.116[b]	16.44[b]	36.53[b]
4	1.120[c]	15.51[c]	37.33[b]
6	1.124[d]	14.36[d]	38.35[c]
CD(*P*=0.05)	**0.002**	**0.10**	**0.41**

Table 2:- Effect of supplementation of WPC on physical properties of lactose hydrolyzed *kulfi*

Note:

- Each value is mean of three trials
- Figures in a column with different alphabets differ significantly
- Control- 70 % lactose hydrolyzed *kulfi* with 7.8 % sugar, 2 % oat flour and 1 % flaxseed oil

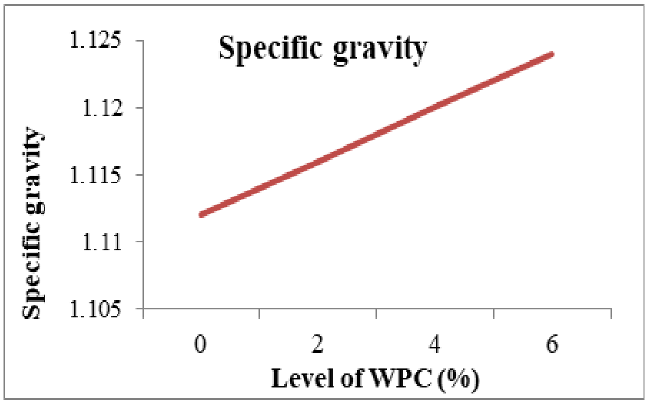

Fig 1:- Effect of supplementation of WPC on specific gravity of lactose hydrolyzed kulfi

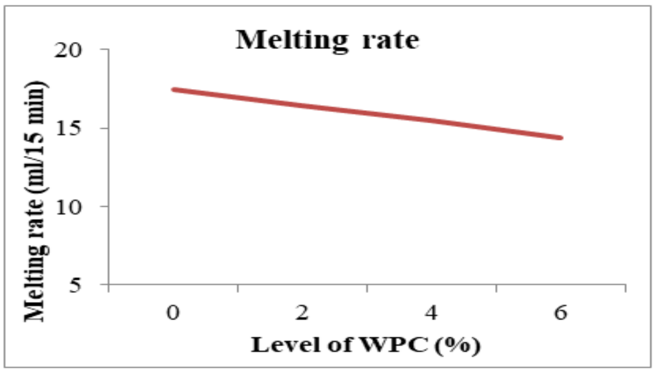

Fig 2:- Effect of supplementation of WPC on melting rate of lactose hydrolyzed kulfi

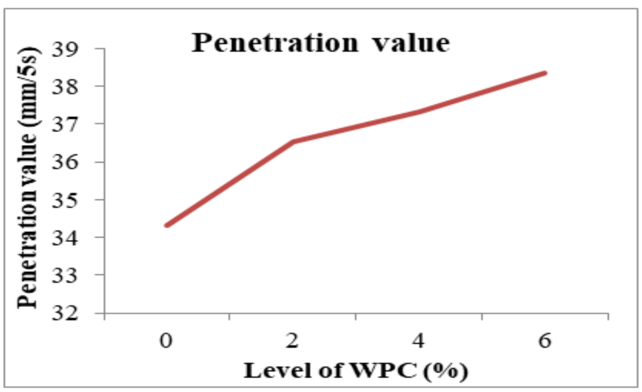

Fig 3:- Effect of supplementation of WPC on penetration value of lactose hydrolyzed kulfi

It was observed that increased levels of WPC addition increased the penetration value significantly in *kulfi*. Increased penetration value is indication of softness of the product. It is well known that whey protein imparts excellent functional properties, especially with respect to emulsifying and water-binding properties, which reduces iciness in the formulated *kulfi*. Similar findings have also been reported by Giri *et al*. (2013) in stevia sweetened *kulfi* enriched with 0, 2, 3, 4 per cent WPC as 28.27, 30.07, 30.96 and 31.81 mm/5s, respectively.

Effect of WPC on sensory attributes of lactose hydrolyzed kulfi The effect of supplementation of WPC on the sensory attributes of *kulfi* is presented in Table 3. The result shows that addition of WPC has significant effect on all sensory attributes. The colour and appearance scores did not show any significant change up to 4 per cent addition of WPC. At 6 per cent level, the colour and appearance scores significantly decreased as compared to control. The decrease in score at 6 per cent level may be attributed to the intense brownish colour developed during condensing of milk for *kulfi* making. Giri *et al*. (2013) reported a decrease in colour and appearance scores in stevia sweetened *kulfi* enriched with WPC as 8.0, 7.95, 7.93 and 7.90 for 0, 2, 3 and 4 per cent levels of WPC, respectively.

It was noticed from the results that the body and texture scores of *kulfi* significantly increased with increased levels of WPC addition. The judges noticed smooth body and texture with higher melting resistance and mellowness with very little feeling of iciness as the level of WPC in the *kulfi* increased. Improved body and texture quality upon incorporation of WPC may be attributed to the increased water binding capacity and excellent emulsifying properties imparted by whey proteins.Giri *et al*. (2013) reported an increase in body and texture scores in stevia sweetened *kulfi* enriched with WPC as 7.4, 7.7, 7.84 and 7.48 for 0, 2, 3 and 4 per cent levels of WPC, respectively.

As the level of WPC increased, the flavour scores also increased up to 4 per cent addition, and then decreased at 6 per cent level of WPC. At all levels of addition of WPC, the flavour scores of *kulfi* samples were greater than that of control. The increase in the flavour score may be attributed to the oil binding capacity of WPC which mask the harsh flaxseed oil flavour. The WPC has excellent ability to act as a binding agent (Marshall, 2004). At 6 per cent level of WPC the flavour scores decreased than that of 2 and 4 per cent levels. This may be due to the detectable whey flavour imparted by the addition of WPC. Giri *et al*. (2013) reported an increase in flavour scores in stevia sweetened *kulfi* enriched with WPC as 8.0, 7.78, 7.57 and 7.38 for 0, 2, 3 and 4 per cent levels of WPC, respectively.

It was observed from the results that there was significant increase of overall acceptability score up to 4 per cent WPC addition followed by significant reduction as the level of WPC addition increased. Up to 4 per cent addition of WPC overall acceptability score increased due to improved body and texture, and flavour scores due to the excellent functional properties of whey protein but above 4 per cent addition of WPC there was detectable whey flavour. Hence the judges adjudged the 4 per cent WPC added *kulfi* as the best. Similar observation was reported by Giri *et al*. (2013) in stevia sweetened *kulfi* enriched with WPC. They observed overall acceptability scores of *kulfi* with 0, 2, 3 and 4 per cent levels of WPC as 7.5, 7.62, 7.82 and 7.55.

Addition of WPC (per cent of kulfi mix)	Colour & appearance	Body & texture	Flavour	Overall acceptability
	Scores on nine point hedonic scale			
0 (control)	7.85^{a}	8.02^{a}	7.25^{a}	7.83^{a}
2	7.85^{a}	8.08^{b}	7.62^{b}	7.88^{b}
4	7.82^{ab}	8.13^{c}	7.86^{c}	7.95^{c}
6	7.80^{b}	8.23^{d}	7.55^{d}	7.83^{a}
CD(*P*=0.05)	**0.01**	**0.01**	**0.007**	**0.006**

Table 3:- Effect of supplementation of WPC on sensory properties of lactose hydrolyzed *kulfi*

Note:

- Each value is mean of three trials
- Figures in a column with different alphabets differ significantly
- Control- 70 % lactose hydrolyzed *kulfi* with 7.8 % sugar, 2 % oat flour and 1 % flaxseed oil

IV. CONCLUSION

The present investigation was carried out to develop lactose hydrolyzed *kulfi* supplemented with WPC. The effect of supplementation of WPC on chemical composition, physical properties and sensory attributes shows that, as the level of WPC increased from 2 to 6 %, moisture, fat, ash and the melting rate decreased whereas the protein content, specific gravity and penetration value increased significantly. 70 % lactose hydrolyzed *kulfi* with 4 % WPC was found better in physico-chemical and sensory attributes than 2 % and 4 % WPC added 70 % lactose hydrolyzed *kulfi*.

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