Bacteriological, Preparation and optimization for lactose hydrolyzed sweet concentrate whey using protein hydroysate in ice creams

Abstract: Whey solids are well known for their nutritional and functional properties and the same has been well documented. Owing to their excellent nutritional and functional properties of whey solids find numerous applications in food and dairy industry The biological components of whey including Lactoferrin, β-Lactoglobulin, α-Lactalbumin, glycomacropeptides and Immunoglobulin. Whey contains valuable lactose and proteins. Almost about 80 percentof whey is wasted without being used in country. Whey carries low total solids and has very low shelf life. Thus, the parameters like water activity (a_w), pH, redox potential, heat treatment etc, which are bacteriostatic, bactericidal are hurdles. These hurdles may govern many preservations process and more than, one hurdle is often responsible for long shelf life of certain food product. Whey concentrate was preserved by combined effect of lowering water activity (0.92-0.94%), low pH (5.2) and addition of potassium sorbate (0.2%), to achieve a storage life of about 3 months. Lactose, if added in higher concentration causes higher sandiness to ice cream; however, this defect could be reduced by hydrolyzing lactose present in whey.

Keywords: Whey proteins, Bacteriological, Preparation, optimization, Ice cream

1. Introduction:

Whey is a potential source of nutritionally rich carbohydrate and protein. Whey is highly perishable and its disposal is of great concern to the dairy industry as it carries high BOD. Preservation of whey by hurdle techniques helps in extending shelf life of whey. Whey solids are preserved by this technique could be used in various food formulations. As and when whey is available, it could be preserved by this technique and used in different dairy and food product formulations. This will show a better way for preservation of whey and its utilization in human chain.

Lactose, a disaccharide composed of the simple sugars glucose and galactose, is the primary carbohydrate in mammalian milk. Lactose represents an enormous energy potential and has a number of unique properties, which can be utilized for further purposes. Lactose is an

economically profitable sugar for the food for the food industry and is a basic raw material for technical applications (Stegmann, 1986)

In food industry, lactose is used in the manufacture of breads, beer, frozen vegetables, salad dressings, soups, cereals, processed meats, confections, cake mixes, and nutritive breakfast and diet drinks (Kumar et al., 2018; Pérez-Escobar et al., 2020). Because of its relatively low sweetness compared to other sugars, lactose may be added to increase osmotic pressure or viscosity or to improve texture without making the product too sweet. For example, if lactose is added to beer, it will not be fermented by the yeast and remains in the product to enhance flavour, viscosity and mouth feel. (Sachdeva*et al*., 1998). It finds similar uses in other beverages and low-calorie foods. In baked goods, as it is a reducing sugar, lactose readily undergoes the maillardreaction, contributing to the formation of the desirable golden-brown crust colour. (Kapil, 1990). Lactose excels in absorbing flavours, aromas, and colouring materials, example wine may be absorbed on to anhydrous lactose for incorporation into cake mixes. Lactose is also used as a carrier for sweetening agents such as saccharin (Jandal, 1989).

Whey proteins have been used for many years as highly nutritious food supplements. The biological components of whey including Lactoferrin, β-Lactoglobulin, α-Lactalbumin, glycomacropeptides and Immunoglobulin, demonstrate a range of immune enhancing properties and has the ability to act as antioxidant, antihypertensive, antitumor, hypolipidemic, antiviral, antibacterial and chelating agents (Marshall, 2004). β-Lactoglobulin is the major protein in whey, which comprised of about 54 per cent and other proteins are α-Lactalbumin 21 per cent, serum Albumin 5 per cent, immunoglobulin 10 per cent and Protease peptone 10 per cent (Huffman, 1996). By virtue of their higher proportion of essential amino acids, the biological value of whey protein is higher as compared to that of other dietary protein (Renner, 1983).

The microbial stability and safety of most traditional as well as of novel foods is based on a combination of several preservative factors, which the microorganism present in the food is unable to overcome. The hurdle concept was first introduced by Listener (1978). The hurdles govern many preservation processes. Intense heat treatment preserves canned food, low water activity (a_w) prevents any microbial growth in dried products and low pH is responsible for prolonged shelf life of fermented foods.

Hurdle technology is a technology by which hurdles (preservation parameters) are employed in suitable combination and every hurdle is used at an optimum level so that damage to the overall quality of food is kept to the minimum. Hurdle technology foods are defined as "products whose shelf life and microbial safety are extended by use of several factors none of which individually would be lethal towards spoilage or pathogenic microbes" (Berwal, 1994).

Whey is a potential source of nutritionally rich carbohydrate and protein. Whey is highly perishable and its disposal is of great concern to the dairy industry as it carries high BOD. Preservation of whey by hurdle techniques helps in extending shelf life of whey. Whey solids are preserved by this technique could be used in various food formulations. As and when whey is available, it could be preserved by this technique and used in different dairy and food product formulations. This will show a better way for preservation of whey and its utilization in human chain.

2. Materials and methods:

2.1 Bacteriological Materials:

2.2 Utensils and Glass wares

Stainless steel vessels of varying capacities and Stainless-steel stirrers were used at various stages of the investigation. Conical flasks, beakers, volumetric flasks, measuring jars of Borosil make were used for chemical and microbiological analysis. Glass wares were cleaned by detergents and sterilized using Hot Air Oven at 160-180^{o}C for 2 h and used for microbiological tests.

2.3 Plate count Agar

Plate count AgarM091S was procured from Hi-Media Laboratories Ltd., Mumbai was used to enumerate total counts.

2.4 Violet Red Bile Agar

Violet Red Bile Agar M049 was procured from Hi-Media Laboratories Ltd., Mumbai was used to enumerate coliform counts.

2.5 Malt extract agar

Malt extract agar M049 was procured from Hi-Media Laboratories Ltd, Mumbai was used to enumerate Yeast and mould counts.

2.6 Chemicals

All chemicals used in this investigation were of analytical grade (AR Grade).

2.7 Equipment's

2.8 Evaporator

Single effect, rising film vacuum evaporator of capacity 35kg water evaporation per hour (Anhydro, Copenhagen, Denmark) was used to condense whey & milk.

2.9 Water activity meter

Water activity meter applied by Hygro-palm with rotronic digital probe having measuring parameters of water activity ranging from 0.000 to 1.000 a_w and temperature between -9.9^{o}C to +99.9^{o}C, with a system accuracy of ± 0.005 a_w + 1.5% of displayed value at 23^{o}C/ 73 ^{0}F was used to measure the water activity of the samples.

2.10 Preparation of plain concentrate whey

Plain whey concentrate was prepared by condensing in a falling film vacuum evaporator. Whey was heated to a temperature of 80^{o}C/5 minutes and cooled to 55^{o}C and condensed in a single stage vacuum evaporator at a vacuum of 635 mm Hg to different levels of total solids (40, 45, 50 and 55 per cent). The concentrated whey samples were cooled to room temperature; the resultant whey concentrates were subjected to various physico-chemical analysis. Then the samples were stored in refrigerated condition until further use.

2.11 Preparation of sweet concentrate whey

Sweet concentrate whey was prepared by addition of sugar followed by condensing in a falling film vacuum evaporator. The best accepted combination of sugar was added to whey. Whey added with sugar at different levels 10, 12, 14 and 16 per cent was heated to temperature of 45^{o}C. Again, whey was heated to a temperature of 80^{o}C/5 minutes and cooled to 55^{o}C and condensed in a single stage vacuum evaporator at a vacuum of 635 mm Hg to different levels of total solids (40, 45, 50 and 55 per cent) and subjected to various physico-chemical analysis. Then the samples were stored in refrigerated condition until further use.

2.12 Process optimization for lactose hydrolyzed sweet concentrate whey

Lactose hydrolyzed sweet condensed whey was prepared by hydrolyzing lactose present in whey followed by addition of sugar and condensing.

2.13 Optimization of process parameters for lactose hydrolysis of whey

Level of enzyme addition being one of the important processing parameters it was optimized to obtain 80 per cent lactose hydrolysis in whey. The enzyme concentration level tried to obtain 80 per cent lactose hydrolysis was optimized by keeping temperature and pH of hydrolysis constant as recommended for the optimum activity of the enzyme.

2.14 Level of Enzyme

A known quantity of whey was taken and adjusted to constant pH of 6.5 by adding 10.0 per cent sodium bicarbonate solution after which the whey was added with enzyme at the rate of 600, 900, 1200 and 1500 units/l and incubated at 37^{o}C for different periods 2, 3 and 4h. At an interval of 30 minutes, the samples were drawn and the extent of hydrolysis was estimated to find out the optimum level of enzyme.

2.15Preparation of lactose hydrolyzed sweet concentrate whey

Eight litres of whey were taken in a clean stainless-steel vessel and adjusted to pH of 6.5 by the addition of 10.0 per cent sodium bicarbonate solution. Then whey was added with lactozyme enzyme at its optimum concentration and incubated at 37^{o}C for the required duration to obtain lactose hydrolysis of about 80 per cent. The resultant lactose hydrolyzed whey was used for lactose hydrolyzed whey concentrate preparation at different levels of sugar (10, 12, 14 and 16 per cent). Lactose hydrolyzedwhey along with various levels of sugar were heated to a temperature of 80^{o}C/5 minutes and cooled to 55^{o}C and condensed in a single stage vacuum evaporator at a vacuum of 635 mm of Hg to various levels (40, 45, 50 and 55 per cent TS). The concentrated whey samples were cooled to room temperature; the resultant whey concentrates were subjected to physico-chemical analysis. Then the samples were stored in refrigerated condition until further use.

2.16 Preparation of lactose hydrolyzed sweet condensed whey added with whey protein hydroysate

Eight litres of whey were taken in a clean stainless-steel vessel and adjusted to pH of 6.5 by the addition of 10.0 per cent sodium bicarbonate solution. Then whey was added with

lactozyme enzyme at its optimum concentration and incubated at 37^{o}C for the required duration to obtain lactose hydrolysis of about 80 per cent. The best combinations were tried in lactose hydrolyzed whey. Lactose hydrolyzed whey added with whey protein hydrolysed and optimum level of sugar was heated to a temperature of 80^{o}C/5 minutes and cooled to 55^{o}C and condensed in a single stage vacuum evaporator.

Vacuum of 635 mm Hg to various levels (40, 45, 50 and 55 per cent TS). The concentrated whey samples were cooled to room temperature; the resultant whey concentrates were subjected to various physical and chemical analysis. Then the samples were stored in refrigerated condition until further use.

2.17 Preparation of standard Ice cream (control), Mixing and processing of ingredients mix.

Fresh whole milk was standardized to have 10 percent Fat, 11 percent MSNF by using fresh cream, whey concentrates and skim milk powder followed by addition of sugar to have 15 per cent sugar level, along with addition of stabilizer and emulsifier of 0.5 per cent to obtain standard ice cream.

Calculated quantities of pasteurized whole milk and cream were mixed in a stainless-steel vessel and heated to 55^{o}C and added with sugar premixed, stabilizer, emulsifier and skim milk powder. All the ingredients were mixed thoroughly at 55 to 60^{o}C. After blending the various ingredients, the mix was pasteurized at 68.5^{o}C for 30 min in a thermostatically controlled water bath with continuous stirring. Immediately after pasteurization, the mix was homogenized by using two stage laboratory model homogenizers (Ranniecopenhagen, Denmark) at pressure of 250 and 50 kg/cm^{2} at I and II stage, respectively.

2.18 Cooling and ageing of the mix

The homogenized mix was cooled immediately to 4^{o}C and aged at this temperature for a period of 12-14h. Aged mix was added with vanilla flavour at the rate of 2.5ml/l and mixed thoroughly at the time of freezing. The mix was loaded into a horizontal batch freezer (Vulcan Laval of 5 l capacity) which was previously cleaned and sterilized. Freezing and whipping was carried out until a semi solid consistency was obtained and was then drawn directly into 50 ml previously sterilized polystyrene cups.

2.19 Hardening of Ice cream

The filled ice cream cups were immediately transferred to a hardening cabinet maintained at -20 to -22^{o}C and hardened for a period of 24-48h.

2.20 Preparation of ice-cream mix by using plain and sweetened whey concentrate

The plain and sweetened whey concentrates were used to replace the skim milk solids of ice cream mix at various levels (20, 30, 40 and 50 per cent). Mix prepared with whey concentrate was processed as per the standard procedure to obtain ice cream. The ice cream prepared with whey concentrate was subjected to various physico-chemical and sensory attribute studies in comparison with control to adjust the extent of replacement of SNF with whey concentrate without sacrificing any of the quality attributes.

2.21 Preparation of ice-cream with lactose hydrolyzed whey concentrate

The lactose hydrolyzed whey concentrate was used to replace skim milk solids of ice cream mix at various levels (20, 30, 40 and 50 per cent). Mix prepared with lactose hydrolyzed whey concentrate was processed as per the standard procedure to obtain ice cream. The ice cream prepared with hydrolyzed whey concentrate was subjected to various physico-chemical and sensory attribute studies in comparison with control to adjust the extent of replacement of SNF with whey concentrate without sacrificing any of the quality attributes.

2.22 Preparation of Ice cream with replacement of skim milk powder by using lactosehydrolyzed sweetened whey concentrate along with added WPH

Lactose hydrolyzed sweetened wheyconcentrate was prepared by lactose hydrolysis and sugar addition followed by condensing in a falling film evaporator. The best accepted combination of sugar and hydrolyzed levels were used. Lactose hydrolyzed whey along with various levels of sugar were heated to a temperature of 80^{o}C/5 minutes and cooled to 55^{o}C and condensed in a single stage vacuum evaporator at a vacuum of 635 mm of Hg to various levels (40, 45, 50 and 55 per cent TS). The condensed whey was cooled to room temperature.

The lactose hydrolyzed and WPH was added to replace skim milk solids of ice cream mix, at various levels (20, 30, 40 and 50 per cent). Mix prepared with whey concentrate added with WPH was processed as per the standard procedure to obtain ice cream. The ice cream prepared with sweetened whey concentrate and WPH was subjected to various physico-chemical and sensory attribute studies in comparison with control to adjust the extent of

replacement of SNF with lactose hydrolyzed whey concentrate and WPH without sacrificing any of the quality attributes.

2.23 Sensory evaluation of samples

Ice cream of both control and experimental samples were judged on 9-point hedonic scale score card by a panel of four judges. Provision was made in the score card for commenting on any possible defect in the product.

3. Results and Discussion

Whey concentrate was used to prepare shelf stable ice cream mix. Whey was added with different levels of sugar followed by condensing to different total solids to prepare the shelf stable ice cream. Similarly, lactose hydrolyzed sweetened whey concentrate along with added WPH and condensing to different levels of total solids to develop the formulated ice cream mix.

3.1 Microbiological quality of whey concentrates and Ice cream prepared by whey concentrate during Storage

The results pertaining to the microbiological quality of whey concentrate that were stored at 30±1^{o}C and 4±1^{o}C is presented in Table-1 and Table -2 respectively.

3.2 Effect of storage on microbiological quality of whey concentrates stored at 30 ± 1^{o}C

The microbiological quality of whey concentrates was stored at 30 ± 1^{o}C presented in Table-2. The initial total bacterial counts of hydrolyzed concentrate and unhydrolyzed was 1.77 and 1.89 log CFU/ml. The total bacterial counts hydrolyzed concentrate and unhydrolyzed after 15, 45, 60 and 90 days of storage at 30 ± 1^{o}C were found to be 1.83, 1.86, 2.01and 2.25 and1.95, 2.12, 2.22 and 2.23 log CFU/ml.

Table 1:Effect of storage on microbiological quality of whey concentrates stored at 30±1°C

Types of whey concentrate	Duration of Storage (Days)									
	0		15		45		60		90	
	TBC	Y&M	TBC	Y&M	TBC	Y&M	TBC	Y&M	TBC	Y&M
	Log cfu/ml									
Hydrolyzed	1.77	0.43	1.83	0.45	1.86	0.47	2.01	0.61	2.25	0.91
Unhydrolyzed	1.89	0.40	1.95	0.39	2.12	0.43	2.22	0.57	2.23	0.76
CD	**0.041**	**0.105**	**0.058**	**0.044**	**0.025**	**0.020**	**0.023**	**0.102**	**0.140**	**0.071**

*All values are average of three trials

The initial yeast and mold counts of hydrolyzed concentrate and unhydrolyzed was found to be 0.43 and 0.40 log cfu/ml. The yeast and mould counts hydrolyzed concentrate and unhydrolyzed after 15, 45, 60 and 90 days of storage at 30±1°C were found to be 0.45, 0.47, 0.61 and 0.91 and 0.39, 0.43, 0.57 and 0.76 log CFU/ml respectively.The coliform counts were found to be nil in hydrolyzed and unhydrolyzed whey concentrates.

3.3 Effect of storage on microbiological quality of whey concentrates stored at 4±1°C

As could be seen from the result presented in Table -2, the total bacterial counts of whey concentrate (Hydrolyzed whey) increased from 1.56 log cfu/ml to 2.23 log cfu/ml after 90 days of storage at 4 ±1°C. The initial total bacterial of unhydrolyzed whey concentrate 1.77 log cfu/ml. After 90 days of storage period at 4 ± 1°C the total bacterial counts were found to be 2.22 logCFU/ml. It is evident from the results presented in the Table-2 the microbial load increased significantly after 90 days of storage period at 30±1°C.

The initial yeast and mould counts of hydrolyzed and unhydrolyzed whey concentrate was found to be 0.15 and 0.18 log cfu/ml. The yeast and mould counts of both concentrates were similar to that initial counts after 15 days of storage at 4±1°C where as it is 0.25 and 0.38 log cfu/ml after 60 days of storage at 4±1°C. The yeast and mould counts hydrolyzed and unhydrolyzed whey concentrate after 90 days of storage was found to be 0.62 logCFU/ml and 0.65 log CFU/ml.

The coliform counts were found to be nil in hydrolyzed and unhydrolyzed whey concentrate.

Table 2:Effect of storage on microbiological quality of whey concentrates stored at 4±1^{o}C

Types of whey concentrate	Duration of Storage (Days)									
	0		15		45		60		90	
	TBC	Y&M	TBC	Y&M	TBC	Y&M	TBC	Y&M	TBC	Y&M
	Log cfu/ml									
Hydrolyzed	1.56	0.15	1.56	0.15	1.96	0.191	1.98	0.25	2.23	0.62
Unhydrolyzed	1.77	0.18	1.77	0.18	2.01	0.195	2.15	0.38	2.20	0.65
CD	**0.069**	**0.064**	**0.038**	**0.043**	**0.101**	**0.070**	**0.022**	**0.032**	**0.042**	**0.019**

*All values are average of three trials

3.4Effect of storage on micro biological quality of Ice cream prepared by whey concentrates stored at -18±1^{o}C

The results pertaining to microbiological quality of ice cream prepared by hydrolyzed and unhydrolyzed whey concentrates which were stored at defreeze temperature presented in Table-3. The initial total bacterial counts of unhydrolyzed concentrate and hydrolyzed ice cream was 5.17 and 5.09 log cfu/ml. The total bacterial counts of both types of ice cream were similar to that initial counts after 15 days of storage at defreeze, there after the increase in total bacterial counts in both types of ice cream was observed. The total bacterial counts unhydrolyzed concentrate and hydrolyzed after 45, 60 and 90 days of storage at defreeze were found to be 5.27, 5.36, 5.58 and 5.24, 5.3, 5.55 log cfu/ml respectively.

The initial yeast and mold count of ice cream prepared by unhydrolyzed and hydrolyzed whey concentrates were found to be 0.8 and 0.78 log cfu/ml. After 15 days of storage the yeast and mould counts were almost same to that of initial counts. However, after 60 days of storage the increase in yeast and mould count was observed. The significant increase in yeast and mould counts was observed in the ice creams prepared by hydrolyzed and unhydrolyzed whey concentrates. The yeast and mould counts of unhydrolyzed concentrate and hydrolyzed after 45, 60 and 90 days of storage was observed to be 1.1, 1.31, 1.55 and 0.96, 1.28, 1.54 log cfu/ml respectively.

The coliform counts of ice cream prepared by hydrolyzed and unhydrolyzed whey concentrates were found to be nil.

Table 3:Effect of storage of micro biological quality of whey concentrates Ice Cream stored at -18±1^{o}C

Types of whey concentrate	Duration of Storage (Days)									
	0		15		45		60		90	
	TBC	Y&M	TBC	Y&M	TBC	Y&M	TBC	Y&M	TBC	Y&M
	Log cfu/ml									
Hydrolyzed	5.09	0.78	5.17	0.85	5.24	0.96	5.3	1.28	5.55	1.54
Unhydrolyzed	5.17	0.8	5.19	0.9	5.27	1.1	5.36	1.31	5.58	1.55
CD	**0.032**	**0.059**	**0.091**	**0.105**	**0.064**	**0.012**	**0.071**	**0.082**	**0.067**	**0.021**

*All values are average of three trials

3.5 Process optimization for lactose hydrolysis of whey

From among various levels of concentration of enzyme tried, the maximum hydrolysis was attained at a concentration of 1500 units/l. It could be observed from the result that at standardized pH of 6.5 and temperature of 37^{o}C, hydrolysis of around 80 per cent was attained at an enzyme concentration of 1500 units/l for an incubation period of 4 h. It was observed that there was significant increase in the degree of lactose hydrolysis as the amount of enzyme concentration per litre of substrate increased from 600 to 1500 units/l. However, as the enzyme concentration increased further from 2.0 ml/l to 2.5 ml/the extent of increase of lactose hydrolysis was insignificant. Similar trends were observed by Vodickova*et al.,* (1984) when the enzyme concentration increased from 1.0 per cent to 2.0 per cent. Hydrolysis of lactose to an extent of 80 per cent has been recommended as optimum for acceptability of product by eventual users (Coton, 1980). Taking in to account the cost factor of obtaining higher hydrolysis 80 per cent hydrolysis was found to be economical (Fox, 1985). Therefore, in our investigation the process optimization was carried out to get lactose hydrolysis of 80 per cent.

3.6 Process optimization for production of lactose hydrolyzed sweetened whey concentrate

From among various levels of sugar levels used, 10 per cent sugar was found to be optimum for hydrolyzed whey concentrate which was 2.0 per cent lesser sugar requirement as compared to the un-hydrolyzed whey concentrate. The reduction in sugar level is the result of

lactose hydrolysis. Lactose is only 15 per cent as sweet as sucrose but upon 100 percent hydrolysis, it becomes 70 per cent as sweet as sucrose at 25^{o}C (Fox, 1985), which was the cause for reduction in the sugar requirement in hydrolyzed whey. Reduction of one percent sugar was reported at 73-78 per cent of lactose hydrolyzed fruit based chhana whey beverage (Ravikrishna*et al.*, 1994). However, in present investigation 2.0 percent reduction was noticed in lactose hydrolyzed sweetened whey concentrate. Geilman (1993) reported 4.66-fold increase in sweetness after 80 per cent lactose hydrolysis which also shows major reduction in the total sugar requirement after hydrolysis. It has been reported by many workers that hydrolysis increases the sweetness (Cotton, 1980; Arndt and Wehling, 1989; Geilman, 1993; Timmermans, 1997). The increase in sweetness upon hydrolysis is due to the release of glucose and galactose which are known to be sweeter than lactose. Hence, there was reduction in sugar requirement (Timmermans, 1997).

3.7 Process optimization for lactose hydrolysis of whey

From among various levels of concentration of enzyme tried, the maximum hydrolysis was attained at a concentration of 1500 units/l concentration. It could be observed from the result that at standardized pH of 6.5 and temperature of 37^{o}C, hydrolysis of around 80 per cent was attained at an enzyme concentration of 1500 units/l for an incubation period of 4h. It was observed that there was significant increase in the degree of lactose hydrolysis as the amount of enzyme concentration per litre of substrate increased from 0.5 to 2 ml/l. However, as the enzyme concentration increased further from 2.0 ml/l to 2.5 ml/l the extent of increase of lactose hydrolysis was insignificant. Similar trends were observed by Veerapandian*et al*, (1998) when the enzyme concentration increased from 1.0 per cent to 2.0 per cent. Hydrolysis of lactose to an extent of 80 percent has been recommended as optimum for acceptability of product by eventual users (Coton, 1980). Taking in to account the cost factor of obtaining higher hydrolysis, i.e.,80 percent hydrolysis was found to be economical (Fox, 1985). Therefore, in our investigation the process optimization was carried out to get lactose hydrolysis of 80 per cent.

3.8 Process optimization for the production of ice cream from hydrolyzed sweetened whey concentrate added with WPH

Lactose hydrolyzed whey concentrate was prepared by lactose hydrolysis by using lactozyme enzyme and added with best level of sugar addition and WPH, followed by vacuum

condensing. This obtained concentrate was added to ice cream mix to replace skim milk solids at different levels.

3.9 Effect of replacement of SNF with lactose hydrolyzed whey concentrate and added with WPH on quality parameters of ice cream

The effect of replacement of SNF with lactose hydrolyzed whey concentrate and WPH on the quality parameters of ice-cream such as melting resistance, whipping rate, overrun and hardness were studied. With the increase in level of replacement from 0 to 50 per cent the melting resistance decreased from 19.25 to 18.00 min. The extent of decrease was found to be significant. A significant decrease in melting resistance of ice cream with increase in levels of replacement of SMP with lactose hydrolyzed whey concentrate and WPH could be ascribed to comparatively weaker gels formed by the WPH as compared to casein gels which are stronger and firmer.

As could be seen from the results WPHs are known to contribute better whipping rate and overrun. To obtain 90 per cent overrun the time require by control sample was 7 min where as it was 4.50 min at 20 per cent level of replacement of SNF with lactose hydrolyzed whey concentrate and WPH. Similarly overrun increased to 109 as a result of replacement of SNF at 50 per cent with lactose hydrolyzed whey concentrate and WPH. This decrease in the whipping time and increase in overrun could be ascribed to improved emulsifying capacity, emulsion stability and foaming stability of whey protein because of partial hydrolysis (Konrad*et al.*, 2004). Similar observations were made on whipping rate and overrun when skim milk solids replaced with whey solids in ice cream preparation (Saritha*et al.*, 1998 and Ruger*et al.*, 2002). The hardness of ice cream decreased with the increase in the level of whey concentrate. The decreased hardness is probably due to replacement of casein with whey protein. Casein is known to form firmer product as compare to whey protein hence the control sample shown penetration value 110, as against 112, 114, 116 and 119 mm/5sec at 20, 30, 40 and 50 per cent replacement. Some of the earlier worker observed decreased hardness of ice cream as a result of replacement of skim milk solids with whey protein concentrate (Tirumalesha and Jayaprakasha, 1998). Hemanth*et al.*, (1998) observed soft body and texture when enzymatically modified protein was incorporated in to the ice cream.

3.10 Effect of replacement of SNF with lactose hydrolyzedwhey concentrate and added with WPH on sensory attributes of ice cream

The replacement of SNF with lactose hydrolyzed whey concentrate and WPH on sensory characteristic of ice cream. Replacement of SNF with lactose hydrolyzed whey concentrate and WPH was found to have positive effect on almost all the sensory attributes of ice cream. The overall acceptability score for replacement of whey concentrates at 20 percent was 8.78 out of 9 where as it was 8.10 out of 9 for control ice cream. Better sensory scores as a result of lactose hydrolyzed whey concentrate and WPH incorporated is probably due to better functional properties of whey concentrate was attribute to the improved functional properties of whey concentrate like emulsifying, gelling and tactical characteristics. With further increase in replacement level 30 to 50 percent, a significant decrease in the score awarded with respect to all sensory characteristics, the decrease in scores with higher levels of replacement could be due to slight bitterness imparted as a result of hydrolysis also probably due to whey flavour and slight increase in iciness, which affected color and appearance, melting quality and flavour of ice cream. Similar observation was made by Hemanth*et al*., (1998) when ice cream was formulated by utilizing enzymatically modified protein.

3.11 Microbiological quality of whey concentrates and whey concentrate based ice cream

The concentrated lactose hydrolyzed and unhydrolyzed whey, hydrolyzed and unhydrolyzed whey concentrate ice cream, which were adjudged based on their acceptance of characteristics when stored both at 30±1^{o}C and 4±1^{o}C. The results obtained are discussed here under.

3.12 Effect of storage on micro biological quality of whey concentrates stored at 30±1^{o}C and 4±1^{o}C

The results pertaining to the microbiological quality with respect to total bacterial counts (TBC) and yeast and mould counts of whey concentrates stored at 30 ± 1^{o}C and 4 ± 1^{o}C. The initial total bacterial counts for both types of concentrates ranged from 1.77 to 1.89 log cfu/ml when stored 30 ± 1^{o}C. With the increase in the storage period a significant increase in the total bacterial counts was observed in the concentrates stored at 30±1^{o}C. However, increase in total bacterial counts for the concentrates stored at 4±1^{o}C was found to be nonsignificant. The total bacterial counts for both types of concentrates after 90 days of storage at 30±1^{0} C and 4±1^{o}C ranged from 2.25 to 2.23 log cfu/ml and 2.23 to 2.20 log cfu/ml respectively. The microbial stability in the concentrated whey could be mainly attributed to the lower water activity (a_w) (0.803 to 0.993) which is known to inhibit the growth of microorganisms.

The microbial stability of the concentrated cheese whey having total solids of 50 per cent and 0.90 water activity (a_w) was challenged with inoculation of *staphylococcus aureus, Osmophillic*and non *Osmophillic*yeasts and various moulds (Leiras et al., 1991). In the present investigation, the condensed whey had a maximum water activity (a_w) of 0.803 which could be the most important parameter in reducing the overall growth of the microorganisms.

The initial yeast and mould counts ranged from 0.15 to 0.18 log cfu/ml for both concentrated whey when stored at 4 ± 1^{o}C. Yeast and moulds may be the major contributors for the higher total bacterial counts. The yeast and mould counts in both types of concentrate when stored at 4 ± 1^{o}C were similar even after 15 days of storage. It has been observed that most of yeast are known to be inhibited in about 0.75 (Range 0.80 to 0.75) (Jayaprakasha*et al*., 1997). Even though yeast and moulds can grow at the water activity (a_w) of the concentrated whey (0.993 to 0.803) as the values were nearer to the value of inhibition of yeast, the extent of growth was very less.

In both the types of concentrates coliform counts was found to be nil since the concentrates are prepared hygienically.

3.13 Effect of storage on micro biological quality of Ice cream prepared by whey concentrates stored at -18 ± 1^{o}C

The results pertaining to the microbiological quality with respect to total bacterial counts (TBC) and yeast and mould counts of whey concentrate based ice cream stored at defreeze temperature. The initial total bacterial counts for both types of whey concentrate ice cream ranged from 5.09 to 5.17 log cfu/ml. The total bacterial counts of both types of ice cream were similar to that initial counts after 15 days of storage at defreeze, there after the increase in total bacterial counts in both types of ice cream was observed. The total bacterial counts hydrolyzed concentrate and unhydrolyzed after 45, 60 and 90 days of storage at defreeze were found to be 5.24, 5.30, 5.55 and 5.27, 5.36, 5.58 log cfu/ml. The microbial stability in the concentrated whey could be mainly attributed to the lower water actively (a_w) (0.803 to 0.993) which is known to inhibit the growth of microorganisms.

The microbial stability of the concentrated cheese whey having total solids of 50 per cent and 0.9 water activity (a_w) was challenged with inoculation of *staphylococcus aureus, Osmophillic*and non-*Osmophilic*yeasts and various moulds (Leiras*et al.,* 1991). In the present

investigation, the whey concentrate had a maximum water activity (a_w) of 0.803 which could be the most important parameter in reducing the overall growth of the microorganisms.

The initial yeast and mould counts of ice cream prepared by hydrolyzed and unhydrolyzed whey concentrates were stored at defreeze temperature was found to be 0.78 and 0.80 log cfu/ml. Yeast and moulds may be the major contributors for the higher total bacterial counts. After 15 days of storage the yeast and mould counts were almost same to that of initial counts. It has been observed that most of yeast are known to be inhibited in about 0.75 (Range 0.80 to 0.75) (Jayaprakasha*et al.,* 1997). Even though yeast and moulds can grow at the water activity (a_w) of the concentrate whey (0.803) as the values were nearer to the value of inhibition of yeast, the extent of growth was very less. The coliform counts of hydrolyzed and unhydrolyzed whey concentrates ice cream was found to be nil since the concentrates are prepared hygienically.

Conclusion

This investigation was aimed at utilizing whey concentrate in the preparation of shelf stable formulated ice cream. A technology has been developed to prepare shelf stable condensed whey and its utilizations in the form of sweetened condensed, lactose hydrolyzed and hydrolyzed whey protein concentrate. The storage study revealed that both concentrates were acceptable up to 90 days of storage at 4±1^{o}C. The microbiological quality with respect to total bacterial counts as well as yeast and mould counts was within the prescribed limits of PFA standard for sweetened concentrated whey even after 3 months of storage. In both the types of concentrates coliform counts were found to be absence since the concentrates are prepared hygienically.

As compared to plain whey concentrate, lactose hydrolyzed whey concentrates and WPH replacement resulted in greater increase in pH and decrease in acidity. The extent of increase in viscosity was slightly lesser as compare to plain whey solids. Similar lactose hydrolyzed whey concentrates and WPH replacement decrease in melting resistance and hardness. Increase in freezing point with increase in replacement. Compared to plain whey concentrate, lactose hydrolyzed whey concentrates and WPH replacement resulted in lesser whipping time and more overrun with increase in the level of replacement.

Formulated ice cream besides increases the mix viscosity resulting in smoother and refreshing nature they can provide potential profit margins, which in turn add to the growth and development of dairy industry.

References:

Stegmann, B., 1986, Lactose and some of its derivatives.*J. Society Dairy Technol*., **39**: 91-96.

Sachdeva, S., Bhattacharjee, P.P. and Singh, S., 1998, Technology of lactose Manufacture- A Review.*Indian J. Dairy Sci*., **51**: 1-12.

Kapil, V., 1990, Lactose manufactures from whey-engineering solutions, *Indian Food Ind*., 27-31.

Jandal, J. M., 1989, Utilization of lactose in pharmaceutical industries. *Indian Dairyman,***41**:127-129.

Marshall, K., 2004, Therapeutic applications of whey protein.*Alternative med rev*., **9**(2): 136-156.

Huffman, L. M., 1996, processing whey protein for use as a food ingredient. *Food Technology,* **50**(2): 49-52.

Renner, E., l983, Milk and dairy products in human nutrition.Volksw, Verlag, Munchen Proc. *6th SIFST Symp. Food Ingredients*, 27-29.

Leistner, L., 1978, Food Quality and Nutrition.W.K Downey (Ed.) pp.553-557.Applied Science Publishers, London.

Berwal, J.s., 1994, Hurdle Technology for Shelf –Stable Food Products. *Indian Food Industry*, **132**:40-43.

Vodickova, M. and Forman, L., 1984, Use of buttermilk in quality improvement of ice cream, *Dairy Sci. Abstr*.,47:3904.

Cotton, G., 1980, The utilization of permeates from the ultrafiltration of whey and skim milk. *Bull. Int. Dairy Fed*., **126**: 23-33.

Fox, P.F., 1985.Developments in dairy chemistry -3, Elsevier Applied Science, London.

Ravikrishna, S., Atmaram, K., Venkateshaiah, B.V. and Kempanna, C., (1994), Utilization of lactose hydrolyzedchanna whey for preparation of beverages. *Indian j. dairy and Biosci***., 5**:20-23

Gielman, W., Schmdit, D., Herewrth, Kennedy, C. and Path, J., 1990, production of frozen dairy products from milk retentate.*Dairy Sci. Abstr*., **52**(2):8476.

Arndt, E.A. and Wehling, R.L., 1989, Development of hydrolyzed and hydrolyzed-isomerized syrups from cheese whey ultrafiltration permeate and their utilization in Ice cream. *J. Food Sci.*, **54**: 880-884.

Timmermans, E., 1997, Lactose derivative: Function and applications. In: Whey proceedings of Second International Whey Conference, held in Chicago, USA, 27-29 October, IDF (1998); 233-250.

Konrad, G., Kleinschmidt, T. and Rohenkohl, H., 2004, Enzymic modification of whey protein concentrate for improvement of foaming and emulsification properties. Part II. Effects on surface properties in model systems.*Deutsche-Milchwirtschaft*.**55**(19): 780-783.

Saritha, M., Ramanjanayulu, G. and Jayaprakasha, H.M., 1998, Effect of whey powder on physico-chemical and sensory attributes of ice cream. *Indian J. Dairy Biosci*, 8:6.

Ruger, P.R., Baer R.J, Naidu., 1986 and Kasperson, K.M., 2002, Effect of double homogenization and whey protein concentrate on the texture of Ice cream. *J. Dairy Sci*., 85:1684

Tirumalesha, A. and Jayaprakasha, H.M., 1998, effect of admixture of spray dried whey protein concentrates and butter milk powder on physico-chemical and sensory characteristics of Ice cream. *Indian J. Dairy Sci*., 51:13.

Hemanth, H.R., Vincentmonteiro, P., RamachandraRao, H.G. and Bhat, G.S., 1998, Effect of enzymatic modified milk proteins on the rheological and sensory properties of ice cream. *Indian J. Dairy and Biosciences*, **9:** 20-25.

Leiras, M.C., Chirife, J. and Alzamora, S.M., (1991), Development of a shelf-stable hydrolyzed concentrated cheese whey. *Lebensmitted-wissenschaft and tecnologie*, 24: 12-16. *Cited in dairy Sci. Abstr*. No.3604, 1991, 53:454

Kumar A, Kathuria D, Sharma SK, Kumar S. Optimizing process to prepare lactose hydrolysed milk by enzymatic treatment. Journal of Hill Agriculture. 2018;9(3):361-9.

Pérez-Escobar LV, Mosquera-Martínez AJ, Ciro-Velásquez HJ, Sepúlveda-Valencia JU, Vargas-Díaz SL. Obtaining a lactosehydrolyzed from nanofiltration of sweet whey: Characterization and process optimization. Revista Mexicana de IngenieríaQuímica. 2020;19(1):445-53.