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INFLUENCE OF STORAGE TEMPERATURES ON THE QUALITY OF CONCENTRATED WHEY

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There is an increasing interest in the use of whey due to its therapeutic and functional properties such as water binding, solubility, gelation, emulsification and foaming. Whey and its components are being incorporated as value-added ingredients in many foods including dairy, infant formulas, sports foods, meat, bakery, beverages and other food products. Whey is highly perishable due to high water activity. This project was designed to develop concentrated whey by an economical evaporation method, and then its quality was evaluated at freezing (-20°C) and refrigerated (4°C) storage temperatures. The results showed that during frozen storage minor changes occurred in pH (4.22-4.12), acidity (2.63-2.72%), total solid (48.68-47.82%) and lactose contents (36.81-36.02%) as compared to the decrease in pH (4.22-3.81), acidity (2.63-2.93%), total solids (48.68-44.30%) and lactose (36.81-33.87%) contents at refrigeration storage. An increase in viscosity and NPN content were observed at both temperatures which might be due to protein denaturation. The protein (4.53%), ash (4.03%) and fat (2.33%) remained constant. The SDS-PAGE patterns indicated a significant denaturation and aggregation of major whey proteins during storage which was intense at freezing temperature.
Keywords: Concentrated whey, protein denaturation, SDS-PAGE, storage, lactose

INTRODUCTION

Whey is the milk serum obtained as a byproduct after casein precipitation from milk during cheese manufacturing (Hoffman and Falvo, 2004). It is produced in larger quantity as its volume accounts 8–9 kg of whey form 1–2 kg of cheese (Atra *et al*., 2005). Whey represents 85-90% of the volume of milk and contains ~ 55% of its nutrients, in which proteins (water soluble), lactose and lipids are the most copious components (Spahn *et al*., 2008). The whey composition usually varies depending on the method of casein preparation. The whey obtained after rennet coagulation of milk is known as sweet whey having pH from 6.00 to 6.5. It contains higher lactose content than acid whey (pH 3.57-4.34) which is obtained by acidification of milk directly by addition of either lactic or mineral acid as occurs in cottage cheese manufacture(Alsaed *et al*., 2013). In general, whey contains whey proteins 0.7 %, fat 0.1%, ash 0.5%, lactose 4.9% and total solids 6.3% (Smithers, 2008).

Most of the whey produced is dumped into rivers, drains and sewers in Pakistan because of the cheapest way of disposal. These methods of disposal reduce the profitability of dairy industry and also responsible for environmental pollution because of higher Biological Oxygen Demand (>35000 ppm) and Chemical Oxygen Demand (> 60000 ppm) due to high organic load (Siso, 1996). Very few industries are converting the whey into powder at small scale in Pakistan.

The significance of whey has been realized after knowing its potential application. Whey protein consists of heterogeneous mixture of secreted proteins, mainly β-lactoglobulin, α-lactalbumin, blood serum protein and immunoglobulin. These contribute 90% of protein and remaining portion contains minor proteins that are proteose peptone (PP), lactoferrin and indigenous enzymes (Chatterton *et al*., 2006; Jovanovic *et al*., 2007). Whey proteins have various functional properties such as thermal stability, gelation, emulsification, foam formation, viscosity, firmness and creaminess to the end product (Foegeding *et al*., 2002) and also contribute therapeutic effect. Whey proteins are an excellent source for the fortification of food with protein and aid to increase the nutritional value of foods products. It also contains vitamins and minerals comprising high bioavailability along with branched-chain amino acids such as valine, isoleucine and leucine (Ha and Zemel, 2003).

Liquid whey is usually not used as such in food as an ingredient, so it is further processed into various products that are more frequently used in the food industry. Whey protein concentrates (WPC), whey powder, hydrolyzed whey protein (HWP), whey protein isolates (WPI), reduced-lactose whey and demineralized whey are the products produced from whey (Marshall, 2004). The whey can be concentrated by three principal methods; evaporation, cryoconcentration also called freeze concentration, ultrafiltration (UF), diafiltration (DF) and reverse osmosis (RO). In dairy industry ultrafiltration (UF) technique has been utilized to produce whey protein concentrates (Damon *et al*., 2003) in developed countries. Membrane filtration is good for the concentration of whey but is expensive

technique and uncommon in Pakistan. The evaporation processing units designed for production of concentrated whey (CW) are comparatively simple and cost effective as compared to other methods mentioned above (Lewicki, 2006).

Whey derivatives such as WPC and CW have wide range of applications in food product due to their high nutritional quality, excellent functional properties and desirable sensory characteristics (De la Fuente *et al*., 2002). Due to its gelation and emulsification properties, WPC has been used in meat products (Yetim *et al*., 2001). Whey is widely utilized in the beverages, bakery, confectionary, desserts and cheese spread (Ha and Zemel, 2003). Whey protein is considered a useful ingredient in infant formula, weight reduction and weight gain diets, protein fortified fruit juices and other healthy foods and drinks (Omole *et al*., 2012). Approximately 50% of cheese whey produced in the world is treated and transformed into various foods and feed products. About half of this amount is used directly in liquid form, 30% as cheese whey powder, 15% as lactose and its byproducts and the rest as whey protein concentrates (Spalatelu, 2012). The market for whey products is a rapidly growing in the food manufacturing industries. New processing technologies enabled the manufacturers to increase the concentration of WPC and CW (Wright *et al*., 2006).

The evaporation is one of the common and well-studied practices for the concentration of liquid systems and mostly used method in the food industry. The commercial importance of whey concentrate is 3 to 40 times higher than the whey powder due to its functional and nutraceutical properties (Spahn, 2008).

Keeping in view the above facts, the present research work was planned to concentrate the cheese whey through evaporation and to study the different physicochemical characteristics during storage at refrigeration and frozen temperatures which ultimately influence the different functional and nutritional properties of concentrated whey.

MATERIALS AND METHODS

The research work was conducted in the Dairy Technology Laboratory of National Institute of Food Science and Technology, University of Agriculture, Faisalabad. Buffalo cheese whey was collected from dairy plant of Noon Pakistan Limited, Bhalwal. The whey was analyzed for pH, acidity, total solids, lactose, minerals, ash, total protein, non-protein nitrogen (NPN) and viscosity determinations, and then it was pasteurized at 65°C for 30 minutes and concentrated at 48% total solids through Rising Film Evaporator (Armfield, UK). CW was packed in jars and stored at refrigeration (4°C) and freezing (-20°C) temperatures.

During storage of two months CW was analyzed for several parameters. The pH was measured by electronic digital type pH meter (Ong *et al*., 2007). Titrimetric method of AOAC 947.05 (2000) was used for acidity determination. Total solids were measured according to method No. 925.23 (AOAC, 2000). The total protein and non-protein nitrogen was analyzed by methods described by International Dairy Federation (1993) and Rowland (1938), respectively. Ash content was determined by igniting sample on flame, then in a furnace at 550°C until silver grey ash is obtain as given in method No. 945.46 (AOAC, 2000). The calcium, sodium and potassium were determined by flame photometer following the method of AOAC (2000). The method described by Marshall (1992) was used to find out fat content by using butyrometer. Furthermore, viscosity of the concentrated whey was measured by means of Brook Field DV-I Viscometer (Farrag *et al*., 2010). The SDS-PAGE was performed by using method described by Laemmlli (1970). All the tests were carried out in triplicates. A complete randomized design was used for the analysis of variance of physicochemical properties of CW. Means were compared at 0.05 level of significance using Least Significance Difference Test (Steel *et al*.,1997).

RESULTS

The physicochemical analysis indicated that whey had 5.5 pH, 0.27% acidity, 0.74% protein, 0.15% NPN, 6.2% total solids, 4.76% lactose, 0.30% fat, ash 0.43%, and 1.6 cP viscosity. The calcium, sodium and potassium contents of whey as determined were 43.13 mg/100 mL, 35 mg/100 mL and 139.66 mg/100 mL, respectively (Table 1).

Table 1. Physicochemical analysis of cheese whey

Parameters	Values	Parameters	Values
pH	5.50	Fat (%)	0.30
Acidity (%)	0.27	Ash (%)	0.43
Protein (%)	0.74	Viscosity (cP)	1.60
NPN (%)	0.15	Ca (mg/100mL)	43.13
Total solids (%)	6.20	Na (mg/100mL)	35.00
Lactose (%)	4.76	K (mg/100mL)	139.66

The statistical analysis of CW presented in Table 2 revealed the significant ($p<0.01$) impact of storage temperature, days and their interaction on the pH, acidity and total solids while a non-significant ($p>0.05$) impact was observed for the protein and fat contents of CW. The NPN, viscosity and lactose contents of the CW were significantly ($p<0.01$) affected by the temperature and storage period but their interaction had a non-significant ($p>0.05$) influence on these parameters except the lactose content which affected significantly ($p<0.05$). The ash, Ca, Na, and K contents of concentrated whey were unaffected ($p>0.05$) during storage at different temperatures.

Table 2. Effect of storage days and temperature on the physicochemical characteristics of concentrated whey

Storage	0 days		15 days		30 days		45 days		60 days	
(days)	4°C	-20°C	4°C	-20°C	4°C	-20°C	4°C	-20°C	4°C	-20°C
pH	4.22a	4.22a	3.93cd	4.18ab	3.85de	4.14ab	3.70ef	3.10b	3.63f	3.98c
Acidity (%)	2.63h	2.63h	2.78e	2.67g	2.94c	2.72f	3.08b	2.78e	3.25a	2.82
Fat(%)	2.33	2.33	2.30	2.30	2.26	2.30	2.23	2.26	2.23	2.26
Protein(%)	4.53	4.53	4.46	4.60	4.38	4.46	4.46	4.53	4.46	4.50
NPN (%)	1.13	1.13	1.16	1.18	1.20	1.24	1.22	1.26	1.24	1.29
Lactose (%)	36.81a	36.81a	35.47abc	36.81a	33.64cd	36.11ab	32.50de	35.47abc	30.95e	34.90bc
Total Solids (%)	48.68a	48.68a	45.93d	48.46a	44.33e	47.86ab	42.78f	47.26bc	39.80g	46.83c
Ash (%)	4.03	4.03	3.96	4.08	4.09	4.05	4.03	4.11	4.09	4.16
Viscosity(cP)	651.33	651.33	653.33	655.67	657.67	659.33	660.33	663.00	662.67	665.67

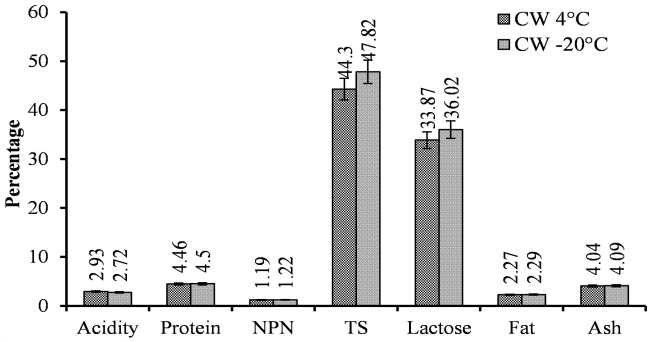

Figure 1. Impact of storage temperatures on the composition of concentrated whey

The statistical analysis of different attributes of CW revealed that during storage period of 60 days, the pH of CW stored at 4°C decreased from 4.22 to 3.63 (0.9) which is higher than stored at -20°C (4.22- 4.98=0.24). Similarly increase in acidity of CW stored at 4°C was 0.26% (3.25-2.63%) higher than frozen CW i.e., 0.19% (2.82-2.63%). The fat contents of concentrated whey at 4°C and -20°C were 2.27 and 2.29, respectively. No significant change was observed in protein content, while the NPN value of CW was higher at frozen temperature (Fig. 1). There was a slight but progressive increase noticed in NPN of CW at both temperatures during storage. The values of NPN for 4°C and -20°C were 1.19% and 1.22%, respectively.

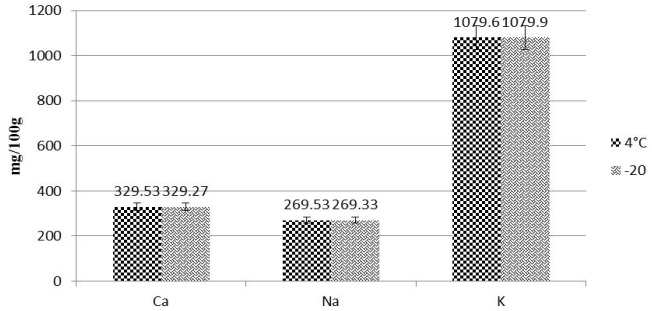

Figure 2. Mineral content in concentrated whey at 4°C and -20°C

Thechanges were minor in total solids and lactose contents at 4°C and -20°C were 44.3% & 47.82%, and 33.87% & 36.02%, respectively (Fig.1). The impact of storage temperatures and duration was non-significant for ash content (Table 2; Fig. 1); hence the ash content of CW was 4.04 and 4.09% at 4°C and -20°C, respectively. The viscosity of CW increased during storage of 60 days at both temperatures (Table 2). The significant higher viscosity (659 cP) was noticed for CW at -20°C than at 4°C (657 cP). The 11.34 cP increase was recorded for at 4°C and 14.34 cP at -20°C during whole period of storage. The results of Ca, Na and K content of concentrated whey, indicated that the effect of temperatures (4 and -20°C) and period (0-60 days) was non-significant ($p>0.05$) on the mineral content. It is further explored in Figure 2 that Ca, Na and K values did not show any change at two temperatures. The mineral contents (Ca, K, Na) content remain constant throughout the storage period.

DISCUSSION

The results of whey composition (Table 1) are in accordance with Johansen *et al*. (2002). Omole *et al*. (2012) investigated the whey composition from different cheese production areas and found that lactose, protein, ash and total solid content of cheese whey ranged from 3.98 to 4.06%, 0.6 to 0.65%, 0.84 to 0.95% and 6 to 6.5%, respectively. The fat content of cheese whey was 0.26% that is less than present finding. The pH and acidity of cheese whey from different cheese producing areas were 5.90 and 0.27-0.36%, respectively. Goyal and Gandhi (2009) compared paneer (acid whey) and cheese whey for minerals. They found calcium, sodium and potassium contents 29.1, 26.0 and 130 mg/100 mL, respectively. De wit (2001) reported that whey constitute about 0.18% NPN which is higher than present findings.

The results regarding the protein and NPN contents are confirmed by the findings of Abdrabo *et al.* (2009) and Soliman *et al.* (2010) who reported that total protein and NPN content increase during frozen at refrigeration

temperature. De la Fuente *et al*. (1997), and Fransson and Lonnerdal (1983) reported a non- significant effect on minerals and ash content. Tacken *et al*. (2009) and Friend *et al*. (1983) observed minor but non-significant changes in fat content.

A decreasing trend observed in total solids and lactose content during storage could be due to the production of higher lactic acid in refrigerated then frozen CW. Findings of this study are in co-ordinance with the results of Sameen *et al*. (2013). The decrease in lactose content during storage is due to its conversion into lactic acid (Goodnaugh, 1976).

The results of viscosity are in support with the work of Soliman *et al*. (2010) and Rattray and Jelen, (1995). Freezing depressed the activity of bacteria greatly as compared to refrigeration temperature. This trend is observed in the findings of Fonseca *et al*. (2006) who find that at -20°C the activity and acidification of *Lactobacillus bulgaricus* decreases maximum. At -20°C lactose conversion might be due to already produced lactase enzyme.

The SDS-PAGE of CW is presented in Figure 3. The extreme left lane indicate the marker while the other lanes from left to right showed the electrophoretic pattern of CW proteins at different storage days. The figure indicates the two consistent bands at 17 and 14 KDa which represent the β-Lg and α-La, respectively. β-Lg is the main protein involved in these interactions basically due to its high concentration and self-association to form dimers or higher ordered polymers at reduced temperatures. At 26 KDa, a band appeared on 30th day of storage (7 lane from left) in CW at -20°C and on 45th day at 4°C, showing the aggregate formation which increased with the passage of time. These aggregates might be of β-Lg and α-La complex. The aggregates formation was higher in intensity in CW stored at freezing temperature and increased in concentration with time. A very thin band showing the lesser intensity was also visible in CW near to the 6 kDa band of the maker, which was assigned as the caseino macro peptide (CMP).

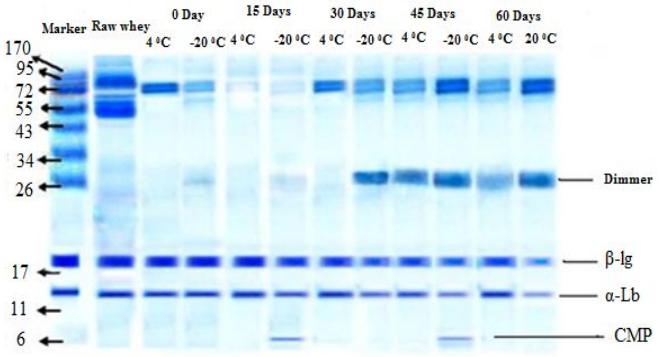

Figure 3. SDS-PAGE of concentrated whey during storage at different temperatures.

The bands appeared at 71-71 KDa position could be the complexes between k-casein, β-Lg and α-La as reported by Jovanovic *et al.* (2007). The study showed the more changes in frozen CW than the refrigerated. The rate of chemical reactions increased at freezing storage due to the concentration effects of freezing, that is, macro molecules are forced together, making interactions more probable with resultant denaturation, insolubilization, aggregation, and precipitation of proteins. Hosseinpour *et al*. (2011) conducted research to investigate the effect of storage at -18, 4, 25, 40 and 60°C on sweet and acid whey proteins by SDS-PAGE. They found that at -18 and 60°C protein aggregates are formed. Similar results but less drastic obtained at 4, 25 and 40°C. Soliman *et al*. (2010) stated that denaturation degree of whey protein solutions increased depending on duration of frozen storage, protein concentration and pH.

***Conclusions*:** It is concluded from the study that liquid whey can be concentrated (preserved) by evaporation under vacuum at 70°C in developing countries without any drastic change in physicochemical attributes. The concentrated whey stored at refrigeration temperature exhibit more changes in physicochemical characteristics than at frozen temperature; however, the slightly higher rate of protein denaturation occurred in frozen storage which can affect the functional properties of the concentrated whey. The both temperatures can be applied for concentrated whey storage depending upon their applications.

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