

# Nutritional Potential and Functionality of Whey Powder Influenced by Different Processing Temperature and Storage

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**Abstract**—Whey is an excellent food ingredient owing to its high nutritive value and its functional properties. However, composition of whey varies depending on composition of milk, processing conditions, processing method, and its whey protein content. The aim of this study was to prepare a whey powder from raw whey and to determine the influence of different processing temperatures (160 and 180 °C) on the physicochemical, functional properties during storage of 180 days and on whey protein denaturation. Results have shown that temperature significantly ( $P < 0.05$ ) affects the pH, acidity, non-protein nitrogen (NPN), protein total soluble solids, fat and lactose contents. Significantly ( $p < 0.05$ ) higher foaming capacity (FC), foam stability (FS), whey protein nitrogen index (WPNI), and a lower turbidity and solubility index (SI) were observed in whey powder processed at 160 °C compared to whey powder processed at 180 °C. During storage of 180 days, slow but progressive changes were noticed on the physicochemical and functional properties of whey powder. Reverse phase-HPLC analysis revealed a significant ( $P < 0.05$ ) effect of temperature on whey protein contents. Denaturation of  $\beta$ -Lactoglobulin is followed by  $\alpha$ -lactalbumin, casein glycomacropeptide (CMP/GMP), and bovine serum albumin (BSA).

**Keywords**—Whey powder, temperature, denaturation, reverse phase – HPLC.

## I. INTRODUCTION

WHEY is a nourishing and precious product from cheese manufacturing. It is 85-90% of the volume of milk. It contains ~55% of its nutrients, in which proteins (water soluble), lactose, and lipids are the most abundant components [1], [2]. The gross composition of whey is important in predicting the properties of whey products. The variation in the composition of milk, the type of bacteria used in the curdling of milk, and production methods determine its potential application and functional attributes [3]. The composition of whey usually varies depending on the method of casein precipitation. Rennet-coagulated cheese produced sweet whey having pH 5.6 and contains higher lactose content than the acid whey which is obtained by milk acidification by the addition of lactic or mineral acid [4], [5].

Whey protein comprises of a mixture of proteins, and major proteins are  $\beta$ -lactoglobulin (<55%),  $\alpha$ -lactalbumin (<24%), immunoglobulins (<15%), BSA (<5%), while glycomacropeptide (GMP/CMP), lactoferrin (LF), and lactoperoxidase (LP) are minor components. Whey protein

remained in soluble form in milk at pH 4.6. Whey protein is heat labile so above 60 °C, the globular structure of it unfolds, and its protein denatured. Main factors influencing the denaturation of whey protein are temperature, time, and pH, while pressure, interfacial forces, and organic solvents have been proved to have some effects. Denaturation of  $\beta$ -lactoglobulin determined the phenomenon of denaturation of whey protein as already reported by [6]. Denaturation occurs in two steps. Initially, unfolding of protein and changes in equilibrium between dimers and monomers are associated with hydrogen bonding. In second step, aggregates formation starts due to intermolecular SH to S-S exchange [7]. BSA does not greatly affect the whey properties due to its relatively low proportion and contains disulfide bond and one free thiol group. Glycomacropeptide (GMP) is a part of k-casein produced during cheese manufacturing by the action of rennet on C- terminal from amino acid residue 106-169 [8].

Generally, functional and nutritional quality is influenced by action of heat in the processing of food and the degree of protein denaturation. Physical, chemical, and structural characteristics of whey protein are also linked to its functional properties [9]. Whey proteins have brilliant functional properties like good solubility, emulsifying, viscosity, flavor binding, and gelation abilities [10]. Different factors like pH, temperature, ionic strength, isolation method, interface with other food components and processing conditions change the functional properties [11], [12].

Technological advances, increased scientific knowledge, and tighter environmental regulations caused the whey utilization in a pleasing way but liquid whey is usually not used in food as an ingredient, so it is further processed into various products that are more frequently used in the food industry. Increasing global demand for whey created an opportunity to resolve a waste disposal issue. This urged to cultivate the market for whey powders, whey lactose, whey proteins, whey protein concentrates (WPC), hydrolyzed whey protein (HWP), whey protein isolates (WPI), reduced-lactose whey, and demineralized whey from the same quantity of whey solids [13]-[15]. Several studies investigated the behavior of whey as an ingredient in beverages, bakery, confectionary items, infant formula, and other healthy foods and drinks [16], [17], [3]. Moreover, its gelation and emulsification properties make it a valuable ingredient for meat products [18].

Different processing steps are used to produce whey powder including filtration techniques, separation technologies such as

reverse osmosis, ultra-filtration, and ion exchange. All these technologies improved the quality of protein but also caused change in functionality such as gelling and solubility properties [19], [20]. Cost comparison for the concentration of whey by several systems including conventional evaporator and reverse osmosis shows that they are more economical than other means. In the current study, influence of different processing temperatures on the physicochemical, functional and protein denaturation of whey were studied. Whey powders produced at different processing temperature were further investigated after one month interval to observe the changes during storage period of 180 days.

## II. MATERIALS AND METHODS

Buffalo cheddar cheese whey used in the present study was acquired from Noon Pakistan Limited, Sargodha Road Bhalwal, Pakistan.

### A. Preparation of Whey Powder

Initially, raw whey and whey concentrate were analyzed for physicochemical analysis including pH, acidity, protein, NPN, lactose, total solids, ash, fat and viscosity. Cheese whey was concentrated by Armfield rising film evaporator up to 48% total solids. The temperature of the evaporator was 70 °C, pressure is 1 bar, and vacuum is 400. Concentrated whey was spray dried by using Armfield spray dryer at different processing temperatures of inlet and 80 °C outlet temperature. Inlet temperatures were adjusted at 160 °C (WP160) and 180 °C (WP180), and qualitative assessment was carried out after one month interval during storage period of 180 days.

### B. Physicochemical Analysis of Whey Powder

The moisture content of whey powder processed at 160 °C and 180 °C was determined by method no. 925.23 and ash content by method No. 945.46 as given in [21]. Electronic digital pH meter of Wellium model-Inolab pH 720, WTW82362, was used for pH determination. Acidity in sample was determined by method No. 947.05 given in [21]. The Soxhlet apparatus was used for the determination of crude fat in sample according to [21] method No. 30-10. Total protein and NPN were determined according to the method as described by [22], [23], respectively. Lactose was determined by the method given in NIRO manual [24].

### C. Functional Properties of Whey Powder

FC and FS of whey powder were calculated according to the method as described by [25]. Turbidity and SI were determined by the method as described by [26], [27], respectively. WPNI is a measure of the heat treatment applied to the whey during processing of whey powder, calculated by [28].

### D. Whey Proteins Analysis Using HPLC

Reverse phase HPLC was used to determine the whey protein contents in whey powder using a method of [29] with some modification. Samples were diluted 5-fold with demineralized water. pH 4.6 of the 1 ml diluted sample was adjusted using 0.1 mL of 10% acetic acid and then further 0.1

mL of 1 M sodium acetate added and centrifuged at 15000 g for 30 minutes. Buffer containing Bis (2-hydroxyethyl) amino-tris (hydroxymethyl) methane (Bis-Tris), sodium citrate and hydrochloric acid, and 20 mg/mL dithiothreitol (DTT) were mixed with diluted sample and 100 µL supernatant. After one hour, 1500 µL buffer containing urea, acetonitrile, and trifluoroacetic (TFA) were added and filtered through a 0.22 µm filter into a vial before further analysis.

### E. HPLC Condition

An Agilent 1100 series HPLC consisted of a pump (Agilent, G1311A), UV-detector (Agilent, G1314A), and a degasser (Agilent, G1379A) operate with software (Agilent ChemStation). A silica-based C-18 RP-HPLC column (250 mm length x 4.6 mm, particle size 5 µm, pore size 30 nm) was used for protein separation. Mobile phase consisting of acetonitrile-water-trifluoroacetic acid (100:900:1) and acetonitrile-water-trifluoroacetic acid (900:100:1) in a gradient mode at a flow rate of 1 mL/min and 20 µL injection volume were used. Detection wavelength of UV- detector was adjusted at 220 nm.

### F. Statistical Analysis

All the experiments were carried out in triplicates, and results were presented as mean±Standard deviation. Two factor-factorial statistical design under completely randomized designed (CRD) was applied on all parameters followed by LSD pair-wise comparison test. All the analysis was performed using Statistix 8.1 Software (Analytical Software, Tallahassee, FL, USA) considering 95% of confidence interval.

## III. RESULTS AND DISCUSSION

### A. Physicochemical Analysis of Liquid Whey and Whey Concentrate

The physicochemical profile of liquid buffalo cheese whey and whey concentrate were shown in Table I. Results have shown that whey contains lactose 4.76%, protein 0.76%, ash 0.43%, fat 0.30%, NPN 0.15%, acidity 0.27%, and total solid 6.20%. The physical attributes under consideration were pH (5.5) and viscosity (1.6 cp). All the results are within the range as reported [3], [30] findings of the researchers have shown that composition of whey varies according to cheese type, season of production, and geographical regions. Similar results have been reported in previous studies [31]-[33].

### B. Physicochemical Analysis of Whey Powder

Results regarding the different processing temperatures (160 and 180 °C) as well as storage on physicochemical parameters of whey powder are shown in Table II. Both treatments and storage days have a significant ( $P < 0.05$ ) effect on the pH, acidity, NPN, total soluble solids, fat and lactose content. On the other hand, protein contents during storage and ash contents with respect to treatments changed non-significantly ( $P > 0.05$ ). Interaction of storage days and treatments for moisture, ash, fat, protein and NPN were non-significant ( $P > 0.05$ ). The moisture has a significant effect on

powder flowability. Increase in moisture leads to decrease flowability due to the increase in liquid bridges and capillary forces acting between the powder particles. In addition, it may also lead to a swear flowability problems due to powder caking [34]. Mean values of moisture content of whey powder (Table II) revealed that there was a progressive increase in moisture content in powder samples throughout the storage period. In the present finding, the moisture of powder processed at 160 °C was higher than at 180 °C, which could be due to an increase in the release of water molecules from whey droplet at higher temperature. The higher moisture content could be due to the higher humidity level and temperature during the production of whey powder in the month of August in Pakistan. The other reason is that the lactic acid is very hygroscopic and thermoplastic and thus very difficult to dry, which could be the reason of higher moisture content [35].

TABLE I  
 PHYSICO-CHEMICAL PARAMETERS OF LIQUID WHEY AND WHEY  
 CONCENTRATE (48%)

Parameters	Liquid whey (LW)	Whey concentrate (WC)
pH	5.5	4.22
Acidity (%)	0.27	2.63
Viscosity (cp)	1.60	651.33
Total soluble solids (%)	6.20	48.18
Lactose (%)	4.76	36.61
Protein (%)	0.74	4.53
NPN (%)	0.15	1.13
Fat (%)	0.30	2.33
Ash (%)	0.43	4.03

pH of powder affected the solubility of calcium and influence the texture [36]. The interaction between processing temperatures of whey powders and storage days revealed the decrease in pH value for WP160 from (5.58±0.006 to 5.40±0.000) and for WP180 from (5.39±0.003 to 5.37±0.009). These results showed the higher decrease (0.18) in pH for WP160 and lower (0.02) in WP180 during the 180 days of storage period. It is obvious from the results of WP180 that no significant change was noticed during the whole period of storage except on the 120<sup>th</sup> day of storage, while in the WP160 a very slow drop in pH was noticed (reported pH > 5.1) for the powder, sweet whey, which is in accordance with this finding, but the pH decreases with the increase in the duration of storage. Reference [37] observed the behavior of whey protein in a wide range of pH (4-6) and reported that the gel formed at pH 4 was less elastic than those formed at 5-6.

Drying of whey, containing high amounts of lactic acid, is difficult and imparts stickiness to the products. Neutralization of the lactic acid whey by sodium hydroxide is recommended by the scientists [38]. The increase in acidity value in WP160 was 0.24% (2.24±0.007 - 2.48±0.009%) and WP180 was 0.15% (2.36±0.009 - 2.51±0.006%). Results showed that the increase in acidity was higher in whey powder processed at 160 °C (WP160) than samples processed at 180 °C during the 180 days of storage.

There is great variation in fat content of whey powder reported by different researchers. Reference [39] analyzed the different whey powder and found that the fat content of whey powder varied from 0.5 to 3.7%. Results of fat content of whey powder (Table II) revealed that there was a minor decrease in fat content in powder samples throughout the storage period. The interaction of storage and temperature showed the highest fat content (4.52±0.006%) in WP160 at 0, 120, and 150 days of storage. The lowest fat content (4.45±0.010 %) was observed in WP160 at 30 days of storage.

The protein content of whey powder remained constant throughout the storage period. No significant decrease was observed during storage and value of protein content varied from 11.29 to 11.28%. The present results are in accordance with the previous finding of [39]. Results of NPN of whey powder (Table II) revealed that there was a progressive increase in NPN of whey powder samples throughout the storage period which is counted as the 0.14% from zero (1.34±0.015%) to 180 days (1.48±0.014 %). However, there was a non-significant increase (0.13 and 0.14% respectively) in NPN value of WP160 (1.38±0.003 to 1.51±0.009 %) and WP180 (1.31±0.006 to 1.45±0.006 %) during the 180 days of storage. The NPN of powders depends on the processing conditions and type of the whey used. NPN was higher than 20% of total nitrogen in various concentrated and dried powders. The interactive effect of temperatures and storage days showed that a decrease in lactose content was observed in both dried whey samples. The decrease in lactose value was observed in WP160 (67.70±0.132 - 62.10±0.050 %) as well as for WP180 (64.30±0.115% - 56.40±0.115%) during storage. These results showed that the minimum decrease in lactose (5.6%) was observed in WP160 during the 180 days of storage, while 7.9% decrease was noted in WP180 during the same period of storage. The interaction of the storage days into temperatures showed that the highest lactose (67.70±0.132%) was found in WP160 at 0 day of storage followed by WP160 at 30 and 60 days of storage, while the lowest lactose (56.40±0.115 %) was observed in WP180 at 180 days of storage followed by WP180 at 150 and 120 days of storage. The decrease in lactose content during storage was due to the production of lactic acid [40]. The decreasing trend of lactose during storage was also examined by [41]. It is obvious from the results that a very minute decrease was observed up to 120 days after which the ash content remained consistent up to 180 days; however, the ash content of whey powder varied from 7.91±0.022% to 7.80±0.017% during the storage. The ash content increased from 0 to 120 days, but afterwards, no significant change was noticed up to 180 days.

### C. Functional Properties of Whey Powder

The results regarding the functional properties of whey powder (FC, FS, turbidity, SI, and WPNI) are mentioned in Figs. 1-5. Whey powder processed at 160 °C (WP160) had a significantly (P < 0.05) higher FC, FS, WPNI and a lower turbidity and SI value as compared to whey powder processed at 180 °C (WP180). During storage of 180 days, a low but progressive decrease was observed in FC, FS, and WPNI,

while turbidity and SI increased. The ability of whey to form and stabilize foams was due mainly to the relationship between monomeric and polymeric species. The monomeric form contributes to the FC, and the polymeric forms contributed to foam stabilization [42]. It has been found that the heating to 70 °C induced modifications in protein related with unfolding and exposure of active sites. These modifications increased the possibility of flocculation and coalescence and affect negatively the foaming properties. References [43], [44] reported that spray-drying temperatures had significant impact on the whey protein denaturation;

hence, they observed that inlet drying temperatures from 160 to 200 °C and outlet from 89-100 °C had no significant effect on the denaturation of β-lactoglobulin. Later on, [45] reported that β-lactoglobulin showed more stability than casein when both were dried at different temperatures in a spray dryer. The insolubility index increases when milk powder was stored for a long time at a high water content and temperature. The solubility of the denatured proteins depends on the pH and calcium concentration of the powder. The SI of powders varies from < 0.1 to 2.37 mL [46].

TABLE II  
 PHYSICO-CHEMICAL ANALYSIS OF WHEY POWDER

Storage days		Moisture (%)	pH	Acidity (%)	Fat (%)	Protein (%)	NPN (%)	Lactose (%)	Ash (%)
0day	160°C	4.34±0.003	5.58±0.006	2.24±0.007	4.52±0.006	11.35±0.010	1.38±0.003	67.70±0.132	7.91±0.035
	180°C	3.88±0.010	5.39±0.003	2.36±0.009	4.47±0.006	11.23±0.000	1.31±0.006	64.30±0.115	7.91±0.035
30days	160°C	4.75±0.012	5.54±0.010	2.30±0.009	4.50±0.010	11.34±0.003	1.39±0.003	66.20±0.058	7.79±0.012
	180°C	3.14±0.995	5.39±0.000	2.40±0.006	4.45±0.010	11.22±0.003	1.31±0.007	63.00±0.153e	7.81±0.035
60days	160°C	4.92±0.012	5.49±0.012	2.38±0.003	4.51±0.010	11.34±0.000	1.41±0.003	65.40±0.100	7.87±0.006
	180°C	4.37±0.012	5.38±0.013	2.44±0.006	4.47±0.006	11.22±0.010	1.34±0.009	61.93±0.067	7.83±0.018
90days	160°C	5.14±0.007	5.47±0.006	2.41±0.009	4.51±0.006	11.34±0.003	1.43±0.006	64.03±0.067	7.83±0.012
	180°C	4.45±0.019	5.38±0.006	2.49±0.003	4.46±0.006	11.23±0.006	1.37±0.006	60.40±0.173	7.86±0.012
120days	160°C	5.19±0.006	5.42±0.012	2.46±0.003	4.52±0.006	11.34±0.007	1.45±0.003	62.80±0.076	7.82±0.012
	180°C	4.82±0.012	5.37±0.009	2.50±0.010	4.47±0.010	11.22±0.010	1.40±0.012	59.10±0.058	7.85±0.017
150days	160°C	5.20±0.006	5.40±0.000d	2.45±0.003	4.52±0.010	11.35±0.003	1.48±0.006	62.10±0.050	7.80±0.012
	180°C	5.05±0.029	5.39±0.007	2.48±0.006	4.47±0.006	11.23±0.006	1.42±0.003	57.70±0.153	7.83±0.027
180days	160°C	5.34±0.012	5.40±0.000d	2.48±0.009	4.50±0.006	11.34±0.000	1.51±0.009	65.70±0.090	7.85±0.023
	180°C	5.12±0.010	5.38±0.006	2.51±0.006	4.46±0.006	11.22±0.006	1.45±0.006	56.40±0.115	7.82±0.009

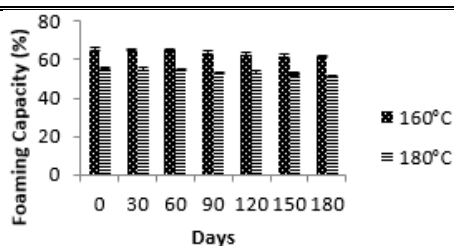


Fig. 1 FC of whey powder

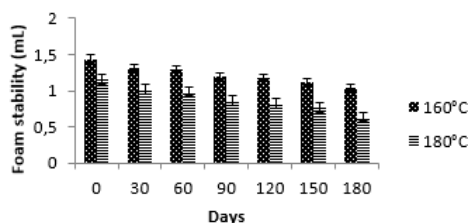


Fig. 2 Foaming stability of whey powder

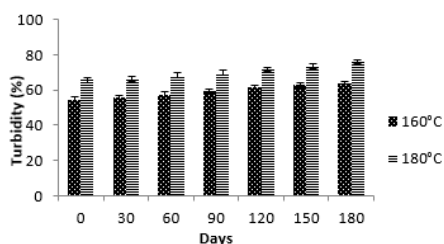


Fig. 3 Turbidity of whey powder

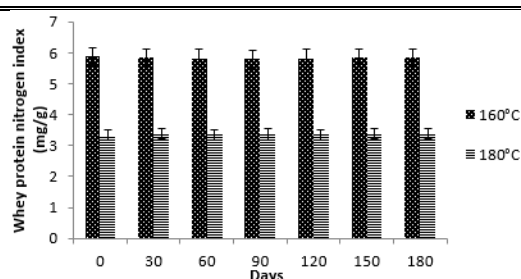


Fig. 4 WPNI of whey powder

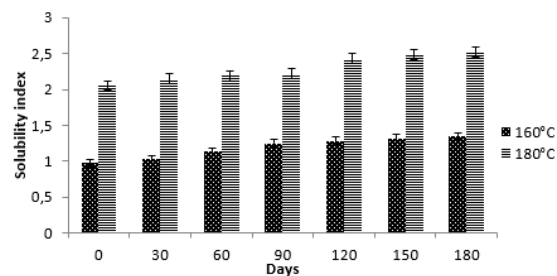


Fig. 5 SI of whey powder

#### D. Whey Proteins Content Using RP-HPLC

The results regarding the effect of temperatures (160 °C and 180 °C) on concentration of α-Lb, β-Lg, BSA, and CMP are presented in Fig. 6. All the whey proteins were significantly (P < 0.05) influenced due to varying processing temperatures. Reference [29] reported that denaturation of whey protein depends upon the concentration, intensity of heating, and

concentration of calcium ions. Heat induces various conformational changes in structure of whey proteins. Unfolding of the protein structure starts at 60-70 °C, while at higher temperature protein aggregates. Susceptibility to denaturation of whey proteins differs from each other [8]. In the present study, the denaturation of  $\alpha$ -Lb and  $\beta$ -Lg was more as compared to BSA and CMP. The contents of whey proteins as reported in the present study are comparable with the previous studies. Reference [47] reported that the content of  $\alpha$ -

Lactalbumin and  $\beta$ -Lactoglobulin in milk was 0.6-1.7g/L and 2-4 g/L, respectively. The rate of degradation and denaturation varies from 30 to 80% during the spray drying; however, more denaturation was found at 160 °C than at 180 °C. Among the whey proteins,  $\beta$ -lactoglobulin is the most abundant protein, so most heat induced changes have been observed on the  $\beta$ -Lg. Reference [48] reported that the peaks during RP-HPLC are of  $\beta$ -Lg due to heat sensitivity and modified the structure by covalent binding with lactose residue

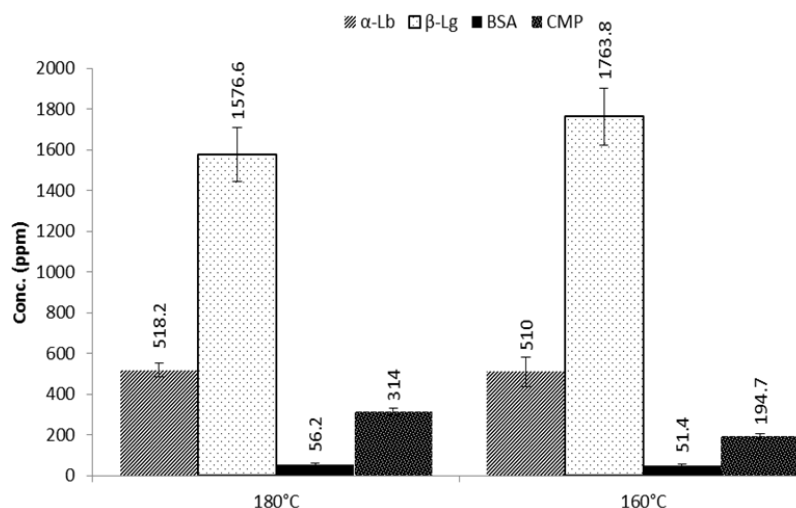


Fig. 6 Effect of temperatures (160 °C and 180 °C) on concentration of  $\alpha$ -Lb,  $\beta$ -Lg, BSA and CMP of whey powder

#### IV. CONCLUSION

Composition of whey determines the quality and type of whey products and its utilization. Processing temperature is one of the key factor influencing the nutritional and functional properties of whey powder. At lower processing temperature (160 °C), whey exhibits good functional properties compared to 180 °C. Concentration of whey proteins and intensity of heating affect the whey protein denaturation. Denaturation of  $\beta$ -Lactoglobulin is more due to its concentration, its monomeric form, and covalent bonding with lactose residues as compared to other whey proteins.

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