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## Physicochemical and Amino Acid Profiling of Cheese Whey

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**Abstract:** In the current exploration, sweet liquid whey was subjected to physicochemical analysis, minerals assay and amino acid profiling. It comprised of total solids, lactose, ash, crude protein and fat as 6.49±0.31, 5.26±0.26, 0.56±0.02, 0.81±0.03 and 0.25±0.01%, respectively. The results regarding mineral analysis indicated that whey contains appreciable amount of potassium, sodium and calcium. Furthermore, amino acid profile elucidated it a balance source of essential and non-essential amino acids. Amongst essential amino acids, highest value was noticed for leucine 97.25±4.68 followed by lysine and threonine 83.98±2.46 and 48.89±2.84 mg/g protein.

**Key words:** Whey, minerals, amino acid profile, protein

### INTRODUCTION

Food industry generates enormous amount of processing byproducts creating disposal problem and an incremental addition in the environmental pollution. Nonetheless, these byproducts are exploitable source of bioactive ingredients. Additionally, supplementation of these byproducts is possible to improve the nutritional profile of the end products. Its annual production is 115 million tons on global scale (Illanes, 2011).

Whey is the milk serum remained after curdling followed by straining. It is a byproduct of cheese or casein production with several commercial uses. It has greenish-yellow color or sometimes in bluish shade depending upon the quality and type of milk used. Whey can be produced from cow, goat, sheep or even camel milk however, cow milk is mostly accepted for its production (Grba *et al.*, 2002). About 9 L of whey is generated for every kilogram of cheese produced; a large cheese making plant can produce >1 million liters of whey daily. Sweet whey is synthesized by removing considerable portion of water from renneted cheese having off-white to cream colored product (Tunick, 2008). The history of whey dates back more than 8,000 years, intertwined with the cheese. Whey invented accidentally when milk was curdled and whey separated naturally. The curd represents the earliest known cheese (Fox and McSweeney, 2004). Previously, whey was fed to domestic animals or livestock, spread on fields as fertilizer, or just thrown out. It was considered as potent pollutant with a Biological Oxygen Demand (BOD) of 35-45 kg/L, whilst 4,000 L of whey from a small creamery holds the polluting strength of sewage from 1,900 people (Athanasiadis *et al.*, 2004). Before the environmental regulations, disposal of whey by dumping in rivers was a frequent practice in the United States (Cryan, 2001).

Whey constituents notably proteins and peptides are helpful to raise its status towards valuable dairy byproduct. It contains multitude of exploitable components for food, medical and biotechnological purposes (Foegeding *et al.*, 2002). Application of contemporary approaches has enabled the food scientists to incorporate whey in various edibles due to its unique nutritional and functional features (Henning *et al.*, 2006). These techniques include proficient and cost effective processes for concentration, transformation, fractionation and dehydration of whey for the development of therapeutic products (De Silva *et al.*, 2003).

Whey proteins are one of the most bioavailable proteins and reservoir of essential amino acids compared to various other protein sources like corn, soy and wheat (Bos *et al.*, 2000). Whey protein based amino acids are readily absorbed and utilized than free amino acid solutions (Daenzer *et al.*, 2002). It is also rich in branched chain amino acids i.e., leucine, isoleucine and valine. These Branched Chain Amino Acids (BCAAs) play pivotal role as metabolic regulators in lipid metabolism as well as in protein and glucose homeostasis (Smilowitz *et al.*, 2005; Zemel, 2004). They are directly metabolized by skeletal muscles, promoting protein synthesis, preserve muscle mass and produce energy during prolonged exercise (Hazen, 2005; Walzem, 2004).

Whey proteins are also a balanced source of sulfur containing amino acids (cysteine and methionine). These amino acids serve a vital role as antioxidants and precursors to intracellular antioxidant glutathione and in one-carbon (1-C) metabolism (Shoveller *et al.*, 2005). It has also proven that whey protein may diminish muscle loss linked with aging, distressing many of the elderly people (Hoolihan, 2005). A study conducted by Paddon-

Jones (2005) delineated that 15 g whey protein isolate daily stimulates muscles synthesis.

Whey protein constituents are essential to attain the required functional properties like solubility, gelation, water-binding, aeration and emulsification in an array of foods (Foegeding *et al.*, 2002). They are Generally Recognized as Safe (GRAS) for food applications and commonly used in dairy products, frozen foods, meat products, infant formula and health foods. They are less expensive than milk protein or casein and impart bland flavor compared to other proteins like soy (Hazen, 2005). Whey protein ingredients are preferred for foam and emulsion stabilization in frothed drinks, ice creams, souffles and other foods. The nutritional value of whey protein has encouraged its use in a range of drinks. Likewise, processed cheese emulsion also contains whey protein to enhance slicing, melting and spreading abilities (Foegeding *et al.*, 2002).

## MATERIALS AND METHODS

The present research work was conducted in the Functional and Nutraceutical Food Research Section, National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad.

**Procurement of raw material:** Sweet liquid whey was procured from Noon Dairies, Bhalwal. The refrigeration conditions were maintained during storage to ensure whey quality.

**Characterization of whey:** Initially, liquid whey was analyzed for various physicochemical parameters including pH, acidity, total solids, fat, crude protein, ash, lactose, mineral analysis and amino acid profile. However whey sample was also estimated for microbial assay i.e., Total Plate Count (TPC).

**pH:** For the pH estimation, electronic digital pH Meter (Inolab pH 720, WTW 82362) was used. The whey sample was taken in a 50 mL flask to measure this trait. After calibration, pH was directly recorded from digital display.

**Acidity:** Whey was analyzed for acidity through the titrimetric method. Acidity was determined by titrating raw liquid whey against N/10 NaOH using phenolphthalein as indicator following the guidelines of AOAC (2006), Method Number 947.05:

$$\text{Acidity (\%)} = \frac{0.009 \times \text{Volume of NaOH used}}{\text{Weight of the sample (g)}} \times 100$$

**Total solids:** Total solids of whey were assessed according to the AOAC (2006) Method No. 925.23. The sample was heated for 15 min in water bath followed by drying in hot air oven at  $100 \pm 5^\circ\text{C}$  till constant weight. The total soluble solids were calculated by the following expression:

$$\text{Total solids (\%)} = \frac{\text{Residue after drying (g)}}{\text{Weight of sample}} \times 100$$

**Fat:** Fat content was calculated by Gerber method following the protocol of Djuric *et al.* (2004). For the purpose, 10 mL of sulfuric acid ( $\text{H}_2\text{SO}_4$ ) was poured into the butyrometer. Milk sample (10.94 mL) and isoamyle alcohol (1-2 mL) was also added. Afterwards butyrometer was closed with a rubber stopper. The contents of butyrometer were mixed carefully by rotating the tube at  $45^\circ$  and placed in a water bath at  $65^\circ\text{C}$  for 5 min. Butyrometer was then immediately centrifuged @1100 rpm for 5 min. The resultant fat percentage was read out from the butyrometer graduated column.

**Crude protein:** Crude protein was determined by Kjeldahl method using Kjeltach Apparatus (Model: D-40599, Behr Labor Technik, GmbH-Germany) following the procedure elaborated in AOAC (2006) Method No. 991.20. Accordingly, 1 mL whey sample was digested in a digestion tube using sulfuric acid. Digested sample was distilled with 40% NaOH followed by titration against 0.1 N HCl. The protein content in milk was estimated by multiplying with factor 6.38.

$$\text{Protein (\%)} = \text{N (\%)} \times 6.38$$

**Ash contents:** Ash in whey was estimated by incinerating the dry samples in a Muffle Furnace (MF-1/02, PCSIR, Pakistan) at  $550^\circ\text{C}$  till grayish white color following the protocol given in AOAC (2006) Method No. 945.46.

$$\text{Ash (\%)} = \frac{\text{Residue after ashing (g)}}{\text{Weight of sample}} \times 100$$

**Lactose:** Lactose content of whey was determined through the enzymatic method as explained in AOAC (2006). For the intention, 1 g milk sample was added in a 100 mL volumetric flask along with 60 mL distilled water followed by mixing and stored at  $50^\circ\text{C}$  for 15 min. Afterwards, 2 mL of Carrez I and Carrez II solutions and 4 mL of 100 mM NaOH was added with subsequent mixing. Then volume was made upto the mark with distilled water. The filtered sample solution (0.2 mL) was taken in cuvette and incubated for 10 min at  $25^\circ\text{C}$ . Later, 2.2 mL of distilled water, 0.2 mL of Ethylenediaminetetraacetic Acid (EDTA) buffer and 0.1 mL of Nicotinamide Adenine Dinucleotide (NAD) were added at  $25^\circ\text{C}$ . The absorbance of solution was measured after 3 min at 340 nm and recorded as  $A_1$ . Then again read the absorbance at same wavelength as  $A_2$  after addition of 0.02 mL of  $\beta$ -galactosidase dehydrogenase. Analytical conditions were:

Wavelength : 340nm  
Cuvette : 1cm  
Final volume : 2.72 mL

The lactose concentration was calculated as:

$$\frac{V \times MW \times A}{\epsilon \times d \times V}$$

Where:

- V = Final volume (mL)
- MW = Molecular weight
- $\epsilon$  = Coefficient of NADH at 340nm
- d = Light path
- V = Sample volume
- A = Absorbance

**Mineral profile:** Liquid whey was analyzed for mineral assay following the guidelines of AOAC (2006). Purposely, Flame Photometer-410 (Sherwood Scientific Ltd., Cambridge) was used to determine sodium and potassium whilst calcium and magnesium through Atomic Absorption Spectrophotometer (Varian AA240, Australia).

**Total plate count:** Total plate count was determined by the procedure of Yousaf and Calstrom (2003). Sample dilutions were prepared using normal saline solution. Each dilution was shifted to separate nutrient agar plates. The plates were spread well and incubated at 37°C for 24 hr. Afterwards, number of colonies were counted by Colony Counter (Galaxy 230, Utech Products Inc., Albany, NY, USA) and used to calculate number of colony forming units/mL of sample (cfu/mL).

**Amino acid profile:** Amino acid profile of whey was performed following the protocol of Walsh and Brown (2000). In this context, whey sample was centrifuged at 5000 g for 15 min at 4°C to separate the fat. Hydrochloric acid (6 M) was added to the sample vial for a final concentration of 5 mg of protein/mL of HCl. Hydrolysis vial was placed in an ultrasonic cleaner and flushed with nitrogen gas before sealing under vacuum. Sample was placed in a heating block for 4 hr at 145°C. Afterwards, sample was removed from the heating block and allowed to cool before filtration through 0.2 µm filter. Sample was dried with nitrogen gas and dissolved in a dilution buffer. The prepared whey sample was analyzed for amino acid profile by running through Automated Amino Acid Analyzer (Model: L-8500 A, Hitachi, Japan). Areas of amino acid standards were used to quantify each amino acid in representative sample.

**Statistical analysis:** The resulting data was subjected to statistical analysis to determine the level of significance (Steel *et al.*, 1997).

## RESULTS AND DISCUSSION

**Characterization of whey:** Characterization of raw material is a key step for the assessment of its essential constituents. With intent, whey was probed for its

Table 1: Compositional analysis of whey

Components	Quantity
pH	5.42±0.24
Acidity	0.29±0.01 (%)
Total solids	6.49±0.31 (%)
Fat	0.25±0.01 (%)
Crude protein	0.81±0.03 (%)
Ash	0.56±0.02 (%)
Lactose	5.26±0.26 (%)
TPC	3.25±0.07 (10 <sup>4</sup> cfu/mL)

Values are expressed as means ± standard deviation

Table 2: Mineral profile of whey

Minerals	(mg/100g)
Sodium	34.26±1.68
Potassium	98.67±4.54
Calcium	24.89±1.24
Magnesium	4.91±0.19

Values are expressed as means ± standard deviation

physicochemical attributes i.e., pH, acidity, total solids, fat, crude protein, ash and lactose. Further, whey sample was also estimated for microbial assay i.e., Total Plate Count (TPC). Alongside, mineral analysis and amino acid profiling of whey are also discussed in the proceeding section for meticulousness regarding its nutritional profile.

**Physicochemical analysis of whey:** The sweet liquid whey was assessed for various quality attributes that indicated pH, acidity, total solids, fat, protein, ash, lactose and total plate count as 5.42±0.24, 0.29±0.01%, 6.49±0.31%, 0.25±0.01%, 0.81±0.03%, 0.56±0.02%, 5.26±0.26% and 3.25±0.07x10<sup>4</sup> cfu/mL, respectively (Table 1).

The results are in accordance with the earlier work of Goyal and Gandhi (2009), reported pH, protein, fat, lactose and total solids; 6.21±0.08, 0.53±0.28, 0.2±0.06, 5.0±0.05 and 6.3±0.12%, respectively. The present results are also corroborated with the findings of Djuric *et al.* (2004) for pH, protein, fat, lactose, ash and total solids contents of liquid whey and they narrated the values 3.63, 0.45, 0.82, 4.69, 0.50 and 7.00% for respective traits. Earlier, Smithers (2009) explored cheese whey for chemical composition and found protein, fat, lactose and total solids i.e., 0.7, 0.1, 4.9 and 6.3%, correspondingly. Likewise, Sakhale *et al.* (2012) characterized liquid whey and observed 0.19% fat, 0.45% protein, 5.73% SNF and 6.12% total solids.

**Mineral profile:** Liquid whey used in this study was also assessed for minerals including sodium, potassium, calcium and magnesium with 34.26±1.68, 98.67±4.54, 24.89±1.24 and 4.91±0.19 mg/100g concentration (Table 2).

Whey contains numerous valuable micronutrients like, sodium, potassium, chloride, magnesium, citrate and phosphate that help to enhance the functionality of whey

Table 3: Amino acid profile of whey

Essential amino acids (mg/g protein)		Non-essential amino acids (mg/g protein)	
Histidine	17.68±0.81	Alanine	47.49±2.82
Isoleucine	45.65±2.73	Arginine	22.33±0.39
Leucine	97.25±4.68	Aspartic acid	104.59±5.20
Lysine	83.98±2.46	Cystein	15.08±0.46
Methionine	25.89±0.95	Glutamic acid	151.30±7.61
Phenylalanine	25.32±0.84	Glycine	16.54±0.39
Threonine	48.89±2.84	Proline	67.64±2.97
Tryptophan	20.45±0.95	Serine	50.69±2.87
Valine	45.64±1.79	Tyrosine	30.08±1.52

Values are expressed as means ± standard deviation

proteins (Zemel, 2004). Whey is also an excellent source of bioavailable calcium that improves bone health. The composition of whey salts expounded low sodium to potassium ratio that is essential for maintenance of blood pressure. Moreover, calcium from the whey is readily absorbed in the intestine, facilitated by the presence of lactose (Smithers, 2009). The results for mineral contents of whey in the present study are in line with the observations of Goyal and Gandhi (2009). They recorded the values for sodium, potassium, calcium and magnesium for cheese whey 35, 130, 48, 5.9 mg/100g. Additionally, Wit (2001) noticed the amount of sodium, potassium, calcium, magnesium and chloride in cheese whey by 50, 150, 60, 10 and 110 mg/100g, respectively.

**Amino acid profile of whey:** Whey proteins contain essential and non-essential amino acids in higher concentration as compared to vegetable protein sources. In the present exploration, amino acid composition exposed that leucine is the major essential amino acid 97.25±4.68 mg/g followed by lysine and threonine with mean values 83.98±2.46 and 48.89±2.84 mg/g, respectively. However, the concentrations of other essential amino acids including valine, isoleucine, histidine, methionine, phenylalanine and tryptophan were 45.64±1.79, 45.65±2.73, 17.68±0.81, 25.89±0.95, 25.32±0.84 and 20.45±0.95 mg/g, correspondingly (Table 3). In contrary, means for non-essential amino acids indicated that glutamic acid was present in highest concentration 151.30±7.61 mg/g followed by aspartic acid 104.59±5.20 and proline 67.64±2.97 mg/g. Likewise, means for alanine, arginine, cysteine, glycine, serine and tyrosine were 47.49±2.82, 22.33±0.39, 15.08±0.46, 16.54±0.39, 50.69±2.87, 30.08±1.52 mg/g protein.

Earlier, Hulmi *et al.* (2010) documented essential amino acids values in whey protein isolate as 61, 122, 102, 33, 30, 68, 18 and 59 mg/g for isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, correspondingly. The results concerning amino acid profile of whey are in conformity with the findings of Etzel (2004), who noticed highest concentration of leucine as 118 mg/g whey protein isolate followed by lysine with mean value 95 mg/g. According to him, in non-essential amino acids, maximum concentration

was recorded for glutamic acid (154 mg/g) followed by aspartic acid (107 mg/g).

Amongst branched chain amino acids, leucine plays an important role in dietary protein metabolism. The breakdown of leucine takes place in the liver and skeletal muscles (Layman, 2003). It undergoes transamination in the muscles by transferring into glutamine or alanine that ultimately converts to glucose in the liver through gluconeogenesis; a unique pathway for the maintenance of blood glucose level (Borsheim *et al.*, 2003). Hence dietary proteins rich in essential and branched chain amino acids particularly leucine provide health benefits that are not usually observed for diets containing protein from other sources (Wolfe, 2002).

The composition of whey strongly depends upon the type and variety of cheese. The observed deviations in the composition, mineral contents and amino acid profile of whey in the current study are probably due to variations in whey, milk source and cheese manufacturing practices.

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