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Investigation of the Functional Properties of Whey Powder Produced by Traditional and Ultra-Filtration Cheese Making

M. Faramarzi [a], ***M. Goli*** [b,c*]
[a] MSc Graduated of the Department of Food Science and Technology, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran.
[b] Associate professor of the Department of Food Science and Technology, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran.
[c] Associate professor, Laser and Biophotonics in Biotechnologies Research Center, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran.

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ABSTRACT: In this research, two types of whey powder from traditional and UF cheese making were compared for structural components and functional properties. The effect of pH on the functional properties of the two whey powders was evaluated. Foam capacity, foam stability, water holding capacity, and emulsifying activity index were the functional characteristics that were evaluated in different pH values for two whey powder. The whey powder obtained from the traditional cheese-making compared to whey powder by UF cheese-making showed a higher percentage in terms of protein, fat, lactose, and salts, as well as the foam capacity, water holding capacity, and emulsion activity index of whey powder obtained from the traditional cheese-making was significantly ($P < 0.05$) more than whey powder obtained from UF cheese-making. However, the foam stability of these two powders did not show a significant difference ($P > 0.05$). This study shows that the higher quality of whey powder produced in the traditional cheese making as compared to whey powder produced by the UF cheese making can compensate for the lower traditional cheese production efficiency compared to UF cheese.

Keywords: *Functional Properties, Traditional Cheese-Making, Ultrafiltration Cheese-Making, Whey Powder.*

Introduction

Whey products have been widely used in food additives over the past 40 years and have a special place. Whey proteins are at the top of the list of important nutrients, which, in addition to unique biological properties, have significant functional properties, such as emulsion capacity, the ability to produce foam with optimum stability, water holding capacity, and ability to produce gel. Some of the advantages of ultra-filtration (UF) cheese making compared to the traditional ones include: maintaining more soluble proteins in the cheese structure and increasing (20-40%) cheese production efficiency, the possibility of using the continuous process of production and the hygienic system, the use of less rennet and starter, less BOD and COD than membrane whey (about 80%), better control of the dimensions of the cheese, neutralizing the pH of whey (Kosikowski, 1979). Whey products are produced and supplied in various ways and

* Corresponding Author: mgolifood@yahoo.com; m.goli@khuisf.ac.ir

often in powder form. Spray drying is one of the best methods for drying heat-sensitive products like proteins because the functional properties of proteins can be affected by various processes. Denaturation is one of the most important structural changes that can be affected by changes in pH and heat. On the other hand, functional properties of proteins are strongly influenced by their structural properties (Huffman *et al*., 2011). In addition to its medicinal applications, whey protein plays an essential role in the development of whey protein-based functional foods. Whey protein is widely utilized as an antibacterial in edible film and as a protective substance to extend food shelf life (Kumar *et al*., 2018). Glycomacropeptide, the glycosylated portion of caseinomacropeptide (CMP) that is present in sweet whey but absent in acid whey, prevents heartburn, reduces gastric acid output, and lowers the risk of heart attack by inhibiting platelet aggregation and fibrinogen binding to platelets (Santos et al., 2018). Whey protein concentrate is considered as fat mimetic having fat like properties and improved water binding, giving mouth feel of fat (Sun et al., 2018). Whey protein is a protein combination with various and different functional qualities, and as such, it has a wide range of possible applications. The two most important proteins are β-lactoglobulin and α-lactalbumin. They account for over 70% of total whey proteins and are in responsible of the hydration, gelling, and surface-active characteristics of whey protein components (Cayot & Lorient, 2017).

Considering the widespread use of whey powder in food products such as cereal, meat, chocolate, and snacks products, in this research, the physicochemical properties of whey powder derived from these methods namely traditional and novel (UF) and their impact on some of the functional characteristics of the powder were investigated and compared.

Materials and Methods

- *Materials*

Initially whey was produced by UF and traditional cheese making processes. In the UF method, the milk after separating stage (GEA, Germany) passed into the UF machine made in the Iranian Novin-sanaat company with the tube cell membrane made of cellulose acetate (Osmonic, USA) with a 20,000-Daltons molecular weight transfer at the pressure of 14 bars and temperature 40 °C. In the traditional method, whey was obtained at 35°C after renneting process and separated from the curd. The whey (traditional and UF) entered into the evaporator concentrated. Condensed whey was transferred from the pump to a spray dryer (air temperature of 160 °C and Output product temperature of 50 °C). The outlet powder had a moisture content of approximately 3 percent. Chemical properties of whey powders obtained are presented in Table 1.

Table 1. Chemical analysis of whey powders

Characteristic	Traditional whey powder	UF whey powder
pH	5.73±0.27	5.92±0.1
Moisture (%)	2.67±0.14	2.77±0.14
Protein %	10.81±0.59	4.22±0.15
Salt (%)	4.02±0.1	3.36±0.26
Acidity (%)	1.28±0.02	1.21±0.09
Fat (%)	3.08±0.27	0.11±0.01
Lactose (%)	76.83±1.03	62.67±1.27

- *Measurement of whey composition*

Moisture, protein and fat, as well as the determination of pH and acidity, water-whey powders were carried out in accordance with the Iranian National Standard No. 164 (ISIRI, 2007).

- *Water holding capacity (WHC)*

First, 100 mg (w_0) of the sample was weighed in vial (w_1), then 1000 ml of distilled water was added to each vial and mixed with the shaker for 2 minutes and stored for 30 minutes at the laboratory temperature, then were centrifuged with 5000g and finally the supernatant was evacuated and the vial weighed (w_2). WHC was calculated according to Eq. 1 (Beuchat, 1977).

$$\textbf{Water holding capacity } (\mathrm{WHC}) \left(\frac{\mathrm{ml}}{\mathrm{mg}}\right) = \frac{W_2 - W_1}{W_0} \quad \textbf{Eq. 1}$$

- ***Foaming properties***

250 mg of each sample were dissolved in 250 ml of distilled water, and the pH was adjusted to 2, 4, 6, 8 and 10. This protein solution was whipped for 3 minutes and poured into a 100 ml graduated cylinder. The total sample volume was taken at the zero minutes for foam capacity, and up to 60 minutes for foam stability (Taheri *et al*., 2013). Foam capacity and stability were then calculated using the following equations:

$$\text{Foam capacity } (\%) = \frac{\text{Volume after whipping (ml)} - \text{volume before whipping (ml)}}{\text{Volume before whipping (ml)}} \times 100 \quad \textbf{Eq. 2}$$

$$\text{Foam stability } (\%) = \frac{\text{Volume after standing (ml)} - \text{volume before whipping(ml)}}{\text{Volume before whipping (ml)}} \times 100 \quad \textbf{Eq. 3}$$

- ***Emulsifying properties***

For determination of emulsifying activity index (EAI), three hundred milligrams of samples were dissolved in 30 ml of deionized water. This solution was mixed with 10 ml of sunflower oil, and the pH was adjusted to 2, 4, 6, 8 and 10. The mixture was homogenized at a speed of 14000g for 1 minute. Aliquot of the emulsion was homogenized and 15 µl were removed by pipette from the bottom of the container at 0 and 10 min after homogenization. Afterward the sample mixed with 5 ml of 0.1% sodium dodecyl sulphate solution. The absorbance of the diluted solution was measured at 500 nm using a spectrophotometer (Jenway, 6305, UK). The following Equation used to calculate EAI (Taheri *et al*., 2013).

$$\text{Emulsifying activity index (EAI) } (m^2/g) = \frac{2 \times 2.303 \times A0}{0.25 \times \text{sample weight (g)}} \quad \textbf{Eq. 4}$$

A_0 is the absorbance measured immediately after emulsion formation.

- ***Statistical analysis***

In order to investigate the effect of manufacturing process type on the functional characteristics of the two types of whey powder, a factorial experiment was conducted in a completely randomized design with 6 replications. Statistical analysis of data from general linear models (GLM) was performed using SAS 8.0 software. Comparison of meanings for each trait was performed by LSD test at a significant level of 5%.

Results and Discussion

- ***Water holding capacity***

The characteristics of water holding capacity in two traditional powders and UF powder have a significant difference ($P < 0.05$), and this characteristic has a higher mean in traditional powder (Figure 1). The pH has a significant effect on the ability of the powder to maintain water, and the highest mean water holding capacity in pH is 2. By increasing the pH to the near-neutral range, the ability to keep whey powder for both traditional and UF showed a downward trend, followed by a rising trend in pH values. Traditional and UF powder show a different and significant water holding capacity and pH also has a significant effect on the ability to preserve water of whey powder with its own changes. While the changes in the traditional and UF powder are identical, the lowest water preservation potential was at pH of 6. Since water holding capacity is a characteristic feature of whey protein (Huffman, 1996), a significant difference in water keeping ability can be attributed to different protein levels in both traditional and UV powder. Proteins can interact with water through a variety of interactions, and they can be related to water. Hydrogen, ion-dipole and bipolar-bipolar interactions are among the mechanisms of absorption and preservation of water molecules in proteins. Interactions between ion-bipolar interactions between water molecules and uncharged amino acids with the energy of about 5 kcal per mole create relatively strong bonds. Proteins also contain uncharged polar amino acids. These molecules are bipolar molecules that water molecules can bind with them by bipolar-bipolar interactions. Hydrogen bands generally have energies ranging from 2 to 6 kcal / m (Whitaker, 1977). In addition to polar groups, as well as dipoles, water can be attached to hydrophobic groups in the structure of capillary tubes of product (Mangino, 1984). Inflation is the absorption of a solvent by a solid. The rate and rate of inflation depend on the number of active sites in the water absorption, the spatial structure of the protein and the external surface of the particles. At near neutral pH (pH = 6), due to the isoelectric conditions, the least interaction between water and protein will occur (Mangino, 1984). Manoi et al., 2008 stated that with the recede of pH from the isoelectric point, the viscosity of the whey solution increases, one of the reasons for which can be due to the increased water holding capacity of whey proteins. The reason for the increase in water absorption in alkaline conditions, although the bonding capacity is higher than the neutral range and close to it, is lower compared to acidic conditions, which can be explained by the fact that under alkaline conditions solubility of whey proteins decrease due to the formation of polymers with a greater molecular weight, resulting in the formation of a gel with a specific structure (Onwulata *et al*., 2006). This gel with its own specific structure will generally be able to maintain less water (Hudson et al., 2000). However, the structure of the gel formed in acidic conditions due to the different structure, in contrast to the conditions of the alkaline, will have far greater water holding capacity (Hudson *et al*., 2000; Ikeda *et al*., 2002).

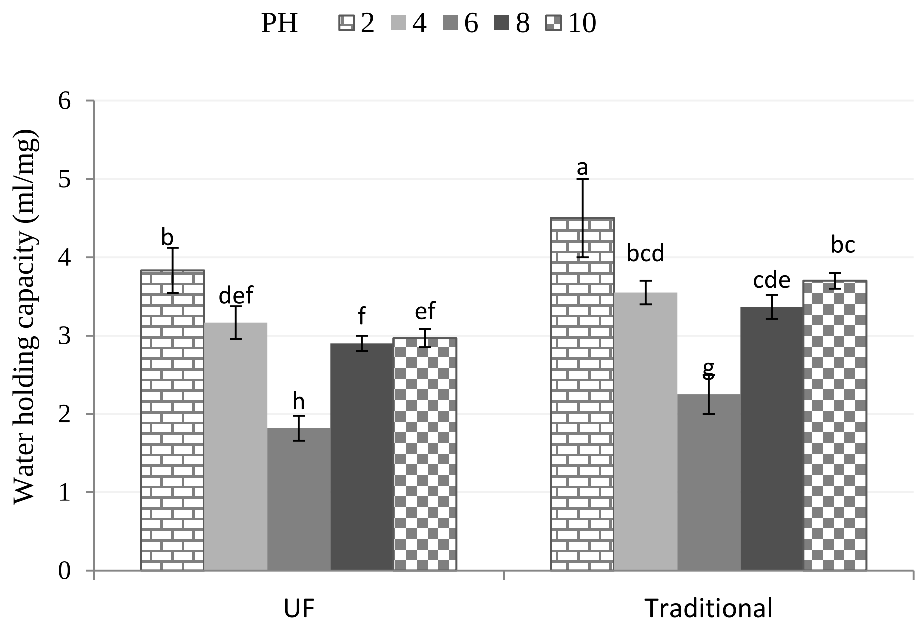

Fig. 1. The effect of pH on the water holding capacity of whey powder obtained from the traditional and the UF cheese making.

- *Foaming capacity*

The foaming characteristics of the two types of traditional and UF powder have a significant difference ($p < 0.05$). In addition, the variation of foamability at different pH values is significant (Figure 2).

Foam capacity has higher values in traditional powder, and there is a similar trend in the foaming capacity of both traditional and UF powder, and in addition, whey powder in acidic and alkaline pH against the near neutral pH had more foaming capacity. In the acidic range, whey powder has the highest foam capacity relative to the alkaline area and close to neutral so that the minimum foaming strength is significantly recorded at pH 6. The slope of the changes in the acidity range is farther than the slope of this variation in the range of the alkaline. Since foaming capacity is a characteristic feature of whey protein, the significant difference observed in the foaming index can be due to a significant difference in the protein content of these two types of whey powder and is justifiable in this regard (Huffman, 1996). According to the expectation, the trend of foaming capacity changes in two traditional and UF powder was equal for different pH and the lowest amount was seen in pH 6. Absorption of the protein from the soluble phase to the fluid and air interface to spontaneously is a key factor in the formation of the foam by the protein. This phenomenon is the highest good viewpoint of thermodynamic (Dickinson, 1986). The most obvious result of adsorption of the protein is a decrease in surface tension so that it ranges from 75 mg /m in water (at room temperature) to 45 mg / m in a concentrated protein solution (Prins *et al*., 1998). This reduction in surface tension provides favorable conditions for the creation of foam in the system. Electrostatic interactions play a very important role in the absorption of proteins. On the other hand, these interactions are influenced by the pH of the system, as this determines the net charge on the protein molecule (Richert, 1979). Mishra *et al*., (2001) showed that the foaming properties of whey powder at pH = 4.6 were higher than that of pH 7. The highest foaming behavior for various proteins has been reported in the range of the iso-electric pH (pH), i.e., the pH of the net charge on the protein is zero. The highest foaming capacity of whey powder was at pH ranges from 4 to 5 (Singh, 2011). More important than the amount of surface tension reduction in foaming capacity is its reduction rate (Wilde *et al*., 1996), which is affected by the rate of surface adsorption of protein in the common air and water surface and the highest rate of surface adsorption in PI was have reported. The reason for this is the minimum molecular repulsion due to the same electric charge (Dickinson, 1986). Fat content has a negative effect on the foaming capacity of whey powder, which decreases with increasing percentage of fat (Phillips *et al*., 1994). However, despite the higher fat content in the traditional powder, the higher foaming capacity was found in the traditional powder than the UF powder. The difference in the percentage of protein in both the traditional and UF powder is such that it has neutralized the effect of the difference in fat percentage.

- *Foam Stability*

The foam stability of whey powder has not been influenced by its type (traditional and UF), although different pH has a different stability in the foam of the product (Figure 3). Unlike the process type, pH has a significant effect on the stability of the foam and the pH stability index has different values. With increasing pH, the stability of the foam was significantly decreased, in other words, there was a reverse correlation between pH and floor stability. The same behavior is observed in both traditional and UF powder under the influence of pH changes. In the acid range, the foam stability is greater than the neutral and alkaline range, so that the maximum is observed at pH 2. In addition, the stability index of the foam has always shown a descending trend with increasing pH. Banavara *et al*. (2003) reported a positive correlation between foaming capacity and foam stability with a correlation coefficient of 0.86. (Kamman, 1997) showed that the addition of acid during egg whites mixing increased the stability of the foam (Li-Chan *et al*., 1989). The reason for such behavior is the proximity of the protein to its iso-electric pH due to the addition of acid (and reduced pH). The agent of production of foam in an egg white solution and whey solution is essentially protein, and there is a similar behavior mechanism. Mishra *et al*., (2001) compared the foam stability of UF whey powder in two pH values of 4.6 and 7. They reported higher foam stability in acid pH. The role of protein in the stability of the foam is due to its water binding so that the higher the protein binding capacity will produce more surface viscosity and reduce the drainage rate of the water from the water and air surface, which means more stabilization of the foam (Nakamura *et al*., 1964).

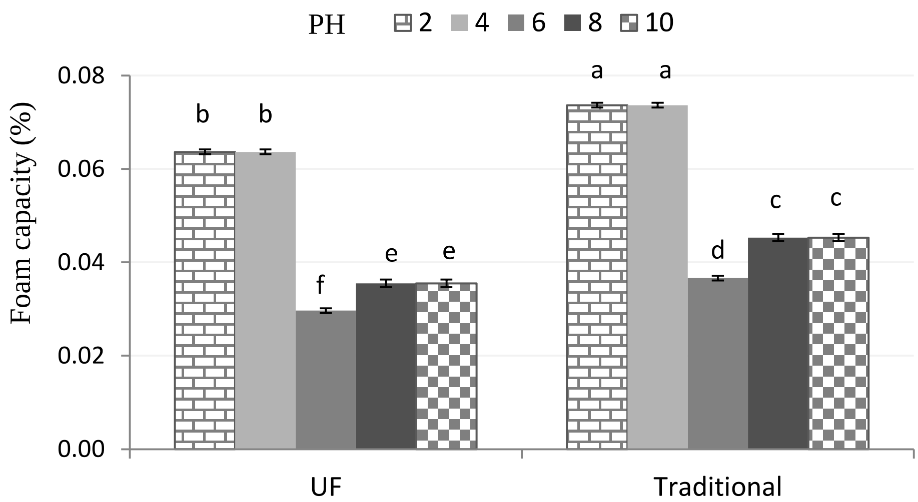

Fig. 2. The effect of pH on the foam capacity of whey powder obtained from the traditional and the UF cheese making.

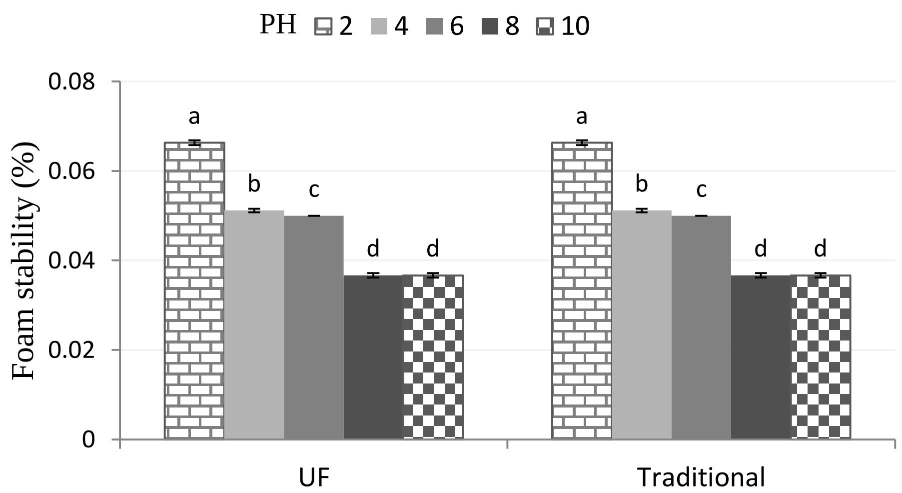

Fig. 3. The effect of pH on the foam stability of whey powder obtained from the traditional and the UF cheese making.

- ***Emulsifying properties***

The results of measuring the emulsifier activity for traditional and UF powder at different pH values are presented (Figure 4). The effect of the type of process on the emulsifier activity of whey powder was significant ($P < 0.05$). In addition, pH has a significant effect on the emulsifier index ($P < 0.05$). The lowest value was recorded at pH of 4. The emulsion ability in whey powder is influenced by whey protein and is, in fact, one of its most prominent functional properties (Huffman, 1996). Zayas (1997) also explains the increase in protein concentration as an agent for increasing the emulsifier capacity. The emulsion capacity of a protein in a food system depends on its ability to reduce the surface tension between the hydrophobic phase and the hydrophilic phase (Sinha, *et al*., 2007). The stability of the emulsion increases with increasing protein concentration. This can implicitly indicate an increase in the emulsion strength of whey powder under the influence of increased protein concentrations (Zayas, 1997). It should be noted that the percentage of protein in the UF powder is about 4% and in the traditional powder is about 11%. Klemaszewski *et al*. (1992) stated that beta-lactoglobulin exhibits a much higher emulsion capacity compared to the bovine serum albumin and alpha-lactalbumin. Since the molecular size of β-lactoglobulin is greater than the other two whey protein (Edwards *et al*., 2014), it can be expected that, during the UF process, a lower percentage of β-lactoglobulin protein than the protein α-lactalbumin and serum albumin can pass the width of the membrane, and therefore the contribution of albumin and α-lactalbumin to the formation of protein of UF powder will be more significant, and from this perspective, in addition to a lower percentage of protein, another reason may be the difference between the traditional emulsion and the powder of UF. Patel *et al*., (1990) observed that the emulsion capacity of whey powder with high-fat content (9-14%) was lower than that of low-fat whey powder (4-5%). However, in the present study, despite higher amounts of fat in traditional powder, the higher emulsion capacity in it than in the UF powder is observed. On the basis of this, it can be stated that the significant difference in protein content in both traditional powder and UF powder and its positive effect on the emulsion capacity is far more than the negative effect of the difference in lipid content of these two powders in the emulsifier characteristics. Damodaran, (2005) attributed the negative effect of fat on the characteristics of the emulsifier of whey powder to the presence of a competitive characteristic of lipid with protein in the absorption in the water and air interface and direct lipid-protein reaction, which all reduced the protein's emulsion activity. Particle size distribution and surface coating of proteins are two important properties of emulsions which formed by proteins. The concentration and protein state, the energy given to the system, pH, temperature, ionic strength, and calcium ion concentration is considered as factors affecting protein coatings (Singh, 2011). Sanmartín (2013) compared the emulsion activity of whey powder in two pH values of 4 and 7, with a higher in pH value of 4. Casper et al. (1999) observed a greater emulsifier activity in pH 8 in comparison to a pH of 3. The reduction in the activity of emulsifying whey protein in the iso-electric pH was attributed to a greater interaction of fat and protein by hydrophobic bonds. (Patel *et al*., 1990) and a positive correlation between emulsion stability and protein solubility. There is a positive correlation between emulsion activity and whey protein solubility. So that in pH from 3.5 to 5.5, the lowest emulsion strength of whey proteins is due to the presence of an isoelectric point in this range and, consequently, to decrease the solubility of these proteins under the influence of isoelectric pH (Walstra *et al*., 1999).

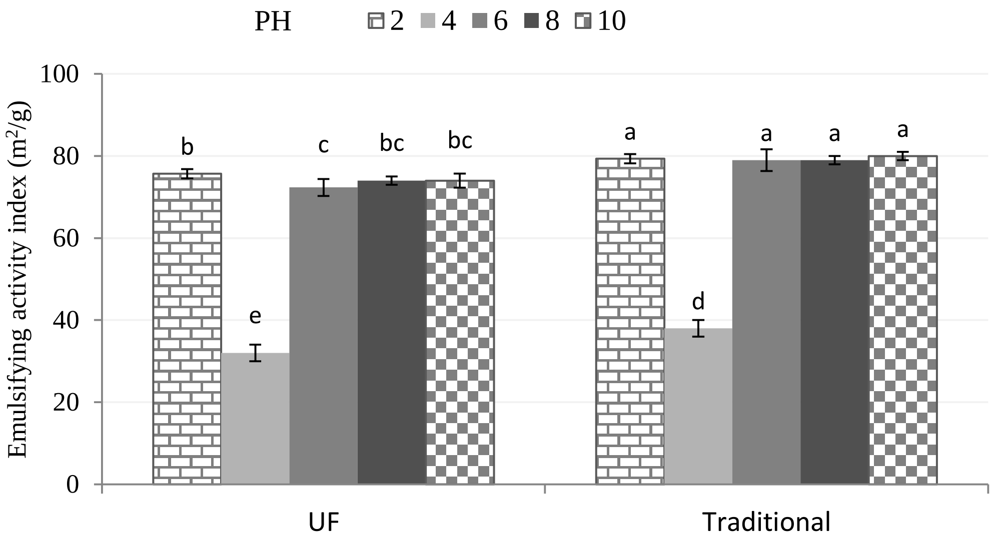

Fig. 4. The emulsifying activity index of whey powder obtained from the traditional and the UF cheese making

Conclusion

Traditional whey powder showed a higher percentage of protein, fat, lactose and salts than UF whey powder. Foaming capacity, water holding capacity and emulsion activity, the quality of the traditional whey powder was better than UF whey powder. The foam stability of these two powders did not show a significant difference. The process of changing the functional properties of the whey powder obtained from the traditional and UF cheese making was influenced by the pH changes, and except for the stability of the foam (It's only in pH between 6 and 8), the trend was the same in other cases. The higher quality of the traditional whey powder compared to UF whey powder can compensate for the lower efficiency of traditional cheese making as compared to UF one.

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