Determination of Whey Proteins structural and Functional Changes Under Heat and Pressure

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Abstract

Background: The effects of heat and high-pressure treatments on whey proteins and micellar caseins have been extensively studied to understand structural and functional changes. Whey protein isolate (WPI) solutions and micellar casein (MC) dispersions were analyzed to observe protein stability and denaturation under varying conditions. Methods: Samples were treated at 500 MPa at different pH levels (3.0, 5.8, and 7.0) and temperatures (from room temperature to -35°C). Protein denaturation was analyzed using spectrophotometry and SDS-PAGE, while secondary structure changes were determined using differential scanning calorimetry. Results: High-pressure treatments caused unfolding of WPI at room temperature, leading to increased surface hydrophobicity and exposed thiol groups. In contrast, low-temperature treatments showed less protein unfolding but induced secondary structural changes, especially in β-sheets. Heating whey proteins increased particle size and zeta potential, with further impacts on emulsion stability at higher temperatures. Conclusion: The application of heat and high-pressure treatments significantly affects whey protein structure, influencing their functionality in food and beverage systems. Optimal processing conditions depend on temperature, pH, and treatment duration.

Significance | This study determined the understanding whey protein structural changes under heat and pressure is crucial for optimizing food processing and functional beverage production.

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Introduction

Whey protein is a highly valued by-product derived from milk during the cheese-making process. After milk undergoes curdling, filtering, and straining, the remaining liquid is whey, rich in proteins that have significant commercial and nutritional value (Creamer, 1996). Whey proteins comprise approximately 20% of the total protein content in bovine milk, the rest being caseins. These proteins are characterized by their globular structure, sensitivity to heat, and resistance to calcium (Creamer, 1996). Whey proteins are further classified into three main types based on their composition: whey protein concentrate, whey protein isolate, and whey protein hydrolysate. These classifications have distinct nutritional and functional properties, which make them suitable for various applications in food and health products.

Whey proteins are made up of several key components, including β-lactoglobulin (β-Lg), α-lactalbumin (α-La), and smaller amounts of serum albumin, immunoglobulins, and proteose peptones (Creamer, 1996). These proteins are not only vital for their nutritional value but also contribute to the functionality of food products, thanks to their ability to form gels, foams, and emulsions. Heat treatments, often used in food processing, significantly impact the structure and functionality

of whey proteins. For example, high-pressure treatments at low temperatures (HPLT) cause distinct changes in the secondary structure of whey proteins, increasing the number of β-sheets while preserving the tertiary structure (Baier et al., 2015). Similarly, high temperatures affect the particle size and physicochemical

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properties of whey proteins, making them more prone to denaturation and aggregation (Quant et al., 2019).

Given their nutritional and functional properties, whey proteins are extensively used in sports nutrition, beverages, and dietary supplements. Understanding the effects of processing conditions such as heat, pressure, and pH on whey protein stability is critical for optimizing their application in various food systems. These proteins exhibit minimal solubility at their isoelectric point (pH 4.5) across various temperatures, leading to denaturation at elevated temperatures (Pelegrine & Gasparetto, 2005). Additionally, the denaturation behavior of whey proteins can be influenced by the interaction with other milk proteins, as seen in dairy products, which provides insights into their functional performance in different applications (Anema, 2020).

The functional versatility of whey proteins makes them a cornerstone in food science, providing an avenue for further research into their potential health benefits and technological applications.

Materials and Methods

Effect of Heat Treatment on Whey Protein

Whey protein beverages (WPB) were prepared to evaluate the impact of heat treatment on the physiochemical properties of whey proteins. The formulation consisted of 5% whey protein isolate, 0.04% potassium sorbate, and pH adjustment to either 3.0 or 7.0 using 0.5 M phosphoric acid. To analyze the heat treatment effects, the beverages underwent a hot-fill treatment at 88°C for 2 minutes. The particle size and zeta potential of the whey proteins were measured using a spectrophotometer. The zeta potential provided insight into the stability of the colloidal particles, while the particle size indicated potential protein complex formation.

Effect of Temperature and pH on Solubility of Whey Protein

The solubility of whey proteins was assessed by varying temperature (40-60°C) and pH (3.5-7.8). Whey protein solutions were prepared, and solubility was determined by measuring the protein concentration in the supernatant after centrifugation at different pH and temperature combinations. Protein solubility was analyzed using UV-visible spectrophotometry. The interaction between pH and temperature was assessed, with a particular focus on pH 4.5, which corresponds to the isoelectric point of whey proteins. Changes in solubility due to protein denaturation at different temperatures were also studied.

Effect of pH, Heat, and Ethanol on Whey Protein Denaturation

A difference-UV spectral method was employed to investigate the effects of pH, heat, and ethanol on whey protein denaturation. Whey protein isolate was exposed to heat treatment at 90°C for 30 minutes at different pH levels, ranging from 2.5 to 7.0, and with ethanol concentrations of 20-50%. The stability and denaturation rates of whey proteins were monitored using a UV-visible spectrophotometer, and the degree of protein denaturation was compared across different treatment conditions. Protein aggregation and conformational changes were inferred based on shifts in the absorbance spectra.

Effect of Heat Treatment on Whey Protein Solubility

Whey protein solubility was evaluated at various temperatures (up to 150°C) in relation to pH and the presence of lactose and calcium salts. Differential scanning calorimetry (DSC) was used to monitor protein denaturation at specific temperatures, while amino acid analysis was employed to detect changes in cysteine residues upon heating. The effect of thermal treatment on the solubility of α-lactalbumin and β-lactoglobulin was investigated by monitoring protein precipitation and solubility under different conditions. (Data not shown).

Results

The effects of heat and pressure treatment on whey proteins reveal significant structural and functional changes. High-pressure-low-temperature (HPLT) treatments caused an increase in the number of β-sheets in whey protein isolate (WPI) while preserving the tertiary structure, and only minor changes were seen in surface hydrophobicity and thiol group accessibility (Baier et al., 2015). At room temperature, however, high pressure treatments resulted in an unfolding of WPI, leading to increased surface hydrophobicity and thiol exposure. Similarly, thermal treatments caused denaturation, with hot-fill treatments (88 °C for 2 min) significantly increasing particle size and zeta potential of whey protein beverages (WPB), especially at pH 3.0 and 7.0 (Quant et al., 2019). See Table 1 and 2.

The heating of whey protein-stabilized emulsions also impacted particle size and shear viscosity, with maximal increases observed at 75°C for 45 minutes (Sliwinski et al., 2003). A study on whey protein solubility showed that both temperature and pH influenced solubility, with minimal values observed at pH 4.5—the isoelectric point of whey proteins—where higher temperatures led to decreased solubility due to protein denaturation (Pelegrine & Gasparetto, 2005).

Ethanol and pH conditions also affected the stability and denaturation rates of whey proteins, with higher stability at pH 3.75 and increased denaturation at pH values greater than neutral (Nikolaidis et al., 2017). Moreover, denatured whey proteins interacted with casein micelles, influencing the functional properties of milk-based products (Anema, 2020). Heat treatment studies on skim milk showed that the denaturation of β-lactoglobulin and α-lactalbumin increased with higher temperature and holding times, with differences in denaturation levels based on heating strategy (Akkerman et al., 2016).

Table 1. Denaturation degree of whey protein for heating skim milk

Temperature Holding Time	Relative RCT (%)	Denaturation of β-lactoglobulin	Denaturation of α-Lactoalbumin
105/4	127.46 ± 13.19	0.79 ± 4.29	4.80 ± 1.21
115/4	138.92 ± 10.56	15.75 ± 11.36	9.23 ± 1.92
130/4	192.26 ± 40.79	25.29 ± 4.86	10.53 ± 5.65
145/4	420.81	39.35 ± 2.83	15.35 ± 2.97

Table 2. Denaturation of composition of whey protein

S.No	Protein	Approx.Content G/L Whey	Total Whey Protein %	Denaturation Temperature
1	α-Lactoalbumin	0.6-1.7	20	61
2	β-lactoglobulin	2.0-4.0	55	82
3	Serum Albumin	0.2-0.4	5	66
4	Immunoglobulins	0.5-0.4	8	72
5	Proteose-peptone	0.2-0.4	12	-
6	Other (casein,glycoprotein	0.1	1	-

Discussion

The findings from these studies demonstrate that heat and pressure treatments have a profound impact on the structural and functional characteristics of whey proteins. The increased surface hydrophobicity and thiol exposure due to high-pressure treatment at room temperature indicate protein unfolding, whereas HPLT treatments induced more subtle structural changes, suggesting a greater potential for preserving functional properties at lower temperatures.

Heat treatment-induced changes in particle size and zeta potential in whey protein beverages highlight the challenges in maintaining the functional stability of whey proteins in acidic or neutral environments. The large aggregates formed in whey protein emulsions after heating, especially at temperatures around 75°C, indicate that temperature control is critical in the preparation of whey protein-based products to ensure the desired functional properties, such as viscosity and texture.

The impact of pH and temperature on solubility underscores the importance of managing these factors during processing to prevent undesirable protein denaturation and aggregation. Additionally, ethanol and ultrasonication methods offer alternative ways to manipulate whey protein stability, suggesting potential strategies for tailoring whey protein functionalities in food applications.

The interaction between denatured whey proteins and other milk proteins, especially casein, provides insights into their role in modifying the textural and functional properties of dairy products. Future studies could focus on optimizing these interactions to improve the performance of whey protein in specific applications, such as bakery or beverage formulations.

Conclusion

In conclusion, while heat and pressure treatments can lead to beneficial structural changes in whey proteins, careful control of processing conditions is essential to preserve the desired functionalities. Advanced analytical techniques and controlled processing conditions are necessary for maximizing the potential of whey proteins in various industrial applications.

Author contributions

N.S. conceptualized the project and developed the methodology. U.N. and I. conducted a formal analysis and drafted the original writing and contributed to the methodology. S.Z. conducted investigations, provided resources, visualized the data and contributed to reviewing and editing the writing.

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Competing financial interests

The authors have no conflict of interest.

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