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# Techniques to improve the functional properties of whey proteins

Devabattini Sharika [1, *], Hemalatha Ganapathyswamy [2], S. Kanchana [3], M.L. Mini [4] and M. Jeya Bharathi [1]

[1] *Department of Food Science and Nutrition, Community Science College and Research Institute, Tamil Nadu Agricultural University, Madurai, India.*
[2] *Department of Food Policy and Public Health Nutrition, Community Science College and Research Institute, Tamil Nadu Agricultural University, Madurai, India.*
[3] *Community Science College and Research Institute, Tamil Nadu Agricultural University, Madurai, India.*
[4] *Department of Biotechnology, Agriculture College and Research Institute, Tamil Nadu Agricultural University, Madurai, India.*

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## Abstract

Whey protein (WP) is a high-quality protein found in milk with a high nutritional value and also distinct physiological functions. Whey protein in its natural state is extremely unstable and hence, many modification techniques have been designed to enhance WP stability for diverse food applications, each with a unique set of characteristics. This paper reviews physical, enzymatic, and chemical methods for modifying whey protein, as well as innovative strategies. The traditional physical methods include thermal processing, texturization, freeze modification, high moisture extrusion, while the enzymatic procedures primarily consist of enzymatic hydrolysis and enzymatic cross-linking, and the ternary system is one of the most commonly adopted chemical processes. Among the novel processing techniques, those most commonly experimented include, high pressure shearing, ultrasound treatment, high hydrostatic pressure, pulsed electric field, and cold plasma technology. This article summarizes the mechanisms of various modification methods of WP structure, texture and other functional properties and their effects on whey protein characteristics, as well as future prospects of development of whey protein modification techniques and its food applications.

**Keywords:** Whey protein; Modification techniques; Functional properties; Food applications; Texturization

## 1 Introduction

Whey from milk is a by-product from the cheese and casein manufacturing sectors. Whey protein (WP) is the most important component of whey and contains a number of components, including β-lactoglobulin, α-lactalbumin, immunoglobulin, and bovine serum albumin, etc. (1). Whey protein is known as "the king of protein" because of its high essential amino acid content and biological value (2). Technological advancements in WP processing such as ultrafiltration, microfiltration, reverse osmosis, and ion exchange have resulted in production of a variety of final whey products such as whey powder, whey protein concentrate (WPC) and whey protein isolate (WPI). Based on its protein concentration, WP concentrate can be classified into four categories namely WPC34, WPC50, WPC75, and WPC80, whereas whey protein isolate signifies a relative protein concentration greater than 90%.

Whey protein has been utilized as an ingredient in many traditional and novel food items due to its high nutritional value and functional qualities such as gelation, emulsification, foaming, and flavor binding (3). These functional characteristics contribute to the textural, sensory, and nutritional properties of a wide range of food products. The nature of WP is unstable, and its high-level structure can be rapidly destroyed during processing and production, resulting in changes in its physical, chemical, and biological functions, limiting its application. Whey protein

* Corresponding author: Devabattini Sharika

modification is essential to change the hydrophobic group distribution, spatial arrangement conformation, and amino acid content, thereby improving or establishing novel functional properties (4).

This paper focuses on different whey protein modification techniques inclusive of physical, enzymatic, chemical methods, novel techniques and its effect on the structure and function of WP which are summarized to provide a reference for enhancing the efficient utilization and economic value of WP resources. These technologies provide new opportunities to expand the usage of whey proteins in food and non-food applications.

## 2 Modification of whey protein by physical methods

Physical modification is the process of changing a protein's high-level structure and molecular aggregation mode using physical methods such as mechanical energy, thermal energy, and acoustic energy. Physical modification has distinct advantages over chemical modification, in that, other than food, no other chemical components are required, and the changed product contains no chemical residues, making it extremely safe. The thermal treatment, texturization, freeze modification, high moisture extrusion, high pressure shearing, ultrasound treatment, high hydrostatic pressure, pulsed electric field, and cold plasma technology are the most common ways of physical modification of whey protein (5).

### 2.1 Thermal treatment

Thermal processing is one of the most commonly applied food processing methods, and it has a significant impact on the physicochemical and functional properties of whey proteins.

Whey protein changes β-lactoglobulin and α-lactalbumin structure during the heating process. The β-lactoglobulin exists in the form of natural dimers at room temperature and when the temperature rises above 60°C, the spherical folding structure opens and stretches, exposing the internal thiol (—SH) and the disulfide bond (S—S) to form thermally denatured aggregates; when the temperature rises above 85°C, the aggregates in S—S are broken and β-lactoglobulin stretches further (6). Although α-lactalbumin has no free—SH, it does contain S—S. After being destroyed, it will be combined with other free-SH protein aggregates to form protein complexes (7), as shown in Fig 1.

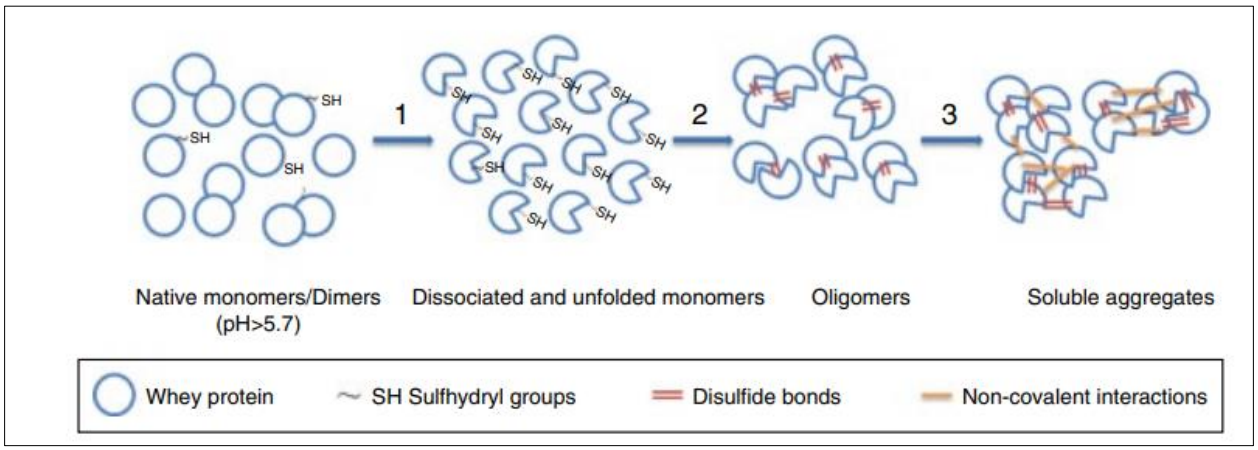



**Figure 1** Schematic representation of formation of whey protein soluble aggregates upon thermal treatment

Whey protein gels are generated by the subsequent aggregation of initial aggregates at increasing protein concentrations. Based on rheological and microstructural features, whey protein gels can be characterized as fine stranded, mixed, or particulate. A schematic representation of the formation of whey protein gel is shown in Figure 2. Whey protein can form primary aggregates of various sizes and forms, including flexible strands and globular aggregates. These basic aggregates are progressively aggregated (also known as secondary aggregation) to produce a gel network. The electrostatic conditions (pH and ionic strength) and protein concentration have a significant impact on the gelation properties of whey proteins. The addition of whey protein soluble aggregates to native whey protein solutions increased gel fracture stress, storage modulus, water holding capacity, and heat induced gel (8).

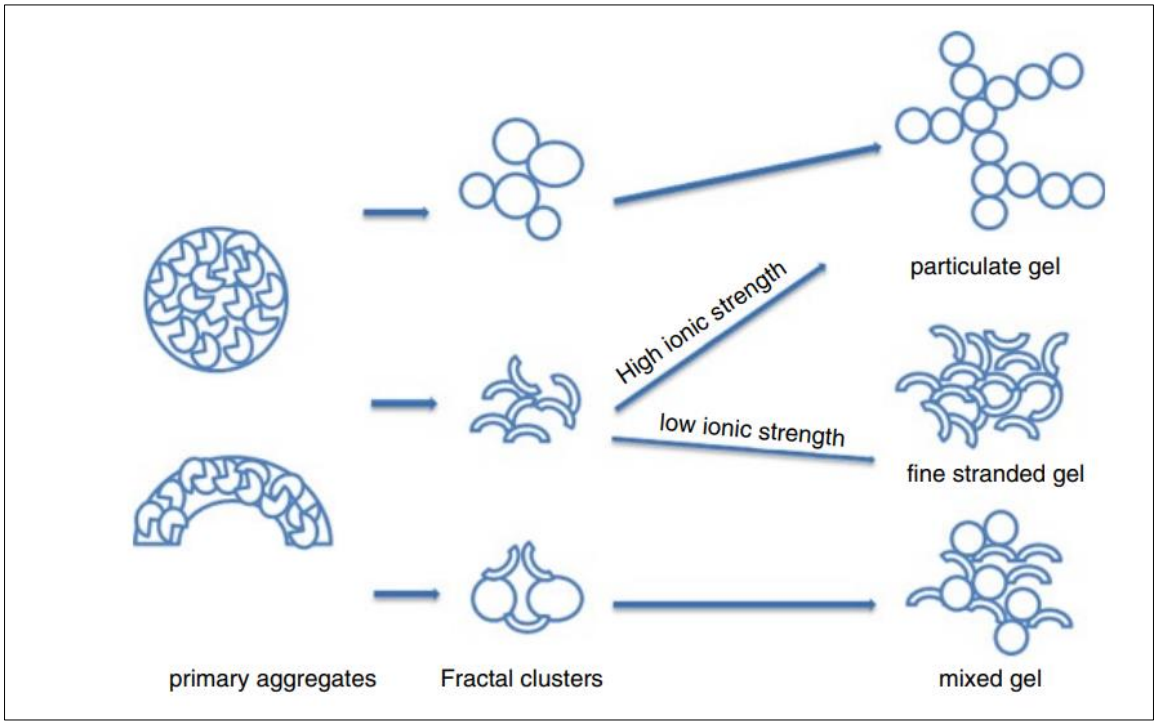



**Figure 2** Schematic representation of the formation of whey protein gels (including fine stranded, mixed and particulate gels) during thermal treatment**.**

The physical and chemical properties of whey protein is altered due to changes in the intrinsic structure throughout the heating process. Thermal modification of whey protein can be optimized to control the characteristics of the aggregates, offering new opportunities for whey protein application. Whey protein aggregate formation is also used as an encapsulating method to protect sensitive food ingredients and bioactive substances. Whey protein aggregates can be used to stabilize emulsions and foams as a good emulsifier and stabilizer.

## 2.2 Texturization

Texturization is a process that uses mechanical shear to unravel the globular structure of native proteins, which can be accompanied by the breakdown of intramolecular connections and the re-alignment of disulfide bonds, with heat or pressure as prerequisites (9). These changes provide whey proteins additional functional properties, resulting in new protein-based food ingredients. The new functional behavior is regulated by altering parameters such as extrusion temperature and moisture level, which are dependent on the extent of texturization and structural change imparted. The texturized whey proteins can be utilized for producing food products with increased functional properties (10).

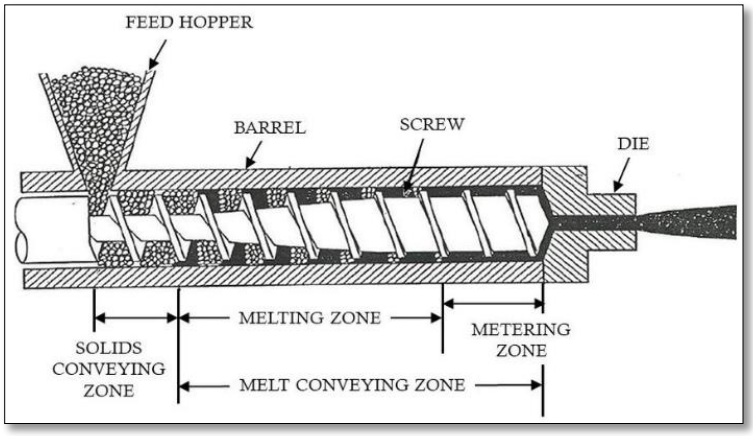
Texturization is not directly assessed, but it is inferred from the degree of denaturation or insolubilization of proteins, which is determined by the difference in rates of moisture uptake between the native and texturized proteins (11). Texturized proteins absorb water at different rates, and it is assumed that these rates are related to the degree of texturization; thus, the insolubility test for denaturation is often used as a substitute for specifically assessing texturization. Protein solubility is influenced by their surface hydrophobicity, which is directly related to the degree of protein-protein interactions, an intrinsic feature of proteins in their denatured form (12&13).

### 2.2.1. Texturization by Twin screw extrusion

An extruder is a device used to texturize proteins by pressing the protein against the fixed heated barrel walls and forcing the molten protein mass through a restriction die that aligns the protein mass in the direction of rotational flow. Extrusion texturization of proteins can occur at temperatures ranging from 50 to 100ºC and with short residence durations (< 2 min) (14).

*2.2.1 Flow chart 1 Twin screw extrusion texturization (14)*

Twin screw extruder – Whey protein concentrate/isolate
↓
Extruder – Consisting of 9-barrel zones
↓
Set the barrel temperature – (1, 2 and 3 -35ºC), (4 and 5 – 75ºC), (6, 7, 8 and 9 - 35ºC)
↓
The die plate is fitted with two circular inserts of 3.18 mm diameter each.
↓
Feed is conveyed into the extruder with a series 6300 digital feeder
↓
The feed screw speed is set at 600 rpm, corresponding to a rate of 3.50 kg/h.
↓
. Water is added into the extruder at the rate of 1.0 L/h with an electro-magnetic dosing pump
↓
Samples are collected after 25 min of processing
↓
Freeze- dried overnight
↓
Stored at 4.4°C until analyzed




**A** B

**Figure 3** A. Twin screw extrusion texturization, B. Texturized whey protein

### 2.2.2. Texturization by reactive supercritical fluid extrusion (SCFX)

The SCFX is an innovative food processing technique that provides expansion *via* direct supercritical fluid carbon dioxide (SC-$CO_2$) infusion. Its unique low-temperature and low-shear conditions caused by high moisture allows heat-sensitive components to be retained. The SCFX process involves general expansion mechanism which can later WP structure and comprises of the following major steps: (a) heat-shear treatment to develop a gas holding matrix, (b) injection of SC-$CO_2$ into the matrix and mixing in the extruder barrel to create a solution that is fully saturated (c) cell nucleation induced by thermodynamic instability due to a sudden pressure drop at the die, and (d) cell growth and extrudate expansion at the die exit as the pressure quenches to the atmosphere level (15).

During SCFX processing, a delicate balance of temperature and shear allows for controlled alteration of WP structure. The SC-$CO_2$ is a chemically inert and environmentally safe solvent which is ideal for food processing. The addition of SC-$CO_2$ provides a more acidic environment while also acting as a blowing agent for surface alteration of WP matrices. It has been hypothesized that a reactive SFCX method in a highly alkaline or acidic environment, combined with regulated shear and temperature in the presence of mineral salts ($CaCl_2$ and NaCl) and SC-$CO_2$, can favorably generate new WP ingredients with distinctive gelling and functional properties, creating a new avenue for WP utilization as a thickening or gelling agent in food formulations (16).

*2.2.2 Flow chart 2. Extrusion by reactive supercritical fluid extrusion*

Wenger TX-52 Magnum co-rotating twin screw extruder
↓
Extruder with 4.5 heads, barrel diameter of 52 mm, L/D ratio 28.5:1
↓
Screw speed of 180 rpm, product temperature 90 °C, and feed rate 35 kg/hr.
↓
The die fitted with two circular inserts of 1.2 mm diameter each.
↓
Modification of pH by injection of HCL/NaOH at mixing zone
↓
Injecting SC-$CO_2$
↓
Maintaining pressure of 10–15 MPa for continuous SC-$CO_2$ flow
↓
Extrusion at 60% (db) moisture content
↓
The extrudates collected and dried at ambient condition (5-7% m.c)
↓
Packed and stored at room temperature

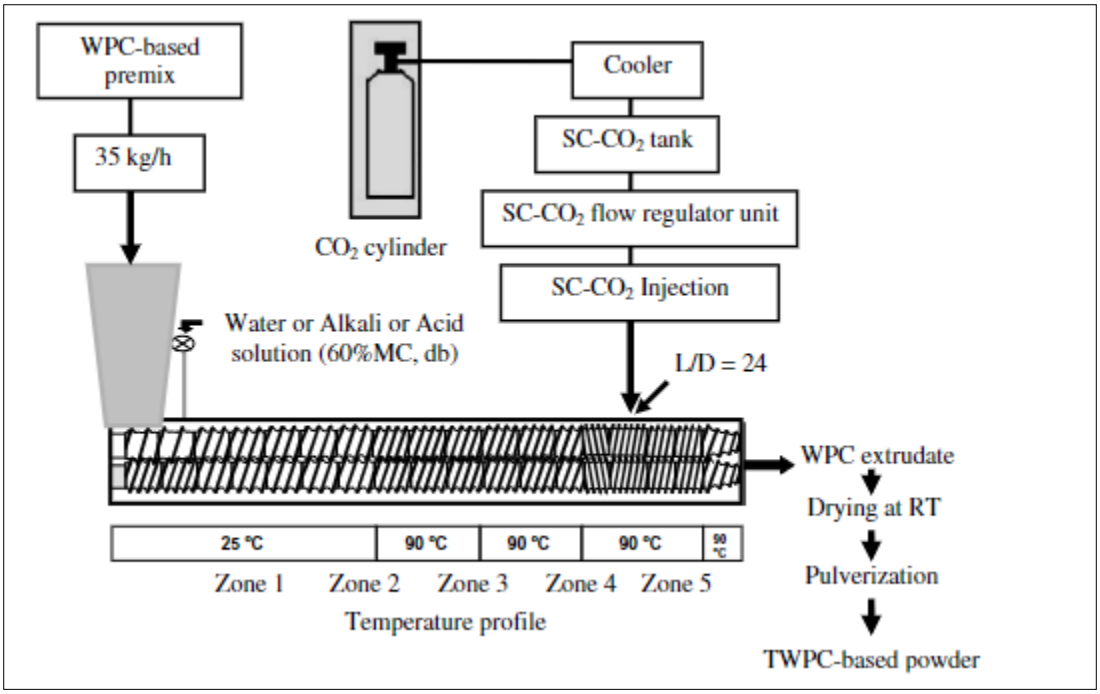



**Figure 4** Schematic of whey protein-based texturization by supercritical fluid extrusion (SCFX), screw configuration, and temperature zones.

### 2.2.3. High moisture extrusion

Primarily in the field of texturization, extrusion cooking at high moisture levels represents a novel and useful technique, clearly different from other continuous protein texturization processes. In order to generate a fibrous texture with improved functional properties, the whey proteins are heated in the extruder under high water conditions of > 50% w.b. and texturized in a cooling die by varying the moisture, temperature, pressure and shear (17). The combination of these process variables results in molecular transformation and chemical reaction of the protein molecules. It was suggested, that the proteins are plasticized inside the extruder and subsequently solidified during passage through the cooling die (18).

A co-rotating twin-screw with 11 mm screw diameter, length to diameter (L/D) ratio of 40 was used for high moisture extrusion with die adapter for attaching the slit cooling die to the extruder. In the screw configuration, only forward elements were used which included eight barrel elements in the extruder barrel. All barrels, with the exception of the first, can be heated and cooled separately. Additionally, the die adapter can be heated. The first barrel element from the system uses a gravimetrically controlled feeder to dose solids, and the third barrel element of the system uses a peristaltic pump to administer water. The 32 mm-long die adapter provides a transition from the screw section to the cooling die. A water-cooled process circulator delivered Thermal HL60, a temperature-controlling liquid, at 10°C to cool the cooling die (125 x 19 x 4 mm). During the extrusion trials, the screw speed was kept constant at 600 rpm. There was always an addition of 1.1 kg/h of water and 0.9 kg/h of a protein mixture. The temperature settings for barrel elements 2 to 5 were TBarrel,2 = 25 °C, TBarrel,3 = 50 °C, TBarrel,4 = 90 °C, and TBarrel,5 = 110 °C. For each material system, the barrel elements 6 to 8 and the die adapter were adjusted to reach one of the three specified material temperatures (115, 125, or 133°C). The die adapter mounted before the cooling die was used to measure the material temperature and die pressure. After the die pressure and material temperature had been stable for at least three minutes, sampling for additional analysis was carried out. After three minutes of pressure monitoring, the average was calculated. The extrudates were torn open, photographed, and then analysed to determine the anisotropic product structure. The extrudates were immediately vacuumed after exit, frozen, and kept at -18°C until use for additional analysis (19).

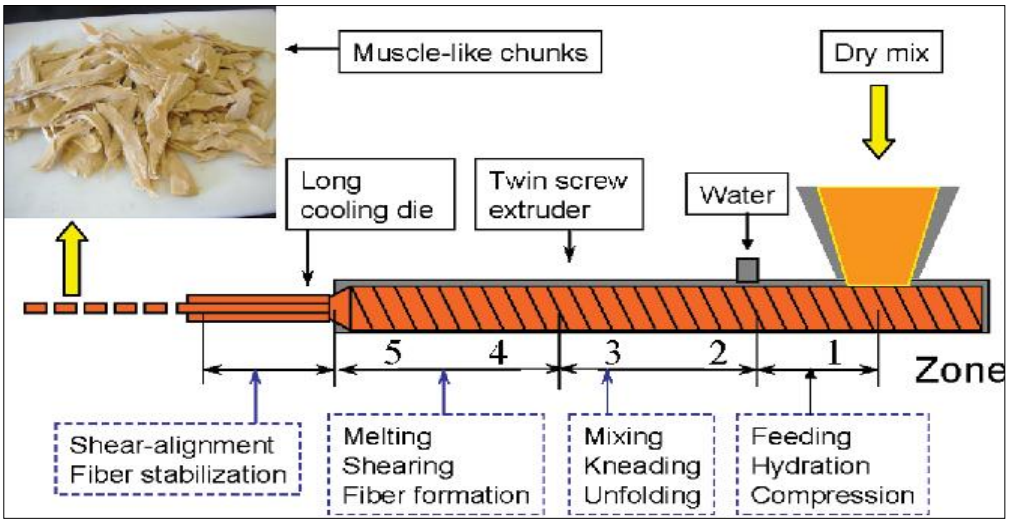



**Figure 5** Schematic of whey protein-based texturization by High moisture extrusion

### 2.2.4. Freeze texturization

Freezing is one of the widely used technologies for food preservation. However, changes in the physicochemical characteristics of food, such as pH, ionic strength, and solute concentration, might result from development and growth of ice crystals. The product undergoes modification as a result of freeze-concentration in the aqueous phase's unfrozen fraction, including textural modifications, protein denaturation, and cell membrane destruction (20). In order to form elongated crystals and stabilize the protein, the aqueous protein mixture is cooled at a very low temperature. After freeze drying, the aligned fibrous proteins which are created exhibit improved functional characteristics (21).

To create 15% protein solutions, the WP concentrate powder was added in the appropriate quantities (20g) with distilled water at room temperature (20–24°C). After that, a magnetic stirrer was used to agitate the solutions for 1 hour. The solutions were acidified from their pH of 6.8 to pH 5.0 as needed using lactic acid (85%). A noticeable rise in viscosity was seen once the pH decreased to 5.0. The samples were divided into 60 mL aliquots and frozen in 80 mL glass beakers. The frozen samples were kept at -17°C in a still freezer or -30°C in a forced draught freezer for 24 hours. After 24 hours, all samples were thawed at the same pace by putting the beakers in a water bath at 20°C for 30 minutes before restoring them to room temperature. The samples were subsequently freeze dried and kept for later analysis (22).

*2.2.3 Flow chart 3. Freeze texturization of whey protein*

Taken 20g whey protein/100g of distilled water (20-24 ºC)
↓
Made to 15% protein solution
↓
Stirred for 1 hour with magnetic stirrer
↓
Adding lactic acid to acidify the solution (from pH 6.8 to 5.0)
↓
Visible increase in viscosity
↓
Frozen as 60 ml aliquots in 80 ml beaker
↓
Frozen samples stored at (-17 ºC in a still freezer or -30 ºC in a forced draft freezer for 24 hours)
↓
The samples are thawed (95% ethyl alcohol for 30 min at room temperature)
↓
Placed in water bath at 20ºC
↓
Freeze drying and ground the samples

**Figure 6** Freeze texturized whey protein

## 2.3 High pressure shearing of whey proteins

The application of high shear and heat has been the basis for production of microparticulated products such as used microfluidization and heat denaturation to improve the solubility of a WP isolate (23).

The application of heat treatment affects the size and shape of proteins, exposes hydrophobic regions, causes aggregation, and ultimately causes the creation of networks (24). Links between or within the network may break under the influence of an applied shear, resulting in cluster fragmentation (25). When whey protein preparations are structurally altered through heat treatment and dynamic high-pressure shearing, the resulting products may have functional properties that are noticeably different from those of the native preparations (26&27).

After protein determination, whey protein retentate samples were adjusted to 10% (wt/wt) protein using appropriate whey permeates. Before being heated and subjected under high pressure, the pH of the resultant solutions was adjusted to 7.0 with NaOH. Prior to being microfluidized at 140 MPa, the sample was heat-denaturized for 20 minutes at 90°C to achieve total WP denaturation. A pilot-scale spray dryer was then used to spray dry the samples. All powders were stored at room temperature in airtight plastic containers until they needed for further analysis (28).

### 2.4 Modification of whey proteins by ultrasound treatment

Due to its unique mechanical ability to change the quality and properties of processed foods, ultrasonication, a relatively new physical processing technology, has attracted considerable attention and offers fascinating opportunities for usage in the food sector (29). Acoustic waves of frequencies higher than 20 kHz, or above the range of human auditory detection, are considered to be ultrasounds (30).

It can be divided into two types: high-frequency (100 kHz-1 MHz) low-intensity ultrasound and low-frequency (low frequency, 20–100 kHz) high-intensity ultrasound. High-intensity ultrasound is employed for altering the physicochemical properties of food, whereas the latter is frequently employed in analytical instruments for evaluating food characteristics (31).

The fundamental effects of high-intensity ultrasound on food systems are related to cavitation and microstreaming currents. Small gas bubbles emerge and implosively collapse in the treated liquid as a result of pressure variations created by acoustic wave transmission through the water during sonication. The properties of food materials are altered physically and chemically by the intense hydrodynamic shear forces, pressures, and temperatures produced by this process (32).

The ability of ultrasonic treatment to alter the secondary structure, solubility, foaming, and gelling properties, as well as the particle size and particle distribution of proteins, has recently been demonstrated in various studies (33).

After being sonicated, black bean protein isolate, according to Jiang (34), became more hydrophobic on the surface. Similarly, amaranth protein isolate treated with high-intensity ultrasound for 30 min showed the best emulsifying qualities, which were attributed to its increased surface hydrophobicity (35).

**Method**

In sodium phosphate buffer solution (10 mM, pH 7.0, PBS), aqueous solutions of WP isolate at a concentration of 5 mg/mL were prepared. The WP isolate solutions were sonicated using an ultrasonic processor at 600 W of sonication power (pulse duration: 3 s on, 4 s off). The ultrasound probe, which had a titanium tip with a 20 mm diameter that vibrated, was inserted into the samples 1.5 cm below the liquid's surface. An ice water bath was used to maintain the temperature of the entire sonication procedure at 3 ± 1ºC. Ground the materials after freeze-drying them for additional examination (36).

*2.4.1 Flow chart 4. Ultrasonic treatment of whey proteins*

Whey protein isolate
↓
Sonication at power of 600 W (pulse duration: 3s on, 4 s off)
↓
Probe was submerged into samples at 1.5 cm below liquid surface
↓
Temperature maintained at 3± 1ºC in an ice water bath
↓
Freeze dried
↓
Ground the samples for further analysis

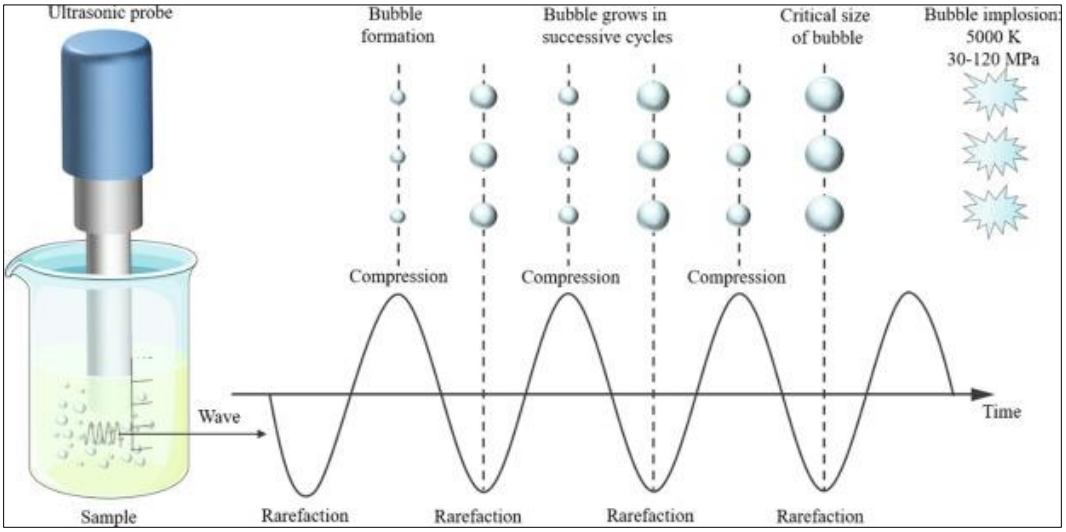



**Figure 7** The acoustic cavitation effect produced by ultrasonication

### 2.5 Modification of whey proteins by high hydrostatic pressure

High hydrostatic pressure (HHP), also referred to as ultra-high pressure (UHP), is a new non-thermal technology used mostly for food preservation. In this method, foodstuffs are subjected to pressure (100-1000 MPa) for a short period of time in order to produce high-quality food, including the inactivation of microorganisms and enzymes, better retention of nutrients, and bringing about alteration of desired food attributes. Through management of pressure-induced changes in food components (such as proteins, polysaccharides, carbohydrates, etc.), high pressure can alter the structure and texture of food products. High pressure is thought to alter proteins by disrupting hydrophobic and electrostatic interactions (37). Application of direct high pressure on proteins can cause structural changes, thus leading to denaturation/aggregation followed by gelation or precipitation depending on protein system (protein nature, concentration, solution condition) as well as the treatment condition (pressure level, duration, and temperature) (38). Proteins can acquire the emulsifying, foaming, and interfacial rheology properties under high pressure (39).

The HHP technique is now considered as a potential alternative for thermal processing. Thermal denaturation at high temperatures, greater than 75°C, generally has a negative effect on functional properties of protein, with significant reduction in emulsifying capacity and foaming properties (40).

Small protein concentrations and pressures of 200 to 300 MPa or more typically cause reversible pressure-induced partial denaturation. In addition to denaturation caused by the unfolding of monomers, aggregation, and gel formation, high pressures greater than 500 MPa have extensive and irreversible effects on proteins (41).

The primary whey protein, β-lactoglobulin, is subjected to relatively high-pressure treatment (greater than 300 MPa for more than 30 min), which causes irreversible denaturation, increasing hydrophobicity, and protein aggregation formation. Pressure treatment simultaneously modifies the structural makeup of proteins and makes them more flexible than heat treatment by exposing previously buried hydrophobic and SH groups.

#### Method

The WP concentrate were subjected to pressure 300 and 400 MPa (0- or 15-min holding time) and 600 MPa (0- min holding time), at an initial temperature of 25°C in a warm isostatic press with a cylindrical pressure chamber (height = 0.25 m, diameter = 0.10 m). The zero holding time corresponds to the come-up time, or the compression time needed to attain a pressure of 300, 400, or 600 MPa (42).

### 2.6 Modification of whey proteins by pulsed electric field (PEF)

The PEF processing is a non-thermal method of preserving food. It uses high voltage electric fields for a short duration (order of microseconds) to make enzymes and microorganisms inactive in order to extend the shelf life of food.

The initial step in protein modification is the unfolding of the protein molecule, which is followed by aggregation. The following aspects may be affected by PEF's impact on protein: Protein polarization results in the following: (i) polarization of the protein molecules; (ii) dissociation of non-covalently linked protein subunits associated with quaternary structure; (iii) protein conformational changes that expose previously buried hydrophobic amino acids or sulfhydryl groups; (iv) polarized structures tend to attract each other by electrostatic forces; and (v) if the duration of the electric pulse is long enough, hydrophobic interactions or covalent bonds may occur (i.e. disulfide bonds) forming protein aggregates (43). Protein concentration, pH, electric field intensity, electrical conductivity, exposure time, and temperature are also linked to protein aggregation in response to PEF (44).

When whey proteins are subjected to PEF, the structure of the proteins is affected by the temperature, number of pulses, protein concentration, and intensity of the electric field. Such conditions can be changed using PEF treatments in order to produce whey proteins with improved functional properties. (45).

### 2.7 Modification of whey proteins by cold plasma technology

The fourth state of matter is referred to as plasma. It is an ionized gas composed of free electrons, ions, atoms, and molecules in their ground or excited states. Electricity, heat, radio waves, or microwaves can all provide the required energy for ionizing the gas. The fact that there are an equal number of positive and negative charges carried by different species can be used to explain how the plasma, despite being created by the ionization of the gas, has a quasi-neutral nature. Plasma can be categorized in a number of ways depending on whether it is generated artificially or naturally.

Thermal plasma and non-thermal plasma are two different kinds of plasma that can be distinguished based on the thermodynamic equilibrium between their constituent species (46). Thermal plasma is plasma that contains strongly ionised species in thermodynamic equilibrium with each other. It is distinguished by temperatures as high as 104 K that are constant throughout all constituent species. Since there is no thermodynamic equilibrium between the ions and unionized species and the electrons, the non-thermal plasma is referred to as cold plasma (47).

According to studies, mild protein oxidation, causes an increase in carbonyl groups and surface hydrophobicity, a decrease in free SH groups, a certain amount of unfolding, and an improvement in foaming and emulsifying capacity which were caused by cold plasma treatment of whey protein isolate model solution under controlled conditions (48).

*2.7.1 Flow chart 5. Whey protein isolate (WPI) modification by cold plasma treatment*

Whey protein isolate added to phosphate buffer (50Mm, pH 6.8) to obtain 2 % (w/v) solution
↓
30 mL WPI solutions in 50 mm petri dishes, packaged in PET trays
↓
Cold plasma treatment –DBD system;
discrete voltage of 70Kv for 1,5,10,15,30 and 60 min
↓
Measurements for proteins: protein carbonyls, free sulfhydryl content, surface hydrophobicity, mean particle size, foaming properties and emulsifying properties.

## 3 Enzymatic method

### 3.1 Enzymatic modification of whey proteins

Enzymatic modification occurs when a specific enzyme interacts with a protein to cause hydrolysis or cross-linking, alteration in protein structure, and enhance protein functionality (49). Enzymatic hydrolysis and enzymatic cross-linking are the two primary enzymatic modifications of whey protein. The polypeptide chain is degraded following the enzymatic hydrolysis of whey protein, which can lead to intramolecular or intermolecular reconnection of whey protein and an improvement in its functional characteristics. The primary purpose of enzymatic cross-linking is to artificially create cross-links in WP, that undergoes protein-like reactions to produce substances with better rheological properties (50).

Millipore water and whey protein were combined while being stirred by an overhead mixer. When the protein powder was visibly hydrated, the mixing process was ceased. The dispersion was then kept at 4°C in a covered container overnight to allow complete hydration.

The protein dispersion was equilibrated to room temperature, and adjusted to the pH ranging from (2.0-8.0) for different enzymes for optimum hydrolysis using sodium hydroxide and hydrochloric acid. After pH adjustment, the temperature of the protein dispersion was equilibrated to 37–60ºC for the hydrolysis for several enzymes, including trypsin, chymotrypsin, and papain. To supply 1 g of enzyme for every 100 g of hydrolyzed protein, enzyme solutions in Millipore water were made. After that, agitated at certain temperature and time in a shaker incubator. After the hydrolysis process was complete, the reactions were stopped by placing the flasks for 10 minutes in a 95°C water bath and freeze dried (51).

*3.1.1 Flowchart 6. Enzymatic modification of whey proteins*

Whey proteins
↓
Mixed into millipore/distilled water
↓
Agitated using magnetic stirrer to hydrate the protein
↓
Stored overnight at 4ºC to allow visible hydration
↓
Equilibrated to room temperature
↓
Adjusted pH range (8.0)
↓
Temperature equilibrated (60ºC)
↓
Adding enzyme solutions protein dispersions (E:P 1:100)
↓
Agitation in shaker incubator at controlled temperature and time
↓
Place flasks in water bath (95ºC, 10 min)
↓
Freeze dry and ground for further analysis

### 3.2 Effect of Trypsin on Enzymatic Hydrolysis of Whey Protein

The exopeptidase derived from animal pancreas known as trypsin is the most widely used proteolytic enzyme and has the highest catalytic specificity (52); it exclusively catalyses the carboxy-terminal peptide bond to produce arginine or lysine as C-For the peptide segment of the terminal residue. Both trypsin and pepsin are exopeptidases with similar properties, although trypsin has a slightly stronger impact on whey protein with difficulty to hydrolyze β-lactoglobulin, making it ineffective in reducing its antigenicity.

The hydrophobicity of whey protein is largely constant during enzymatic hydrolysis. This is due to the low concentration of hydrophobic amino acids in the hydrolysate and the fact that the specific catalytic site of trypsin is the peptide bond at the carboxyl terminus of basic amino acids (arginine and lysine). High, hydrophobicity will not change greatly.

According to Chobertwait's (53) findings, trypsin treatment of whey protein revealed that the hydrolyzed form of the protein was pH-neutral. The hydrolysate has a substantially stronger emulsifying activity than whey protein, has higher solubility at all values compared with control whey protein, and has an enhanced emulsifying capacity in an alkaline environment.

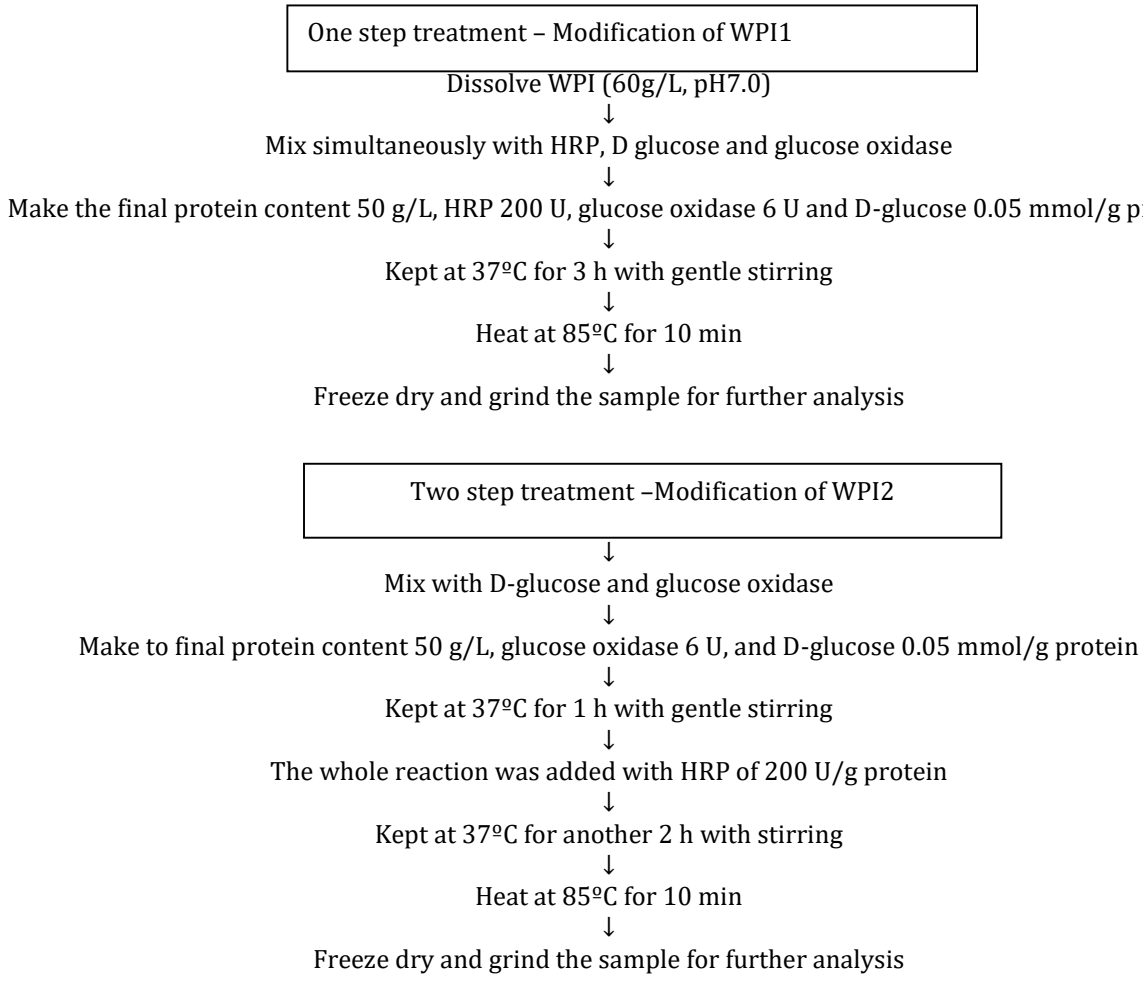
## 4. Chemical modification

Chemical modification is a method of modifying proteins by acting on them using chemical reagents to break peptide bonds, disulfide groups, and hydrophilic and lipophilic groups. Chemical modification offers a very broad range of applications; and not only is the process simple, but the effect of the modification is also significant. Proteins can be chemically altered primarily by processes including glycosylation (Maillard reaction), phosphorylation, acylation, deamidation, and acid modification.

*3.2.1 Modification of whey proteins by ternary system*

Protein crosslinking can be induced *via* a ternary system made up of Horseradish peroxidase (HRP), glucose oxidase, and D-glucose. Two approaches (i.e., one- and two-step treatments) allow this ternary system to change proteins strategically. In the one-step method, D-glucose, HRP, and glucose oxidase are all concurrently supplied to protein substrates, and the entire reaction system is then allowed to run for a predetermined amount of time to carry out both glucose oxidation and protein crosslinking. In the two-step method, protein substrates are simultaneously added with glucose oxidase and D-glucose to carry out glucose oxidation; next, the reaction system is combined with HRP to carry out protein cross-linking. It was noted that the one-step technique was more effective in causing protein crosslinking than the two-step approach. Only a few protein ingredients, including caseinate, whey protein isolate, and SPI, had their properties modified by this ternary system (54).

*3.2.2 Flow chart 7. Modification of whey protein isolate (WPI) by ternary system*




## 5. Changes in properties of modified whey proteins

### 5.1. Gel

Whey protein will form a three-dimensional network structure once it has been sufficiently denaturized. This process, known as gelation, is frequently characterised by gelation. The number of disulfide bonds increases and the hydrophobic interaction increases during the cross-linking process, enhancing gelation. Enhanced gelation can improve the water holding capacity, viscosity and tissue state of whey protein, which plays a significant role in the production of pastries and meat products. In particular, during the transportation of yoghurt, enhanced gelation can significantly enhance syneresis and the precipitation of whey protein.

### 5.2. Solubility

The term "protein solubility" denotes a protein's capacity to dissolve in a solvent. In general, protein molecules with a strong water content are more conducive to dissolution. As the solubility increases, the emulsifying and foaming properties will also increase accordingly and these properties play a very significant role in food processing.

### 5.3. Foaming and foam stability

The terms "foam ability" and "foam dimension" define the way protein products whip and foam. Foam stability is the capacity for stability maintenance. During the foaming process, whey protein will be adsorbed to the air-liquid interface to form a film which reduces interfacial tension. Eventually, foam will be formed from numerous small bubble groups. The effervescent properties of whey protein can give food products an appealing appearance and an excellent mouthfeel.

### 5.4. Emulsion ability and Emulsion Stability

Emulsification is the process through which oil and water in protein combine to produce an emulsion. Emulsification stability is the capacity for maintaining a stable emulsification. Both hydrophilic and hydrophobic groups can be found in whey protein. Whey protein has good water solubility because the hydrophilic groups are spread across the surface of the protein. The hydrophobic regions of the protein are in the oil phase during emulsification, whereas the hydrophilic region is in the water phase.

When homogenous, the whey protein molecules and fat globules are joined together to form an extremely stable protein. To achieve the goal of a homogeneous emulsion, the balls create an aggregation phenomenon. In order to make the water phase and oil phase uniform into an emulsion and maintain a stable state to prevent precipitation in a short time, emulsifiers with good emulsifying properties need to be added during the preparation process of many foods, such as milk and cakes.

## 6. Conclusion

The lifestyle and food consumption behavior of people world over has changed rapidly along with the social economy. New requirements for diverse food type and quality are in demand and those that have been introduced need to be significantly improved. Because whey protein is a high-quality protein, processing and modification can enhance important functional properties. The applicability is still limited, nevertheless, because of the single technology's modest impact on the modification of whey protein. New research on whey protein products and novel processing technologies are urgently required. Based on the current state of research, it could be possible to process whey protein with improved functional properties using number of technologies, including physical, enzymatic, and chemical approaches. Innovative change in the processing routine of traditional whey protein products would provide new ideas for the development of high-quality and personalized foods. Future product development can be focused on the requirements of enhancing human nutrition intake, preserving the health of intestinal flora, and regulating body functions to provide nutritious food that consumers actually require.

## Compliance with ethical standards

*Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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