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OBTAINING A FAT SIMULATOR BASED ON DENATURIZED WHEY PROTEINS

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Abstract. This study examines key approaches to using non-traditional raw materials in the restaurant industry. The primary aim is to adjust dietary patterns, specifically by reducing the fat content in emulsion sauce formulations. We utilized information retrieval as a research method to assess the current state of the problem, and the technological implementation involved microparticulation. The paper outlines methods for producing modified whey protein concentrates. It also justifies the feasibility of a novel approach to protein concentrate production and details a developed technology for obtaining microparticulates, which act as a fat mimetic, through thermo-acidic coagulation. The advantages of this new technology include the use of common restaurant equipment (e.g., a thermomixer), reduced raw material and labor costs, and the ability to use freshly prepared microparticulates in small quantities. Finally, the developed microparticulate technology has been successfully incorporated into the ingredient composition for preparing a low-calorie emulsion sauce. Based on an analysis of existing technologies and experimental data, it has been shown that whey protein microparticulate can be used both as a food fortifier and as a fat mimetic. Currently, the high demand for low-fat products has spurred the development of microparticulation technology, which is one of the latest innovations in utilizing dairy whey. Protein microparticulation is a process involving thermal treatment followed by mechanical processing, typically using whey proteins as raw material. This results in the formation of small protein particles, ranging from 0.5 to 10 µm in size, resembling milk fat globules, with the purpose of being used as a fat replacer.

It is proposed to use the process of microparticulation of whey proteins to give culinary dishes (emulsion sauces) a creamy flavor that compensates for the unpleasant taste of the finished product.

Keywords: microparticulate, protein denaturation, fat simulator, nanotubes, functional ingredients, food technology quality.

Introduction. Formulation of the problem

It's important to note right away that behind this term (microparticulation), still relatively unknown even to niche professionals, lies a completely straightforward concept. It refers to the isolation of whey proteins through ultrafiltration or boiling (heat denaturation), followed by targeted heat and mechanical treatment for microgranulation, including in the form of nanotubes.

Generally, when working with the polypeptide chain of whey proteins as nanoclusters, the intricacy of the microparticulation operation can be visualized in the scheme shown below.

This specific operation imparts unique properties to what's known as «albumin milk» and unlocks new possibilities for using whey proteins. Abroad, this technology is well-developed and has been implemented at the level of fat replacers.

The objective of this research is to analyze and develop technological processes for the production of microparticulate – a finely dispersed protein filler used as a fat substitute in the food industry. This aims to improve the texture, nutritional value, and reduce the caloric content of products. Additionally, the goal is to analyze the informational field of scientific developments, practical production, and application of whey protein microparticulate globally.

Research tasks:

1. To conduct a review of literature sources regarding the use of fat replacers in the food industry.
2. To investigate the composition and technological methods for producing fat mimetics.
3. To examine the theoretical aspects of modifying whey proteins from dairy whey under the influence of temperature (denaturation).

4. To propose an alternative technology for producing a fat mimetic based on whey proteins using the method of thermo-acid coagulation and concentration of these proteins.

The study also involves examining technologies for mechanical, thermal, and chemical processing of proteins to obtain microparticulate, optimizing microparticulation processes to obtain a homogeneous protein filler, determining textural, rheological, and organoleptic characteristics, assessing the impact of particle size on the perception of the final product, investigating the possibilities of replacing fats without losing flavor and structural characteristics, evaluating the physicochemical properties of the obtained particles, researching the influence of various protein sources (whey, egg, plant protein) on the properties of the final product, and its application in dairy products, meat products, baked goods, and sauces.

Analysis of recent research and publications

Based on available information, the most popular protein-based fat mimetic currently is Simplesse-100, derived from denatured whey protein concentrate (DWPC). It's known [1] that Canadian inventors Norman S. Singler, Sean Yamamoto, and Joseph Latella filed a U.S. patent application for Simplesse-100. Simplesse is a dairy co-product obtained from egg white and/or whey protein concentrate through a microgranulation process. Compared to actual fat, Simplesse-100 has a caloric value that's three times lower. However, like other protein-based fat mimetics, Simplesse cannot withstand heating above 100°C and therefore cannot be used for frying [2]

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On the global market, fat mimetics are available based on carbohydrates, proteins, fats, or combinations thereof. Carbohydrate-based fat mimetics are derived from gums, agar, modified food starch, or cereal fibers. These carbohydrate fat replacers absorb water and mimic the volume and texture of fat. They are used in the production of meat delicacies, spreads, soups, salad dressings, glazes, and frozen desserts. However, these substitutes are not suitable for frying [2]. Cerestar Deutschland GmbH (Germany) proposed Snowflake-01906 [3], a food fat substitute resulting from the enzymatic breakdown of potato starch with a high maltodextrin content. Herkules introduced Slendin, a low-calorie fat substitute with pectin as its base, obtained from citrus peels with added sucrose [3]. Additionally, Oatrim, an oat maltodextrin containing 1-12% soluble beta-glucan fiber particles depending on the oat grain type, has also been developed as a fat replacer. Fat mimetics based on sucrose polyesters and acids have also been developed. Procter & Gamble, for instance, created the first prototype of a "fake fat" called Olestra®, which is a mixture of hexa-, hepta-, and octa-esters of sucrose and natural C12-C18 fatty acids. L.P. Kleman and co-authors also patented a low-calorie fat substitute based on esters [4]. Furthermore, Souza Queirós M. et al. proposed a fat substitute [5] that includes a hydrophilic insoluble base (matrix) surrounded by a hydrophobic layer of natural fatty acids.

Low-calorie fat substitutes based on polyvinyl oleyl, trioxymetalalkane esters, triglyceride mixtures, siloxy esters, and acylated amino acid esters have also been proposed [6].

Protein-based fat mimetics are produced by heating and microgranulating whey proteins and/or egg white, or a mixture of egg and whey proteins with xanthan gum. They are not suitable for baking or frying because at high temperatures, the proteins denature, their structure is destroyed, and they lose their ability to mimic fat.

Due to their technological functionality, whey protein concentrates are used as ingredients in new product formulations across various sectors of the food and beverage industry, as well as replacements or alternatives to other traditional ingredients. Different applications of whey derivatives are described below:

- animal feed production: using dry whey as a raw ingredient to enrich nutrient content.
- dairy product manufacturing: for example, developing cheeses based on whey protein coagulation in the presence of calcium. these cheeses are known as ricotta, ricottone, and ricotta cheese.
- sweet and sour whey production: for its protein content, used in protein shakes for athletes to support muscle hypertrophy-related metabolism. also utilized in the production of fermented and non-fermented beverages, and soft drinks.
- whey protein isolation: for use in infant formula due to its nutritional characteristics.
- lactose crystal production from whey: achieved through a series of processes widely used in the pharmaceutical industry for drug coating and at a dietary level due to its lower sweetening value, low hygroscopicity, and lower solubility compared to sucrose.
- utilization of whey in baked goods (cake bases, cookies, nutritional bars), confectionery (chocolate candies), meat products, fish, pasta, and creams as an emulsifier, stabilizer, and water-binding agent.

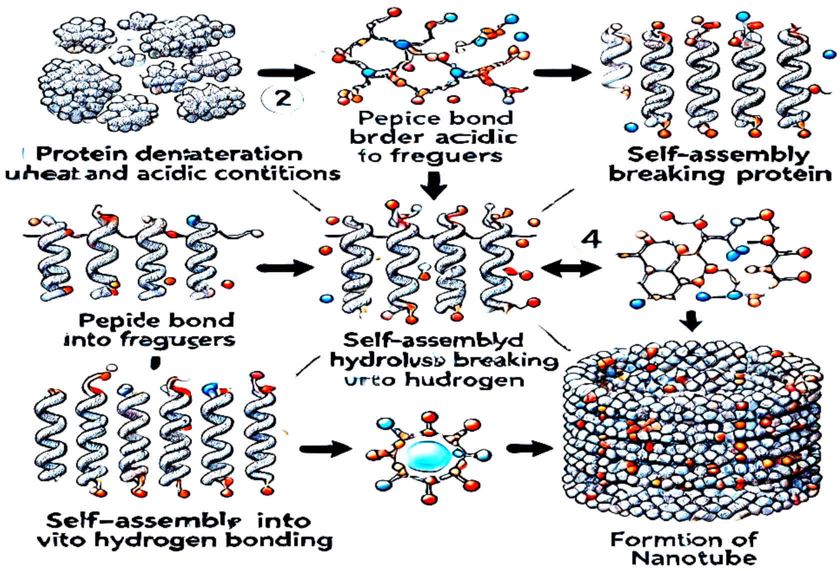

Fig. 1. Formation of nanotube structure from whey protein globules

Well-known brands of protein-based fat mimetics include Simplesse, Dairy-Lo, K-Blazer, ULTRA-BAKETM, ULTRA-FREEZETM, and Lita®. The production technology for Dairy-Lo® involves controlled thermal denaturation of milk. ULTRA-BAKETM, on the other hand, is made from plant proteins.

The composition and nutritional value of Simplesse-100 do not differ from standard whey protein concentrate. No substances that would alter its origin are used in the production technology of Simplesse-100. The composition and properties of this fat mimetic are presented in Table 1 [7].

Whey protein microparticulate (WPM) is a natural ingredient made from whey protein concentrate (WPC, approximately 35–55% protein content) [8]. It's widely used in the food industry due to its high nutritional value, desirable sensory characteristics, and functional properties. Whey proteins are primarily composed of β-lactoglobulin (approximately 3.2 g/L), α-lactalbumin (approximately 1.2 g/L), bovine serum albumin (approximately 0.4 g/L), and immunoglobulins (approximately 0.7 g/L) [9]. Additionally, whey proteins are a source of amino acids essential for muscle synthesis, thereby increasing the demand for incorporating WPM into food products (Çakır-Fuller, 2015) [8].

Microparticulation is a method that uses high-pressure dynamic shearing, with or without heating, to process whey protein concentrate. Through micronparticulation, whey protein concentrate (WPC) proteins can aggregate and denature, forming submicron-sized protein particles (0.5–10 μm). This process improves their functional properties such as emulsification, gelling, and foaming. In fact, microparticulated whey protein (MWP) has been found to behave like fat particles; therefore, it can be used as a fat replacer in several dairy products, as well as other fat-containing food products (e.g., cheeses, ice creams, yogurts, desserts, dressings, and sauces) [10]..

Table 1 – Properties of the Fat Mimetic *Simplesse*-100

Product characteristics	Characteristic
Chemical properties	
Mass fraction of protein, %	53,0
Mass fraction of fat, not more than, %	4,5
Mass fraction of lactose, %	36,0
Mass fraction of moisture, not more than, %	4,0
Organoleptic properties	
Appearance	Powder
Color	White to light cream
Taste	Slight taste of boiled milk
Technical properties	
Dosage	0,5-5,0
Labeling	*Simplesse*-100
Shelf life	18 months in a dry, cool place

Regarding micronparticulated whey protein (MWP) as a fat replacer, Stankey et al. (2017) reported on a commercial MWP that acts as an inert filler in the cheese matrix during the production of low-fat Cheddar cheese. This improved cheese yield and created a softer texture without compromising the sensory or overall quality of the cheese. Similarly, Torres, Janhøj, Mikkelsen, and Ipsen (2011) reported good sensory and rheological characteristics in low-fat yogurts containing MWP, and Sturaro, DeMarchi, Zorzi, and Cassandro (2015) found a stable composition for low-fat CaciottaMWP is used in low-fat cheeses to mimic fat properties by disrupting the protein matrix and providing a sensation of lubricity and creaminess (Stankey et al., 2017). The perception of fat-related sensory properties, such as creaminess, provided by MWP has been found to result from reduced friction through the microparticulates in liquids, primarily due to the lubrication by spherical proteins (Liu, Tian, Stieger, van der Linden, & van de Velde, 2016) [11].

At this stage in the scientific research of whey protein microparticulates, the following production technology has been developed:

The process involves collecting cheese whey, separating milk fat (cream) and casein dust, followed by pasteurization. Next, ultrafiltration is used for fractionation. The whey protein concentrate is then thickened in a vacuum evaporator, ensuring minimal protein coagulation, to a dry matter content that allows for successful microparticle formation. The microparticles are formed by heating the whey protein concentrate above its denaturation temperature under high shear conditions, which are achieved using a homogenizer.

When solutions of heat-coagulable proteins are heated, the constituent molecules denature (unfold) and then begin to aggregate into a single entity. Instead of forming a spatial network of coagulated protein, Simplesse-100 proteins form microparticles and never create a gel [12].

The process of molecular aggregation starts with dimers, and upon further heating, can continue until all protein molecules combine. However, it's been found that this spontaneous tendency to aggregate can be halted within micrometric limits by applying a shear force of sufficient intensity during the heating process. This process can be described as the simultaneous execution of a combined homogenization and pasteurization process [13].

As a raw material for microparticulated whey proteins, either dry whey protein concentrate (WPC), pre-dissolved in water, or WPC obtained through ultrafiltration is used.

The WPC solution is first pre-heated in a plate heat exchanger and then further heated to a higher temperature in a tubular heat exchanger. The product is held at this high temperature for a specific period in a coiled holding tube, where denaturation and aggregation of whey proteins occur, along with inactivation of microflora. Subsequently, the proteins are mechanically processed in a homogenizer to achieve the desired particle size [14].

Particles larger than 3 μm in an aqueous dispersion are perceived as powdery, chalky, or sandy (as particle size increases). Particles smaller than 2 μm do not cause similar associations. Instead, the tongue senses the overall characteristics of the fat mimetic dispersion as oiliness, which is typical for «oil-in-water» emulsions (like full-fat mayonnaise).

In addition to an upper threshold, there's also a lower one. A dispersion of particles smaller than 0.1 μm is perceived as a true solution. Thus, we can speak of a "unique interval" of 0.5 – 2.0 μm, within which microparticles impart an oily consistency to the product. However, even ultrafine particles within this «unique interval» that have needle-like, rod-like, or angular shapes do not create the necessary slipperiness for fat imitation. Instead, they tend to cluster, resulting in a sensation of larger agglomerates. Therefore, to create a high-quality fat mimetic, its particle sizes must be within the 0.5 – 2.0 μm range and have a spherical or near-spherical shape [13].

In the production of whey protein microparticulates, soluble protein molecules are denatured and aggregated under precisely controlled conditions. This yields a highly stable form of whey protein that can no longer agglomerate or gel upon heating. The product retains its functional properties even under the high temperatures of pasteurization and aseptic processing.

The microparticles remain unaggregated and continue to function as a dispersed phase in virtually all products, regardless of pH value, even at the isoelectric point of the proteins [14]. Denatured whey protein concentrate microparticles effectively stabilize emulsions and whipped textures because they possess the exact size required for «oil-in-water» or «air-in-liquid» systems [13]. Denatured whey protein concentrate can stabilize emulsions under elevated processing temperatures, such as ultra-high-temperature (UHT) treatment. This stabilization is achievable without the increase in viscosity typically observed when using other emulsifiers [15].

Standard whey protein concentrates typically gel and alter the texture of emulsions or aggregate and lose their stabilizing properties.

In milk-protein products and fermented milk beverages, denatured whey protein concentrate particles contribute to the formation of the casein curd; they are incorporated into the protein matrix, functioning similarly to the fat globules they replace [16]. Their ability to evenly distribute water within the casein matrix promotes the development of an elastic structure, preventing gaps in the cheese, which positively influences ripening and the formation of a short texture [17].

The American company, Avonmore Cheese Inc., produces Mozzarella cheese with one-third the fat and one-third the calories compared to traditional cheeses of this type. To maintain the typical organoleptic properties of the cheese, low-calorie Mozzarella is made with the addition of a Simplesse blend, which includes milk and egg proteins, vegetable gum, lecithin, sugars, and food acids.

Canadian scientists [18] have developed a method to improve the functional and technological properties of whey proteins based on heat treatment. To obtain soluble polymers, this method involves heat treating a whey protein dispersion (8% protein concentration) with the addition of low concentrations of calcium (0–4 mmol) at 95 °C for 15 minutes and a pH of 6.5–8.5. Such conditions allow for the modification of over 95% of native whey proteins into soluble polymers. An exception is the treatment option at pH 6.5 and a calcium concentration of 4 mmol, where a significant amount of protein precipitates.

Australian scientists [19] have developed a technology for obtaining a solid fat replacer for meat products based on whey protein concentrate with an increased content of β-lactoglobulin. This technology for obtaining a solid fat replacer is based on a method of fractionating whey protein concentrates to obtain products enriched with α-lactalbumin and β-lactoglobulin, respectively. This solid fat replacer has been used in Australia during the development of new types of exclusive meat products under the brand Hans Continental Smallgoods (HCS).

In their work on whey protein microparticulation [20], Spanish researchers proposed investigating the microparticulation process using WPC-60 as a raw material. For this purpose, they studied the influence of pH, whey protein concentration, ionic strength, homogenization pressure, and the number of passes through the homogenizer. The goal was to obtain protein microparticulates with a wide range of particle sizes, viscosities, and percentages of protein microparticles, with the aim of incorporating them into various dairy products as a fat replacer to improve the organoleptic properties of low-fat products.

The following conclusions were drawn from this research:

- The selected study variables increased with an increase in WPC protein concentration and ionic strength. The latter reduces electrostatic repulsion between proteins, promoting their aggregation.
- The size of the aggregated protein increased with increasing pH, as the activity of the thiol group of β-Lg increased, facilitating the formation of disulfide bridges between proteins.
- During heat treatment, higher viscosity was obtained at pH 5.5. This is explained by its proximity to the isoelectric point of β-Lg (pH 5.1), where protein charges are neutral, thereby facilitating their aggregation.

The percentage of protein aggregation during heat treatment increased with decreasing pH. This is because the protective effect of lactose on the thermal denaturation of proteins diminishes, facilitating their unfolding and subsequent aggregation, leading to the formation of higher molecular weight β-Lg associations.

Mechanical treatment had a greater shear effect on larger aggregates; therefore, in contrast to thermal treatment, larger microparticles were obtained at pH 4.

Under working conditions of pH 4.0-7.0, whey protein concentrations of 3-11% by mass, ionic strength of 0-90 mM CaCl, pressure of 300-700 bar, and 1-5 passes through the homogenizer, microparticles were obtained with particle sizes ranging from 0.70-8.20 μm, viscosity at 100 s^{-1} from 1.48-82.17 cP, and a microparticle protein content of 5.08-40.32%.

Thanks to the conducted research and the obtained results, the incorporation of this type of microparticles into products such as cheese, yogurt, ice cream, milkshakes, and other food products is viable. They can replace fat and provide new functional/technological and organoleptic properties (greater elasticity and texture, improved taste perception and creaminess).

At the Department of Restaurant and Health Food Technology at ONTU (Odesa National University of Technology), whey protein microparticulates were obtained using a method involving boiling whey proteins and applying strong mechanical shear with a disperser under the following technological conditions:

– whey protein boiling temperature: 87-90°c, ph 4.6, for 40 minutes.
– protein cooling and settling: 1 hour at 20-25°c.
– permeate separation from whey protein: via drainage.
– protein concentrate separation: via filtration.
– protein washing with water: 3 times, to remove lactose and adjust ph to 6.3-6.5.
– protein concentrate separation (second filtration).
– obtaining whey protein concentrate with a dry matter content of 24%.
– micronparticulation of whey protein concentrate: at 95-97°c, under the action of a disperser with a rotation speed of 30,000-40,000 min^{-1}[10].

As a result of micronparticulation, we obtain protein particles smaller than 2 μm, which impart an oily and smooth consistency to the product. Particles larger than 2 μm, however, give the product a floury taste and consistency [21]. A patent for this process has also been obtained [22].

The resulting microparticulate was used in the development of a recipe and technology for mayonnaise-type sauce [23].

Thus, whey protein microparticulates can be used for the complete or partial replacement of fat in various food products: culinary dishes, mayonnaise, confectionery and bakery products, and dairy products.

The application of this novel fat mimetic offers the following advantages:

– increased efficiency and environmental friendliness in processing dairy raw materials.

– increased yield of finished products.

– enhanced smooth consistency.

– improved texture.

– intensified flavor.

– development of a full-bodied taste in the finished product.

– reduced cost of the finished product.

– reduced calorie content of the finished product.

– expansion of the range of low-fat products, giving them a rich taste, glossy, creamy consistency, and consequently, increasing consumer demand.

– increased nutritional and biological value of new products with a more than twofold reduction in calorie content, giving them a functional direction [21].

Based on the information presented, it appears we can consider all known methods of protein extraction from whey as stages of micronparticulation. These methods involve denaturation through heat treatment, followed by the coagulation and aggregation of denatured particles by altering the medium's reaction (pH adjustment) or by adding coagulants. This includes over 100 substances like calcium chloride, pectin, chitosan, and tannin.

A particularly interesting approach is the isolation of whey components using modified flocculants. This also extends to protein extraction via membrane and biomembrane methods, with subsequent adjustments during further processing (denaturation or structuring).

Further targeted and controlled processing – combining heat and mechanical action to form nanoclusters, such as nanotubes – allows for the creation of a fundamentally new product (or additive) with functional applications, including fat mimetics. This entire process can therefore be considered as microgranulation.

Fractions of Whey Proteins

Whey proteins are the proteins that remain in solution after casein has been precipitated during cheese production. They constitute 20% of the proteins in cow's milk and contain sulfur-containing amino acids [24]. They are distinguished by their high biological value and quality, meaning a greater ability to provide the body with nitrogen, thanks to a high concentration of branched-chain amino acids (isoleucine, valine, and leucine) and essential amino acids compared to other protein sources.

Five main groups can be distinguished within the composition of whey proteins:

- β-lactoglobulin (β-Lg)

β-lactoglobulin (β-Lg) is the most prevalent protein in whey, making up approximately half of all proteins (50-55%) [25] with a concentration of 3 g/L [26].

It consists of 162 amino acids, has a molecular weight of 18.4 kDa, and an isoelectric point (the pH value at which the protein has no net charge) ranging from 4.6 to 5.2, depending on the genetic variant and various impurities [27]. There are common genetic variants, most notably A and B, which differ by two amino acids. Variant A has valine at position 118 and asparagine at position 64, while variant B has alanine and glycine, respectively.

This is a globular protein, meaning its polypeptide chains are tightly folded into a compact spherical or globular shape, leaving hydrophilic groups exposed [28]. Denaturation of β-Lg occurs at 78°C [28, 29].

Its specific biological function is unknown, though the most widely accepted theory is that it acts as a transport vehicle capable of interacting with various hydrophobic molecules, particularly retinol and fatty acids. This property, likely linked to its biological function, also contributes to its good emulsifying properties [24].

Like any protein, β-Lg comprises a series of structures: primary, secondary, tertiary, and quaternary.

The primary structure is defined by the sequence of amino acids linked by peptide bonds. This sulfur-containing protein has two disulfide bridges between four cysteine amino acids, which provide its stability. Additionally, there's a fifth free cysteine amino acid with an exposed thiol group (-SH). Research suggests this group is responsible for the protein's, and thus milk's, antioxidant capacity; it also plays a crucial role in the quaternary structure for aggregation [27].

The secondary structure describes how the polypeptide chain folds in space due to the formation of hydrogen bonds between atoms forming the peptide bond. This allows the polypeptide chain to adopt conformations with lower free energy, making them more stable. This protein has two distinct secondary structures: the α-helix and the antiparallel β-pleated sheet.

The tertiary structure is characterized by the spatial folding of the polypeptide chain, within which the ligand-binding site is located (Fig. 1).

Fig. 1. Structural scheme of β-lactoglobulin [30]

Finally, the quaternary structure is determined by the association of different proteins with one another, forming various aggregates. The protein can form different aggregates depending on the conditions it's subjected to [27].

- α-Lactalbumin (α-La)

α-Lactalbumin (α-La) is the second most abundant protein in whey (20-25%) [25], with a concentration of 0.7 g/L, and is the most common protein in human milk [26]. It's a protein consisting of a single polypeptide chain of 123 amino acids with a molecular weight of 14.2 kDa [30]. Its very compact tertiary structure is globular and held together by four disulfide bridges, featuring both an α-helical region and a β-sheet region (Fig. 2) [31]. It's an acidic protein with an isoelectric point in the range of 4.5-4.8 [31] and a denaturation temperature of 62°C [28, 29].

Fig. 2. Structural scheme of α-lactalbumin

Its primary function is to participate in lactose biosynthesis, as α-lactalbumin, along with the enzyme galactosyltransferase, is a component of the enzyme lactose synthase [32].

A calcium ion is attached to α-La, which is essential for maintaining its structure and its activity as a regulator of galactosyltransferase. The removal of calcium leads to the formation of a structure called a "molten globule," an intermediate denaturation state where the protein retains its secondary structure but has a poorly defined tertiary structure [24].

From a nutritional perspective, α-lactalbumin is important due to its high tryptophan content, which is a precursor to serotonin, a neurotransmitter that regulates appetite. α-La also contains cysteine and branched-chain amino acids, which are crucial for muscle tissue development [33].

- Immunoglobulins (IG).

Immunoglobulins (IG) are glycoproteins whose function is to bind antigens. They constitute 10-15% of the proteins in milk whey [25], with a concentration of 0.6 g/L [26]. They have an average molecular weight of 150 kDa, and their isoelectric point ranges from 5.5-8.3 [30, 31]. Their denaturation temperature is 72°C [28, 29].

The basic Y-shaped structure consists of two light chains and two heavy chains (Fig. 3). The light chains are composed of 200 amino acids, while the heavy chains consist of 400 amino acids. Each light chain is connected to a heavy chain by a disulfide bridge, while the two heavy chains, which are glycosylated, are connected to each other by two disulfide bridges.

Five types of immunoglobulins are known: IgM, IgA, IgG, IgD, and IgE. IgG is the most prevalent, accounting for 70% of whey immunoglobulins. Immunoglobulins provide a passive defense against microbial infections [30].

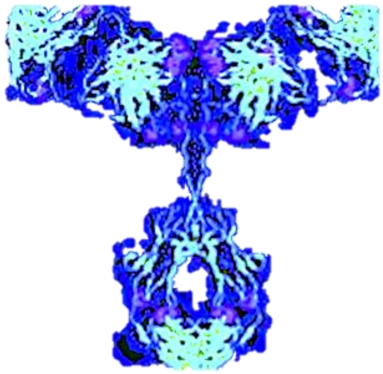

Fig. 3. Structural scheme of immunoglobulin

- Bovine Serum Albumin (BSA)

Bovine Serum Albumin (BSA) is a sulfur-containing protein that makes up 5-10% of the total [34] and is found at a concentration of 0.5 g/L [35]. It's a relatively large protein, with a chain consisting of 583 amino acids, a molecular weight of 69 kDa, and an isoelectric point between 4.7 and 4.9. It also has a denaturation temperature of 64°C.

Its three-dimensional structure (Fig. 4) consists of three domains, each comprised of 10 helical segments. Its secondary structure in its native form is predominantly α-helical and contains 17 disulfide bridges. Furthermore, depending on the pH, BSA exhibits various isomerization states.

The most important property of BSA is its ability to bind ligands, including long-chain fatty acids insoluble in plasma, which are transported by this protein.

Fig. 4. Structural scheme of BSA

- Lactoferrin

Lactoferrin is another protein present in whey, found in low proportions (1-2%) with a concentration of 0.10 g/L. It has a polypeptide chain of 689 amino acids, a weight of 77 kDa, and an isoelectric point at pH 9.

Lactoferrin consists of two lobes (N- and C-lobes) (Fig. 5), corresponding to the N-terminal and C-terminal halves of the molecules, respectively. These lobes are divided into two domains (N1, N2, C1, and C2), connected by a three-turn helix [30].

Fig. 5. Structural scheme of lactoferrin.

Lactoferrin is an iron-binding protein that performs functions such as iron absorption, antimicrobial activity, and regulation of iron metabolism [30].

Based on the information described above, Table I presents the differential characteristics of the main sulfur-containing proteins, considering their proportion in the total amount, concentration (g/L), molecular mass (MM), isoelectric point (pI), and denaturation temperature for those proteins for which these data are known.

Microparticulation is defined as a process in which a protein concentrate, typically whey protein, undergoes thermal (protein denaturation and protein aggregation) and mechanical (microparticulation of protein aggregates through a homogenizer) treatment (Fig. 10) to form particles of similar size (0.1-10 μm). These particles resemble milk fat globules (Fig. 11), which facilitates their incorporation into protein matrices, thus serving as a potential substitute for fat globules, and can be included in various fat-free dairy products [36].

The first stage of the microparticulation process is thermal treatment. Applying heat treatment to whey proteins or protein concentrates leads to changes in their size and shape, exposure of hydrophobic regions, and consequently, the formation of an aggregate network [39].

The denaturation of β-Lg during heat treatment occurs in several stages, described below [40]:

- Reversible changes in the protein structure occur at temperatures of 40-55°C.
- When β-Lg reaches a temperature close to 60°C, the protein structure partially unfolds into a "molten globule" state. This process accelerates at temperatures around 90°C, but the protein can revert to a reversible state, returning to its native form, if the temperature drops below 60°C.
- Aggregation ultimately occurs at temperatures around 90°C, which promotes the exposure of β-Lg's thiol groups, leading to the protein becoming unstable and associating with more proteins. The resulting aggregates are thus completely irreversible.

Figure 8 shows a schematic model of β-Lg with a thiol group in its native globular form (A) and in a denatured state (B) after heat treatment.

The denaturation process of β-Lg is illustrated in Figure 9, which schematically shows the native form of the whey protein (1), the reversible state stage of the whey protein during heat treatment (2), and the final irreversible aggregates (3).

The next stage in the protein microparticle process is mechanical treatment, where, under the action of shear forces from a homogenizer, bonds between and within the network formed during the thermal stage are broken. This prevents the formation of a gel-like matrix and generates small aggregates of whey proteins.

Research materials and methods

A patent search and literature review of the problem under consideration were conducted using internet resources and the repository of the Odesa National University of Technology (ONTU) scientific and technical library.

Table 2 – Main characteristics of whey proteins.

Whey Proteins	Concentration (g/L)	% of Total protein	*MM* (kDa)	*IP*	Denaturation temperature °C
β-Lactoglobulin	3,0	50-55	18,4	4,8-5,1	78
α-Lactalbumin	0.7	25-30	14,2	4,5-4,8	62
Immunoglobulins	0,6	10-15	150	5,5-8.3	72
BSA	0,5	5-10	69	4,7-4,9	64
Lactoferrin	0,10			8,5-9	-

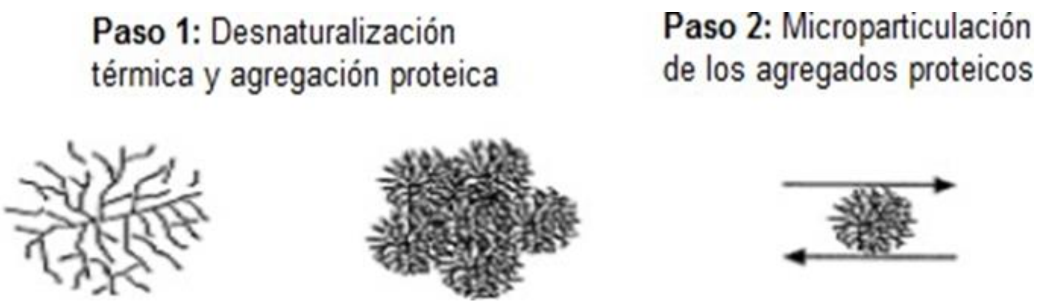

Fig. 6. Stages of protein microparticulation process [37].

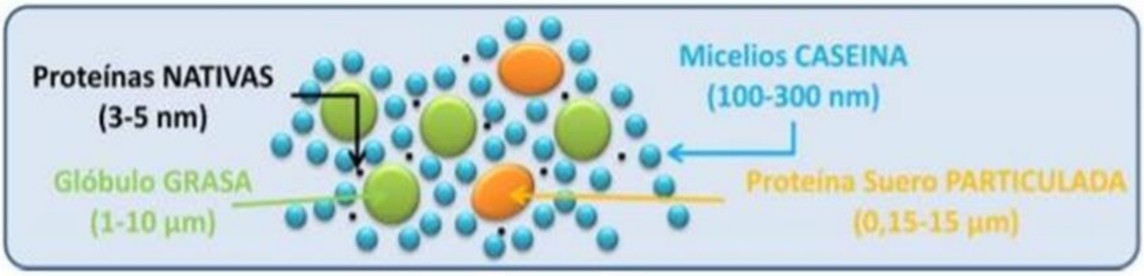

Fig. 7 – Differences in the sizes of native whey proteins compared to microparticles and the similarity in size of the latter to fat globules [38].

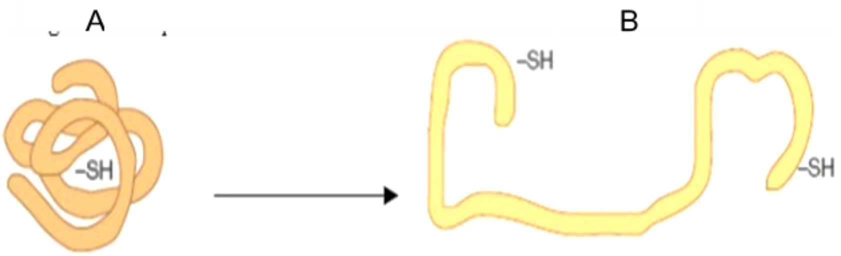

Fig. 8 – Schematic representation of β-Lg in its native globular form (A) and in a denatured state (B).

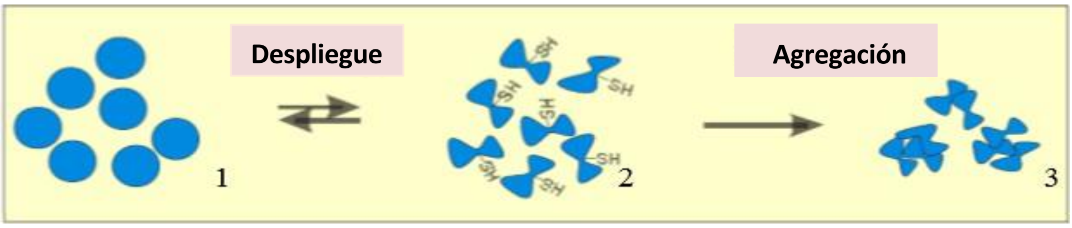

Fig. 9 – Scheme of the β-Lg denaturation process by thermal treatment

Results of the research and their discussion

From the analysis of literature sources, it can be concluded that microparticulate is widely used in the production of various food products. Many different technologies for its use to improve the organoleptic characteristics of reduced-fat food products have been patented. All proposed technologies utilize expensive energy-intensive equipment – ultrafiltration units, plunger homogenizers, vacuum evaporators, and spray drying apparatuses.

The resulting whey protein microparticulate, produced by the reviewed technologies, is a homogeneous, opaque, moderately viscous white liquid with a clean milky taste and aroma. The color of the microparticulate is due to the scattering of light by particles of agglomerated protein, which are characterized by a high degree of dispersity and are similar in size and shape to fat globules. Microparticulation changes the aroma of the UF-concentrate, leading to the leveling of the specific organoleptic characteristics of the whey. Depending on the composition and purposes of further use, whey protein microparticulate can be compared to whole milk or cream in terms of its physicochemical indicators (Table 3) and properties.

Table 3 – Physicochemical parameters of whey protein microparticulates

Parameter Name	Value for Microparticulate			
	Rennet Whey with Concentration, %		Acid Whey with Concentration, %	
	4	13	4	13
Titratable acidity, °T	19	25	60	75
Active acidity, pH	6,3	6,2	4,5	4,4
Dynamic viscosity, mPa·s	10,1	27,0	9,7	25,3

The chemical composition of microparticulate allows it to be considered a food component with functional properties, which is advisable for use in food products, including emulsion sauces. A significant proportion of proteins, peptides, and amino acids gives the microparticulate bifidogenic properties. The milk sugar in the microparticulate also acts as a prebiotic component, as it is not absorbed in the upper gastrointestinal tract and has a stimulating effect on the body's natural microflora. Reaching the large intestine unchanged, lactose is broken down by the intestinal microflora (mainly bifidobacteria), which produces hydrolase-type enzymes. It is used by this microflora as an energy source and is utilized into CO2 and organic acids, lowering the pH of the intestinal environment and inhibiting the development of harmful microorganisms.

For the production of microparticulate in small enterprises, it is proposed to modify this technology and adapt it to the conditions of production in restaurant establishments.

The use of whey protein microparticulate in the production of emulsion sauces is characterized by numerous advantages. Using rennet whey as a base for obtaining microparticulate allows for increased efficiency in milk processing, contributing to environmental friendliness and production profitability. The unique structure and consistency of the microparticulate, as well as its high viscosity, allow for the exclusion of stabilizing systems from the recipe of low-fat products. The resulting product is characterized by reduced caloric content, yet has a rich taste and a smooth, creamy consistency.

Conclusion

From the conducted research, it is evident that the problem of using fat mimetics in the food industry is relevant. Work on improving technologies for obtaining fat mimetics is being carried out worldwide, and the results of this work are being implemented in the production of renowned global brands associated with food production.

The application of whey protein microparticulate as a fat mimetic in the production of emulsion sauces in restaurant establishments has been substantiated.

The proposed technology for obtaining whey protein concentrate and the microparticulation method using a thermoblender will allow adapting the production of fat mimetic in the conditions of restaurant establishments.

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ОТРИМАННЯ ІМІТАТОРА ЖИРУ НА ОСНОВІ ДЕНАТУРОВАНИХ БІЛКІВ МОЛОЧНОЇ СИРОВАТКИ

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Анотація. Розглянуто основні напрямки використання нетрадиційної сировини для ресторанного господарства з метою корегування режимів харчування у бік зниження масової частки жиру в технологіях емульсійних соусів. Інформаційний пошук було використано як метод дослідження стану проблеми, а технологію реалізовували методом мікропартикуляції. У роботі наведено способи виробництва модифікованих концентратів сироваткових білків. Обгрунтована доцільність застосування підходу відмінного від існуючих щодо виробництва білкового концентрату та розроблена технологія отримання мікропартикуляту як імітатора жиру шляхом термокислотної коагуляції. Перевагами нової технології є застосування розповсюдженого в закладах ресторанного господарства обладнання, а саме (термоміксер) зменшення сировинних і трудових ресурсів, можливість використання свіжовиготовленого мікропартикуляту у невеликому обсязі. Розроблено технологію мікропартикуляту, який уведено в композицію інгредієнтів для приготування емульсійного низькокалорійного соусу. На основі аналізу існуючих технологій та експериментальних даних показано, що мікропартикулят сироваткових білків може бути використано як збагачувач харчових продуктів та як імітатор жиру. У наш час високий попит на продукти з низьким вмістом жиру дав поштовх розвитку технології мікропартикулювання, як однієї з останніх інновацій використання молочної сироватки. Мікропартикуляція білка - це процес, що складається з термічної обробки з подальшою механічною обробкою, зазвичай з використанням сироваткових білків як сировини, в результаті якого утворюються дрібні білкові частинки з розміром від 0,5 до 10 мкм, схожі на глобули молочного жиру, з метою використання їх у якості замінника жиру. Запропоновано використати процес мікропартикуляції білків молочної сироватки з метою надання кулінарним стравам (емульсійним соусам) флейвора вершковості, що компенсує недолугий смак готового виробу.

Ключові слова: мікропартикулят, денатурація білків, імітатор жиру, нанотрубки, функціональні інгредієнти, якість технології харчування.