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Review Article

Whey protein: a contemporary gadget for nutraceuticals delivery

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ABSTRACT

Nutraceuticals (NC) often face challenges concerning their stability and bioavailability, limiting their health benefits. Nano-delivery systems (NDS), offer a promising solution by enhancing the protection, stability, and bioavailability of these compounds. Food-grade materials such as whey proteins (WP) are optimal for creating NDS due to their low toxicity, biodegradability, and functional properties that facilitate their ability to carry bioactive compounds (BC) and NC. Considering the significant nutraceutical value of WP, this review emphasizes the main functional properties of WP, its ability to bind NC, especially the hydrophobic ones and other molecules, form gels, act as an emulsifier, and provide barrier protection. The review also evaluates the benefits of WP-lipid conjugates over single-component lipid or protein carriers, as well as the various hybridization techniques utilized in the development of these systems. In conclusion, WP serves as a highly adaptable molecule for constructing a broad range of carriers engineered to hold various NCs, such as vitamins, essential minerals, and phytochemicals, which are prone to degradation or low bioavailability.

Keywords: *Whey protein; nutraceuticals; functional properties; whey-protein lipid conjugates, hybridization techniques.*

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1. Introduction

Biopolymers sourced from natural origins have been extensively employed to create targeted and controlled drug delivery systems (DDS) **[1]**. Among these biopolymers, protein-based polymers are emerging as a promising and versatile vehicle for selective drug targeting, enhancing the delivery and therapeutic properties of drugs, while improving their stability, and bioavailability **[2]**. For instance, collagen, gelatin, albumin, casein, and whey, have been extensively studied for their ability to deliver drugs, nutraceuticals as well as bioactive peptides **[3]**.

Whey protein (WP) **Fig. 1**, a secondary product derived from cheese production, alludes to a protein mixture found in the liquid fraction that forms after cow's milk is acidified to a pH of 4.6, causing casein to precipitate as curds **[4]**. This protein mixture primarily includes α-lactalbumin (α-la), β-lactoglobulin (β-lg), bovine serum albumin (BSA), and several immunoglobulins. Commercial WP are categorized based on their protein concentration: whey protein concentrate (WPC) contains 50–85% protein, whereas WPI has a protein content exceeding 90% **[5]**. Milk proteins possess a range of functional properties that are influenced by

environmental factors for instance pH, ionic strength, and temperature **[6]**. These proteins can bind or encapsulate hydrophobic BC through various mechanisms. The functional attributes that enable the application of milk proteins as nanocarriers for BC include their ability to bind hydrophobic NC and other molecules or ions, surface activity, aggregation, gelation, and interactions with other polymers **[7]**. WP can be employed to create various nanoscale delivery systems, embracing nanoparticles, gels, films, fibers, and nanoemulsions **[8]**. Each of these carriers exhibits specific properties, making them well-suited for different applications in pharmaceutical products.

Currently, natural-source medications and health-promoting foods are increasingly preferred over synthetic options in medicine and nutrition. These products, known as nutraceuticals (NC), combining "nutrition" and "pharmaceuticals", are foods or food components with bioactive phytochemicals that provide therapeutic benefits and can help prevent or treat diseases. NCs offer essential nutrients like vitamins, lipids, proteins, carbohydrates, and minerals, and their role has expanded to include disease risk reduction and symptom management. However, their potential applicability has been limited by their low bioavailability, which frequently results in rapid excretion before therapeutic effects can be achieved. Numerous studies suggest that whey protein, particularly β-lg, can bind various hydrophobic NC, including Curcumin **[9]**, Lycopene **[10]**, β-carotene **[11]**, and vitamin D **[12]**. Accordingly, we, herein, focused on the use of WP as a special carrier-forming system used to maximize the efficacy of NC **[13]**.

This review focuses on the functional attributes investigated in various studies, particularly WP's ability to interact with hydrophobic NC and other molecules. It examines WP's role in gel formation, emulsification, and barrier protection, which are critical to its functionality in delivery systems. Furthermore, the review provides a comprehensive evaluation of the advantages of WP-lipid conjugates, demonstrating their superiority over single-component carriers composed solely of lipids or proteins. It also discusses the different hybridization methods employed to develop these systems.

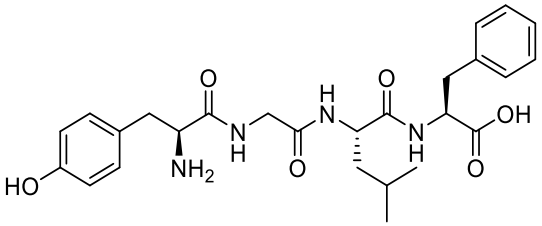

Fig. 1. Chemical structure of Whey protein (L-tyrosyl glycyl-L-leucyl-L-phenylalanine)

2. Whey protein: a promising biopolymer for drug delivery

2.1. Functional properties of whey protein

Fig. 2 shows some of the functional properties that enable the use of WP as a potential biopolymer. Understanding these attributes is highly beneficial in the development of WP-based nutraceutical delivery systems that can provide enhanced protection and stability for encapsulated compounds **[14]**.

2.1.1. Binding ability to nutraceuticals and other compounds

Binding NC is one of the most critical determinants of the efficacy of the carrier. In this respect, WP, particularly β-lg, had been reported to be an excellent candidate. β-lg, a member of the lipocalin protein family, contains three specific binding sites for hydrophobic compounds: the internal cavity of the β-barrel, a surface channel between the α-helix and β-barrel, and an external site adjacent to the residues Trp19 and Arg124 **[15]**. Hence, the primary mode of interaction between β-lg and NC is

through hydrophobic attraction **[16]**. Consequently, increasing the hydrophobicity of β-lg enhanced its binding affinity and encapsulation efficiency for these bind various NC, including retinol **[12, 17]** fatty acids **[18]** and polyphenols **[15]**.

Electrostatic interactions between WP and NC constitute also significant determinants of binding affinity. One key factor influencing these interactions is the pH, as it alters the ionization state of the proteins and NC, thereby affecting their mutual attraction or repulsion **[12]**.

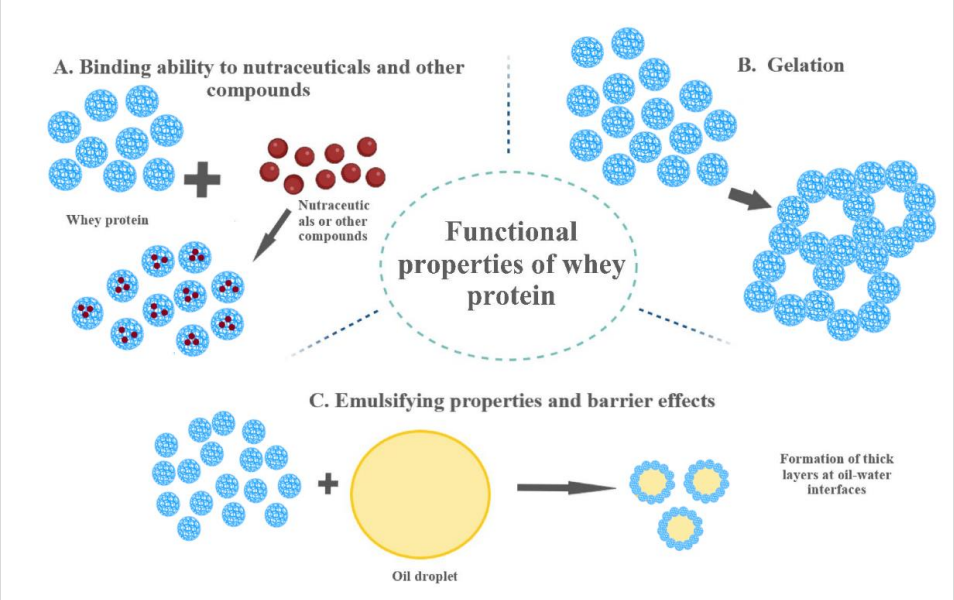

Fig. 2. Schematic representation of some whey protein functional properties

2.1.2. Gelation

WP are recognized for their exceptional gel-forming properties and are widely utilized as gelling agents in the food industry **[19]**. One conventional method for achieving heat-induced gelation involves heating WP solutions or whey-containing food products to temperatures above 80 °C **[20]**. Additionally, the spray-drying process often utilizes heat treatment at temperatures exceeding 100 °C, which is a prevalent technique for producing delivery systems **[21]**. WP may partially or completely unfold during this heat treatment, exposing hydrophobic regions. This unfolding enhances hydrophobic interactions and facilitates the formation of disulfide bonds between WP molecules, creating gel networks, including various sizes of NDS **[14]**.

Nevertheless, there are constraints associated with traditional heat-induced gelation methods and spray-drying for the fabrication of WP-based NDS, as heat treatment can compromise heat-sensitive BC and NC **[22]**. Alternative strategies, such as cold-set gelation **[23]** and processing at sub-ambient temperatures **[18]** can effectively address this issue.

Furthermore, enzyme-induced cross-linking of proteins offers a straightforward and effective method for yielding NDS without relying on heat treatment, presenting fewer toxicity affairs compared to chemical cross-linking agents like glutaraldehyde **[24]**.

2.1.3. Emulsifying properties and barrier effects

WP are considered highly effective emulsifiers due to their ability to adsorb at oil-water interfaces, forming robust layers that help stabilize emulsion droplets. This stabilization reduces lipid separation and prevents the coalescence of the droplets, thereby enhancing emulsion stability **[25]**. One key component of WP, β-lg, is a globular protein that is initially rigid and less surface-active compared to more flexible milk proteins like caseins **[23]**. However, by subjecting β-lg to heat above 60 °C before emulsification, partial unfolding occurs, which increases its emulsifying capabilities. This thermal treatment exposes active groups such as hydrophobic parts and free sulfhydryl groups, enhancing their surface-active properties.

Moreover, WP exhibits a range of functional properties beyond emulsification. Their antioxidant activity, metal-chelating capacity, and gel-forming potential make them valuable for the encapsulation of BC and NC. When used as encapsulating materials, WP provides a protective barrier that boosts encapsulation efficiency and shields active compounds from oxidation. The antioxidant properties of WP arise from the presence of free sulfhydryl groups and aromatic amino acids **[26]**.

2.2. Types of whey protein-based carriers

In this section, different bioactive carriers that can be produced from WP are discussed, including their morphology **Fig. 3**, preparation, structure, and properties.

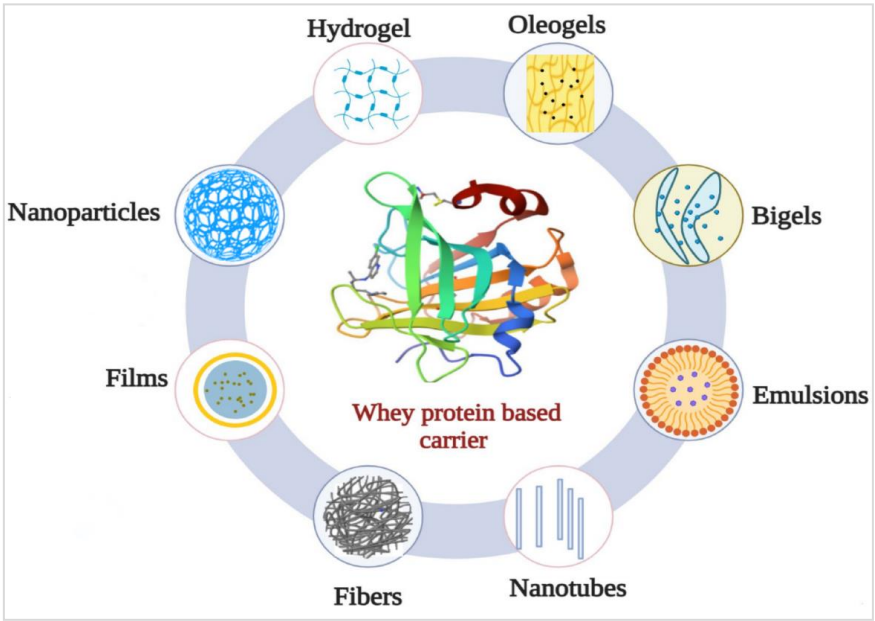

Fig. 3. Various forms of carriers for whey protein-based delivery systems

2.2.1. Nanoparticles

WP-based nanoparticulate systems have been extensively studied for their aptitude as carriers of BC in the food and health field. These nanoparticles are small spherical structures (<1000 nm), primarily composed of crosslinked

WP molecules, with the occasional inclusion of other biomolecules. In food media, charged particulates generated through various interfacial mechanisms interact with each other and with the surrounding medium. The zeta potential is a key parameter used to examine these electrical interactions, and the dimensions and zeta potential of the biomolecular nanoparticles are commonly measured using the dynamic light scattering technique **[27]**.

Various methods have been used for the synthesis of WP nanoparticles, such as antisolvent precipitation, direct self-assembly, and electro-spraying. For instance, bioactive propolis extracts encapsulated within WP nanoparticles through the spray-drying technique exhibited enhanced water dispersibility and stability **[28]**. Similarly, loading of soy isoflavones using a pH-driven tactic has shown improved stability and bioavailability, meanwhile, olive leaf phenolics encapsulated through the electro-spraying technique yielded small particles ranging from 230 to 660 nm in size, achieving high encapsulation efficiency and enhanced water dispersibility **[29]**.

Additionally, composite nanoparticles can be formed by combining WP with other biopolymers, such as polysaccharides or different proteins. These combinations alter the properties and functionality of the nanoparticles, improving their ability to encapsulate bioactive agents **[30]**. For instance, Curcumin was encapsulated in protein-based composite nanoparticles made from hydrophilic WPI and hydrophobic zein using an alcohol-free, pH-driven method. A mass ratio of 8:2 (WPI to zein) resulted in nanoparticles of about 90 nm and significantly increased curcumin solubility to over 0.65 mg/mL. These nanoparticles exhibited excellent storage stability and re-dispersibility. The inclusion of zein enhanced thermal stability and curcumin retention at 80 °C compared to using WPI alone **[9]**.

WP nanoparticles, or their composites, typically range in size from 58-600 nm, demonstrating high loading efficiency for bioactive candidates, and making them promising for applications in the encapsulation and protection of hydrophobic compounds. **Table 1** shows studies of WP-based biopolymeric nano-particulates intended for the loading, protection, and conveyance of bio-actives.

Table 1. Summary of whey protein-based nanoparticles

Series	Protein/Others	Fabrication Technique	Particle Size(nm)	Encapsulation Efficiency (%)	BC or NC	Promising advantage	Refs.
1	WP nanoparticles	Denatured by ethanol	58–126	___	___	Particle size modulation	**[31]**
2	WP nanoparticles	nano fibrillated	78.20–270.44	63.84–84.33	Propolis extract	Regulated release during gastrointestinal digestion	**[28]**
3	WPI	Precipitation ultrasonication method	320	___	Indomethacin	Enhance the dissolution profile and evaluate the stabilizing efficacy of WPI.	**[32]**

4	WPI/ Zein protein	pH-driven method	90		Curcumin	The solubility of curcumin was significantly enhanced to over 0.65 mg/mL	**[9]**
5	WPC	electrospraying	232.3–659.8	36.66- 83.66	Olive leaf phenolics	Encapsulation efficiency improved at elevated WP levels	**[29]**
6	WBC	Calcium ion crosslinking	184–195.6	13.7–28.8	Mandarin peel extracts	Controlled release of flavonoids and antioxidants was ensured	**[33]**
7	zein–WP nanoparticles	pH-driven method	About 410	_	_	Improved in vitro antioxidant activity.	**[34]**
8	WBI/short linear glucan core-shell nanoparticles	self-assembly	70–150	Over 90%	curcumin	Improved sustained release and anti-oxidative activities	**[35]**
9	WPI	Ethanol desolvation method	100-350	50- 65	Lycopene	Enhanced bioavailability, therapeutic effectiveness, and safety profile	**[10]**
10	WPI	Ethanol desolvation method	98- 103	32- 57	Zinc	Improved drug loading capacity and cytotoxicity	**[36]**

* Nanofibrillated whey protein was obtained by heating native whey protein isolate (WPI) at 80 and 90 °C under acidic conditions.

2.2.2. Hydrogels, oleogels, and bigels

Gels are semisolid materials composed of a three-dimensional network of polymers or particles that immobilize a fluid phase. Edible gels are categorized based on the type of fluid they contain; hydrogels (water-based), oleogels (oil-based), or bigels (a combination of water and oil). The creation of these gels relies on the specific polymers or particles used to form the network, and techniques like coagulation, evaporation, or crosslinking can be utilized to establish the gel structure **[37]**. The size of the gels can vary, with methods allowing for the production of both macroscopic and microscopic gels. For example, microgels can be generated by inoculating a protein mix into an appropriate crosslinking medium.

WP-based hydrogels form a three-dimensional system of cross-linked polymers that trap water via hydration and capillary forces **[38]**. These hydrogels are stabilized by covalent and other interactions, including hydrogen bonds, hydrophobic interactions, electrostatic forces, and van der Waals interactions. Due to their structure,

they have been explored for encapsulating and delivering BC, protecting them from degradation.

Research done by Zhan et al., on WP–carrageenan composite hydrogels for curcumin encapsulation highlighted an improvement in curcumin's physical stability and a higher encapsulation capacity compared to WP gels alone. The composite hydrogels facilitated enhanced colonic delivery of curcumin (87%), significantly outperforming WP hydrogels (31%) under simulated digestive conditions **[9]**.

WP-based hydrogels also exhibit strong water retention properties, making them suitable for high-moisture food systems, and their natural biodegradability renders them environmentally friendly. However, their mechanical weakness and susceptibility to pH and temperature variations limit their practical applications. Future studies should aim to improve the physical properties and shelf life of these to fulfill the requirements of various food systems **[39]**.

Oleogels are three-dimensional networks formed by an oleogelator, usually a polymer or particle that contains an oily phase. Oleogelators are categorized as (i) low molecular weight oleogelators (LMWOG) and (ii) biopolymeric gelators. LMWOGs are amphiphilic, forming 3D networks like inverse and cylindrical micelles, lamellar structures, and bilayers **[40]**. Common oleogelators include waxes such as candelilla wax **[41]**, fatty acids **[42]**, lecithin **[43]**, and combinations like fatty acids/alcohols **[44]**. Unlike hydrogels, which use polymer networks to trap water, oleogels rely on minute amphipathic molecules that self-assemble capturing liquid oils via capillary forces **[45]**.

Since proteins are mainly hydrophilic, incorporating them into a hydrophobic oil phase poses a challenge. Recently, various techniques have been developed to produce protein-based oleogels, often in combination with polysaccharides. These oleogels can be synthesized through different techniques, including emulsion-, foam-, and hydrogel-templating approaches **[35]**. WP-based oleogels have been developed for encapsulating fish oil, enhancing its oxidative stability **[46]**. Additionally, incorporating polyphenols into these oleogels further improves the oxidative stability of polyunsaturated oils **[47]**. Polyphenols, like tyrosol and hydroxytyrosol, not only stabilize extra virgin olive oil (EVOO) but also influence the oleogels' digestibility and bioaccessibility **[48]**. WP-based oleogels have been designed to replace fats while simultaneously encapsulating BC, achieving both fat-lessening and boosting nutritional value. Nevertheless, challenges remain in achieving the desired quality, consistency, and steadiness required for their effective usage as fat substitutes.

Bigels are structured as interpenetrating networks of oleogels and hydrogels, which impart distinctive optical, textural, and stability properties. This combination allows for the simultaneous encapsulation of both hydrophilic and hydrophobic BC. WP-based bigels have been investigated for applications in the food and health fields. For example, bigels have been formulated by merging oleo-gel emulsions with WP-based hydrogels. These systems exhibited solid-like properties across a wide temperature range, with textural properties tunable by adjusting the ratio of oleo-gel to hydrogel. Likewise, bigels have been developed using an oleo-gel made from aggregated WP in sunflower oil combined with a hydrogel formed from heated WP solutions. WP-based bigels have been particularly effective in encapsulating and protecting probiotics during simulated gastrointestinal digestion **[49]**. By combining the advantageous properties of both hydrogels and oleogels, WP-based bigels offer strong structural

and functional potential. However, challenges remain in formulating these systems, particularly concerning the stability of the oil-water interface, which is crucial for their development and broader application.

Collectively, these previous studies demonstrate that WP can be employed to develop various gelling systems, including hydrogels, oleogels, and bigels, that hold potential as transporters for a wide extent of BC. However, further research, such as in-vivo studies and safety evaluations, is required to fully understand their efficacy and safety for practical applications.

2.2.3. Films, fibers, and nanotubes

WP can be utilized to create films, fibers, and nanotubes due to their ability to interact differently based on the ecological and system settings. WP-based films are produced using methods similar to conventional polymer films, including casting, compression molding, and extrusion. However, films made exclusively from WP tend to be hard and non-elastic, resulting in limited elasticity and low barrier characteristics. Other bio-based materials are often incorporated into WP films to improve their mechanical performance.

WP are especially suitable for creating edible films or coatings, offering an eco-friendly alternative to petroleum-based plastics and reducing environmental impact **[50]**. For instance, WP films combined with soybean extracts have been shown to possess excellent transparency, as well as antioxidant and antimicrobial activities **[51]**. In another study performed by Shen and colleagues, composite films made from WP, cellulose nanocrystals, and essential oils exhibited strong antioxidant and antimicrobial activity. These attributes could be particularly beneficial for food packing, especially for items like meat, seafood, fruits, and vegetables. These composite films showed significant antimicrobial activity, with 77 and 80% increases against *Staphylococcus aureus*, and *Salmonella enteritidis* respectively **[52]**.

WP films are valued for their biodegradability, tastiness, and effective oil and gas blockade. However, issues with mechanical power, water sensitivity, and processing complications remain obstacles to their broad manufacturing adoption.

WP has additionally been utilized to produce fibers capable of encapsulating, protecting, and controlling the release of BC. The antimicrobial efficacy of WP fibers against *Staphylococcus aureus* showed inhibition zone diameters of 20.0, 2.5, and 35.5 mm, while for *Escherichia coli* (ATCC 25922), the inhibition zone diameters were 11.5, 17, and 24 mm **[53]**. Nanofibers fabricated from WP using electrospinning techniques have been shown to capture and limit the release of biomolecules. Composite fibers have been created by mixing WP with different materials such as soy lecithins, starch, chitosan, and dextran **[54]**.

The large surface area to volume ratio of WP-based nanofibers enhances their ability to encapsulate and deliver bioactive molecules, making them suitable for applications in DDS and scaffolds. Nevertheless, the production of these nanofibers necessitates specialized apparatus, for instance, electrostatic spinning apparatus, and the high expense associated with large-scale manufacturing presents a barrier to broader progress and practical application **[39]**.

Certain WPs are capable of self-assembling into nanotubes in optimal circumstances, enabling the loading, safety, and precise liberation of BC. WP nanotubes are hollow cylindrical structures with variable lengths and two open ends, allowing bioactives to be trapped inside and their dissolution patterns to be

controlled. These nanotubes are capable of disassembly by altering the pH, making them ideal for applications that require pH-triggered release. For example, scientists have successfully incorporated caffeine in WP nanotubes, achieving high entrapment effectiveness and a loading capacity of approximately 10%. These caffeine-based nanotubes also demonstrated strong endurance to lyophilization **[55]**.

Maldonado et al. could entrap curcumin within WP–polysaccharide nanotubes, such as α-la –chitosan and BSA–κ-carrageenan complexes. The encapsulation efficiency of curcumin ranged from 40% to 45%, and curcumin-based nanotubes demonstrated Anticancerous activity in cell culture models **[56]**. Nanotubes based on WP offer a large definite surface area, enabling excellent adsorption and encapsulation of bioactive molecules. However, similar to nanofibers, the high production costs present a significant challenge for large-scale manufacturing and broader commercial application

2.2.4. Emulsions

WP possesses excellent emulsifying properties, making them ideal for creating a conveyance platform grounded on emulsions. WPs are particularly effective in forming nano-emulsions or o/w emulsions or nano-emulsions by stabilizing oil droplets with a protective protein coating. For instance, emulsions formed by WP have been utilized to incorporate β-carotene, meaningfully enhancing its water solubility and improving its stability **[57]**. WP particles have been used to stabilize high internal-phase Pickering emulsions (HIPPE) for β-carotene encapsulation, leading to enhanced stability and bioavailability **[58]**. Likewise, WP-based HIPPEs were employed to incorporate lignocellulosic xanthophylls and enhance their bioavailability **[59]**.

WP–chitosan-coated oil droplets have been used to encapsulate lycopene, improving its dispersibility in water and its chemical stability **[60]**. Additionally, Emulsions have been coated with WP-polysaccharide layers to safeguard and encapsulate β-carotene **[61]**. WP particles have led to stable Pickering emulsions along with successful encapsulation and protection of NC-like curcumin **[62]**.

These studies highlight the ability of WP to serve as particle-based or molecular surfactant in 0/W or nano-emulsions, effectively encapsulating, protecting, and delivering hydrophobic bioactives. WP-based emulsions provide good stability and shelf-life, making them valuable for use in food and health fields. Nevertheless, producing these emulsions often necessitates high-energy methods, such as ultrasonication or high-pressure homogenization, to achieve optimal emulsion stability.

However, producing these emulsions often necessitates high-energy methods, such as high-pressure homogenization or ultrasonication, to attain optimal stability.

3. Whey protein-lipid nanohybrids

Lipid-based nano-formulations, commonly used in clinical applications, offer several advantageous properties, including their GRAS (generally recognized as safe) status, biocompatibility, biodegradability, scalability for large-scale manufacturing, and excellent emulsifying functionality **[63]**. Examples of these lipid-based delivery systems include liposomes, emulsions, microemulsions, nanoemulsions, multiple emulsions, solid lipid nanoparticles (SLN), nanostructured lipid carriers **[64]**, and hollow solid lipid nanoparticles **[65]**. However, despite their potential, lipid-based nanocarriers face various challenges that hinder their progress. One major issue is their colloidal and biological instability, which arises from interactions with

serum proteins and nonspecific binding to cells like lymphocytes, erythrocytes, and endothelial cells. Additionally, lipid nanocarriers often undergo increased clearance by the reticuloendothelial system **[66]**, primarily due to opsonization by complement proteins, fibronectin, and immunoglobulins, which is linked to their hydrophobicity, rigidity, and surface charges.

In contrast, food proteins are another class of biopolymers widely utilized in food industries due to their stability, biocompatibility, biodegradability, and multifunctional characteristics **[67]**. The surface activity, structural properties, and antioxidant capabilities of proteins enhance their utility as nanocarriers **[68]**. As a result, this section seeks to evaluate the benefits of lipid-protein conjugates, particularly lipid-WP conjugates, over single-component lipid carriers, as well as examine the different techniques employed to develop these hybrid systems and previous studies.

3.1. The benefits of protein-lipid nanohybrids

The coupling of fats and proteins results in formulations that integrate the beneficial properties of both, while also overcoming several limitations of traditional or lipid- or protein-based nanocarriers. For example, lipid-protein conjugated DDS exhibit superior attributes, such as enhanced cytotoxic effects and improved therapeutic performance **[69]**. In addition to providing targeted delivery, lipid or protein coatings also contribute to improved drug stability, further highlighting the advantages of conjugation **[70]**. Studies have demonstrated that lipid-protein nanoparticles enable controlled delivery of NC, which extends circulation time and increases their bioavailability **[71]**. Additionally, these conjugates can boost the remedial value of drugs by enhancing dissolution, facilitating direct delivery to target sites, and reducing toxicity to healthy cells **[72]**.

3.2. Hybridization techniques

3.2.1. Chemical conjugation

An amide bond is formed during chemical conjugation between the carboxyl and amine moieties of a lipid and protein respectively. The primary difference between chemical reactions and further techniques is the formation of a covalent bond in the former. In contrast, other approaches depend on non-covalent interactions, including electrostatic forces **[73]**, hydrophobic interactions **[74]**, or a combination of hydrophilic and hydrophobic interactions, as observed in emulsions **[75]**. To enhance the efficiency of protein-lipid conjugation, the lipid's carboxylic group undergoes activation before the formation of the amide bond. Since proteins contain both amine and carboxyl groups, allowing them to self-polymerize, it is essential to activate the lipid’s carboxylic group before conjugation to ensure optimal binding **[76]**. Carbodiimides, particularly 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), are widely used because they are compatible with the hydrophilic nature of proteins and produce water-soluble by-products, which simplify purification. They assist in forming amide bonds between carboxylic and amine groups, common in lipids and proteins. In a liposome formulation, Furumoto et al. employed N-glutarylphosphatidyl ethanolamine (NGPE) to introduce a carboxylic functionality for amide bonding with albumin's amino groups. NGPE's carboxylate was initially activated with EDC, stabilized using N-hydroxysuccinimide (NHS), and residual EDC was neutralized with 2-mercaptoethanol to prevent activation of albumin's carboxylic groups and avoid self-polymerization **[77]**.

3.2.2. High pressure homogenization

In this method, a mixture of protein and lipid is exposed to forceful turbulence and shear force that breaks the lipid phase into small droplets.

Proteins form a macromolecular layer around the droplets, stabilizing them and reducing their tendency to coalesce **[78]**. A two-step homogenization process has been effectively employed to produce WPI-coated nanoemulsions and β-lg-coated lipid nanoparticles. Cooling the homogenizer during this process is crucial to prevent solvent evaporation and to avoid thermal denaturation of the proteins **[79]**. β-carotene-loaded WPI-SLN conjugates were synthesized using the homogenization-evaporation method. The results showed that the particle size was effectively controlled, ranging from 100 to 200 nm which improves β-carotene cellular uptake **[11]**.

3.2.3. Electrostatic coating

Proteins can carry a negative or positive charge depending on the pH concerning their isoelectric point (pI). At pH values above their pI, proteins are negatively charged, while at pH values below their pI, they are positively charged. This property allows lipid nanocarriers to be electrostatically coated with oppositely charged proteins, leading to the formation of protein-lipid nanohybrids **[80]**. Frenzel and Steffen-Heins developed a whey protein (WP) coating for negatively charged liposomes derived from a soy phospholipid isolate (67% purity) to encapsulate quercetin. This coating significantly enhanced the rigidity and stability of the liposomal bilayer. The electrostatic coating method improved liposome stability, allowing them to maintain their size during simulated gastric digestion, whereas uncoated liposomes became destabilized and expanded under the same conditions **[81]**.

3.2.4. Desolvation

Water solubility of the proteins is reduced by adding a desolvating agent, such as ethanol or acetone, to an aqueous protein solution. This reduction triggers phase separation and induces a conformational shift in the proteins from an extended state to a coiled structure, leading to the formation of nanoparticles **[82]**. Gelatin-coated hybrid lipid nanoparticles were prepared using a two-step desolvation process. Initially, gelatin was dissolved in distilled water, followed by precipitation of high molecular weight gelatin using acetone as the desolvating agent. The HMW gelatin was then redissolved in distilled water. Subsequently, the drug was dissolved in dimethyl sulfoxide and combined with lecithin in methanol. After evaporating the methanol, acetone was used to precipitate the drug-loaded lipid-protein conjugates **[83]**.

The properties of the obtained systems depended on the hybridization technique adopted. Each method of hybridization can alter the structural and functional properties of the protein-lipid nanohybrids, such as particle size, surface charge, stability, and drug-loading efficiency, which in turn affects their overall performance as drug delivery vehicles. Thus, selecting an appropriate hybridization technique is essential for tailoring the nanohybrids to meet specific therapeutic requirements.

Conclusion

In this review, we explored the multifaceted functionality of WP, highlighting their promising role as versatile building blocks in the design and formulation of nanocarriers for BC. They serve as highly adaptable molecules for constructing a broad array of systems including nanoparticles, gels, films, and others. These carriers are engineered to hold various bioactive substances, such as vitamins, essential minerals, phytocompounds, and probiotics, which are often prone to degradation or poor bioavailability in their free forms. Encapsulating these materials within whey protein-based systems enhances their water solubility, and chemical stability during food manufacturing, supply, and storing, as well as their availability in vivo.

Abbreviations

NC, Nutraceuticals; Nano-delivery systems, NDS; Whey proteins, WP; Bioactive compounds, BC; Whey protein isolate, WPI; Whey protein concentrate, WPC; β-lactoglobulin, β-lg; solid lipid nanoparticles, SLN; High internal-phase Pickering emulsions, HIPPEs.

Declarations

Consent to publish

All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Availability of data and material

All data generated or analyzed during this study are included in this published article in the main manuscript.

Conflict of Interest

The authors assert that there are no conflicts of interest.

Competing interests

The authors have no financial or non-financial benefits to relate.

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Author contribution

Conceptualization was performed by Rihab Osman and Mai. M. Soliman. Data preparation and collection of the draft was performed by Habeba. N. Hassanin and revision of the first draft was performed by Rihab Osman, Mai. M. Soliman, and Tayseer. M.Nawawy. All authors have read and approved the final manuscript.

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