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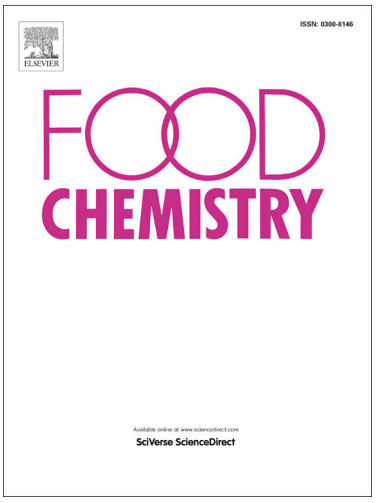

Review

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Interactions between caseins and food-derived bioactive molecules: A Review

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Abstract

Caseins are recognized as safe for consumption, abundant, renewable and have high nutritional value. Casein molecules are found in different aggregation states and their multiple binding sites offer the potential for delivering biomolecules with nutritional and/or health benefits, such as vitamins, phytochemicals, fibers, lipids, minerals, proteins, peptides, and pharmaceutical compounds. In the present review, we highlight the interactions between caseins and food-derived bioactive molecules, with a special focus on the aggregation states of caseins and the techniques used to produce and study the particles used for delivering. Research on interactions between caseins-minerals and casein-pharmaceutical molecules are not included here. This review aims to support the development of new and innovative functional foods in which caseins can be used as designed delivery systems.

Keywords: Casein, delivery systems, nanovehicle, carrier, encapsulation.

1. Introduction

Bioactive molecules are defined as compounds that are able to interact with one or more components in live tissues and produce health benefits (Biesalski et al, 2009). These compounds are not essential to primary nutritional needs but they have been shown to provide beneficial effects when ingested in moderation (Guaadaoiu, Benaicha, Elmajdoub, Bellaoui, & Hamal, 2014). The health benefits associated with bioactive compounds include anti-cancer, anti-inflammatory and anti-obesity properties, a reduced risk of heart disease, and better eye health (Campos, 2018).

Although bioactive molecules offer health benefits, they are sensitive to environmental conditions including high temperatures, extreme pH values, exposure to light and/or oxygen, and enzyme degradation (Sinela et al., 2017; Mahmoodani, Perera, Abernethy, Fedrizzi, & Chen, 2018). Moreover, some bioactive molecules demonstrate low solubility in aqueous or lipid media (Rezaei, Fathi, & Jafari, 2019). The scientific challenge in food processing and formulation is therefore how to find ways to add, protect and deliver bioactive molecules using food products (Tripodi, Lazidis, Norton, & Spyropoulos, 2019). To succeed, the following requirements must be met: (*i*) A significant interaction must take place between the food component and the bioactive molecule; (*ii*) the food component must maintain and protect the biological activity of the bioactive molecule; (*iii*) the food component must deliver the bioactive molecule to the physiological target in the ingesting organism (Nowak, Livney, Niu, & Singh, 2019). Caseins show promise because they can bind hydrophobic, hydrophilic and charged molecules, they can interact with other biopolymers, and they can stabilize emulsions, form gels, and, to some extent, retard oxidation (Damodaran, Parkin, & Fennema, 2008; Horne, 2020). Moreover, caseins are recognized as safe for consumption, are easy to prepare on an industrial scale, and offer a high biological value for a relatively low production cost (Abd El-Salam & El-Shibiny, 2012; Sabliov, Chen, & Yada, 2015).

Caseins represent about 80 % of total cow milk proteins. They are rheomorphic proteins that exhibit an open and flexible conformation (Holt, Carver, Ecroyd, & Thorn, 2013; Lucey & Horne, 2018). There are four primary casein molecules: α-$_{S1}$, α-$_{S2}$, β and κ-caseins. The mass proportion of casein molecules in milk are 30, 10, 36 and 14 % for α_{S1}, α_{S2}, β and κ–caseins, respectively (Davies & Law, 1980). These four caseins have

different amino acid sequences and exhibit additional heterogeneous behaviors due to two post-translational modifications - phosphorylation, for all casein molecules, and glycosylation, for κ-casein (Holland, 2009). In milk, the casein molecules are naturally aggregated in the presence of calcium phosphate forming casein micelles (CMs). Caseins represent about 94 % of CMs dry matter. The remaining 6 % correspond to minerals, primarily colloidal calcium phosphate (CCP) and trace amounts of magnesium and citrate (Holt, Carver, Ecroyd, & Thorn, 2013). CMs have an average diameter of about 200 nm, precipitate at pH 4.6 and have a porous structure that contains about 3.3 g water / g protein (Huppertz et al., 2017; Dalgleish, 2011).

Due to their unique structural and physicochemical properties, caseins have been used as vehicles for bioactive molecules over the past decade (Ranadheera, Liyanaarachchi, Chandrapala, Dissanayake, & Vasiljevic, 2016). Various molecular arrangements of caseins have been studied, including: isolated and purified casein molecules, particularly β-casein; sodium caseinate (CasNa); re-assembled casein micelles (rCMs); native casein micelles (CMs); and casein nanoparticles. The structural aspects of these molecular arrangements have recently been reviewed by Nascimento et al. (Nascimento, Casanova, Silva, Teixeira, & Carvalho, 2020) and Rehan et al. (Rehan, Ahemad, & Gupta, 2019).

The objective of the present study is to review the interactions that occur between caseins and food-derived bioactive molecules such as vitamins, polyphenols, lipids and proteins. We have also covered the aggregation states of casein molecules and the techniques used to produce and study the particles thus formed. Table 1 summarizes bioactive molecule types, casein molecular arrangements, and methods used to study casein-bioactive interactions. This review aims to support the development of new and innovative functional foods in which caseins can be used as a designated delivery system.

2. Casein delivery systems

There are many ways to create casein aggregates that can work as a delivery system. This review will focus on the delivery systems formed by CMs, rCMs, CasNa and pure casein fractions. A summarized representation of casein delivery systems discussed in this paper is depicted in Figure 1. Although all these systems present casein molecules

as the fundamental units, their preparation and the encapsulation strategy of the bioactive compounds vary between them. Different types of casein products can be produced from milk by distinguishing ways, such as isoelectric precipitation, membrane filtration and rennet coagulation (Badem & Uçar). In this sense, the main forms of casein aggregates used to encapsulate bioactive molecules are described below, focusing on the characteristics that are important for the molecules' delivery.

The use of microfiltration techniques to perform the separation of CMs leads to structures very close to those that are naturally found in milk, which is used called native casein micelle (O'Mahony and Fox, 2013). In this case, raw milk is centrifuged to remove fat and dirty. After that, the skimmed milk is microfiltrated using a membrane of molecular cut off that allows the separation of the other milk constituents from CMs. The CMs-rich fraction is dialyzed to remove lactose and salts, is reconcentrated by microfiltration, and then can be spray dried (Schuck, 1994). In general, the strategy used to encapsulate bioactive compounds using native CMs is simple and consists of rehydrating the powder of CMs in a specific medium and environmental conditions, followed by the addition of the bioactive compound (Zhou, S., Seo, S., Alli, I., & Chang, Y. W. 2015; Haratifar, S., Meckling, K. A., & Corredig, M. 2014a), which can be further spray-drying, depending on the aim of the study (Nogueira et al., 2020; Khanji et al., 2018a). Since CMs present a porous supramolecular structure (O'Mahony and Fox, 2013), whose pores are several times larger than the sizes of bioactive molecules, there is always the possibility of the bioactive compounds interact internally in the CMs in a liquid media (Nascimento et al., 2020b). In general terms, the most the bioactive molecule is in the core of the casein aggregates higher is the protective effect (Jarunglumlerta, K. Nakagawab, S. Adachi, 2015). Naturally, the CMs works as a carrier system for delivery minerals for newborn, but CMs also can be modified to improve their physicochemical stability and their capacity of encapsulation (Nogueira et al., 2019; Nascimento, 2020b).

Caseinates also have been employed to develop delivery systems. Caseinates are produced by treating acid precipitated casein with alkali substances, such as NaOH, $Ca(OH)_2$, KOH, which are subsequently spray-dried (Badem, & Uçar, 2017). Sodium and calcium caseinate are the main forms industrially produced. The former is more soluble than the latter (Thomar, P., Nicolai, T., Benyahia, L., & Durand, D. 2013), and the majority

of studies uses sodium caseinate as delivery systems (Ghayour et al., 2019; Penalva et al., 2015; Casanova et al., 2018). The caseinates are composed of all casein fractions, however, they do not form a supramolecular structure as in CMs. Thus, the approach to develop a delivery system changes in accordance with the casein fundamental units, *i.e*., the CMs is the supramolecular structure that can be modified, but it is already "constructed", while the CasNa is composed by the fractions that make CMs, but they can interact to form a different structure. When suspended, the organization of the casein molecules depends on environmental factors, such as pH, ionic strength, presence of divalent cations and temperature (HadjSadok, Pitkowski, Nicolai, Benyahia, & Moulai-Mostefa, 2008). When the rearrangements of CasNa have a higher degree of organization that is close to the structure of native CMs, the systems are called reassemble casein micelle (rCMs) or reconstructed casein micelle (Knoop, Knoop, & Wiechen, 1979). Generally, the production of rCMs consists of the addition of tri-potassium citrate, K_2HPO_4 and $CaCl_2$ in CasNa suspensions (Semo, Kesselman, Danino, & Livney, 2007; Knoop, Knoop, & Wiechen, 1979). The type and concentration of the salts affect directly the characteristics of the rCMs, mainly their size and stability (Loewen, Chan, & Li-Chan, 2018). Besides the addition of specific salts, rCMs have been constructed by crosslinking of casein fractions with transglutaminase enzyme after CMs disruption by alkaline agent (Duerasch, Wissel, & Henle, 2018) and also by submitting CMs to high-pressure treatment followed by the addition of calcium and phosphate ions (Menéndez-Aguirre et al., 2011). Despite the use of casein aggregates composed of all casein fractions, delivery systems containing only α- and β-caseins also have been proposed (He, Xu, Zeng, Qin, & Chen, 2016; Zhang et al., 2014; Bourassa, Bariyanga, & Tajmir-Riahi, 2013). There are several methods to produce casein pure fractions, in general, the fractions have to be isolated from CMs and followed by purification processes, which were reviewed by Atamer et al. (2016). It is worth mentioning that β-casein is a protein that naturally presents the hydrophobic C-terminal well separated domain from the hydrophilic N- terminal domain, and this characteristic allows the association of β- casein with hydrophobic bioactive compounds and the formation of soap-like micelles (Swaisgood, 2003).

As discussed here, many casein delivery systems can be applied to encapsulate bioactive compounds. Thus, the best type of casein system used to encapsulate a specific bioactive compound cannot be simply predicted. The most suitable strategy for encapsulating such compound with casein molecules or aggregates needs to be confirmed by experimental results. However, independently of the adopted strategy, it is important to consider the chemical interactions between caseins and bioactive molecules, which are reviewed in the next section.

3. General aspects of caseins and bioactive molecules interactions

Caseins can interact with a wide variety of bioactive molecules (Tavares, Croguennec, Carvalho, & Bouhallab, 2014) by different means such as hydrophobic, hydrophilic, and electrostatic interactions. Most of the studies that use casein systems to deliver bioactive compounds focus on the flowing topics in higher or low extensions: promotion of the encapsulation (Ghatak & Iyyaswami, 2019); measuring of the binding constants (Bourassa et al., 2013); observation of changes in the protein structure by the complex formation (He et al., 2016); studying the biomolecule stability under storage conditions (Yi, Fan, Yokoyama, Zhang, & Zhao, 2016); incorporating the vehicle in a food matrix (Loewen, Chan, & Li-Chan, 2018); investigating the changes that occur in the food properties (rheological, physicochemical and sensorial changes) (Moeller, Martin, Schrader, Hoffmann, & Lorenzen, 2018); evaluation of the bioaccessibility of the bioactive compound in vitro or in vivo (Cohen et al., 2017).

The strength of the interaction between caseins and the bioactive molecules is usually measured by fluorescence spectroscopy. In caseins, the change in the intrinsic fluorescence of tryptophan caused by the presence of additional molecules is used to determine the binding constants. The reduction in the intrinsic fluorescence can be a result of simple collisions between the caseins and the bioactive compound (dynamic quenching) or by complexation between these two (static quenching). Thermodynamical models are applied to determine which phenomenon occurs, and also to determine the binding's constants in the case of complexation (Lakowicz, 2013). Each molecule has different binding constants and sites and it varies when changes in pH, ionic strength and temperature occur (Casanova et al., 2018). Thus, the knowledge of these constants and

how they change according to environmental parameters is a useful tool to develop a delivery system. The interaction of the bioactive molecule with the caseins does not guarantee a suitable delivery system. Consequently, investigations that aim to evaluate modifications in the protein structure or conformation caused by the complex formation are required, since these modifications can directly impact the characteristics of the food systems (Guri, Haratifar, & Corredig, 2014). At a molecular level, spectroscopy analysis such as circular dichroism (CD), FTIR, and SAXS are valuable tools to follow the protein changes (Semenova et al., 2016; Antonov et al., 2017; Arroyo-Maya et al., 2016). However, changes in the protein structure after complexation with the bioactive molecule do not always occur (Gorji et al., 2015). Accordingly, the absence of a general rule makes necessary the study of those interactions and their micro and macro consequences in the carrier and food system.

The delivery systems can be applied in different fields as pharmaceutical, agricultural, and food. Generally, the encapsulated bioactive molecule is expected to increase its stability when applied in the food system in comparison with the free bioactive compound (Moeller et al. 2018; Kumar et al., 2016). However, good encapsulation efficiency and increased stability of the bioactive compound in a model system do not ensure the same results in a real food system. The complexity of a real food system can add new variables that can destabilize the carrier, invalidating its protective effect. Thus, It is possible that the enrichment of food with the encapsulated bioactive has the same results of stability as it was applied without the carrier material (Loewen, Chan, & Li-Chan, 2018). Another point to take into consideration concerning the application of a carrier system in food is its digestion. As a protein, caseins are degraded by pepsin and pancreatic enzymes (Cohen et al., 2017) which can cause the release of the encapsulated bioactive that can be metabolized. Generally, in vitro methods are applied to evaluate the capacity of caseins to protect and release bioactive compounds. Despite being a simplification of in vivo studies, there have been reported similarities in the casein degradation comparing in vivo and in vitro methods (Miralles et al., 2021). However, it lacks more in vivo studies concerning the bioactive molecules, and if encapsulation plays an important role in their activity.

Due to the difficulty in making comparisons among the published papers, once the parameters change according to casein aggregation, strategy of encapsulation, and type of bioactive compounds, a more detailed description of the recent papers published in the field is presented in the following sections.

4. Casein-vitamin interactions

Vitamins are a group of diverse, organic, nutritionally essential compounds that may induce health issues when deficiencies in the human body occur. Some vitamins are sensitive to light, oxygen, pH, and temperature (Gazzali, 2016), which is why different methods have been proposed to create protein-vitamin systems that protect the vitamins and improve their bioavailability (Katouzian & Jafari, 2016).

4.1. Vitamin A

Vitamin A molecules are liposoluble, unsaturated molecules found in different forms, including retinoic compounds and provitamin A carotenoids. Interactions between CasNa and lutein, an oxygenated carotenoid, have been studied by Yi et al. (Yi, Fan, Yokoyama, Zhang, & Zhao, 2016) using UV and fluorescence spectrometry (FS) and circular dichroism (CD). The authors observed that the lutein solution's turbidity decreased when CasNa was added, an effect attributed to lutein binding to casein molecules. However, the binding between lutein and CasNa had little impact on the caseins' secondary structures. According to the fluorescence results, caseins interacted with lutein by hydrophobic interactions with a constant association magnitude of 10^5 M^{-1}, and stoichiometry of about one bound lutein molecule per casein molecule. The interactions enabled the caseins to protect the lutein molecules against oxidation and decomposition during 16 days of storage at 25 °C. The chemical stability of β-carotene also increased after encapsulation with caseins, however, the protection degree varied depending on the casein aggregation (Jarunglumlerta, K. Nakagawab, S. Adachi, 2015). β-carotene is a carotenoid precursor of vitamin A which is used as a colorant in the food industry. Light, heat treatment and oxygen exposure can all cause degradations in β-carotene. As discussed previously, the structure of the casein aggregates impacts directly the vitamin-protein interactions. Jarunglumlert et al. (Jarunglumlerta, K. Nakagawab, S. Adachi,

2015) compared CasNa aggregates at pH 6.0 and rCMs on the encapsulation efficiency and chemical stability of β-carotene. The strategy consisted of stirring the protein suspensions added of β-carotene from 0 to 120 h. After complex formation was completed, the suspensions were spray-dried and the β-carotene's stability was evaluated over 21 days. The authors showed that the CasNa aggregates formed at pH 6.0 more efficiently encapsulated β-carotene molecules compared to rCMs. This result was attributed to a denser casein aggregate structure formed by CasNa compared to the rCMs structure. Also, it was found that longer complex formation times improved encapsulation efficiency for CasNa aggregates, which was not the case for rCMs aggregates. The higher binding efficiency reflected in the stability of β-carotene during 21 days of storage at 60°C. The results of these studies demonstrate the caseins' potential as a carrier for liposoluble vitamins, which can be further improved by modulating the chemical environment in a simple way, *i. e.*, by controlling pH, salt types and their concentrations and complex formation time.

Other strategies to increase encapsulation efficiency of casein systems were used by Blayo et al. (2014). Ultra-high-pressure homogenization at 14 °C or 24 °C, and isostatic high pressure at 14 °C or 34 °C for 15 min, both at 300 MPa were applied to encapsulate retinyl acetate in CMs. The authors evaluated the amounts of retinyl acetate in the CMs by their precipitation with ammonium sulfate. The authors showed that 2 – 5 nmol of retinyl acetate were carried per mg of precipitate casein. However, retinyl acetate concentrations in the control samples were similar to those found in the high-pressure treated samples, regardless of the type of high-pressure treatment. Therefore, the interactions between retinyl acetate and CMs caseins are not influenced by high-pressure technologies. In other words, solubilizing the retinyl acetate in native CMs suspension was enough to spontaneously encapsulate it.

In addition to systems composed solely of bioactive molecules and caseins, other biopolymers have been used to improve the protective role of caseins (Nascimento et al, 2020a). Jain et al. (Jain, Thakur, Ghoshal, Katare, & Shivhare, 2016) developed a carrier with gum tragacanth (a natural gum obtained from the dried sap of several species of Middle Eastern legumes in the *Astragalus* genus*)* to protect and allow for sustained release of β-carotene via a coacervation of casein. At a protein/gum ratio of 2/1, the

optimal pH for complex coacervation was pH 4.3. At this pH, the intensity of electrostatic interaction was at its maximum. According to the authors, the particle size of the β-carotene-loaded coacervates ranged around 159.7 ± 2.2 nm. The coacervates presented low porous surfaces with no cracking, the coacervation yield was 82.5 ± 0.4 %, and the entrapment efficiency 79.4 ± 0.5 %.

4.2. Vitamin B

The interaction between isolated β-casein and folic acid, a hydro-soluble synthetic B vitamin, has been investigated by Zhang et al. (Zhang et al., 2014) using FS, absorption spectroscopy and CD. The authors reported that folic acid binds to β-casein by hydrophobic interaction with a dissociation constant of ~10^5 M^{-1}. They also showed that binding of folic acid to β-casein inhibited the vitamin's photodecomposition. Penalva et al. (Penalva et al., 2015) manufactured casein nanoparticles for oral delivery of folic acid. These nanoparticles were prepared using a coacervation process and stabilized with either lysine or arginine. Briefly, CasNa was suspended in pure water with lysine or arginine. Then, a solution of folic acid was added, followed by the addition of calcium chloride. The system was ultrafiltrated for purification, then spray-dried. The casein nanoparticles that formed presented a mean diameter close to 150 nm and folic acid content of 25 µg/mg of casein. In vitro and in vivo release studies showed that the oral bioavailability of the folic acid was around 52% when it was administered along with casein nanoparticles, *i.e.* 50% higher than in an aqueous solution. The results demonstrate the protective role caseins can play for water-soluble vitamin B.

These findings pointed to the potential use of caseins as a way to carry and protect folic acid. However, there is still little information available about the casein delivery of B vitamins.

4.3. Vitamin D

High-pressure treatment was applied to CMs to increase the loading capacity of Vitamin D_2. The high-pressure treatments (0.1, 200, 400 and 600 MPa) combined with temperature variations (10 - 50 °C) were able to create rCMs with casein fractions that reassemble after been released from CMs (Menéndez-Aguirre et al., 2014). Contrary to

the results found by Blayo et al. (2014) for retinyl acetate. The authors observed that the vitamin D_2 load per casein increased from 2.2 ± 0.2 μg/mg (CMs) to 10.4 ± 0.2 μg/mg (rCMs) when 600 MPa at 50 °C was applied to a suspension of CMs. These findings highlight the suitability of high-pressure treatments for incorporating hydrophobic vitamin D molecules into rCMs. However, vitamin stability studies still need to be carried out before any recommendations can be made. In another study, a higher loading capacity for vitamin D in rCMs was achieved using response surface methodology to find the best salt concentrations (Loewen, Chan, & Li-Chan, 2018). Loewen et al. (Loewen, Chan, & Li-Chan, 2018) found the optimal vitamin D loading (13.8 – 14.6 mg/mg rCMs) using 4.9 mM phosphate, 4.0 mM citrate and 26.1 mM calcium. The vitamin D stability also was evaluated. The powders from rCMs presented greater vitamin D preservation levels than the CasNa control powders during ambient (25 °C and 25 – 50 % humidity) and accelerated (37 °C and 75 % humidity) storage for 96 hours. However, when applied in fluid milk, it was observed that after 21 days of storage at 4° C under light exposure, vitamin D loss was not different for fluid milk with rCMs powder (loaded with vitamin D) and the control samples of fluid milk with direct addition of vitamin D (Loewen, Chan, & Li-Chan, 2018).

Usually, the delivery systems are used to fortified foods aiming the application in the food industry. The use of rCMs as a way to deliver vitamin D was also tested for fat-free yogurt production. Fat-free yogurt was enriched with vitamin D_3 encapsulated in either rCMs or Polysorbate-80, a synthetic emulsifier (Levinson, Ish-Shalom, Segalb, & Livney, 2016). The yogurt samples were compared for in vivo bioavailability of vitamin D_3. In vivo bioavailability of vitamin D_3 was evaluated by clinical trial and no significant difference was observed between the two enrichment methods. In another study also with yogurt, an original approach was used by Moeller et al. (Moeller, Martin, Schrader, Hoffmann, & Lorenzen, 2018) to encapsulate and protect vitamin D_2 in native CMs. The authors induced vitamin D2 encapsulation in an alcoholic solution by mixing the solution with an acidified suspension of native CMs at 2 °C and pH 5.5. Subsequently, the pH of the suspensions was adjusted to neutral. The suspensions were then spray-dried or freeze-dried. It was observed the maintenance of vitamin D2 content for 4 months of storage after the encapsulation. The authors also observed an increase in the in vitro

bioavailability of vitamin D2 encapsulated in CMs, *where* 90% vitamin D2 (in CMs) remained active compared to only 67% in the free vitamin D2 yogurt samples. According to the authors, this result suggests that vitamin D2 can remain available in the lumen, but in vivo experiments must be done to confirm it.

Cohen et al. (Cohen, M. Levi, U. Lesmes, M. Margier, E. Reboul, Y. D. Livney, 2017) studied the use of rCMs as vehicles for vitamin D and evaluated the vitamin retention during simulated digestion and posterior in vitro bioavailability. The results showed that rCMs improved protection of Vitamin D_3 during simulated digestion and demonstrated a significant increase in vitamin retention for 1 h under gastric conditions. Vitamin absorption by Caco-2 cells from digested rCMs was similar to free vitamin absorption. However, the bioavailability of the vitamin combined with rCMs was four times higher than that of the free vitamin.

The results above show that casein organization directly influences caseins' capacity to encapsulate and protect bioactive molecules. In addition to native CMs, developed rCMs represent another way to retain fat-soluble molecules, such as vitamin D. It is also important to note that both methods - homogenization and salt addition for rCMs production have shown positive results for protecting vitamin D. These principles offer potential as future research topics on enriching dairy products with other fat-soluble vitamins.

5. Casein-polyphenol interactions

Polyphenolic compounds are included in functional foods for their antioxidant, anti-inflammatory, antimicrobial, anti-amyloid, and anti-tumor properties (Martins, Barros, & Ferreira, 2016). Chemically speaking, polyphenolic molecules are characterized by the presence of one or several phenolic groups in their structure (Jia, Dumont, & Orsat, 2016). However, polyphenolic compounds are low soluble in aqueous solutions, which can lead to poor bioavailability and limit their clinical effectiveness. In addition, polyphenols present low stability when exposed to different pH values, light and high temperatures (Faridi Esfanjani & Jafari, 2016). One way to increase their solubility and maintain their stability until ingestion is to encapsulate them in caseins. In this sense, polyphenols' fluorescence, UV and visible spectra absorption are important elements for studying casein-polyphenol interaction.

5.1. Curcumin

Curcumin has low intrinsic toxicity and is credited with antioxidant, anti-inflammatory, antimicrobial, anti-amyloid, antitumor and anticancer pharmacological properties (Maheshwari, Singh, Gaddipati, & Srimal, 2006; Ono, Hasegawa, Naiki, & Yamada, 2004). Khanji et al. (Khanji et al., 2018a) used FS analysis to demonstrate that interactions in the solutions were primarily hydrophobic. From pH 7.4 to pH 5.0 during the gelation process, the binding site numbers varied from 1.25 to 1.49, and the binding constant varied from 3.9 to $7.5x10^4$ M^{-1}. In another study with the purified casein fractions, Bourassa et al. (Bourassa, Bariyanga, & Tajmir-Riahi, 2013) founded that curcumin and α- and β-caseins formed complexes through hydrophilic and hydrophobic interactions with binding constants comprised between 10^5 and 10^4 M^{-1}. Bound curcumin molecule counts were 1.43 per α-casein molecule and 1.27 per β-casein molecule. These interactions were stabilized by a hydrogen bonding network with a free binding energy of −8.89 kcal per mol of α-casein and −10.70 kcal per mol of β-casein.

Besides the complexation of curcumin with CMs, in the study conducted by Khanji et al. (Khanji et al., 2018a), it was also observed that the ζ-potential value of CMs was not changed by curcumin addition. Acid gelation was examined using oscillation rheology and static multiple light scattering at 20 and 35 °C and led to similar behaviors for native and curcumin-doped CM suspensions. The authors demonstrated that the colloidal and functional properties of CMs remained unchanged when doped with curcumin during acidification. These results are interesting because they showed that the industrial process (at lab scale) to produce acid milk gels is not disturbed by the presence of curcumin molecules. Khanji et al. (Khanji et al., 2018a), studied CMs – curcumin interactions in powder systems. The sample was prepared by mixing 8 L of rehydrated CMs (15.5 % dry matter) with 290 mL of ethanolic curcumin solution (1 mg/mL) for 1 h. The sample was then centrifuged (5000 rpm, 10 min) and spray-dried. The mixture was atomized in the drying chamber at an inlet temperature of 180 °C. The powder particles were separated from the drying air at an outlet temperature of 90 °C. Powders were analyzed by small-angle x-ray scattering (SAXS) to determine possible CMs structure changes following interactions with curcumin. No differences in the internal CMs structure

were observed after interaction with curcumin. In addition, the curcumin's antioxidant activity, monitored with ABTS and FRAP, was preserved for 60 days of storage at 40°C and remained at ~ 82 % and ~ 84 % levels, respectively. These findings open new possibilities for curcumin-doped CMs powders.

Ghayour et al. (Ghayour et al., 2019) investigated interactions between curcumin and quercetin with CasNa and studied their respective re-assembled micellar nanostructures or casein nanoparticle formations. During the study, CasNa was dispersed in buffer solutions at pH 7.4. Curcumin and quercetin were prepared in absolute ethanol at 200 and 600 µM, respectively. Spectrofluorometry was used to determine quercetin and curcumin binding, which were 0.96 and 0.78, respectively. The binding constants were 3.2×10^4 for quercetin and $0.92 \times 10^4\ M^{-1}$ for curcumin. The changes in the relative viscosity of the samples during the re-assembly process confirmed the formation of micellar nanostructures. In addition, the authors revealed the entrapment efficiency was greater than 90 % for both systems.

The interactions and gelation properties that occur between curcumin, CMs, Lactobacillus delbrueckii bulgaricus (Lb) and Streptococcus thermophilus (St) were evaluated by Khanji et al. (Khanji et al., 2018b). CMs were obtained by milk microfiltration then spray-dried. Suspensions were prepared at 50 g/L in a buffer at pH 7.4. Analysis by FS was carried out to investigate interactions between curcumin and St and Lb. A decrease in fluorescence intensity, from 1.7 A.U to 1.20 and 1.40 A.U confirmed the quenching process. The gelation process was studied using particle size analysis with multiple light scattering (Turbiscan) and rheometry. The results showed that curcumin adsorption did not affect Lb and St growth or milk acidification rate. For the first time, the authors demonstrated that curcumin interacted with lactic bacteria without modifying its growth or milk gelation properties.

Kumar et al. (Kumar et al., 2016) examined *(i)* curcumin preparations in oil-in-water nanoemulsions stabilized with CasNa and *(ii)* a subsequent addition of these to ice cream. The authors dissolved different concentrations of CasNa (1 – 7 %) in aqueous solutions with milk fat (5 – 9 %), curcumin (0.12 – 0.6 %) and medium-chain triglycerides (1 – 5%). The nanoemulsions were obtained by homogenization and their physicochemical properties were studied. The optimal ratio was observed when milk fat was at 8 %,

medium chain triglycerides were at 2 %, curcumin was at 0.24 % and CasNa was at 6 %. In these conditions, the nanoemulsions presented a particle size of ~ 330 nm, a ζ-potential of ~ − 44 mV with an encapsulation efficiency of ~ 97 %. An in vitro release of curcumin under simulated digestion revealed that nanoemulsions remained stable against pepsin digestion (5.25 % release of curcumin) and pancreatic action (16.12 % release of curcumin). Following these experiments, ice cream was prepared by mixing the dry ingredients (skim milk powder, stabilizer, agar emulsifier and sugar) with milk and cream. The mixture was then homogenized and the O/W nanoemulsions were added. The ice cream base was homogenized, pasteurized and stored overnight at 5 °C. The next day, mango flavor was added to the ice cream mix and transferred to a batch freezer. The ice cream with added nanoemulsions was put to a taste test with a panel of volunteers. Results revealed no significant sensory attribute difference between the control ice cream sample and the ice cream sample prepared with a curcumin nanoemulsion. The authors suggest ice cream may be an ideal food system for delivering curcumin nanoemulsions.

Encapsulation technology which utilizes the pH-dependent solubility properties of curcumin and the self-assembly properties of CasNa was explored by Pan et al. (Pan, Luo, Gan, Baekcand, & Zhong, 2014). Curcumin was deprotonated by dissolution in a CasNa dispersion at pH 12 at 21 °C for 30 min. Capsule creation was achieved by re-associating CasNa after the neutralization of the dispersion (pH 7.0), as confirmed by dynamic light scattering (DLS) and analytical ultracentrifugation. The bioactivity capacity of curcumin to restrict the proliferation of human colorectal cancer cells (HCT-116) and human pancreatic cancer cells (BxPC3) was tested. Results showed that curcumin encapsulated in casein nanoparticles demonstrate greater anti-proliferation activity for both HCT-116 and BxPC3 cells compared to free curcumin pre-dissolved in a polar solvent.

5.2. Anthocyanins

Anthocyanins are plant pigments found throughout the natural world whose color depends on the environment's pH. The pigments can assume a range of colors from blue to red, passing through shades of purple and orange (He & Giusti, 2010). Aside from their use as colorants, anthocyanins have garnered attention for their effectiveness as bioactive compounds (Diaconeasa, Leopold, Rugină, Ayvaz, & Socaciu, 2015). Several

works have attributed health characteristics to anthocyanins. These include reduced risk of heart disease, stroke, cancer, and obesity (Wrolstad, 2004). Anthocyanins' potential use in food formulations is limited by their high susceptibility to degradation (de Moura, Berling, Germer, Alvim, & Hubinger, 2018). The pigments are prone to degradation in the presence of light, oxygen, ascorbic acid and other factors. Therefore, it is essential to find technologies that can protect and maintain their benefits during destabilizing conditions (de Moura et al., 2018).

Malvidin-3-O-glucoside is the primary anthocyanin present in grape skin anthocyanin extract. The molecule contains several phenolic cycles that are sensitive to oxidation. He et al. (He, Xu, Zeng, Qin, & Chen, 2016) studied the interactions between malvidin-3-O-glucoside and α- and β-caseins. Using FS analysis, the authors showed that α- and β-caseins bind to malvidin-3-O-glucoside *via* both hydrophilic (van der Waals forces and hydrogen bonding) and hydrophobic interactions, with binding affinities ~ 10^3 M^{-1}. FTIR and CD indicated the caseins' secondary structures changed after binding. The authors also showed that a casein concentration of 0.1 mg/mL promoted a decrease in anthocyanin degradation rates of 37.61 %, 18.40 %, and 29.37 % in solutions at pH 6.3 under thermal (80 °C / 2 h), oxidation (0.005 % H_2O_2 / 1 h) and photo illumination (5000 Lux / 5 d) treatments, respectively. It was concluded that complex malvidin-3-O-glucoside - caseins can be used as natural colorants in food systems that may be exposed to light or high temperatures with the preservation of the biological activity of anthocyanin molecules.

The interaction mechanisms that occur between cyanidin-3-O-glucoside (C3G) and CasNa nanoparticles at pH 7 and 2 were studied by Casanova et al. (Casanova et al., 2018) using FS and DLS. The authors found a complex formation between C3G – CasNa with static interaction. C3G interacted with two sets of binding sites with association constants of 10^6 and 10^5 M^{-1}. Electrostatic interactions were predominant at pH 7, while hydrophobic effects were the main force at pH 2. DLS analysis showed a slight modification in the CasNa without any alteration in its surface charge. The complexation of C3G molecules and CasNa occurred within the internal casein structure of the particles. The authors proposed CasNa as a putative nanocarrier for anthocyanins in soft drinks when an acidic pH is needed. Nascimento et al. (Nascimento et al., 2020b)

investigated the effect of C3G-rich jaboticaba (*Myrciaria cauliflora*) extract on the rheological properties of CMs hydrogels. The addition of jaboticaba extract decreased the hydrogels' elasticity and produced hydrogels with larger pore sizes compared to control samples. The authors proposed the use of transglutaminase (an enzyme that promotes the formation of covalent bonds between glutamine and lysine residues) as a way to balance out the fruit extract's effect on the hydrogels' structure. The transglutaminase would also modulate the release rate of the encapsulated anthocyanins. The anthocyanin release was evaluated for three pH values, and the authors found a higher release rate at pH 7.0. These results highlight the potential use of CMs hydrogels for controlled anthocyanin release in the small intestine.

Interactions between pelargonidin, an anthocyanidin present in pomegranate fruit (Noda, Kaneyuki, Mori, & Packer, 2002) and β-lactoglobulin, WPI, and CasNa were investigated by Arroyo-Maya et al. (Arroyo-Maya, Campos-Terán, Hernández-Arana, & McClements, 2016). FS experiments demonstrated that pelargonidin quenched milk proteins. The authors showed that the secondary structure of all proteins evaluated was not significantly affected by pelargonidin. Analysis of fluorescence data indicated that β-lactoglobulin and caseinate, but not WPI, bound the pelargonidin at both pH 7.0 and 3.0 with a binding constant of $1.0 \times 10^5\ M^{-1}$.

5.3. Resveratrol

Resveratrol is a polyphenol commonly found in grapes and peanuts (Karthikeyan, Prasad, Ganamani, & Balamurugan, 2013). Studies attribute certain health benefits to resveratrol. These include anti-cancer, anti-inflammatory, neuroprotective and anti-diabetes activity (Bastianetto, Menard, & Quirion, 2015; Varoni, Faro, Sharifi-rad, & Iriti, 2016; Szkudelski & szkudelska, 2015; Bonnefont-Rousselot, 2016). Many authors have attempted to incorporate resveratrol into food systems (Pando, Beltrán, Gerone, Matos, & Pazos, 2015; Davidov-Pardo & Mcclements, 2014; Sessa et al., 2014). However, the polyphenol's poor water solubility presents a drawback and a carrier agent is necessary for its incorporation into a food product.

Insertion of resveratrol into an oil phase followed by casein stabilization is another way to encapsulate resveratrol. A CasNa / maltodextrin conjugate was developed by

Consoli et al. (Consoli et al., 2018). A Maillard reaction using a wet-heating procedure was used to induce the conjugate formation. The conjugates that formed after different reaction times (3, 6, 9, 12 and 24 h) were used to stabilize resveratrol emulsions dispersed in palm oil. The authors showed that longer reaction times lead to the formation of higher molecular weight conjugates. These conjugates increased emulsion stability because they could cover a larger area than smaller conjugates. At the same time, higher reaction times also led to higher antioxidant activity. The authors concluded that CasNa / maltodextrin conjugates formed by the Maillard reaction can be used to stabilize emulsions containing resveratrol and retain the resveratrol‘s antioxidant properties.

Cheng et al. (Cheng, Fan, Liu, Wusigale, & Liang, 2020), dispersed resveratrol in sunflower oil and added CasNa with pectin or gum arabic to stabilize the O/W emulsion. The authors observed a decrease in wavelength intensity in the resveratrol emission spectra due to a change in the resveratrol environment which became more hydrophobic after the CasNa addition. The presence of gum Arabic did not influence the emission spectra, however, increasing the concentration of pectin gradually decreased the resveratrol’s fluorescence intensity. In addition, the CasNa associated with pectin increased the stability of resveratrol after 42 days of storage with stability levels at 84 % compared to 76 % of the CasNa sample without the carbohydrate. The association between CasNa and pectin improved protection against degradation for resveratrol.

5.4. Others polyphenols

Further investigations have been carried out on the relationships between caseins and other types of polyphenols compounds. Zhou et al. (Zhou, Seo, Alli, & Chang, 2015) studied interactions between caseins and two major phenolic acid compounds in cocoa: protocatechuic acid and coumaric acid. This study was performed in a model system, then compared to caseins extracted from milk and white chocolate. Electrophoresis analysis revealed that interactions between caseins and phenolic acids were induced by incubation at 55 °C, with a formation of hydrophobic interactions and hydrogen bonding. Moeiniafshari et al. (Moeiniafshari, Zarrabi, Bordbar, 2015) investigated the interactions between naringenin, a nutraceutical flavanone present in tomatoes and citrus fruits, and β-casein. Using fluorescence quenching methods, the authors found the constant of the

complex at 10^5 M^{-1} whereas a thermodynamic analysis showed that the interaction was spontaneous with contributions of van der Waals forces, hydrogen bonds and hydrophobic interactions. The interactions between rosmarinic acid, a phenolic acid found in certain members of the Lamiaceae family, and α-s1, β and k-casein were investigated by Ferraro et al. (Ferraro et al., 2015). The analysis was performed in water at pH 3.0 and 4.5 and showed that hydrophobic, hydrogen bonding and dipole-dipole interactions occur, and the stabilization of these interactions is pH-dependent.

Several studies have been done in the interaction of tea catechin, especially epigallocatechin-gallate (EGCG), with CMs (Haratifar, Meckling, & Corredig, 2014a; Haratifar, Meckling, & Corredig, 2014b; Haratifar & Corredig, 2014) and Guri et al. (Guri, Haratifar, & Corredig, 2014). The authors showed that EGCG was able to bind to casein *via* both hydrophilic and hydrophobic interactions. A binding constant between CMs and EGCG was calculated to be between 10^{-4} and 10^{-3} M^{-1}. However, these interactions increased the milk gelation time after rennet addition probably because these interactions limited the access of the chymosin to the κ-casein fraction (Haratifar & Corredig, 2014). The bioavailability of this complex in fighting colon cancer cell HT-29, was also investigated. The authors showed that CMs binding did not affect the bioavailability of EGCG, and the in vitro model showed a decrease in proliferation of cancer cells without any reduction in their bioavailability, thus confirming that CMs are an appropriate delivery system of phenolic acid.

Gorji et al. (Gorji et al., 2015) studied Interactions between resveratrol and β-casein. A static interaction with a binding constant of 7.33 x 10^5 M^{-1} was observed by FS analysis. In addition, the authors suggested the presence of hydrogen bond formations between the compound and - NH_2, - OH and - SH groups that are located near the tryptophan residue (position 143). However, CD analysis revealed that resveratrol did not cause any significant change to the proteins' secondary structures.

6. Casein-lipid interactions

Lipids are energy sources and membrane constituents (Calder, 2015). The structural characterization of the interaction between caseins and lipids is important to

understanding how lipids may be transported by caseins and few studies have covered this topic.

Cheema et al. (Cheema, Mohan, Campagna, Jurat-Fuentes, & Harte, 2015) clarified associations between hydrophobic molecules and native CMs to provide a better understanding of their biological distribution in raw milk. Hydrophobic and hydrophilic extractions followed by ultra-performance liquid chromatography analysis were performed on protein fractions obtained from size exclusion fractionation of raw skim milk. The authors showed that hydrophobic compounds, including phosphatidylcholine, lyso-phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin, demonstrated strong associations exclusively with CMs.

The associations that occur between lipids, such as cholesterol (CHOL), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), dioctadecyldimethyl - ammoniumbromide (DDAB) and dioleoylphosphatidylethanolamine (DOPE), and α- and β-caseins were studied by Bourassa et al. (2013) using FTIR, FS, CD and molecular modeling. Structural analysis showed that lipids were bound to casein primarily *via* hydrophobic interactions, with a constant association that ranged from 10^3 and 10^4 M^{-1} and binding sites that varied from 0.7 to 1.1 lipid per protein. Docking calculations showed different binding sites for α- and β-caseins with free binding energies varying from −10 to −13 kcal/mol. According to the authors, casein conformation was altered by lipid interactions and yielded a reduction of α-helix and β-sheet and an increase in random coil and turn structures, suggesting a partial protein unfolding.

Panja et al. (Panja, Khatua, & Halder, 2018) explored the changes that occur in CMs microenvironments when fatty acids are present. Using fluorescence analysis, the authors observed that the unsaturation of fatty acids affects the CMs structure in contrast to hydrophobic interaction forces, which is followed by a decrease in electrostatic interactions of various amino acids. Alterations in these forces are responsible for an increase in aggregate size, modifications in secondary protein structures, and different CMs morphologies. Fluorescence lifetime imaging microscopy (FLIM) analysis indicated that the CMs microstructures become more compact when unsaturated fatty acids are present. According to the authors, the results provide useful information on the binding properties of fatty acids and may help evaluate other fatty acid behaviors.

The particles formed between covalent conjugates of CasNa and maltodextrins and either soy phosphatidylcholine liposomes or soy lysophosphatidylcholine micelles in an aqueous medium at pH 7.0 were investigated by Semenova et al. (Semenova et al., 2016) using DSC, ESR and SAXS. A high encapsulation value (> 95 %) was found for these soy phospholipids formed by the conjugates. More highly-soluble complex particles formed with higher densities and higher thermodynamic affinities for an aqueous medium compared to the control samples. The results have shown that CasNa can carry hydrophobic compounds.

7. Casein-protein interactions

Different studies have described interactions between caseins and various proteins. In general, the objectives of these studies have been to create new assemblies with various techno-functionalities such as texturing and emulsifying (Broyard & Gaucheron, 2015). In the present review, we discuss only research carried out on proteins with health benefits, i.e., lysozyme and lactoferrin.

Lysozyme is a small globular enzyme that contains 129 amino acids. It has bacteriostatic effects that inhibit gram-positive bacteria through the cleavage of the glycosidic bonds between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan layer (Wu et al., 2019). Lysozyme's isoelectric pH is close to 11 and consequently, at a neutral pH, the protein's charge is positive and interactions with negative parts of casein molecules remain possible. Antonov et al. (Antonov, Moldenaers, & Cardinaels, 2017) studied lysozyme's structural and morphological complexation using CasNa and native CMs. Their results showed that lysozyme forms complexes with caseins from pH 7.0 up to pH 11. Lysozyme binding with CasNa and CMs leads to a disruption of the lysozyme's secondary structure.

Lactoferrin is a globular glycoprotein that is widely present in secreted bodily fluids, such as milk, saliva, tears, and nasal secretions. Lactoferrin exhibits antibacterial, antiviral, antifungal, anti-inflammatory and anti-carcinogenic properties (Wang, Timilsena, Blanch, & Adhikari, 2019). Anema and de Kruif (Anema & (Kees) de Kruif, 2016) investigated the phase separation and composition of coacervates of lactoferrin and caseins. The authors showed that optimum complexation occurs at pH 6.55 and is characterized by maximum turbidity. Two different behaviors for coacervates were

observed: the kinetics of complex formation between lactoferrin with κ or β - casein is rapid and appears to occur through a nucleation and coalescence process. However, the kinetics of complex formation between lactoferrin and α_s– casein is much slower. The complex formations between caseins and proteins with health benefits offer potential as a way to protect the proteins' bioactive characteristics and also incorporate and deliver other molecules with nutritional and/or health benefits in the near future.

8. Conclusions and perspectives

Caseins constitute the major protein group present in milk. For the past several years, the formation of complexes between caseins and bioactive compounds has received attention in the academic sector because it represents an effective way to encapsulate and protect the biological activity of bioactive molecules. However, caseins' aggregation states can influence their encapsulation and protective properties. In general, different types of casein aggregates may be sensitive to physicochemical environmental conditions and consequently restrict their application in food development. Cross-linking agents offer one method for overcoming these limitations. Cross-linked caseins present higher stability than non-modified caseins when exposed to high temperatures, acid conditions and light (Casanova et al., 2017; Nogueira et al., 2019). Future studies on the in vivo effects of bioactive molecules delivered by caseins are recommended.

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Highlights

- Caseins as natural nanocarriers to deliver bioactive molecules.
- Tailored the degree of the interactions.
- Possibility to create innovative functional foods.

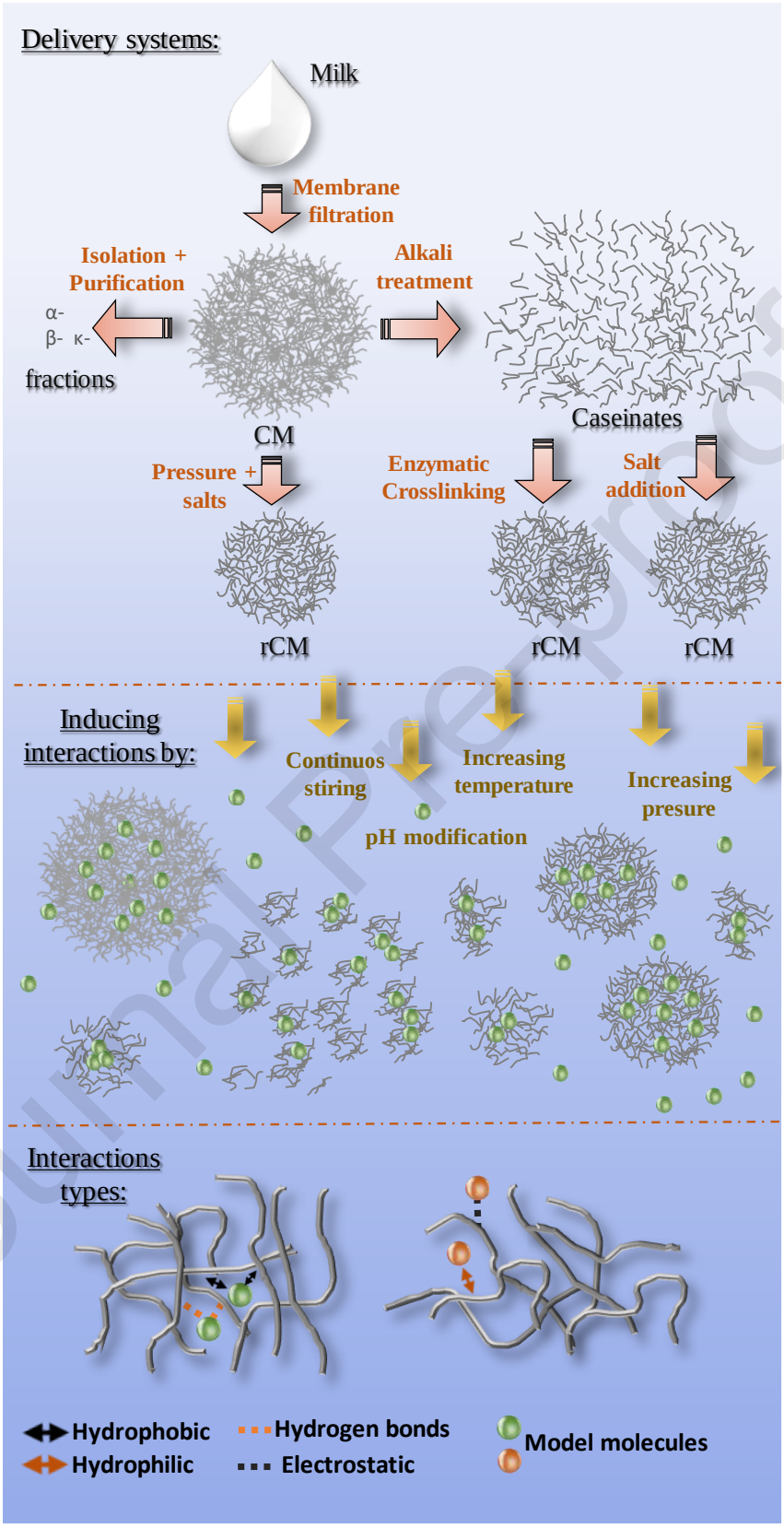

Figure 1. Schematic representation of casein delivery systems.

Table 1. Type of bioactive molecules, casein types and methods employed to study the casein-bioactive interactions. Sodium caseinate - CasNa; re-assembled casein micelles - rCMs; native casein micelles - CMs; casein nanoparticles.

Group	Molecule	Casein organization	Techniques	Interactions	Reference
vitamins	vitamin A	CasNa	UV-Vis spectroscopy, CD, FS	Hydrophobic $5 \times 10^5\ M^{-1}$	(Yi et al., 2016)
		rCMs	Column gel filtration, DLS		(Loewen et al., 2018)
		rCMs	TEM, SAXS		(Jarunglumlerta et al., 2015)
	lutein	CMs and CasNa	DLS, FS	Hydrophobic $10^5\ M^{-1}$	(Mantovani et al., 2020)
	Retinyl acetate	CMs	PCS, DLS		(Blayo et al., 2014)
	β-Carotene	casein / gum tragacanth	FTIR, SEM, CLSM		(Jain et al., 2016)
	Vitamin B9 (folic acid)	β-casein	FS, AS, CD	Hydrophobic $10^5\ M^{-1}$	(Zhang et al., 2014)
		CasNa	In vivo and in vitro release, SEM,	Hydrophobic	(Penalva et al., 2015)
	vitamin D_3	rCMs	Column gel filtration, DLS		(Loewen et al., 2018)
		rCMs	simulated digestion		(Cohen et al., 2017)
		rCMs	rheological and sensorial analysis		(Levinson et al., 2016)
		β-casein	FS	$5.8 \times 10^4\ M^{-1}$	(Bahri et al., 2019)
	vitamin D_2	CMs	RP-HPLC		(Menéndez-Aguirre et al., 2014)
		CMs	DLS, TEM, simulated digestion, sensory evaluation		(Moeller et al., 2018)
polyphenols	curcumin	α- casein	FTIR, CD, FS, molecular docking	Hydrophilic and hydrophobic; $2.8 \times 10^4\ M^{-1}$	(Bourassa et al., 2013)
		β-casein	FTIR, CD, FS, molecular docking	Hydrophilic and hydrophobic; $3.1 \times 10^4\ M^{-1}$	(Bourassa et al., 2013)
		β-casein	FS	$23.5 \times 10^4\ M^{-1}$	(Bahri et al., 2019)
		CasNa	FS,	Hydrophobic; $13.98 \times 10^4\ M^{-1}$	(Ghayour et al., 2019)
		CasNa	DLS, cancer cells HCT-116		(Pan et al., 2014)
		CMs	SAXS		(Khanji et al., 2018a)
		CMs	FS, DLS, SAXS	Hydrophobic; 0.6×10^4 to $6.6 \times 10^4\ M^{-1}$	(Khanji et al., 2015)
		CMs	DLS,FS, simulated digestion	Hydrophobic;	(Benzaria et al., 2013)

			0.56×10^4 to 1.12×10^4 M^{-1}	
	CMs	FS		(Khanji et al., 2018b)
	CasNa	DLS, *In vitro* release properties		(Kumar et al., 2016)
anthocyanins	α- and β-casein	FS, FTIR, CD	Van der Waals forces or hydrogen bonding 0.51×10^3 M^{-1}	(He et al., 2016)
	CasNa	DLS, FS	Electrostatic forces van der Waals bonds hydrophobic $105 - 10^6$ M^{-1}	(Casanova et al., 2018)
	CMs	DLS, rheological analysis		(Nascimento et al., 2020b)
	CasNa	FS, CD		(Arroyo-Maya et al., 2016)
resveratrol	β-casein	CD, FS	Hydrophobic 7.33×10^5 M^{-1}	(Gorji et al., 2015)
	CasNa	SDS-PAGE, rheological analysis		(Consoli et al., 2018)
	CasNa	DLS, FS		(Cheng et al., 2020)
protocatechuic acid, coumaric acid	CMs	SDS-PAGE, HPLC		(Zhou et al., 2015)
rosmarinic	α-s1, β and k-casein	DLS, FTIR, DSC	Hydrophobic, hydrogen bonding and dipole – dipole	(Ferraro et al., 2015)
EGCG	CMs	Human colorectal cancer cells (HT-29)		(Haratifar et al., 2014a)
	CMs	SDS-PAGE, cell proliferation assays		(Haratifar et al., 2014b)
	CMs	HPLC, rheology		(Haratifar & Corredig, 2014)
	CMs	adenocarcinoma cells (HT-29), SDS-PAGE		(Guri, et al., 2014)
EGCG, EGC, EGC, procyanidin A1, procyanidin B1, procyanidin B2	α- casein	FS	8.36×10^3 M^{-1} 1.66×10^3 M^{-1} 1.32×10^3 M^{-1} 2.15×10^3 M^{-1} 1.81×10^3 M^{-1} 1.64×10^3 M^{-1}	(Bohin et al., 2014)
EGCG, EGC, EGC, procyanidin B1, procyanidin B2	β-casein	FS	13.9×10^3 M^{-1} 1.35×10^3 M^{-1} 1.50×10^3 M^{-1} 2.11×10^3 M^{-1} 1.56×10^3 M^{-1}	(Bohin et al., 2014)
naringenin	β-casein	UV-Vis spectroscopic, FS, DLS	Hydrophobic interactions, van der	(Moeiniafshari et al., 2015)

				Waals forces and hydrogen bonds; 10^4 M^{-1}	
	quercetin	CasNa	FS	Hydrophobic 4.88 x 10^4 M^{-1}	(Ghayour et al., 2019)
	genistein	α- casein	FTIR, CD, FS, molecular docking	Hydrophobic; 1.8 × 10^4 M^{-1}	(Bourassa et al., 2013)
		β-casein	FTIR, CD, FS, molecular docking	Hydrophobic; 3.0 × 10^4 M^{-1}	(Bourassa et al., 2013)
	p-coumaric acid	β-casein	FTIR, CD, FS, molecular docking	4.8 × 104 M−1	(Kaur et al., 2018)
	ferulic acid	β-casein	FTIR, CD, FS, molecular docking	2.9 × 10^4 M^{-1}	(Condic et al., 2019)
ipids	phosphatidylcholine, lyso-phosphatidylcho-line, phosphatidylethanolamine, and sphingomyelin,	CMs	UPLC-HRMS		(Cheema et al., 2015)
	CHOL, DOTAP, DDAB, DOPE	α- caseins	FS, FTIR, molecular simulation	Hydrophilic and hydrophobic; 1.0 × 10^4 M^{-1} 1.5 × 10^4 M^{-1} 2.0 × 10^4 M^{-1} 5.0 × 10^3 M^{-1}	(Bourassa et al., 2014)
		β-caseins		Hydrophilic and hydrophobic; 1.0 × 10^4 M^{-1} 2.1 × 10^4 M^{-1} 1.7 × 10^4 M^{-1} 1.5 × 10^3 M^{-1}	Bourassa et al., 2014)
	4-(Dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4H-pyran, 8-anilino-1-naphthalenesulfonic acid	CMs	DLS, FESEM, CD, TCSPC, CLSM		(Panja et al., 2018)
	soy phospholipids	CasNa	DSC, ESR, SAXS		(Semenova et al., 2016)
proteins	lysozyme	CMs, NaCas	turbidity measurements, DLS, ELS, CLSM, fluorescence anisotropy, CD		(Antonov et al., 2017)

lactoferrin and lysozyme	CMs	SDS-PAGE, DLS	(Anema & (Kees) de Kruif, 2015)

CD - circular dichroism, FS - fluoresce spectroscopy, *TEM - transmission electron microscopy*, SAXS - small angle x-ray scattering, PCS - photon correlation spectroscopy, DLS - dynamic light scattering, FTIR - Fourier transformed, SEM - scanning electron microscopy, CLSM - confocal laser scanning microscopy, AS - adsorption spectroscopy, RP-HPLC - liquid chromatography and reverse-phase separation, HPLC - High performance liquid chromatography, UPLC-HRMS - ultra-performance liquid chromatography-high resolution mass spectrometry, FESEM - f*ield emission scanning electron microscopy, TCSPC - time-correlated single-photon counting,* ESR - electron spin resonance, *ELS - electrophoretic light scattering.*

Declaration of competing interest

The authors declare that there are no conflicts of interest.