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Colloidal properties of milk and plant-based milk alternatives: A structural perspective

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Plant-based milk alternatives (PMAs) are less nutritious and more difficult to standardise than dairy milk. Their low colloidal stability is one of the major challenges hindering efforts to enhance their nutritional value by increasing components such as protein and lipids. This property often leads to undesirable phenomena such as phase separation, sedimentation, and creaming, all of which affect product acceptability, manufacturing processes, and even digestibility. This article outlines the colloidal properties and structural characteristics of protein particles and fat globules/oil droplets in both cow milk and PMAs, highlighting the differences in their behaviours during processing and digestion. It also presents strategies to formulate PMAs with colloidal properties that more closely resemble those of cow milk, particularly in terms of stability under processing conditions. Finally, it proposes the use of hybrid protein particles containing a combination of plant and milk proteins, which exhibit unique structural features, improved functional properties, and distinct digestion behaviours, to improve PMA characteristics.

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Introduction

Plant-based milk alternatives (PMAs) are an option for consumers who cannot consume cow milk (e.g. those with lactose intolerance and/or protein allergies) and/or wish to reduce dairy consumption for ethical, environmental, or religious reasons [1]. PMAs can be obtained from numerous plant materials, but the most common in the market are those made from soybeans, rice, oats, coconuts, and almonds. Depending on their source, PMAs can provide substantial amounts of proteins, fibre, phenolic compounds, unsaturated fatty acids, and bioactive compounds such as phytosterols and isoflavones [2]. Furthermore, PMAs do not contain certain components traditionally found in cow milk, such as cholesterol, saturated fatty acids, and lactose, which could be detrimental for health. However, PMAs are nutritionally deficient compared to cow milk; they contain lower levels of proteins, and these are of low quality and lack key essential amino acids [3,4]. The colloidal stability and palatability of PMAs are remarkably different from those of cow milk, and it is very challenging to mimic such dairy attributes, which are desired by consumers and the food industry [5].

From a colloidal science perspective, PMAs are oil-in-water emulsions of plant materials prepared by processing plant raw material into a paste by mechanical force, to remove coarse particles or break them down to smaller particles, and subsequent homogenisation [6]. Most proteins in PMAs exist as aggregates of various particle sizes, and these particles can either be adsorbed at the interface of oil droplets or dispersed in the aqueous phase. Other particles in PMAs can originate from starch, fibre, and other cellular materials, which tend to be dense and can aggregate and sediment over time. Lipids can be present as oleosomes (lipid storage organelles in plants) or small oil droplets fabricated by processing. The size of the oil droplets in these emulsions depend on the emulsifying agents in the PMA formulations, such as proteins and other additives. The total number of droplets and their size and arrangement in space can all change over time [7]. The stability of the emulsion depends upon the droplet size and degree of flocculation. In contrast, cow milk is a nature-designed oil-in-water emulsion mainly composed of milk fat globules dispersed in a serum containing caseins, whey proteins, lactose, and minerals. Proteins such as caseins exist in micellar particles (casein micelles), which remain colloidally stable in native form and under food processing conditions. In cow milk, the particle size distribution and degree of flocculation is altered by processing steps such as homogenisation.

The colloidal stability of PMAs is lower than that of cow milk. In principle, the protein particles and other components in PMAs lack a mechanism to maintain colloidal stability similar to that of proteins in cow milk. It is often necessary to incorporate additives, such as stabilisers, and optimal storage conditions to maintain colloidal stability during the product's shelf life. The casein micelles in cow milk have a unique structure that is stable and very resistant to processing conditions. Furthermore, whey protein (20 %) is water-soluble, despite the denaturation and aggregation that happens under heat treatment.

Many studies have highlighted the need for improving the solubility and stability of plant proteins in PMAs through various modifications [8]. Efforts are being made to develop more colloidally stable systems by gaining a better understanding of the structures, stability mechanisms, and colloidal properties of plant proteins. Homogenisation and heat treatment are necessary during the production and processing of cow milk and PMAs. However, these processes change the properties and structures of proteins and lipid droplets in all types of milk, and may thereby cause gelation and/or sedimentation. Processing can also influence the behaviour of PMAs and milk in the gastrointestinal track and subsequent nutrient delivery. However, there is limited understanding on the digestion and absorption of components from PMAs and cow milk, which are related to their colloidal behaviours under gastrointestinal conditions [9].

This review presents the current understanding on the differences between cow milk and PMAs, focusing on their properties, stability, and digestibility from a colloidal perspective and specifically examining protein particles and fat globules or oil droplets. It explores efforts to enhance the formulation and production of PMAs to more closely mimic the colloidal characteristics of cow milk, such as high stability during processing and storage. Additionally, the review considers how these advancements influence consumer acceptance and the overall consumption of plant-based milk.

Proteins in milk and PMAs

Colloidal properties of proteins in milk

Cow milk contains approximately 3–4% protein. Of this, around 80 % is caseins, and 20 % is whey protein. Whey proteins include β-lactoglobulin, α-lactalbumin, and bovine serum albumin. These proteins are water-soluble and generally exist as globular molecules in milk. In contrast, caseins, owing to their unique amino acid composition, mostly form stable, colloidal particles dispersed in milk, known as casein micelles. These micelles are structurally and functionally stable, both internally and within the milk serum [10]. Their diameter ranges from 50 to 500 nm, with molecular weights from 10^6 to 10^9 kDa. They consist of numerous casein molecules along with ∼6 % calcium phosphate and other metal ions. Casein micelles are considerably stable under processing steps such as commercial homogenisation and heat treatment. They do not coagulate upon heating at 140 °C for <20 min at pH 6.7. The size of casein micelles does not change under homogenisation pressures up to 200 MP and temperatures up to 50 °C [10].

The high stability of casein micelles is attributed to their unique structure, which has been debated for decades, with several models being proposed. However, one consistent feature across models is that κ-casein, being more hydrophilic, resides on the surface of the micelles [11]. Through hydrophobic interactions and calcium phosphate nanocluster linkages, casein molecules interconnect to form the various proposed structures. It is believed that the hydrophilic C-terminal region of κ-casein protrudes from the micelle surface, forming a layer about 5–10 nm thick, giving the micelle a 'hairy' appearance. This hairy layer functions as an ionic brush, contributing significantly to micelle stability owing to a zeta potential of approximately −20 mV and steric stabilisation, which prevents the micelles from interacting and aggregating in milk. This surface architecture, dominated by κ-casein, not only stabilises the micelles sterically but also helps maintain the structural integrity of the micelle interior.

Caseins can form stable micelles with each other because of their basic amino acid composition and sequence. An important feature of the amino acid sequence of caseins is that the hydrophobic and hydrophilic residues are not distributed uniformly; caseins are categorised as amphipathic proteins based on the segregation of their hydrophilic and hydrophobic regions [12]. β-Casein is considered more hydrophobic than α_{S1}-casein, α_{S2}-casein, and κ-casein. This feature, coupled with their open flexible structure, enables caseins to exhibit good surface activity and foaming and emulsifying properties, making them the functional protein of choice for many applications. Moreover, because of their open structure, caseins have a high specific volume and, consequently, form highly viscous solutions. Another unique and important feature of caseins, especially β-casein, is the large number of Pro residues, which greatly affect casein structure. The presence of Pro residues disrupts the formation of α-helices and β-sheets [13]. Hence, α_{S1}-, α_{S2}-, and β-caseins have very few secondary and tertiary structures. In contrast, κ-casein has a distinct secondary structure and a strong capacity for polymerisation. Caseins are considered rheomorphic or intrinsically disordered proteins, meaning that they have open conformations with a backbone containing flexible side chains. Therefore, caseins adapt their structure to environmental needs

[14]. However, if the hairy layer of micelles is removed during processing, such as through specific hydrolysis of κ-casein, the colloidal stability of the casein micelles is destroyed, and they aggregate. Casein micelles can be coagulated by decreasing the pH to <5, at which the colloidal calcium particles dissolve and the surface charge becomes close to the isoelectric point (pI) of the milk protein.

Colloidal properties of proteins in PMAs

PMAs are suspensions or emulsions containing water-soluble extracts from some plant materials, most commonly oilseeds, cereals, pseudocereals, seeds, and/or legumes. The production steps of these extracts are similar, and include soaking, blending, cooking and grinding, starch hydrolysis, and homogenisation/heat treatment. The composition and concentrations of components in PMAs depend on the plant source, processing conditions, and component colloidal stability. Low stability hinders the increase in concentration of components in PMAs, which limits their nutritional value.

The proteins in PMAs are released from plant cells and protein bodies (PBs) when they are disrupted by soaking and grinding. The extent of disruption influences the size of protein particles in the extract and PMAs. Large particles denser than water (sediment) are likely to be undissolved protein, starch, fibre, and other cellular material. Protein in particle form is important for the colloidal stability of PMAs. The structure and colloidal properties of protein particles and oil droplets are dependent on the natural structure and behaviours of PBs during the processing steps of PMAs.

Structure and characteristics of PBs

Natural PBs are subcellular organelles occurring in the storage tissues of seeds. Depending on the plant species, they range in diameter from 1 to 20 μm, and can contain various structural inclusions. These spherical organelles contain an amorphous protein matrix bounded by a single membrane. Structural inclusions embedded in the matrix may be one to several protein crystalloids and phytin globoids [15]. The PB structure observed in plant cells does not reflect the structure and properties of the proteins in suspension of PMAs. Intact PBs have been successfully isolated using differential centrifugation or density gradient centrifugation methods involving aqueous or nonaqueous extraction media [16]. The choice of methods to isolate PBs from a certain seed tissue depends on the ontogenic stage of the organelles within the tissue. For example, PBs have been isolated from hemp seeds using efficient environmental-friendly extraction via a sonication-assisted aqueous enzymatic method with density gradient centrifugation [17]. Hemp PBs are membrane-bound and contain a large crystalloid, globoids, and other minor constituents. Detailed microscopic examination has shown that hemp PBs exhibit a spherical shape with an average diameter of about 4.6 μm. Their structure consists of a protein crystalloid and several phytin globoids, all surrounded by a proteinaceous matrix and a single membrane. SDS-PAGE has revealed that the globulin edestin is the most abundant storage protein in hemp PBs. Dispersions of hemp PBs exhibit excellent colloidal stability only at neutral pH; they aggregate and/or solubilise at other pH levels. These PBs also exhibit irreversible structural changes in response to pH variation. Specifically, little to no swelling of the particles is observed at pH 5 (around the pI of the hemp protein). However, when the pH shifts away from the pI, swelling, rupture, and eventual dissolution of the particles are pronounced, under both extreme acidic and alkaline conditions [17].

Formation and evolution of protein aggregates during PMA processing

The formation of protein aggregates in PMAs begins during the initial soaking and grinding stages, which are designed to break down plant tissues and release intracellular proteins into the aqueous phase. These steps are widely applied to a range of raw materials, including seeds, nuts, and legumes [2]. During these steps, cellular structures such as PBs and storage vacuoles swell, soften, and are disrupted, thereby liberating proteins [18]. The mechanical forces generated during wet grinding also expose proteins to other intracellular components, such as polysaccharides, lipids, and fibre, promoting the formation of large, heterogeneous protein−polysaccharide complex matrices [19,20]. Following soaking and grinding, these complexes may include protein aggregates, fat globules, fragmented plant cells, and other particulate debris [21].

These initial complexes typically have particle sizes ranging from a few micrometres to tens of micrometres with low zeta potential values, indicating limited surface charge and weak electrostatic repulsion [1]. Therefore, large aggregates may associate with fat droplets, air bubbles, or other suspended solids, leading to flocculation, phase separation, or sedimentation of the final PMA product [1]. To be specific, Mu et al. [22] reported that soybean-sourced PMAs exhibit a mean particle size of ∼2.47 μm, which increases to ∼7.96 μm after 28 days of storage, due to protein aggregation. Similarly, in a recent study on peanut-sourced PMAs, the average particle size after soaking and grinding was found to be ∼48.9 μm [23]. Low particle sizes of the suspension are essential to maintain PMA stability. Therefore, further processing techniques are necessary to break down complexes in PMAs.

Protein aggregates from isolated plant proteins

Plant protein isolates and concentrates, commonly used in commercial PMA formulations as functional and

nutritional ingredients to enhance the amount of protein, are typically obtained through salt or alkaline extraction followed by isoelectric precipitation [5,7]. Alkaline extraction combined with isoelectric precipitation is the most widely used method; however, it often leads to irreversible aggregation [24]. The insoluble protein aggregates can further combine with other particles present in PMAs (e.g. starch and fibre), resulting in large, poorly dispersible complexes in aqueous systems. This frequently causes sedimentation, thereby reducing the physical stability of the suspension [25]. During actual PMA production, the solubility and aggregation behaviour of these pre-existing protein aggregates vary depending on subsequent processing steps such as homogenisation and heat treatment [26]. The poor dispersibility of plant protein isolates and their aggregates can severely limit their functionality in PMAs unless appropriate modification strategies are applied.

Stability of protein particles after mechanical and thermal treatments

Following dispersion, treatments such as homogenisation and heating play crucial roles in developing the acceptable dispersion and emulsion properties that enhance stability and functionality in PMAs [27]. Typically, high-pressure homogenisation (HPH) is applied to produce stable PMAs; this process breaks down large protein aggregates and reduces the average particle size to a few hundred nanometres or several micrometres [19]. He and Xu [28] applied HPH at 30 MPa for 10 min to PMAs derived from adzuki beans, adlay, and oats. The treatment significantly reduced the particle size ($D_{4,3}$) from ∼29.15 to ∼14.03 μm, ∼15.39 to ∼5.50 μm, and ∼5.52 to ∼2.98 μm in adzuki bean, adlay, and oat PMAs, respectively. This reduction in particle size enhanced long-term stability, as protein aggregates and oil droplets were mechanically disrupted into finer particles, forming a more uniform and possibly more stable colloidal dispersion [29].

As with conventional milk, heat treatment is commonly applied after homogenisation to extend the shelf life and ensure the microbiological safety of PMAs [30]. However, heating time and temperature must be carefully controlled, as excessive heat may induce protein aggregation and compromise colloidal stability. Bernat et al. [31] investigated the effects of thermal treatment on almond and hazelnut milk at two conditions: 85 °C for 30 min and 121 °C for 15 min. They observed that both treatments significantly increased the particle size, with more pronounced aggregation under the higher temperature condition. Specifically, the particle sizes ($D_{3,2}$) of almond milk and hazelnut milk increased from ∼5.2 to ∼6.5 μm to ∼24.5 and ∼17.9 μm, respectively. This is because higher temperatures induce more protein denaturation, which leads to aggregate formation through disulfide crosslinking and hydrophobic association [32]. Therefore, although homogenisation and heat treatment are both essential for production of PMAs, processing conditions must be carefully optimised to avoid adverse effects such as protein re-aggregation and oil droplet recoalescence. Many efforts have been carried out to fabricate and modify protein aggregates to structures which can stabilise the protein particles and oil droplets during processing and storage, by mimicking the structures of protein particles in milk.

Strategies to modify and stabilise plant protein aggregates in PMAs

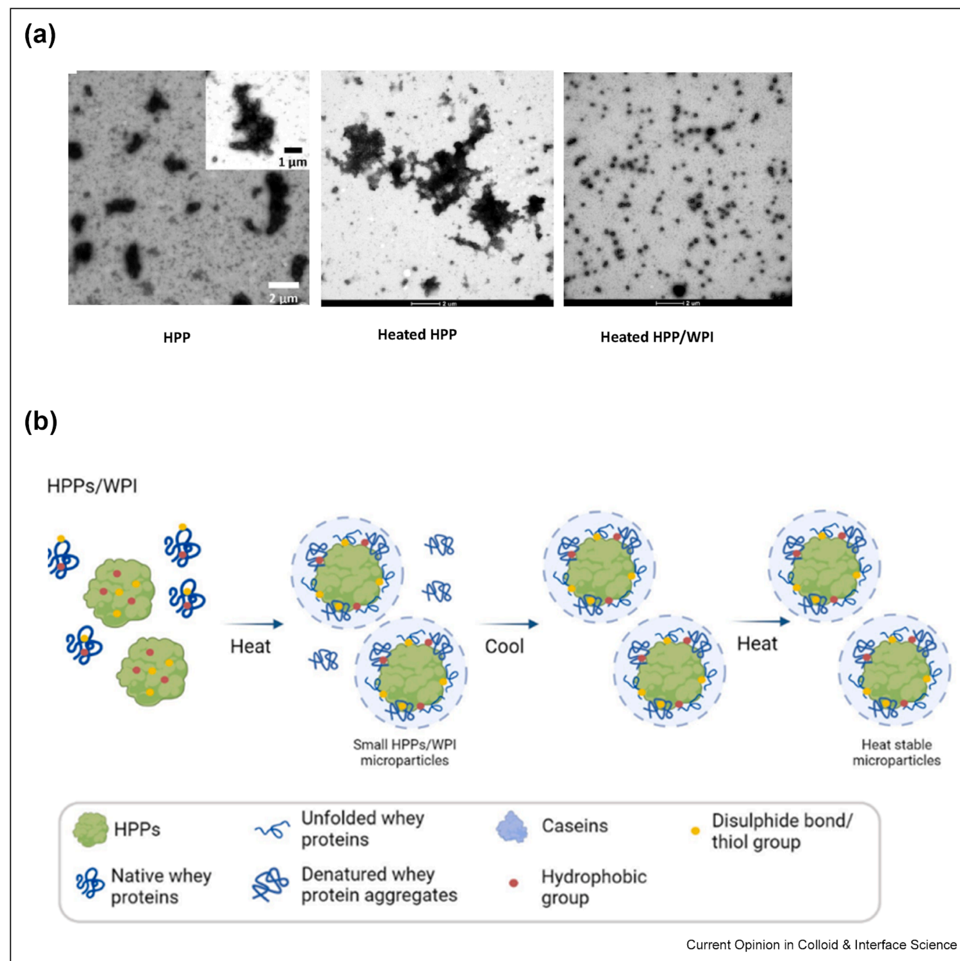
Engineering protein interactions for colloidal stability

A variety of physicochemical strategies have been developed to improve the stability and techno-functionality of PMAs. These include ultrasonication [33], HPH [21], and ultra high-pressure homogenisation (UHPH) [34]. For example, Salve et al. [33] ultrasonicated a peanut-based milk analogue at 200−400 W (20 kHz for 3 min); this treatment significantly reduced the particle size (D_{50}) from ∼0.29 μm (untreated) to ∼0.02 μm (400 W). Consequently, no visible phase separation or sedimentation was observed in the ultrasonicated peanut PMAs after 48 h of storage at 6 °C. Vogelsang-O'Dwyer, Sahin [21] homogenised blue lupin, white lupin, and soy PMAs at 18 and 78 MPa, and demonstrated that the increase in homogenisation pressure reduced the particle size ($D_{4,3}$) from a few micrometres to below one micrometre, resulting in improved colloidal stability across all sources. Similarly, Sidhu and Singh [34] applied UHPH at 276 MPa to soy-based PMAs for 12.48 s; this treatment significantly reduced the particle size ($D_{4,3}$) from ∼129.86 to ∼19.74 μm and prevented phase separation during 28 days of storage at 4 °C. The improvement was attributed to the formation of smaller and maybe more stable particles that suppressed further aggregation of suspended protein aggregates and solids [35]. However, the authors did not propose a mechanism behind the stabilisation against protein aggregation after further treatment.

Hybrid casein/whey protein and plant protein to produce stable protein particles

Researchers have studied combinations of plant and milk proteins to enhance the techno-functional properties of plant proteins. For example, a hybrid protein system combining hemp and whey proteins at a 1:1 ratio effectively inhibited further protein aggregation during continuous heat treatment (95 °C for 20 min), suggesting its potential for application in PMA formulations [36]. The authors employed a microparticulation method to produce hybrid microparticles by complexing hemp protein and whey protein isolate (WPI) via heating at 95 °C for 20 min with microfluidisation. These hybrid microparticles exhibit higher heat stability compared with that of hemp protein particle (HPP)

Figure 1



(a) Transmission electron microscopy images of unheated/heated hemp protein particles (HPPs) and heated HPP/WPI. **(b)** Schematic representation of possible mechanisms by which HPPs/whey protein interact during heat treatment (95 °C, 20 min) and cooling. Adapted from Ma et al., 2025 [36].

dispersions (Fig. 1a). When the HPPs and WPI are co-heated, the denatured whey proteins may either self-aggregate or interact with the HPP surfaces. The association of WPI on the surface of HPPs can restrict further HPP aggregation as denatured WPI occupies some reactive sites on the HPP surfaces (Fig. 1**b**). The resulting HPP/WPI particles are stable because of the irreversible nature of the disulphide bonding between HPPs and WPI. Chuang et al. [37] reported that the solubility of hemp protein improves upon blending it with sodium caseinate and treating with a pH-cycling process, wherein the pH is adjusted to 12 and the reaction is conducted for 1 h, followed by neutralisation to pH 7. Nanoparticles composed of hemp protein and caseinate (Z-average diameter ≈ 130 nm, zeta potential ≈ −17 mV) formed after the pH cycling, and the solubility of hemp protein increased from <20 % to >80 %. The nanoparticles were heat-stable because the Z-average diameter was only 229 nm after heating (90 °C, 30 min). These soluble and heat-stable hybrid protein nanoparticles were then used to increase the plant protein content in beverages and emulsions [36]. From an application perspective, Samarathunga et al. [38] formulated plant milk analogues using various ratios of Spirulina protein concentrate (SPC) and milk protein concentrate (MPC), with total protein, oil, and lactose concentrations of 3.3 %, 3.5 %, and 5 %, respectively. Compared to the other tested ratios, the hybrid formulation containing 25 % SPC and 75 % MPC exhibited superior physicochemical and sensory properties, demonstrating the feasibility of using hybrid protein systems to improve PMA characteristics.

Glycosylation and phosphorylation

Glycation (or glycosylation) is a particularly important chemical reaction in food applications, as it is generally recognised as a safe method [39]. It is a process often associated with the early stages of the Maillard reaction,

involving the covalent bonding of ε-amino groups in proteins with carbonyl groups from reducing sugars. This reaction can produce protein–polysaccharide complexes and other glycoconjugates, which can significantly modify the physicochemical and functional properties of plant proteins [40,41]. The outcomes of glycation-induced protein modification are highly dependent on the reaction conditions, including the temperature, sugar type, reaction time, pH, and protein-to-glucan ratio [41]. For example, Schneider et al. [42] implemented an endogenous Maillard-induced glycation strategy by reacting pea protein isolate with maltodextrin derived from pea starch. The resulting partially glycated purified PPI exhibited dramatically enhanced solubility (up to ∼90 %), improved thermal stability under acidic conditions, and high digestibility, demonstrating its potential for application in PMAs.

Chemical phosphorylation, once widely applied in the 1980s, has recently regained attention, particularly for modifying plant proteins such as those from cereals. This process involves the covalent attachment of phosphoryl groups (PO_3^-) to reactive amino acid residues in protein molecules. Residues containing –NH, –OH, or –SH side groups (e.g. serine, threonine, tyrosine, and histidine) can undergo phosphorylation [43]. The degree of phosphorylation is primarily influenced by the protein type, the phosphorylating agent, and the reaction conditions. In the food industry, the most commonly used phosphorylating agents include sodium tripolyphosphate (STP), sodium trimetaphosphate (STMP), and phosphorus oxychloride ($POCl_3$) [44]. STP and STMP are most effective under alkaline conditions (pH > 9) and moderate temperatures (35–70 °C), whereas $POCl_3$ functions under milder conditions. Phosphorylated proteins exhibit high thermal and pH stability (up to 120 °C and pH 2.0–10.0), making them suitable for a wide range of food applications [45]. For instance, Guo, Liu [46] treated 10 % (w/v) soy protein isolate (SPI) with STP and applied the phosphorylated SPI to stabilise oil-in-water emulsions with an oil fraction of 25 % (v/v). The emulsions prepared with modified SPI exhibited a significantly enhanced emulsifying ability (increasing from ∼25 to ∼38 m^2/g) and improved stability (Emulsifying Steadiness Index increasing from ∼18 to ∼90 min), compared to those stabilised with native SPI. These findings highlight the potential of phosphorylated SPI for application in PMAs.

Lipids in milk and PMAs

For both milk and PMAs, the colloidal state and stability of lipids in an aqueous medium represent another consideration beyond the stability of protein particles. To retain lipid stability in PMAs, wherein lipids exists in emulsions, fat or oil droplets are stabilised in a suspension liquid by breaking plant tissue and processing natural oils in plant seeds into smaller, stable fat/oil droplets. These droplets in the emulsion decide the appearance, colour, texture, and shelf life of PMAs.

Fat globules in milk

Bovine milk contains approximately 4–5% fat. The fat in cow milk exists in the form of globules ranging in diameter from 0.1 to 10.0 μm; their size influences the stability of milk in creaming, flocculation, coalescence, and some aspects of lipolysis [47]. Each fat globule of milk is surrounded by a thin protective layer, generally called the milk fat globule membrane (MFGM). The mass of this membrane is about 2 % that of the whole fat globule. The membrane (approximately 10 nm thick) consists of a complex mixture of proteins, phospholipids (PLs), glycoproteins, triglycerides, cholesterol, enzymes, and other minor components [48]. All interactions between fat and plasma must occur across this membrane. Many properties of milk, such as its stability and acceptability are directly related to this unique membrane system. The MFGM is markedly affected by processing treatments such as cooling, heating, and homogenisation [49].

When milk is homogenised, the fat globules are broken up and reduced in size from 1–10 μm to 0.2–0.5 μm. Smaller fat globules are more resistant to creaming and coalescence. The size reduction of fat globules involves the destruction of the original MFGM; subsequently, the fat globule surface is largely covered by micellar casein and whey protein [50]. The fat globules covered by the original MFGM and those within casein micelles elicit differences in the properties and functionality of milk. For example, the fat globules covered by casein micelles mostly resemble large casein micelles in their properties, and tend to interact with the other components in milk serum during processing and digestion steps such as heat treatment, acidification, renneting, and coagulation induced by pepsin in the stomach [51]. The micellar caseins adsorbed on the surface of fat globules show greater reactivity in aggregation reactions than casein micelles in serum [52].

The surface charge of fat globules, as estimated by zeta potential, is ∼ –13 to –14 mV in unhomogenised bovine milk [53] and ∼ –20 mV after homogenisation [53]. From a colloidal stability point of view, milk fat globules with high zeta potentials are electrically stabilised, whereas those with low zeta potentials tend to coagulate or flocculate [54]. The stability of milk fat globules against flocculation is governed not only by electrostatic repulsion but also by steric repulsion provided by the highly hydrophilic moieties of glycoproteins protruding from the globule membranes. It has been reported that hydrolysis of these glycoproteins by papain causes flocculation of milk fat globules [55]. In addition, Shimizu et al. [55] reported that removal of

the polar heads of PLs in milk fat globule membranes by phospholipase C results in oiling-off; hence, it appears that the polar heads of PLs also play an important role in the stability of milk fat globules against coalescence.

Oil bodies (OBs) in PMAs

OBs of plants

In plants, triacylglycerols (TAGs) are stored in OBs, which are present as discrete organelles within plant cells [56]. The diameters of OBs in oil seeds and some fruits are about 0.6–2.0 μm and up to 10–20 μm, respectively [57,58]. Similar to milk fat globules, OBs consist of a core of up to 95 % (w/w) TAGs that is stabilised by a 2–4 nm thick membrane [59].

The interfacial membrane contains anchored proteins, i.e., oleosins, caleosins, and steroleosins; a PL monolayer; glycosyl groups; and some minor bioactive components [60]. The average composition of an OB membrane (OBM) is 33.0–49.1 % PL and 50.9–67.0 % protein [56]. Oleosins (15–26 kDa), caleosins (25–32 kDa), and steroleosins (33–50 kDa) are three kinds of membrane proteins, accounting for 80–90 %, 10 %, and 1–10 % of the total membrane protein content, respectively. OBs are resistant to digestion by phospholipase A2 and phospholipase C but are digested by trypsin [61]. This suggests that the PL monolayer on the surface of an OB is entirely covered by membrane proteins in such a way that the OB is not accessible by phospholipases [61]. This conformational structure of the OBM components enables electrostatic interactions between negatively charged PLs, i.e., phosphatidylserine and phosphatidylinositol, and positively charged amino acid residues [56].

Formation and stability of OBs in PMAs

Natural OBs are not disrupted by soaking and grinding during milk extraction and processing at neutral pH and mild conditions, whereas cells and protein storage vacuoles are. The extrinsic proteins, including globulins and albumin, bound to intact OBs can be removed by washing during extraction. Soaking is a key step in avoiding OB damage during grinding; soaking time in water influences the extent of OB damage during grinding [62]. OBs obtained from rapeseeds soaked for less than 8 h are damaged because incomplete hydration makes the matrix constituents highly rigid. Longer soaking times of 16–24 h have traditionally been used for the extraction of OBs [63]. However, the optimal soaking time should be determined for each seed, because long soaking times might lead to seed germination. OBs remain intact after grinding, with the protective membranes on their surface dispersing the lipids and shielding them from degradation caused by environmental stresses. The intactness and stability of OBs are dependent on the degree of grinding, which influences the extent of cellular disruption [64]. For example, coconut milk is prepared by pressing grated coconut at pH 6.2; the OBs in freshly extracted coconut milk range in size from 2 to 20 μm and show minimal flocculation [65]. At the natural pH of extraction, the OB suspension in coconut milk has a net charge of −16 mV [65]. The charged surfaces of OBMs have pI values between 4.8 and 6.5, depending on the source; thus, they provides OBs a negative surface charge that leads to electrostatic repulsions [66]. In addition, the presence of oleosins at the interface provides OBs with steric hindrance, protecting the PLs against the action of phospholipases and the OBs from coalescence or aggregation [67].

OBs are generally homogenised to reduce their size from the native >5 μm to ∼0.5 μm for retaining stability in PMAs during processing and storage. Although homogenisation can reduce the OB size to <0.5 μm, which is similar to that of homogenised cow milk, the behaviour of such OBs, especially the colloidal stability, is still different from that of fat globules in cow milk because of the differences in their surface layers. During homogenisation, plant proteins or protein particles and exogenous protein/emulsifiers adsorb onto the newly created OB interface to form a new surface layer incorporating the original OB membrane materials, which influences the stability of OBs and interactions with other components during further processing and storage. Oil droplets coated by many types of plant-based emulsifiers are susceptible to aggregation if they are heated to a high temperature and for a long enough time. In particular, oil droplets coated by many plant-based proteins, such as those from lentils, peas, and faba beans, often aggregate when they are heated above the thermal denaturation temperature of the proteins [68].

Strategies to modify and stabilise OBs in PMAs

Emulsification of oil using OBM materials in PMAs

OB instability is often observed during heat treatment in PMA processing owing to the aggregation of plant protein particles at the surface of the oil emulsions. A promising strategy for stabilising PMA emulsions is to use reconstituted emulsions made with OBMs, leveraging their superior emulsifying and nutritional properties.

Several studies have investigated the emulsifying properties of oleosins, caleosins, steroleosins, and PLs either individually or in combination. Both purified and recombinant OB proteins, in combination with PLs, have been the focus of many studies on creating ‘reconstituted’ or ‘artificial’ OBs [69]. In these studies, OB proteins, PLs, and oil were mixed to create reconstituted OBs that stabilised similar to natural OBs. Unlike steroleosins, caleosins and oleosins were shown to individually stabilise reconstituted OBs in the presence of PLs to mimic the OBMs in natural OBs [70].

The structural configurations of these OB proteins are expected to influence their ability to stabilise emulsions. A central hydrophobic domain with a proline knot motif appears to be the structure required for OB stabilisation; as steroleosins contain a proline knob, but not a proline knot, they cannot successfully stabilise an emulsion. Although the central domain is required, it is not the only structure responsible for stabilisation. Reconstituted OBs stabilised only by the central hydrophobic domain of oleosins are as unstable as those containing no protein, indicating that the stability is dependent on the interaction of several domains between and within oleosins [71].

PLs are also necessary for the formation and stabilisation of reconstituted OBs. The importance of the presence of PLs along with OB proteins in relation to emulsion stabilisation has already been shown [61,71,72]. Reconstituted OBs emulsified by PLs in the absence of oleosins contain smaller droplets, but coalescence occurs readily because of insufficient repulsive electrostatic forces and the subsequent breakdown of the thin interfacial film. When oleosins are used without PLs to emulsify oil droplets, the droplets do not coalesce, but flocculation occurs because of the hydrophobic and van der Waals attractive forces present between oleosin molecules [72]. These results imply that both oleosins and PLs are necessary to stabilise reconstituted OBs synergistically. They interact with each other on OB surfaces through electrostatic interactions, i.e., the positively charged residues of oleosins interact with the negatively charged parts of PLs [56], forming a thick membrane that is resistant to coalescence. Additionally, the hydrophobic interactions and the presence of hydrogen bonds between proteins and PLs offer extra stability to the reconstituted OBs by improving molecular flexibility and viscoelasticity [72]. An important question that arises at this point is the ratio of these components that should be used when reconstituting an OB. To obtain an optimal stabilisation effect, a PL to oleosin ratio close to that in native OBs is desirable [61,72].

Some factors should be considered for the synthesis of artificial OBMs and stabilisation of reconstituted OBs, e. g. the configurational structure of proteins might change once they are extracted from an OB because of the extraction medium to which they are exposed, which might influence the adsorption and emulsifying properties. Capuano, Beaudoin [73] stated that the oleosin structure may be altered after extraction because oleosins are very hydrophobic and form aggregates that can be dissolved only with chaotropic agents or strong detergents. In contrast, Peng, Lin [74] reported that oleosins in native and reconstituted OBs behave similarly in terms of emulsification, by proving that the central hydrophobic anchoring segment is present in both cases after proteinase K digestion. Similarly, Tzen and Huang [61] reported that oleosins may have similar structures in both native and reconstituted OBs, as reflected by their similar sizes and stabilities.

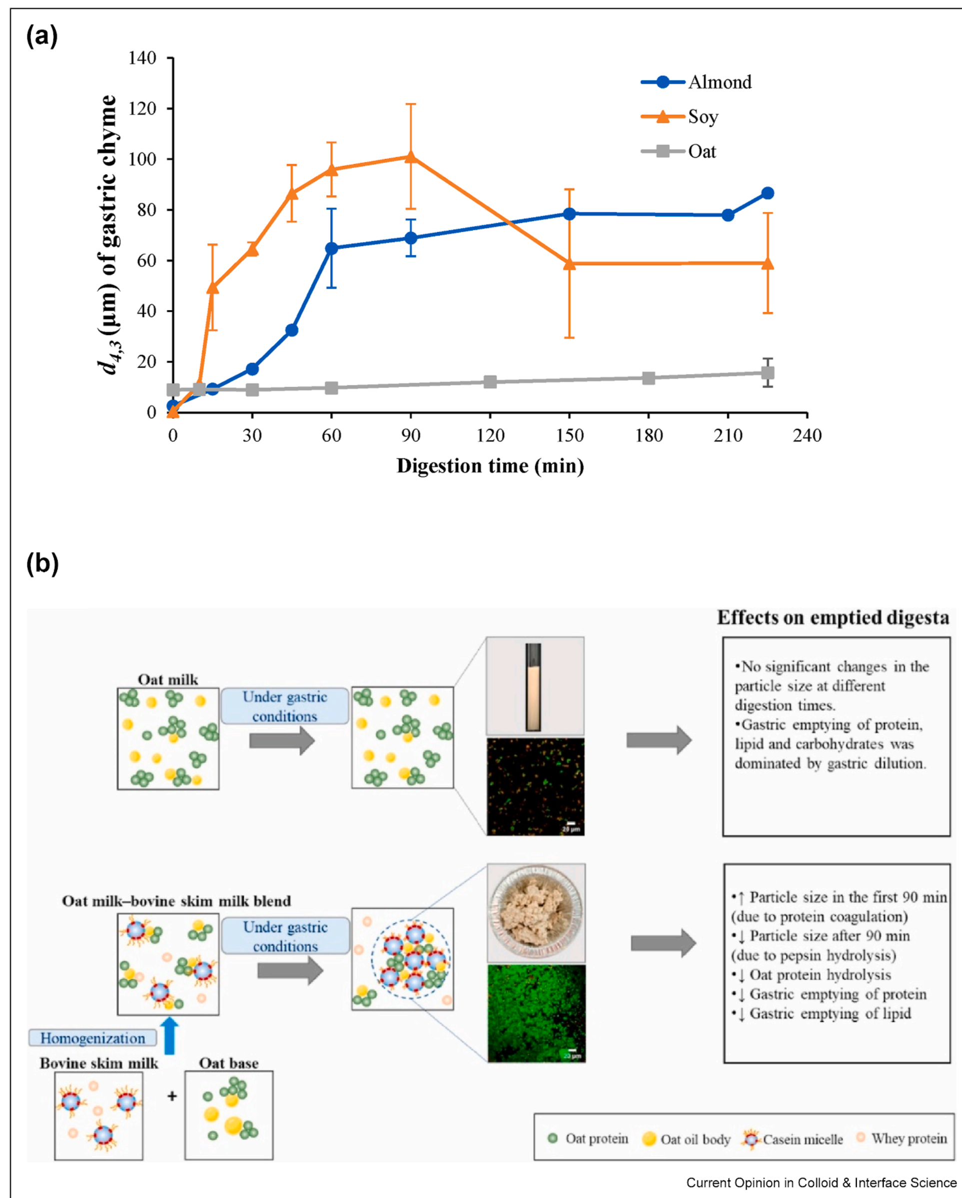
Colloidal behaviour of milk and PMAs during digestion: impact on gastric emptying and digestion rate

Comparisons between cow milk and PMAs have drawn the attention of researchers, not only due to the marked differences in their physicochemical properties but also in their digestion and absorption. Gastric digestion plays a critical role in controlling digestion kinetics. The intragastric stability and food structure/matrix changes during the gastric digestion of milks have important effects on the gastric emptying rate of proteins and lipids and the digestion in the intestines. Liquid foods are generally considered to stay in the stomach for a shorter time because they do not have complex structures to be broken down. However, some liquid foods can remain long in the stomach because of their colloidal instability under gastric conditions; some causes for this phenomenon are creaming of oil/fat, aggregation of proteins, and high viscosity of carbohydrates [75—77]. These changes in liquid foods are induced mainly by interactions between the food components and physiological fluids or among the food components under gastric conditions [78].

Colloidal behaviour of cow milk under gastric conditions

The colloidal behaviour of milk casein micelles in the gastrointestinal tract is different from that of the individual caseins, because casein micelles are coagulated both by pepsin and low pH conditions [77], both of which are present in the stomach. Casein micelles coagulate to form a structured curd before the pH in the stomach drops to a level that can lead to aggregation of the micelles during gastric digestion [51]. This suggests that the milk clotting induced by pepsin is the major mechanism for coagulation of casein micelles during the gastric digestion of milk. Any variation in the casein and mineral composition in milk, depending on the animal source, affects the coagulation properties and the formation of the coagulum [79]. Even slight changes in the ratio of the individual caseins and the specific genetic variants of each casein can affect the enzymatic coagulation properties of milk [80]. Coagulation of milk micelles because of the loss of electrostatic and steric repulsion due to κ-casein is dependent on the level of *κ*-casein cleavage [81]. The proteolysis of *κ*-casein also leads to a significant decrease in the zeta potential of casein micelles, which may enhance their sensitivity to the environmental stress in the stomach and promote their colloidal coagulation during gastric digestion. Gastric conditions in the presence of Ca^{2+} levels with ionic strength may promote the aggregation of casein micelles as induced by pepsin proteolysis [80]. The

Figure 2



(a) Changes in average diameter ($D_{4,3}$) of gastric chyme of almond milk, soymilk, and oat milk during gastric digestion in the human gastric simulator. **(b)** Schematic diagram of gastric behaviours of oat milk and oat milk–bovine skim milk blend. Adapted from Wang et al., 2022 [86,89].

presence of calcium ions facilitates the formation of the coagulum by connecting micelles as a bridge and inducing isoelectric conditions [10].

The structure of the coagulum formed under the same gastric conditions differs for milks subjected to various treatments or containing other components along with casein micelles. Skim milk heated at 90 °C for 20 min forms a loose, fragmented-network-structured curd with more larger voids than those in unheated milk, which forms a firm, dense-structured clot [51]. This phenomenon has also been observed in whole milk; the presence of fat globules in the clot does not alter the formation of a more open, looser, and more fragmented curd upon heating whole milk under gastric conditions [82]. Homogenisation leads to the formation of coagula with fragmented and crumbled structures, unlike the coagulum formed from raw whole milk, but a larger fraction of the protein and more fat globules are incorporated into the coagula [83]. Heat treatment of

homogenised milk enhances the looseness of the gastric coagula. Homogenisation causes a marked increase in the number of fat globules by reducing the fat globule size, which leads to the adsorption of caseins and whey proteins onto the surface of the fat globules, thereby reducing the size of the MFGM [84]. The large number of fat globules that embed in the clots when they form during digestion may block the network of the protein matrix in homogenised milk, which results in the looser and fragmented structure [83,85]. These structures formed in the early stages of digestion, induced by pepsin hydrolysis, influence the subsequent protein hydrolysis during gastric digestion and the delivery of protein and fat globules to the next stage of digestion. They also influence the delivery of other nutrients that are involved in the formation of the gastric clot into the next digestion step because of the delay in the rate of disintegration of the clot structure when other foods are digested along with milk.

Colloidal behaviours of PMAs under gastric conditions

Pepsin hydrolysis does not induce the coagulation of plant proteins in PMAs, but the decrease in pH during gastric digestion can induce their aggregation into large particles. Pepsin exhibits high activity for the hydrolysis of protein aggregates both in aqueous phases and at the surfaces of OBs at low pH [86]. Protein hydrolysis by pepsin leads to the destabilisation and coalescence of plant OBs, ultimately resulting in creaming and phase separation of PMAs, with a lipid layer floating on top of the stomach contents. In this processing, different plant proteins demonstrate various behaviours.

For example, almond milk is a typical gastric-unstable emulsion in which OBs are dispersed uniformly in an aqueous phase containing protein (Fig. 2). Almond OBs are stabilised by a monolayer of PLs embedding oleosin proteins [87]. When gastric pH drops to 5.26 from the original 6.36, almond OBs flocculate and proteins aggregate rapidly to formed large aggregates (Fig. 2a) [88]. The OBs then coalesce quickly due to pepsin hydrolysis of the interfacial proteins, and this accelerates the creaming process in the human gastric simulator, producing an upper layer rich in lipids and a lower aqueous phase consisting of proteins. This layering substantially delays gastric emptying of lipids, whereas proteins are emptied gradually. The findings of gastric digestion of almond milk highlight the role of the interfacial properties of OBs in controlling the gastric colloidal stability and food structure of PMAs. Oat milk is a relatively stable emulsion system under gastric conditions, as no significant physical destabilisation has been observed under dynamic pH changes and no considerable changes in particle size occur during digestion [89]. The protein and lipid contents are relatively homogeneously dispersed in oat milk throughout *in vitro* gastric digestion, and both are emptied gradually.

Another remarkable difference in the intragastric microstructures among PMAs is that almond milk exhibits extensive coalescence, whereas soymilk and oat milk do not notably coalesce [88–90]. One possible explanation for this observation is that the curd particles in soymilk and the protein aggregates in oat milk act as physical barriers, protecting plant OBs from the attack of pepsin. However, it is more likely that the interfacial membranes of soy and oat OBs have superior stability against protein hydrolysis by pepsin.

Gastric colloidal behaviour of mixed milk systems

A study applied the coagulatory nature of dairy protein in gastric digestion to manipulate the digestion outcome of plant- and dairy-based milk blends [89]. The milk blend was prepared by mixing oat milk with bovine skim milk (1:1, v:v). As expected, compared to oat milk, the milk blend showed a considerably different digestion behaviour by coagulating in the first few minutes of gastric digestion, which was induced by coagulation of the casein micelles due to the hydrolysis of κ-casein by pepsin. Proximate and SDS-PAGE analyses indicated that the curd particles consisted of not only dairy proteins (mainly caseins), but also some specific oat proteins and lipids. The coagulation remarkably delayed gastric emptying of both proteins and lipids since the curds were larger than the sizes required to empty from the stomach (Fig. 2**b**). It was also found that the oat proteins in the curds were protected from attack by the added pepsin. This study clearly shows that the addition of dairy protein to plant-based milk can significantly modify the gastric structure, protein hydrolysis, and macronutrient delivery of PMAs (Fig. 2**b**). On the other hand, plant components also altered the structure of the coagulated dairy protein networks. This work demonstrates that milk proteins can be effectively applied to design food structure and manipulate the gastric colloidal stability and digestion kinetics of PMAs.

Conclusions

The differences between PMAs and dairy milk stem from their distinct physicochemical properties, particularly the low stability of protein particles and oil droplets in PMAs. High levels of protein and fat can lead to physical issues such as sedimentation and phase separation. This instability arises because the proteins in PMAs typically originate from water-insoluble PBs, which exist as large particles. Unlike the structured casein micelles in milk, these particles lack a stable architecture and tend to aggregate further during processing and storage. Studies comparing the protein particle structures in milk and PMAs highlight the importance of designing particles that resist aggregation and remain stable. One promising approach involves the

synthesis of hybrid protein particles, which exhibit improved thermal stability, enhanced surface adsorption, and the ability to create more stable dispersions and emulsions suitable for PMA applications. Additionally, particle structure plays a key role in digestion by influencing the dynamics of how milk and plant proteins are broken down and absorbed.

Author contributions

Writing—original draft preparation, Y. H.; writing—review and editing, A. A., and H. S.; conceptualization, writing—original draft preparation, A.Y.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. McClements DJ, Newman E, McClements IF: **Plant-based milks: a review of the science underpinning their design, fabrication, and performance**. *Compr Rev Food Sci Food Saf* 2019, **18**:2047–2067.

2. Aydar EF, Tutuncu S, Ozcelik B: **Plant-based milk substitutes: bioactive compounds, conventional and novel processes, bioavailability studies, and health effects**. *J Funct Foods* 2020, **70**, 103975.

3. Collard KM, McCormick DP: **A nutritional comparison of cow's milk and alternative milk products**. *Acad Pediatr* 2021, **21**: 1067–1069.

4. * Beckett EL, Cassettari T, Starck C, Fayet-Moore F: **Dairy milk: there are alternatives but no equivalents**. *Food Sci Nutr* 2024, **12**:8470–8482.
This commentary contrasts dairy milk with plant-based milk alternatives, arguing there are alternatives but no equivalents. This paper details non-equivalence in nutrient density and quality and matrix-driven bioavailability. The piece urges dietary guidance and marketing to reflect these differences and notes that environmental impacts should be interpreted per nutrient contribution.

5. ** Hu Y, Ye A, Cheng L, Lee SJ, Yang Z: **Recent progress in fabrication, characterization and application of functional protein aggregates derived from plant proteins**. *Crit Rev Food Sci Nutr* 2025, **1**:40.
This review surveys recent advances in creating functional aggregates from plant proteins, detailing how physical, chemical, and biological methods to modify microstructure and techno-functional performance of plant proteins. By integrating spectroscopy, microscopy, and rheology, the review links aggregation states to emulsification, gelation, and foaming, and spotlights applications in beverages, emulsions, and whipped cream.

6. Arbach CT, Alves IA, Serafini MR, Stephani R, Perrone ÍT, de Carvalho da Costa J: **Recent patent applications in beverages enriched with plant proteins**. *NPJ Sci Food* 2021, **5**:28.

7. Dickinson E, Stainsby G: *Advances in food emulsions and foams*. 1988.

8. Hu Y, Cheng L, Gilbert EP, Lee SJ, Yang Z: **Impact of thermosonication at neutral pH on the structural characteristics of faba bean protein isolate dispersions and their physicochemical and techno-functional properties**. *Food Hydrocoll* 2024, 110140.

9. ** Yang M, Yang Z, Everett DW, Gilbert EP, Singh H, Ye A: **Digestion of food proteins: the role of pepsin**. *Crit Rev Food Sci Nutr* 2025:1–22.
This work reviews how pepsin governs the first stage of gastric protein digestion across milk, meat, egg, and plant proteins, showing that protein source, composition, processing, and co-ingredients determine hydrolysis kinetics and amino-acid release. This review maps design levers for high-protein foods that optimize nutrition with lower allergenic potential.

10. Horne DS: *Casein micelle structure and stability. Milk proteins: from expression to food*. Academic Press; 2009.

11. Chandrapala J, Huppertz T: *Structure of casein micelles*. Casein; 2024:37–49.

12. Lucey JA, Horne DS: **Perspectives on casein interactions**. *Int Dairy J* 2018, **85**:56–65.

13. Swaisgood HE: *Chemistry of the caseins*. 1992.

14. Holt C, Carver JA: **Quantitative multivalent binding model of the structure, size distribution and composition of the casein micelles of cow milk**. *Int Dairy J* 2022, **126**, 105292.

15. Müntz K: **Deposition of storage proteins**. *Plant Mol Biol* 1998, **38**:77–99.

16. Huang A: *Protein bodies*: *Cell components*. Springer; 1985: 134–144.

17. ** Do DT, Ye A, Singh H, Acevedo-Fani A: **Protein bodies from hemp seeds: isolation, microstructure and physicochemical characterisation**. *Food Hydrocoll* 2024, **149**, 109597.
This study isolates hemp seed protein bodies via sonication-assisted aqueous enzymatic extraction and maps how pH (2–13) governs their colloidal stability and structural integrity. Microscopy shows ~4.6 μm spherical organelles comprising a protein crystalloid and phytin globoids within a protein matrix and single membrane. Proteins dispersions are stable near neutral pH, but they will aggregate, swell, rupture, or dissolve under acidic/alkaline extremes. The work positions HPBs as pH-responsive building blocks for food structuring applications.

18. Kizzie-Hayford N, Jaros D, Zahn S, Rohm H: **Effects of protein enrichment on the microbiological, physicochemical and sensory properties of fermented tiger nut milk**. *LWT* 2016, **74**: 319–324.

19. * Daryani D, Pegua K, Aryaa SS: **Review of plant-based milk analogue: its preparation, nutritional, physicochemical, and organoleptic properties**. *Food Sci Biotechnol* 2024, **33**:1059–1073.
This paper reviews plant-based milk analogues from manufactural, nutritional, physicochemical, and sensory aspects. It maps how matrix composition, fortification, and processing affect stability and acceptability, and outlines remedies for typical deficits via formulation, fermentation, and advanced homogenization. This work is a practical guide to designing milk analogues with improved nutrition, stability, and consumer appeal.

20. Gao X, Dai Y, Cao J, Hou H: **Analysis of the effect mechanism of wet grinding on the film properties of pea protein isolate based on its structure changes**. *Innov Food Sci Emerg Technol* 2023, **89**, 103474.

21. Vogelsang-O'Dwyer M, Sahin AW, Zannini E, Arendt EK: **Physicochemical and nutritional properties of high protein emulsion-type lupin-based model milk alternatives: effect of protein source and homogenization pressure**. *J Sci Food Agric* 2022, **102**:5086–5097.

22. Mu Q, Su H, Zhou Q, Xiao S, Zhu L, Xu X, *et al.*: **Effect of ultrasound on functional properties, flavor characteristics, and**

storage stability of soybean milk. *Food Chem* 2022, **381**, 132158.

23. Dai T, Shuai X, Chen J, Li C, Wang J, Liu W, *et al*.: **Whole peanut milk prepared by an industry-scale microfluidization system: physical stability, microstructure, and flavor properties**. *LWT* 2022, **171**, 114140.

24. Luo L, Cheng L, Zhang R, Yang Z: **Impact of high-pressure homogenization on physico-chemical, structural, and rheological properties of quinoa protein isolates**. *Food Struct* 2022, **32**, 100265.

25. * Reyes-Jurado F, Soto-Reyes N, Dávila-Rodríguez M, Lorenzo-Leal A, Jiménez-Munguía M, Mani-López E, *et al*.: **Plant-based milk alternatives: types, processes, benefits, and characteristics**. *Food Rev Int* 2023, **39**:2320–2351.

This paper reviews plant-based milk alternatives across sources and processing routes, showing how different routes govern stability, particle size, and sensory quality. Links matrix composition and processing to nutrition and while discussing allergen/anti-nutrient mitigation and reported health effects. Identifies the effects of formulation levers, fortification, enzyme treatments, high-pressure, ultrasound on PMAs to alternative bovine.

26. * Wei X, Dou N, Wang G, Tan Z, Tian Z, Ren J, *et al*.: **Physicochemical and rheological properties of soybean oil body fermented milk: impacts of high pressure homogenization**. *LWT* 2024, **199**, 116090.

27. ** Hu Y, Cheng L, Yang Z: **Impact of various physical treatments on physicochemical and microstructural characteristics of vegetable oil-based whipped cream stabilised by faba bean protein isolate**. *Food Hydrocoll* 2025, **163**, 111084.

This research investigates how physical treatments reshape the physicochemical profile and microstructure of vegetable oil–based whipped cream stabilized by faba bean protein isolate. By comparing processing routes, this study links microtructure to tecono-functionalities to design plant-based whipping creams.

28. He A, Xu B: **High-pressure homogenisation improves food quality of plant-based milk alternatives**. *Int J Food Sci Technol* 2024, **59**:399–407.

29. Wang Q, Jiang J, Xiong YL: **High pressure homogenization combined with pH shift treatment: a process to produce physically and oxidatively stable hemp milk**. *Food Res Int* 2018, **106**:487–494.

30. Thakur N, Gupta N, Sood M, Bandral JD, Singh J, Bhat A, *et al*.: **Plant-based milk alternatives: an emerging segment: a**. *Pharm Innov* 2022:2752–2758.

31. Bernat N, Chafer M, Rodriguez-Garcia J, Chiralt A, González-Martínez C: **Effect of high pressure homogenisation and heat treatment on physical properties and stability of almond and hazelnut milks**. *LWT* 2015, **62**:488–496.

32. * Li D, Ma Y, Acevedo-Fani A, Lu W, Singh H, Ye A: **Heat-induced modifications of pea protein: implications for solubility and digestion behaviour**. *Curr Res Food Sci* 2025, 101173.

This work examines how heat treatment across pH tunes pea protein behavior, showing that alkaline heating increases solubility and decreases particle size. Also, heated pea proteins will digest faster in the gastric phase. This research links process conditions to digestibility and dispersibility, guiding formulation of high-protein beverages and plant-based nutrition

33. Salve AR, Pegu K, Arya SS: **Comparative assessment of high-intensity ultrasound and hydrodynamic cavitation processing on physico-chemical properties and microbial inactivation of peanut milk**. *Ultrason Sonochem* 2019, **59**, 104728.

34. Sidhu JS, Singh RK: **Ultra high pressure homogenization of soy milk: effect on quality attributes during storage**. *Beverages* 2016, **2**:15.

35. Poliseli-Scopel FH, Hernández-Herrero M, Guamis B, Ferragut V: **Sterilization and aseptic packaging of soymilk treated by ultra high pressure homogenization**. *Innov Food Sci Emerg Technol* 2014, **22**:81–88.

36. ** Ma S, Ye A, Singh H, Acevedo-Fani A: **Heat-induced interactions between microfluidized hemp protein particles and caseins or whey proteins**. *Food Chem* 2025, **463**, 141290.

This study probes heat-induced interactions between microfluidized hemp protein particles and milk proteins (i.e. whey, casein), showing that co-heating at 95 °C for 20 min suppresses hemp aggregation: particle size drops from ~27.5 μm (hemp alone) to ~3.8 μm with whey and ~1.9 μm with casein. SDS-PAGE indicates irreversible disulfide bonding with whey versus reversible, chaperone-like association with casein, infirming the mechanisms for stabilizing hemp–dairy hybrid microparticles.

37. Chuang C-C, Ye A, Anema SG, Loveday SM: **Hemp globulin forms colloidal nanocomplexes with sodium caseinate during pH-cycling**. *Food Res Int* 2021, **150**, 110810.

38. * Samarathunga J, Le TPL, Gabard M, Strazdins K, Rens J, Adhikari B: **Formulation and characterization of hybrid milk containing bovine and Spirulina proteins**. *Sustain Food Technol* 2025.

This work develops hybrid milk formulations by blending Spirulina protein concentrate with milk protein concentrate at 0–100% replacement. Microfluidization yields fine fat globules, but storage enlarges droplets. The higher SPC shifts color greenish-brown, raises viscosity, and lowers thermal stability, aligned with decreased β-sheet/increased random coil structure. The blend of 25% SPC / 75% MPC most closely matches the bovine-protein control, positioning SPC–MPC hybrids as a practical route to plant-forward "hybrid milk."

39. Doost AS, Nasrabadi MN, Kassozi V, Dewettinck K, Stevens CV, Van der Meeren P: **Pickering stabilization of thymol through green emulsification using soluble fraction of almond gum–Whey protein isolate nano-complexes**. *Food Hydrocoll* 2019, **88**:218–227.

40. Higa FA, Nickerson MT: **Plant protein-carbohydrate conjugates: a review of their production, functionality and nutritional attributes**. *Food Res Int* 2023, **39**:750–771.

41. Zhang Q, Li L, Lan Q, Li M, Wu D, Chen H, *et al*.: **Protein glycosylation: a promising way to modify the functional properties and extend the application in food system**. *Crit Rev Food Sci Nutr* 2019, **59**:2506–2533.

42. Schneider AA, Bu F, Ismail BP: **Enhancement of pea protein solubility and thermal stability for acidic beverage applications via endogenous Maillard-induced glycation and chromatography purification**. *Curr Res Food Sci* 2023, **6**, 100452.

43. Hu Z, Qiu L, Sun Y, Xiong H, Ogra Y: **Improvement of the solubility and emulsifying properties of rice bran protein by phosphorylation with sodium trimetaphosphate**. *Food Hydrocoll* 2019, **96**:288–299.

44. Akharume FU, Aluko RE, Adedeji AA: **Modification of plant proteins for improved functionality: a review**. *Compr Rev Food Sci Food Saf* 2021, **20**:198–224.

45. Li C-P, Enomoto H, Hayashi Y, Zhao H, Aoki T: **Recent advances in phosphorylation of food proteins: a review**. *LWT* 2010, **43**:1295–1300.

46. Guo Y, Liu C, Ma Y, Shen L, Gong Q, Hu Z, *et al*.: **Study on the structure, function, and interface characteristics of soybean protein isolate by industrial phosphorylation**. *Foods* 2023, **12**:1108.

47. Keenan T, Dylewski D: **Intracellular origin of milk lipid globules and the nature and structure of the milk lipid globule**. *AdvAdvan Dairy Chem-2 Dairy Chemistry-2: Lipids* 1995, **2**:89.

48. Huppertz T, Uniacke-Lowe T, Kelly A: **Physical chemistry of milk fat globules**. *Advan Dairy Chem* 2020, **2**:133–167. Lipids: Springer.

49. Ye A, Anema S, Singh H: **High-pressure–induced interactions between milk fat globule membrane proteins and skim milk proteins in whole milk**. *J Dairy Sci* 2004, **87**:4013–4022.

50. Ye A, Anema SG, Singh H: **Behaviour of homogenized fat globules during the spray drying of whole milk**. *Int Dairy J* 2007, **17**:374–382.

51. Ye A, Cui J, Dalgleish D, Singh H: **Formation of a structured clot during the gastric digestion of milk: impact on the rate of protein hydrolysis**. *Food Hydrocoll* 2016, **52**:478–486.

52. McKenna NJ, Lanz RB, O'Malley BW: **Nuclear receptor coregulators: cellular and molecular biology**. *Endocr Rev* 1999, **20**:321–344.

53. Michalski M-C, Briard V, Michel F: **Optical parameters of milk fat globules for laser light scattering measurements**. *Lait* 2001, **81**:787–796.

54. Zou X-Q, Guo Z, Huang J-H, Jin Q-Z, Cheong L-Z, Wang X-G, *et al.*: **Human milk fat globules from different stages of lactation: a lipid composition analysis and microstructure characterization**. *J Agric Food Chem* 2012, **60**:7158–7167.

55. Shimizu M, Yamauchi K, Kanno C: **Effect of proteolytic digestion of milk fat globule membrane proteins on stability of the globules**. *Milchwissenschaft* 1980, **35**:9–12.

56. Tzen JT, Cao Y, Laurent P, Ratnayake C, Huang AH: **Lipids, proteins, and structure of seed oil bodies from diverse species**. *Plant Physiol* 1993, **101**:267–276.

57. Ross JH, Sanchez J, Millan F, Murphy DJ: **Differential presence of oleosins in oleogenic seed and mesocarp tissues in olive (Olea europaea) and avocado (Persea americana)**. *Plant Sci* 1993, **93**:203–210.

58. Tangsuphoom N, Coupland JN: **Effect of heating and homogenization on the stability of coconut milk emulsions**. *J Food Sci* 2005, **70**:e466–e470.

59. Napier JA, Stobart AK, Shewry PR: **The structure and biogenesis of plant oil bodies: the role of the ER membrane and the oleosin class of proteins**. *Plant Mol Biol* 1996, **31**: 945–956.

60. Weiss J, Ahmad T, Zhang C, Zhang H: **A review of recent progress on high internal-phase Pickering emulsions in food science**. *Trends Food Sci Technol* 2020, **106**:91–103.

61. Tzen J, Huang A: **Surface structure and properties of plant seed oil bodies**. *J Cell Biol* 1992, **117**:327–335.

62. Zhang P, Bari VD, Briars R, Taher ZM, Yuan J, Liu G, *et al.*: **Influence of pecan nut pretreatment on the physical quality of oil bodies**. *J Food Qual* 2017, 3864126.

63. De Chirico S, di Bari V, Foster T, Gray D: **Enhancing the recovery of oilseed rape seed oil bodies (oleosomes) using bicarbonate-based soaking and grinding media**. *Food Chem* 2018, **241**:419–426.

64. Nikiforidis CV, Kiosseoglou V: **Aqueous extraction of oil bodies from maize germ (Zea mays) and characterization of the resulting natural oil-in-water emulsion**. *J Agric Food Chem* 2009, **57**:5591–5596.

65. Tangsuphoom N, Coupland JN: **Effect of thermal treatments on the properties of coconut milk emulsions prepared with surface-active stabilizers**. *Food Hydrocoll* 2009, **23**: 1792–1800.

66. Zhou Lz, Chen Fs, Hao Lh, Du Y, Liu C: **Peanut oil body composition and stability**. *J Food Sci* 2019, **84**:2812–2819.

67. ** Şen A, Acevedo-Fani A, Dave A, Ye A, Husny J, Singh H: **Plant oil bodies and their membrane components: new natural materials for food applications**. *Crit Rev Food Sci Nutr* 2024, **64**:256–279.
This review surveys plant oil bodies and their membrane materials as natural, clean-label emulsifiers capable of stabilizing bioactives. Centers on extraction technologies, interfacial functionality, and interactions with other food components, comparing sources and outlining practical challenges for scale-up and use. Positions oil bidy membrane materials as promising building blocks for food emulsions and delivery systems.

68. Qamar S, Bhandari B, Prakash S: **Effect of different homogenisation methods and UHT processing on the stability of pea protein emulsion**. *Food Res Int* 2019, **116**:1374–1385.

69. Chang MT, Tsai TR, Lee CY, Wei YS, Chen YJ, Chen CR, *et al.*: **Elevating bioavailability of curcumin via encapsulation with a novel formulation of artificial oil bodies**. *J Agric Food Chem* 2013, **61**:9666–9671.

70. Chen MC, Chyan C-L, Lee TT, Huang S-H, Tzen JT: **Constitution of stable artificial oil bodies with triacylglycerol, phospholipid, and caleosin**. *J Agric Food Chem* 2004, **52**: 3982–3987.

71. Li M, Murphy DJ, Lee K-HK, Wilson R, Smith LJ, Clark DC, *et al.*: **Purification and structural characterization of the central hydrophobic domain of oleosin**. *J Biol Chem* 2002, **277**: 37888–37895.

72. Deleu M, Vaca-Medina G, Fabre J-F, Roïz J, Valentin R, Mouloungui Z: **Interfacial properties of oleosins and phospholipids from rapeseed for the stability of oil bodies in aqueous medium**. *Colloids Surf B Biointerfaces* 2010, **80**: 125–132.

73. Capuano F, Beaudoin F, Napier JA, Shewry PR: **Properties and exploitation of oleosins**. *Biotechnol Adv* 2007, **25**:203–206.

74. Peng CC, Lin IP, Lin CK, Tzen JT: **Size and stability of reconstituted sesame oil bodies**. *Biotechnol Prog* 2003, **19**: 1623–1626.

75. Araiza-Calahorra A, Glover ZJ, Akhtar M, Sarkar A: **Conjugate microgel-stabilized Pickering emulsions: role in delaying gastric digestion**. *Food Hydrocoll* 2020, **105**, 105794.

76. Steingoetter A, Buetikofer S, Curcic J, Menne D, Rehfeld JF, Fried M, *et al.*: **The dynamics of gastric emptying and self-reported feelings of satiation are better predictors than gastrointestinal hormones of the effects of lipid emulsion structure on fat digestion in healthy adultsâ€" A bayesian inference approach**. *J Nutr* 2017, **147**:706–714.

77. Ye Q, Ge F, Wang Y, Wu P, Chen XD, Selomulya C: **Digestion of curcumin-fortified yogurt in short/long gastric residence times using a near-real dynamic in vitro human stomach**. *Food Chem* 2022, **372**, 131327.

78. Singh H, Ye A, Horne D: **Structuring food emulsions in the gastrointestinal tract to modify lipid digestion**. *Prog Lipid Res* 2009, **48**:92–100.

79. Ye A, Cui J, Carpenter E, Prosser C, Singh H: **Dynamic in vitro gastric digestion of infant formulae made with goat milk and cow milk: influence of protein composition**. *Int Dairy J* 2019, **97**:76–85.

80. Yang M, Ye A, Yang Z, Everett DW, Gilbert EP, Singh H: **Effect of ingestion temperature on the pepsin-induced coagulation and the in vitro gastric digestion behavior of milk**. *Food Hydrocoll* 2023, **139**, 108550.

81. Yang M, Ye A, Yang Z, Everett DW, Gilbert EP, Singh H: **Kinetics of pepsin-induced hydrolysis and the coagulation of milk proteins**. *J Dairy Sci* 2022, **105**:990–1003.

82. Ye A, Cui J, Dalgleish D, Singh H: **The formation and breakdown of structured clots from whole milk during gastric digestion**. *Food Funct* 2016, **7**:4259–4266.

83. Ye A, Cui J, Dalgleish D, Singh H: **Effect of homogenization and heat treatment on the behavior of protein and fat globules during gastric digestion of milk**. *J Dairy Sci* 2017, **100**:36–47.

84. Ye A, Anema SG, Singh H: **Changes in the surface protein of the fat globules during homogenization and heat treatment of concentrated milk**. *J Dairy Res* 2008, **75**:347–353.

85. Mulet-Cabero A-I, Mackie AR, Wilde PJ, Fenelon MA, Brodkorb A: **Structural mechanism and kinetics of in vitro gastric digestion are affected by process-induced changes in bovine milk**. *Food Hydrocoll* 2019, **86**:172–183.

86. Wang Y, Li Z, Li H, Selomulya C: **Effect of hydrolysis on the emulsification and antioxidant properties of plant-sourced proteins**. *Curr Opin Food Sci* 2022, **48**, 100949.

87. Beisson F, Ferté N, Voultoury R, Arondel V: **Large scale purification of an almond oleosin using an organic solvent procedure**. *Plant Physiol Biochem* 2001, **39**:623–630.

88. Wang X, Ye A, Singh H: **Structural and physicochemical changes in almond milk during in vitro gastric digestion:**

impact on the delivery of protein and lipids. *Food Funct* 2020, **11**:4314–4326.

89. Wang X, Ye A, Dave A, Singh H: **Structural changes in oat milk and an oat milk–bovine skim milk blend during dynamic in vitro gastric digestion**. *Food Hydrocoll* 2022, **124**, 107311.

90. Wang X, Ye A, Dave A, Singh H: **In vitro digestion of soymilk using a human gastric simulator: impact of structural changes on kinetics of release of proteins and lipids**. *Food Hydrocoll* 2021, **111**, 106235.