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Effect of food-grade biopolymers coated Pickering emulsions on carotenoids' stability during processing, storage, and passage through the gastrointestinal tract

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The use of Pickering emulsions (PE) as a delivery system of carotenoids and related hydrophobic compounds has arisen great interest in the scientific community due to their nontoxicity, safety, and long-term stability against different environmental conditions. In this line, designing suitable food-grade biopolymers for their use as stabilizers in carotenoid-loaded PE is an emerging research trend with many challenges. Thus, this review describes the recent advances in using proteins, polysaccharides, and protein–polysaccharide complexes as stabilizing agents of carotenoid-loaded PE. Besides that, this review provides a critical updated understanding of the effects of processing and storage on the stability of carotenoids encapsulated in PE, which are critical to support PE applications. The sustained-release behavior of PE in the gastrointestinal tract (GIT) is a hot topic that was also discussed, as well as the challenges for the applications of PE in the food industry.

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Introduction

Emulsions are thermodynamically unstable systems consisting in two nonmiscible liquid phases, generally oil and water. They can be kinetically stabilized by the addition of surfactants, that is, amphiphilic compounds that reduce the disperse phase/continuous- phase interfacial tension. In general, surfactants are natural or chemically synthesized compounds, and those employed in food products should be carefully analyzed and approved by the international regulatory organizations (e.g. European Food Safety Authority (EFSA), Food and Drug Administration (FDA)).

The awareness of consumers regarding the traceability and the carbon footprint of the products they ingest has challenged technologists to develop stable emulsions without using surfactants. This way emerged the concept of Pickering emulsions (PE) in which solid particles have to partially wet both oil and aqueous phases through a combination of steric, electrostatic, and hydration repulsions [1]. The partial wettability is determined by calculating the three-phase contact angle (θ), which should be close to 90° to ensure irreversible adsorption of the PE-type particles. Such particles irreversibly adsorb to the oil–water interface, forming a physical barrier that prevents coalescence, flocculence, and Ostwald ripening effects. For this reason, PE do not require the employment of surfactants, which is their main advantage over the conventional ones. Particle size is another important parameter to consider when developing PE, as it must be significantly smaller than emulsion droplets in order to ensure a protective barrier around the droplets. In addition, the compounds employed to form the particles are generally biodegradable and generally recognized as safe (GRAS), which not only ensures the safety consumption but also fulfills the requirements of plant-based consumers, also concerned about green production. The particles are irreversibly adsorbed on the interface in an eco-friendly process, which provides both physicochemical stability (also against oxidation and during long-term storage) and nutritional advantages, as they are not degraded during digestion, thus enhancing the bioaccessibility of the encapsulated compounds.

Carotenoids are hydrophobic compounds, naturally occurring in fruits and vegetables, and responsible for their pigmentation (from yellow to red). Besides being attractive alternatives to synthetic colorants in the food industry, carotenoids are also functional compounds that offer many health benefits [2]. However, carotenoids are sensitive to temperature, pH, oxygen, light, salt concentration, and other components of the food matrices and thus, can be degraded during food processing, long-term storage, and passage through the gastrointestinal tract (GIT). These disadvantages decrease their solubility in aqueous-based foods and thus, their bioavailability. Therefore, the use of carotenoids in nutraceutical and pharmaceutical products is limited. In this context, the development of PE, including food-grade biopolymers as stabilizers, can be an interesting strategy to deliver physicochemically more stable carotenoids to their target sites. It is interesting to point out that most of the articles dealing with PE as carotenoids' carrier systems have been published in the last two years and are mainly focused on the encapsulation of β-carotene, astaxanthin, lutein, and lycopene, these two latter in a lesser extent. The role of proteins, polysaccharides, and protein/polysaccharides' complexes as PE stabilizers is addressed in this work. The review provides an updated critical understanding of the effects of processing, storage, and gastrointestinal environment on the physicochemical properties of carotenoid-loaded PE stabilized using food-grade biopolymers. As the release behavior of PE-containing carotenoids is one of the main aspects to be evaluated when designing an appropriate delivery system, it is also discussed as well as the challenges that have to be addressed for an actual application in the food industry. As a whole, this work attempts to broaden the knowledge about the advantages and eventual disadvantages associated with the high stability of PE used as carotenoids' delivery systems.

Food-grade biopolymers to stabilize carotenoid-loaded Pickering emulsion

The increasing public concern about environmental protection has encouraged both the industry and academics to search for eco-friendly biopolymers with additional advantages, including renewability, cost-effectiveness, biocompatibility, biodegradability, and clean label [3]. Thus, the new research trend is to develop food-grade materials to be used as stabilizers of PE in food industrial applications. A PE-type particle should have a partial wettability both in oil and water phases to be properly adsorbed on the interface, and a size significantly smaller than that of conventional emulsion droplets [4]. In addition, food-grade biopolymer particle-coated PE should be stable during processing, long-term storage, and passage through the GIT, as well as control the release of their content in the small intestine. In this context, proteins and polysaccharides have shown great potential to stabilize PE (Table 1). The network formed in the space among PE-type particles in the continuous phase, the dense interfacial film, and the capillary pressure generated at the interface between adjacent particles are the primary mechanisms responsible for PE stabilization [5,6].

Proteins have good emulsifying properties and interfacial adsorption capacity due to their amphiphilic nature. They can stabilize PE not only through electrostatic repulsion and steric hindrance but also by forming a physical barrier at the oil/water interface that avoids oil droplets' coalescence (Figure 1) [7•–9]. The amphiphilic compounds employed to stabilize PE should have a partial and stable wettability in both oil and water phases, which ensures their efficient and irreversible interfacial adsorption [4]. In this regard, proteins should have strong structural stability when adsorbed to the interface [7•]. These properties limit the type of food proteins that are suitable for stabilizing PE. Therefore, different treatments aiming to modify the structure of native proteins are commonly applied to overcome these problems and enable their use as PE stabilizers. Among them, thermal treatment [7•,10], antisolvent precipitation [11], solvent evaporation, and pH adjustment [12] are the most common methods. Thermal treatments applied to soy glycinin (SG) protein at temperatures higher than those of denaturalization converted SG into a PE-type particle with improved emulsifying properties and stability against coalescence and creaming [7•]. The heating significantly reinforced the internal structure of soy protein-based nanoparticles by changing the pattern of the intraparticle interactive forces to hydrophobic interaction and disulfide bonds [7•]. This background explains the increasing number of works showing the potential of different protein-based particles as food-grade PE stabilizers. In particular, proteins from plants (e.g. lupin, pea, zein, and SG) and animal sources (e.g. whey protein (WP), ovalbumin (OV), sea bass, mackerel, lactoferrin, and cod) have been proposed as PE stabilizers of carotenoids' delivery systems (Table 1) [7•,10,12]. The controlled-release capacity of PE depends on the chemical nature of the protein. For example, Liu et al. [7•] observed that the release of β-carotene from PE stabilized by plant-based proteins (e.g. SG) was lower than that from PE prepared with WP, but similar to that from PE formulated with sodium caseinate. The research trend is to use proteins from plants' origin because of their reduced contribution to the environmental footprint in comparison with those of animal origin and of the increased weight of the vegan/vegetarian food market. In addition, plant-derived proteins have less digestive capacity than animal ones [13], representing an excellent option for delaying lipid digestion, particularly in the gastric phase. Inspite of these physiological and environmental advantages, the technological properties of plant proteins as PE stabilizers should be improved to

Table 1

Recent advances in designing food-grade biopolymer-coated PE as carotenoids' delivery systems.

Biopolymer	Source	Biopolymer modification	Lipid core	Encapsulated carotenoid	Main stability outcomes	Bioaccessibility/bioavailability findings	References
Protein							
	OV	Acid–heat	Candelilla wax mixed with soybean oil 0.7% w/w	Astaxanthin	OV emulsion was more stable during the freeze–thaw process than conventional emulsion	Higher fatty acid release and astaxanthin bioaccessibility in the OV emulsion (27.4%) than control (13.3%)	[42]
	Sea bass protein (SBP)	Acid precipitation + heat	Corn oil	Astaxanthin	High astaxanthin encapsulation efficiency (> 80%). High AST retention in SBP emulsion during storage at 50 °C or room temperature and in the presence of light or oxygen when 4% of SBP particles were applied	The astaxanthin bioaccessibility increased (51.17%) by increasing the SBP particle concentration up to 4% w/w	[34]
	Pea	Alkali extraction–acid precipitation + microfluidization	Soy oil (0.2% w/w)	β-carotene	Increasing the oil fraction promoted the formation of gel-like emulsions. This structure provided protection of β-carotene during digestion	β-carotene bioaccessibility was 24%	[12]
	SG	pH adjustment + thermal treatment (100 °C for 30 min)	Soy oil 0.2% (w/w)	β-carotene	Increasing protein concentration and oil fraction ratio produced a gradual strengthening of the gel-like network in the emulsions related to the adsorbed proteins at the interface	The oil fraction affected β-carotene bioaccessibility. The strong gel-like network limited carotene bioaccessibility	[7•]
	Gliadin (GL)	Antisolvent procedure + microfluidization	Corn oil	β-carotene	Good thermal stability in GL emulsion. High β-carotene retention after thermal treatment (90 °C, 3 min and 70 °C, 30 min). Higher β-carotene retention (> 90%) after 28 d of storage at 4 °C in GL emulsion with the highest particle concentration	β-carotene bioaccessibility was about 40%	[36]
Polysaccharides							
	CH+ CS	CS: acid hydrolysis	Corn oil (1 mg/mL)	β-carotene	Higher β-carotene retention when exposed CH–CS emulsion to UV light and storage than that stabilized with Tween- 80. The β-carotene retention in CH–CS emulsion remained unchanged during thermal treatments. High β-carotene protection during 30 d of storage (55.67–68.85%). Lipid oxidation was delayed in CH–CS emulsion		[34]

Table 1 (*continued*)

Biopolymer	Source	Biopolymer modification	Lipid core	Encapsulated carotenoid	Main stability outcomes	Bioaccessibility/bioavailability findings	References
	ST	Ultrasonication + high-pressure homogenization	Olive oil	Carotenoids from olive oil	Thermal and freeze–thaw stability of ST emulsion High antioxidant activity of ST emulsion	-	[19•]
	CH + guar gum (GG)	pH adjustment	Linseed oil	Astaxanthin	Optimal physical stability of CH–GG emulsion was obtained at pH 6.0 and oil fraction of 0.6 during six months of storage at 4 °C. Better lipid oxidation stability was found in CH–GG emulsion than in that prepared with Tween-80. Higher astaxanthin retention in CH–GG emulsion (85.9%) than that of CH alone (77.85%) during 30 d at 37 °C	-	[23]
	Cellulose nanocrystals (CN)	Heat	Soybean oil with beeswax	β-carotene	Higher freezing–thawing and physical stability in CN–oleogel emulsion than control. Higher droplet size but better stability against pH (4–8) and salt level (0.05–0.60 M) in CN–oleogel emulsion than control. Improved β-carotene stability in CN–oleogel emulsion during long-term storage	Higher β-carotene bioaccessibility (68.17%) in CN emulsion than control (53.15%)	[43]
	Sugary maize dendrimer-like glucan (SMDG)	Esterification with OSA	Medium-chain triglyceride (MCT) oil (0.1% w/v)	β-carotene	High β-carotene encapsulation efficiency of SMGD emulsion (> 89.6%). Higher β-carotene retention (> 70%) after 4 weeks of storage than emulsion formed with oil. High antioxidant activity of SMDG emulsion	Higher fatty acid release and β-carotene bioaccessibility (24.9%) than control	[39]
Protein–polysaccharide complexes							
	WP + CH	WP: high pressure CH: pH adjustment	MCT oil (0.8%, w/w)	Lycopene	WP–CH emulsion was stable against ionic strength and pH range (2–8). Higher lycopene retention was found in WP–CH emulsion when stored at 25 °C instead of 55 °C. Lycopene in low particle concentrations degraded during storage time and when exposed to light	Lycopene release in the GIT was increased in WP–CH emulsion containing low particle concentrations	[38]

Table 1 (*continued*)

Biopolymer	Source	Biopolymer modification	Lipid core	Encapsulated carotenoid	Main stability outcomes	Bioaccessibility/bioavailability findings	References
	Zein-PGA + lactoferrin (as emulsifier)	Solvent-evaporation coprecipitation	MCT oil (1.5% carotene)	β-carotene	Zein-PGA emulsion containing lactoferrin showed higher physical, light, and ionic strength stability than Zein-PGA alone. Zein-PGA emulsion containing lactoferrin in low concentration protected β-carotene against thermal degradation. Enhanced storage stability in Zein-PGA emulsion containing lactoferrin (28 d, 55 °C)	Higher β-carotene bioaccessibility in Zein-PGA containing low lactoferrin concentration. Excessive lactoferrin concentration in the water phase could interact with bile salts and form precipitates, reducing the bioaccessibility of β-carotene	[26]
	PP+ HMP	PP: pH shifting +heating	Corn oil (0.1%, w/w)	β-carotene	Higher β-carotene retention (68.3%) in PP-HMP emulsion after 30 d of storage at pH 6 than that containing PP alone.	Lower lipid digestion and β-carotene bioaccessibility in PP-HMP emulsion (25.82%) than using PP alone (31.4%) but higher than corn oil	[28]
	WGN + XG	Gluten: pH modification + high-pressure microfluidization	Corn oil	β-carotene	WGN-XG emulsion was stable against a wide pH range (4–8), salt concentrations (0–1000 mM NaCl), and thermal treatments (65 °C for 30 min and 90 °C for 30 min). WGN-XG emulsion protected β-carotene degradation during one month of storage at 25 and 37 °C	Higher β-carotene bioaccessibility in WGN-XG emulsion (38.2%) than XG alone (20%)	[27]
	LPA + maltodextrin	Alkali extraction–acid precipitation + heat	Sunflower oil (0.5% w/w)	Astaxanthin	The oxidative stability and encapsulation efficiency were improved by increasing LPA concentration. Higher astaxanthin retention was observed in powders obtained after spray drying at 140 and 160 °C when prepared at the highest LPA concentration. Better astaxanthin retention during storage was observed in LPA emulsion than control	The astaxanthin bioaccessibility was about 80% for emulsion with the highest LPA concentration	[31••]
	β-lactoglobulin (LG) + gum Arabic (GA)	LG: pH adjustment + heat + acid precipitation GA. Acid treatment	MCT oil (1% w/w)	Lutein	LG–GA emulsion had high stability against flocculation and coalescence during 12 weeks of storage. High lutein retention (> 70%) in LG–GA emulsion after 12 weeks of storage at 25 °C and exposition to UV light	-	[37]

Table 1 (*continued*)

Biopolymer	Source	Biopolymer modification	Lipid core	Encapsulated carotenoid	Main stability outcomes	Bioaccessibility/bioavailability findings	References
Other complexes							
	SMP + PC	SMP: pH adjustment	Soybean oil	Astaxanthin	SMP-PC emulsion exhibited higher astaxanthin stability against light and temperature (4 °C and room temperature) during storage (28 d) than that containing SMP alone. Higher adhesion and thermal stability when incorporated into surimi products	SMP-PC emulsion showed higher astaxanthin bioaccessibility (40.16%) than control (16.31%) but was similar to SMP alone	[33••]
	Zein + Adzuki bean seed coat polyphenol (ABSCP)	pH adjustment	Corn oil	Astaxanthin	Zein emulsion containing ABSCP showed higher stability against the ionic strength, UV light irradiation, and thermal degradation (50–90 °C for 30 min) than Zein emulsion alone	No significant differences in astaxanthin bioaccessibility were observed between Zein emulsion containing or not ABSCP (55.66–58.56%)	[29]
	LG + (-) epigallocatechin- 3-gallate (EGCG)	LG: pH adjustment + heat	MCT oil (2% w/w)	Lutein	LG-EGCG emulsion showed smaller droplet size after 30 d of storage than emulsion prepared with native LG. Higher lutein retention (90%) in LG-EGCG PE after 30 d of storage than emulsion containing native LG	-	[30]
	Zein + PGA + curcumin (costabilizer)	Microfluidization	MCT (1.5% w/w)	β-carotene	β-carotene stability was obtained at optimal conditions: 2.0% (w/v) of particle concentration, 100 MPa, and 60 °C. Improved β-carotene photostability	Particle concentration (0.5–3.0% w/v), microfluidization pressure (0–150 MPa), and temperature (30–70 °C) modulated the bioaccessibility of curcumin and β-carotene	[41]

achieve similar results as those from animal origin. As far as we know, none of the published results get deep insights on the molecular mechanisms explaining the better performance of proteins from animal origin. This supports the need of more research works to better understand how the physicochemical properties, the structures of the employed proteins, and their interaction with the digestive environment determine the ability of PE to release carotenoids in the intestine for their absorption.

Polysaccharides have been also used as PE stabilizers, starch (ST), cellulose, Arabic gum, and chitin and CH being the most studied ones due to their abundance, biocompatibility, cost-effectiveness, and biodegradable properties (Table 1) [14]. Similar to proteins, polysaccharide-derived particles irreversibly adsorb to the oil/water interface and create a physical barrier, mainly attributed to the electrostatic repulsion and steric hindrance that ensures the formation of stable PE (Figure 1) [15]. However, polysaccharides in their native state have a large size and hydrophilic characteristics, with poor capacity to adsorb to the oil/water interface. Thus, some modifications should be applied to enhance the emulsifying properties of the polysaccharide-derived particles [16]. Chemical modifications include hydrolysis with inorganic acids (sulfuric acid, hydrochloric acid, and phosphoric acid) and esterification with octenyl succinic anhydride (OSA), the latter being the most widely applied in the food industry [16,17]. Modification with OSA involves the replacement of hydroxyl groups with hydrophobic alkenyl groups from OSA to increase the amphiphilic properties of polysaccharides and thus, their interfacial adsorption capacity and stability in environmental conditions [18••]. Although the FDA (USA) has authorized the chemical modification of polysaccharides with up to 3% (w/w) OSA, consumers still demand clean-labeled ingredients [18••]. Thus, more eco-friendly strategies, including the use of physical methods, are preferred to modify polysaccharide characteristics and make them suitable for PE stabilization following the Green Chemistry concept. Physical treatments, including ultrasound [19•], high-pressure homogenization [19•], milling [20], and nanoprecipitation [21], have been used to reduce the particle size of ST and cellulose and thus, improve the particles' emulsifying properties without producing chemical wastes. In the case of CH, CH-derived particles suitable for PE can be produced by self-aggregation (deprotonation of amino groups at high pH) [22], complexation with negatively charged moieties of biopolymers (commonly proteins and polysaccharides) [23,24], or hydrophobic modification [25].

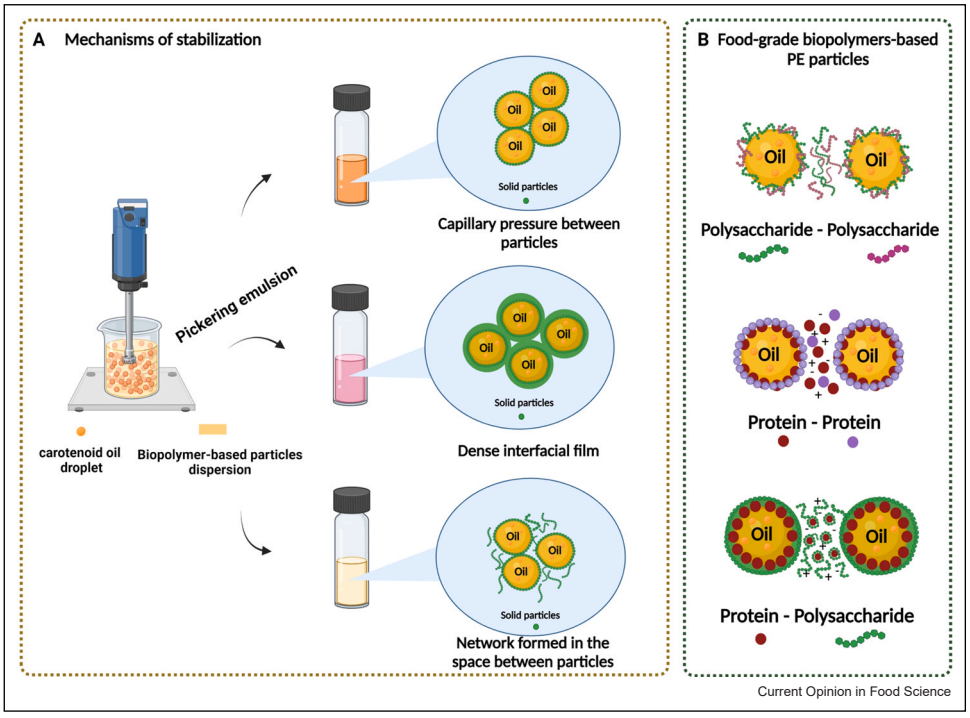
The emulsifying properties of polysaccharides can be enhanced when they interact with other molecules, such as proteins, other polysaccharides, and surfactants (Table 1). For example, the electrostatic attraction of zein protein (isoelectronic point about pH=6.2) with propylene glycol alginate (PGA) (dissociation constant about pH=3.5) at pH= 4 precludes coalescence, thus generating stable PE for prolonged periods [26]. In addition, zein complexation with other biopolymers leads to a multilayered thicker interface that protects the encapsulated carotenoids from oxygen, free radicals, and pro-oxidants from the bulk phase (Figure 1) [26]. In this regard, when compared the stability of PE-containing hybrid particles formed by wheat gluten (WGN) and xanthan gum (XG) with that of emulsions just containing WGN, the former showed not only greater stability toward a wide range of pHs and salts' concentration but also to β-carotene degradation after 30 d of storage at 25 and 37 °C [27]. In addition, β-carotene bioaccessibility was significantly higher in the emulsion containing WGN/XG (38.2%) than in the emulsion having gluten (20%). XG could avoid the aggregation of oil droplets in the gastrointestinal fluids and thus, allow their interaction with the lipase enzyme, releasing β-carotene into the micellar phase [27]. On the other hand, PE-containing pea protein (PP) isolates and high methoxyl pectins (HMPs) as binary particles exhibited higher stability toward pH variations and better protection of β-carotene during long-term storage in comparison with emulsions prepared just with PP [28]. However, the complexation of PPs with HMPs significantly affected the β-carotene bioaccessibility, which was much lower than that of the PE just containing PPs [28].

In this context, solid nano- and microparticles derived from food-grade biopolymers are opening up new opportunities for the development of long-term stable food emulsions. Chemically modified proteins and polysaccharides may form a physical barrier, delaying prooxidant diffusion and protecting carotenoids embedded in oil droplets from the effects of processing and storage conditions (oxygen, pH, light, and temperature). The superior physical structure of PE over conventional emulsions is also related to the fact that the thickness of the interfacial layer coating the oil droplets is comparable to the size of the stabilizing biopolymers. In addition, the development of biopolymers with antioxidant properties may additionally improve the protective properties of PE particles.

Carotenoid stability in the Pickering emulsions during processing, storage, and passage through the gastrointestinal tract

In order to extend the application of PE to the food industry, the stability of carotenoids encapsulated in PE should be tested against different environmental stresses, including food processing conditions (temperature, pH, oxygen, light, and salt concentration), long-term storage (time and temperature), and the passage through the GIT (different pH and salt concentrations in the oral, gastric, and intestinal phases)

Fig. 1



Mechanisms for Pickering emulsion stabilization (A) using food-grade biopolymers (proteins, polysaccharides and their interaction) as solid particles (B).

(Figure 2). Considering the hydrophobic nature of carotenoids, their limited water solubility, and susceptibility to oxidation, PE-containing carotenoids should resist the physiological and physicochemical conditions of the gastrointestinal environment and ensure their sustained release in the small intestine [2].

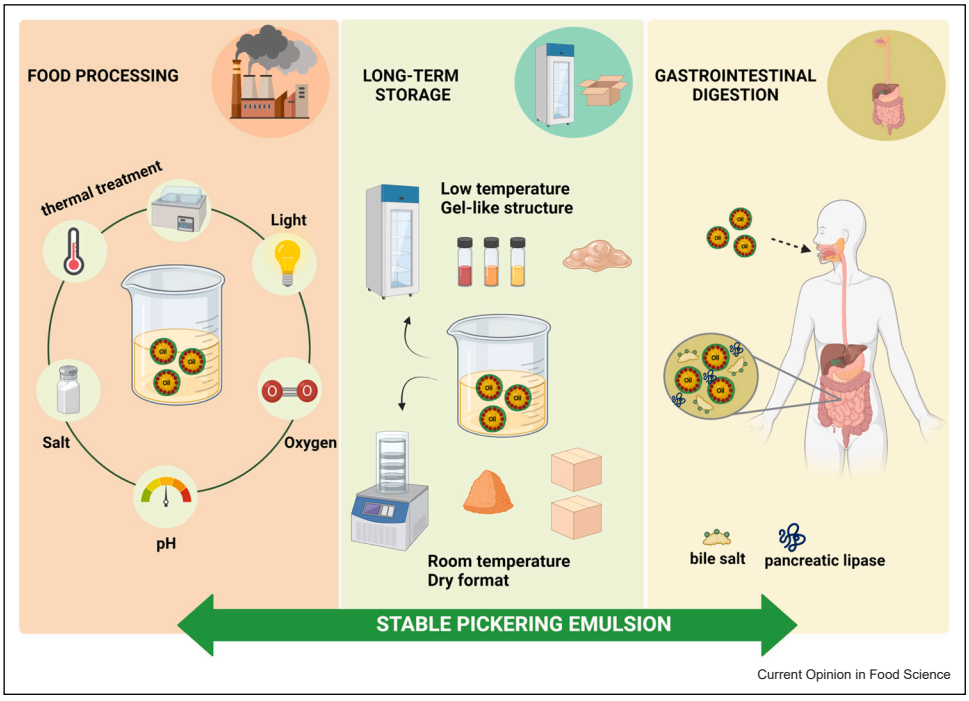
Temperature

Thermal processing is the most applied preservation technique in the food industry; therefore, temperature is a crucial parameter determining not only the food quality and shelf life but also the carotenoids' stability (Table 1). Protein denaturation associated with thermal treatments leads to an exposure of the hydrophobic residues, increasing the size of the oil droplets in PE assembled by proteins [26,29]. Consequently, the attraction of hydrophobic residues conducts to the aggregation and coalescence of oil droplets. In contrast, PE assembled by binary/ternary particles are thermally more stable than those prepared by reducing the droplet size of biopolymers. The small droplet size is associated with higher emulsion stability as a result of the reduction of the creaming rate and of the strengthening of electrostatic repulsive forces between oil droplets, which precludes collapse [30]. For example, the astaxanthin retention in emulsions prepared with zein-procyanidins (PC) and further submitted to thermal treatments (25–90 °C) was higher than that in emulsions prepared just with zein, which highlights the superior properties of using hybrid particles as PE stabilizers. Similarly, Burgos-Díaz et al. [31••] observed high astaxanthin retention in the spray-dried PE assembled by lupin protein aggregates (LPA) combined with maltodextrin at 140 and 160 °C. The thermal stability of astaxanthin was associated with the small size of the oil droplets in the emulsion prepared at the highest lupin-based particle concentration. Likewise, PE assembled by the complexation of two polysaccharides (e.g. chitosan hydrochloride (CH) and carboxymethyl starch (CS)) protected β-carotene from thermal degradation, the droplet size, and emulsion integrity remaining unchanged after applying three different thermal treatments (37, 60, and 90 °C) [32]. Zhou et al. [33••] incorporated astaxanthin-loaded Spanish mackerel protein (SMP)–PC PE into surimi products, observing that heating at 95 °C for 30 min did not lead to phase separations nor to structural changes of the surimi's gel, indicating the potential of adding astaxanthin-loaded PE to food products.

Light

Photostability should be also considered when engineering PE to deliver photolabile carotenoids. PE stabilized by hybrid particles are more stable toward UV irradiation than those prepared with just one of the

Fig. 2



Factors affecting the stability of PE during food processing, storage, and gastrointestinal digestion.

biopolymers, and even more stable than conventional emulsions formed with bulk oil (Table 1) [32–34]. The complexation between two or three components could form a multilayered structure, leading to a thicker interface and thus, reducing the light transmission, and precluding carotenoid degradation [26]. In addition, the smaller droplet size of PE stabilized by binary/ternary particles (compared with that of emulsions prepared with only one biopolymer) could contribute to their higher photostability [29].

Ionic strength

Ionic strength is another factor affecting the stability of PE incorporated into food products or exposed to the human gastrointestinal conditions. Therefore, studying the effect of different salt concentrations on the stability of carotenoid-loaded PE is mandatory (Table 1). The presence of salts, even at low concentrations, altered the droplets' size and zeta potential of PE stabilized with WGN-based particles, leading to aggregation and creaming [27]. The incorporation of NaCl may cause electrostatic screening that changes the droplets' charge and reduces the electrostatic repulsion [29]. However, when PE was stabilized with WGN/XG complexes, greater stability toward different salt concentrations was observed as a result of the increased water-phase viscosity and of the thicker barrier created by both polymers, which increased the spatial repulsion [27]. The spatial repulsion resulting from a high salt concentration environment was also responsible for the better protection of carotenoids loaded in hybrid PE when compared with those loaded in PE formed by a single biopolymer [29].

pH

The physical stability of carotenoid-loaded PE toward wide ranges of pH is another important aspect to be taken into account when formulating food products that should be stable both in processing and digestion conditions. In general, when proteins are adsorbed to PE, their stability toward pH is limited to values close to their isoelectric point, with a significant decrease of their electrostatic repulsion, leading to protein aggregation [28]. In this regard, the proteins–polysaccharides' interaction provides again a possible solution to improve the stability of PE regardless of pH variations. The strong and thick barrier formed between both biopolymers at the oil/water interface and the generated steric repulsion could explain such improvement [28]. Regarding carotenoid stability, pH variations slightly affected β-carotene retention during storage of PE stabilized with PPs and HMPs. The pigment was better retained at pH 6 than at pH 3 [28].

Long-term storage

The stability of carotenoid-loaded PE during storage is another crucial issue to be taken into account when determining the food shelf life. In this sense, most

studies have focused on developing PE to preserve carotenoid integrity during long-term storage (Table 1). Generally, the lower the storage temperature, the higher the carotenoid retention in long-term storage [23,27]. It is well-known that high temperatures lead to carotenoids' structural alterations and degradation. In addition, PE stabilized by biopolymers were more effective to preclude chemical degradation of β-carotene or astaxanthin along storage than conventional emulsions formed in bulk oil [23,28,35,36]. In turn, oil fraction affects the carotenoid retention during storage. The higher the oil fraction, the higher the carotenoid (e.g. lutein, β-carotene) retention along storage. This can be explained considering the formation of closely packed oil droplets in a gel-like network that could retard the diffusion of free radicals, pro-oxidants, and oxygen into the droplets [32,37]. Similarly, the concentration of the solid particles also determines the carotenoid stability during long-term storage, and the higher the particle concentration, the higher the carotenoid (e.g. lycopene, astaxanthin) retention [31••,34,38]. In this case, increasing the biopolymer-derived particle concentration could form a dense and charged interfacial film that protects the encapsulated carotenoid from the external oxidative environment through steric hindrance, hydrophobic binding, and electrostatic repulsion among droplets [39]. Different studies suggested that PE stabilized by hybrid particles show higher carotenoid retention during storage than those formed by a single biopolymer [23,30,33••]. The interaction among biopolymers creates a strong and thick barrier around the carotenoid-loaded oil droplets that prevents the access of oxidative agents from the environment into the droplets [27]. In addition, the antioxidant activity of some biopolymers could also contribute to protect carotenoids from oxidative degradation during storage.

Passage through the gastrointestinal tract

PE have gained great attention for their potential application as carriers of different bioactive compounds, this supports their use beyond the formulation of food products, namely to develop nutraceutical and pharmaceutical ones. An effective delivery system should allow the digestion of lipids (disperse phase), enabling the formation of mixed micelles, necessary to solubilize and transport carotenoids through a mucus layer to their absorption site (intestinal epithelial cells) [40]. In addition, carotenoid-loaded PE should have enough residence time in the intestine to enable the activity of the digestive enzymes, leading to hydrolysis reactions. Therefore, understanding the release capacity of PE and their stability in the gastrointestinal environment is of great importance when analyzing the effectiveness of PE carotenoids' delivery systems.

The bioaccessibility of carotenoids encapsulated in PE is significantly higher than that of emulsions formed in bulk oils due to the larger active interfacial area created by the smaller PE droplets. This results in more binding sites for lipase and bile salts (Table 1) [7•,26]. For this reason, the droplets' size has a key role in lipid digestion and thus, in the bioaccessibility of carotenoids. Furthermore, the thick layer formed by PE particles not only coats the carotenoid-loaded oil droplets but also forms a 3D network structure between droplets that protects carotenoids from degradation when exposed to gastrointestinal conditions. As a result, a highly effective encapsulation system for unstable dietary carotenoids can be developed, with applications in the food and nutraceutical industries.

Controversial results about the effect of solid particles' concentration on carotenoid bioaccessibility were found in the literature. On the one hand, some works showed that the carotenoid bioaccessibility improved as the particle concentration increased [31••,34,35]. It was ascribed to the reduction of oil droplets' size resulting from the rupture of larger droplets and the increased surface area (with more binding sites for lipase), facilitating the lipid's digestion and the transfer of carotenoids into micelles [35]. In contrast, other reports [7•,32,38,41] suggest that the PE-type particles irreversibly adsorbed to the multilayer interface limit the available surface to bind lipase, and thus, restrict the degree of lipolysis [26,32]. Hence, creating a more porous interfacial barrier could accelerate lipid digestion and improve carotenoids' release without forgetting stability issues. The oil fraction is another parameter determining carotenoids' bioaccessibility. The lower the oil fraction, the higher the carotenoids' bioaccessibility [7•]. Increasing the oil fraction turns the liquid emulsion into a gel-like structure with increased viscosity and viscoelasticity, capable to trap the oil droplets within this network and make them inaccessible to lipase molecules [32]. In this context, optimizing solid particle concentration and oil fraction is critical for developing PE that improve carotenoids' bioaccessibility. The controversial results reported in the literature in the last years underline the need of additional research to shed light on this novel topic.

In turn, PE stabilized by protein–polysaccharide complexes showed lower carotenoids' bioaccessibility than those formed by protein alone [28]. This was attributed to the rigid interfacial barrier created by the adsorbed particles that limits lipid digestion and thus, the formation of the micelles required to solubilize carotenoids [32]. Additionally, the use of polysaccharide-based particles not digestible by the human gastrointestinal enzymes in the PE formation precludes their desorption by bile salts and thus, delays the lipid digestion. Therefore, special attention should be paid on these studies where the gel-like network or the interfacial architecture limits lipid digestion and affects the proper release of

carotenoids in their absorption site. This background also emphasizes the importance of thoroughly understanding the mechanisms of carotenoids' release from PE formed by food-grade biopolymers during intestinal digestion. More research is needed to examine the addition of carotenoid-loaded PE to food products, as well as alternatives to the gel-like format of PE for ease of transportation, storage, and incorporation into various food systems. Furthermore, the safety and compatibility of carotenoid-loaded PE should be deeply investigated both *in vitro* and *in vivo*.

Current challenges for applying carotenoid-loaded Pickering emulsions in the food industry: future perspectives

Although there have been advances in the formation of edible carotenoid-loaded PE, there are still some barriers to their commercialization. To achieve that goal, the understanding of the latest technology and its application in materials science should improve and create more effective and innovative formulations that offer greater benefits in food production and processes. This would result in higher product quality, better nutritional profiles, and more sustainable and efficient manufacturing methods.

Despite these challenges, the future perspectives of carotenoid-loaded PE in the food industry are promising. Consumer awareness of the carbon footprint of the products they consume appears to be an additional support for transforming PE into a well-established process in the food industry. Carotenoids are natural pigments with antioxidant properties that provide health benefits. The use of PE as a delivery system for carotenoids can enhance their bioavailability and stability, increasing their potential to be used as functional ingredients in various food products. However, there is still a lack of thorough research on the toxicity and allergy of biopolymers used as solid particles in PE, which raises concerns about their use in food applications [5]. As a consequence, another challenge is producing a consistent and reproducible product that meets regulatory standards and consumer expectations.

The cost-effectiveness of the production process represents an additional demand. The use of natural and sustainable materials in the formulation of PE can raise manufacturing costs and make the product less competitive in the market. As a result, research efforts should be directed toward creating cost-effective production methods without compromising the products' quality and safety.

In addition, more research is needed to define the best conditions for ensuring processing and storage stability, as well as carotenoids' stability in the upper part of the GIT and lower stability in the intestine, to enable the release of the encapsulated carotenoids. Deeper understanding of the physicochemical mechanisms underlying these behaviors, as well as research into other proteins/polysaccharides suitable for the production of PE (e.g. wettability), will provide knowledge to support the use of PE to encapsulate carotenoids other than the most well-studied ones (β-carotene and astaxanthin).

According to the information reported and discussed in this work, it is clear that the formulation of PE is a novel approach involving interdisciplinary aspects that are not only related with physicochemical aspects of emulsions and structural analysis but also with nutritional and physiological processes that cannot be disregarded. In addition, as PE have potential applications going beyond the food industry (e.g. nutraceutical, pharmaceutical industries), researchers and industrialists should be prepared to eventual requirements of the international organizations (e.g. FDA, EFSA), especially in what concerns safety issues.

Conclusion

PE represents an emerging approach with great potential for the food, nutraceutical, and pharmaceutical industries, but still requiring fundamental and applied research. Fundamental research is critical to understand the physicochemical mechanisms explaining the behavior of such systems and applied research will enable the expansion of PE applications beyond carotenoids. In this regard, it is remarkable that more than 80% of the articles dealing with PE have been published in the last two years and all of them report the encapsulation of carotenoids (e.g. β-carotene, astaxanthin, lycopene, and lutein, these two latter with a lesser extent). The potential of PE makes it a suitable technique to encapsulate other hydrophobic compounds of nutritional or pharmaceutical interest, such as polyunsaturated fatty acids [docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and other omega-3 compounds].

The great stability of PE is a technological advantage because of the possibility of preparing emulsions without using surfactants. In addition, the simplicity and eco-friendliness character of the method enable a simple and sustainable scaling-up. The possibility of using plant-based proteins and naturally occurring polysaccharides (e.g. ST, pectins) not only allows their incorporation into food products but is also in line with the consumption pattern/requirements of vegan population. Furthermore, solid particles formed by proteins and polysaccharides have interesting biological activities, such as antioxidant, antimicrobial, anti-inflammatory, and so on, which can be used to enhance the functionality of emulsions. The main advantage of PE formed by proteins, polysaccharides, and protein–polysaccharide

complexes is related with their greater stability during processing, long-term storage, and gastrointestinal digestion due to the irreversible adsorption of PE particles to the oil/water interface and the formation of a 3D thick network that prevents oil droplets from coalescing. However, temperature, light, pH, salt concentration, and storage conditions can have an impact on carotenoid-loaded PE stability if particle concentration and oil fraction are not properly optimized.

Although PE ensure the safe arrival of carotenoids to their absorption target (upper part of the intestine), such stability slows down the digestion of lipids, which results in a slow release of carotenoids in the intestine. These two aspects should be carefully considered to adopt a compromise solution for generating stable emulsions without precluding the release of the encapsulated compounds.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare there is no conflict of interest.

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