Digestion fate and food applications of emulsions as delivery systems for bioactive compounds: challenges and perspectives

Eleonora Loffredi[a*] and C. Alamprese[a]

[a]*Department of Food, Environmental and Nutritional Sciences (DeFENS), Università degli Studi di Milano, Via G. Celoria 2, 20133 Milan, Italy*

*E-mail: eleonora.loffredi@unimi.it

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Different emulsion systems can be used to deliver bioactive compounds: single, multiple, gel, Pickering, and high internal phase emulsions. Each of them offers advantages and challenges in terms of stability, encapsulation efficiency, and bioactive compound availability during digestion. Moreover, the different emulsion types can affect the quality characteristics of the food products in which they are used, as well as the bioactive compound availability in the complex final matrix. This review provides an overview of the most recent works in this field, divided into two main sections. The first section is focused on *in vitro* digestibility of emulsion-based delivery systems to highlight the factors that can affect the digestion process and bioactive compound accessibility and availability. The second section is dedicated to the applications of delivery systems in food products, underlining the need for a better evaluation of the emulsion effects on food properties and on the fate of bioactive compounds in the complex matrix. Challenges and perspectives for emulsion-based delivery systems are presented, giving suggestions for future research.

Keywords: double emulsion; gel emulsion; Pickering emulsion; high internal phase emulsion

Introduction

Studies concerning emulsion science and technology have increased in the last fifteen years, including fields such as petroleum[1], material[2] and chemical[3] sciences, as well as drug delivery[4]. Due to the increasing industry demand for new tailored food products, many publications have also been realized in the food science and technology field. Emulsions consist of two immiscible liquids (usually oil and water), with one of the phases dispersed as small droplets in the other. They are quite widespread in several food products, such as beverages, creams, sauces, sausages, desserts, mayonnaise, butter, and margarine, providing desirable mouthfeel, texture, and flavor profile.

Two types of single emulsions can be produced: an oil-in-water (O/W) system when the oil droplets are dispersed within the continuous water phase; a water-in-oil (W/O) emulsion, if the continuous phase is oil and water droplets are the dispersed phase[5]. Single emulsions are formed by homogenizing given percentages of oil and water phases together, by applying different technologies. If a single O/W or W/O emulsion is homogenized again with an oil or water phase, double emulsions are obtained (O/W/O or W/O/W). Indeed, double (or multiple) emulsions are "emulsions of an emulsion". In an O/W/O system, water droplets contain dispersed oil droplets, whereas in a W/O/W emulsion the oil droplets have water droplets dispersed within them[6].

Emulsions are thermodynamically unstable and can break down due to different physical mechanisms, such as droplet aggregation and gravitational separation. Many efforts have been done in the research of food-grade surfactants[7, 8] or biopolymers[9] able to stabilize the dispersed phase droplets and prevent physical instabilities. Other strategies might be the addition of food-grade solid particles, such as in Pickering emulsions, or the gelling of the continuous phase (such as in emulsion gels) (Fig. 1). In the latter systems, the final structure of the emulsion can result in a different texture,

also affecting the properties of the final food products[10]. Moreover, when the volume ratio of the internal phase is equal to or above 74% a super concentrated emulsion is obtained, defined as high-internal phase emulsion (HIPE), with tightly packed droplets and interfacial compounds interacting to form networks[11]. The scientific literature also reports studies that combine all the different types of emulsions previously cited to create the HIPE version, such as Pickering HIPEs[12], O/W HIPEs with gelled oil phase, or with a double emulsion morphology[13].

An interesting aspect of emulsions is their ability to act as delivery systems for bioactive compounds, protecting them against oxidation and degradation, improving their solubility and bioavailability during the digestion process, and promoting human health[14]. In this case, during the emulsion design, the different stabilizing abilities of emulsifiers and biopolymers must be considered, because they can affect the release of the delivered bioactive compound. For instance, Li et al.[15] demonstrated that the use of sodium tripolyphosphate as a stabilizer for whole egg gel emulsions improved their physical and chemical stability and also provided an efficient delivery system for β-carotene, able to protect it against chemical degradation and to modulate its release during digestion. Thus, when designing new emulsions as delivery systems, for better tailoring it is important to investigate digestive mechanisms and interactions using *in vitro* or *in vivo* tests.

When used as a new ingredient for developing nutraceutical-enriched or low-fat food products, the emulsion formulation greatly impacts the final food structure. The work by Moriano et al.[16] about the application of a W/O/W double emulsion in reduced-fat biscuits has shown the importance of a multidisciplinary approach in developing a new food product with the desired technological, nutritional, and sensory properties.

However, many studies in the literature tend to consider emulsions only from a macro

and microscopical point of view, without taking into consideration their fate during digestion and the technological, nutritional, and sensory effects in real foods. Thus, the aim of this work was to systematically review the effect of different emulsion types on their digestibility and on the accessibility and availability of bioactive compounds encapsulated in the emulsions. Moreover, for the first time this review also considers the quality implications of emulsions used as bioactive compound carriers in real food products. The review is divided into two sections; at first, recent studies about digestion fate of different emulsion types are explored, highlighting the importance of the formulation and structure to obtain an efficient carrier for bioactive compounds; then, the effects of the different types of emulsions in real food applications are reviewed, featuring the need for deeper studies exploring all the important aspects for the promotion of new delivery systems for bioactive compounds.

In vitro digestibility evaluation of different emulsion systems

During lipid digestion, bile salts and lipases are absorbed on the surface of lipid droplets so that lipolysis can occur. However, lipolysis can be delayed by several factors such as, in the case of emulsions, droplet size and composition as well as emulsifier type and amount. Moreover, because each type of emulsion-based delivery system has its own digestion mechanism and release process, it is fundamental to develop standardized and validated *in vitro* methods to compare the performance of these systems[17]. Hereby, some studies for several emulsion-based systems are presented and summarized in Table 1, in which the digestibility is evaluated using different laboratory tests, including the international standardized protocol INFOGEST[18] and its update INFOGEST 2.0[19]. INFOGEST protocol is based on human physiological conditions, and it was developed to harmonize the *in vitro* digestion procedure so that different research laboratories can

compare their results more easily; in detail, this procedure gives the concentrations of the key gastrointestinal (GI) constituents to use (e.g., enzymes and their activity, bile salts concentration), the reaction times, and some recommendations for a correct execution of the protocol.

Single emulsions

The most studied emulsion-based delivery system is the simplest one; indeed, studies on single emulsions can be easily found in the literature, particularly investigating the carotenoid accessibility during digestion. Because carotenoids are lipophilic compounds, their bioaccessibility is very low, especially in aqueous environments such as intestinal juices. However, even if a lipid source might enhance their bioaccessibility during the digestion process[20], the intestinal lipase may interact differently with the lipids depending on the emulsion oil and interfacial composition or oil droplet size, influencing the oil digestibility, the micelle formation, and thus the carotenoid bioaccessibility. So, the purpose of the study by Salvia-Trujillo et al.[21] was to investigate how the emulsion droplet size influences the lipid digestion rate and the micelle formation under *in vitro* GI conditions following the INFOGEST standardized protocol. To do so, emulsions with different droplet sizes (large, 15.1 µm; medium, 1.9 µm; small, 0.7 µm) were tested after going through the digestion process. The authors found that the emulsions with droplets below 1 µm showed a faster and complete lipid digestion at the early stages of the intestinal phase compared to large-droplet emulsions. Also, the kinetics of lipid hydrolysis was influenced by the droplet sizes; indeed, with small and medium droplets the hydrolysis of triacylglycerols (TAGs) was complete after two hours of digestion, whereas the conversion of monoacylglycerols (MAGs) to free fatty acids (FFAs) and glycerol was faster in emulsions with small droplets. This

131 can be explained by the higher interfacial area of the small droplets, favoring the lipase
132 attack of TAGs and MAGs. Carotenoid bioaccessibility (i.e., the fraction of carotenoids
133 in the micelle phase over their initial concentration in the emulsion) during digestion
134 was also affected by the droplet size of the emulsions. Indeed, the concentration of α-
135 and β-carotene increased until reaching plateau values depending on the droplet sizes:
136 28 and 20% respectively in the case of large droplets, compared to 63 and 66% with
137 small droplets. Another study on carotenoids, compared the lycopene protection ability
138 of an O/W emulsion delivery system with respect to hydrogel beads[22]. An appropriate
139 delivery system should increase the low bioavailability of lycopene due to its
140 hydrophobic nature and poor chemical stability. Actually, lycopene undergoes chemical
141 degradation during storage, thus a delivery system should also protect it towards this
142 phenomenon. Indeed, the authors demonstrated a higher stability of lycopene in delivery
143 systems than in solution, with an even better performance of alginate beads over
144 emulsions at higher temperatures. The simulation of the GI tract, performed according
145 to a protocol very close to INFOGEST, but with slight modifications (e.g., presence of
146 mucin in simulated salivary fluid, SSF)[23], demonstrated that, besides the lower
147 bioaccessibility of the non-encapsulated lycopene, performances of the emulsion were
148 better than those of the alginate beads (20.2% accessibility vs 15.6%) due to a higher
149 extent of lipid digestion and FFAs release able to form complex mixed micelles with a
150 better solubilizing effect towards lipophilic bioactive compounds.
151 When choosing emulsion ingredients, it must be considered that they will affect not
152 only the technological properties and the stabilization mechanism of the emulsion itself
153 but also its behavior when passing through the GI tract. In particular, the interaction
154 between proteins and polysaccharides can favor the emulsion stability during digestion,
155 as demonstrated by Boonlao et al.[7] for different concentrations of whey protein isolate

(WPI) and xanthan gum (XG) in an emulsion-based delivery system for astaxanthin oleoresin. Following the *in vitro* digestion test proposed by Shrestha et al.[24], the authors found that during the oral phase the presence of XG prevented the oil droplet flocculation better than the use of WPI alone. However, during the gastric and intestinal phase there was an increase in the mean droplet diameter, associated with flocculation phenomena, even if lower when XG was added. Furthermore, the rate of FFAs release in emulsions without XG was higher than that in WPI-XG-stabilized emulsions; thus, the authors state that the XG influence on lipid digestion is clear and due to restricted access of bile salts and lipase to the droplet surface, inhibiting the formation of micelles. Lastly, after the final stage of the *in vitro* digestion, the authors found 46.2% astaxanthin released from the emulsion without XG, but only 12.6% from the WPI-XG-stabilized system and these results are extremely connected to the ability of XG to restrict the bile salt and lipase access to the droplets, which let the astaxanthin remain in non-digested droplets.

As the mentioned studies showed, the rate and extent of lipid digestion are related to the ability of bile salts and lipase to get in contact with lipid droplet surface, which can be affected by emulsion instability during the GI process. Flocculation is the main instability process that occurs during digestion, so Wang et al.[25] investigated its influence during dynamic gastric digestion of O/W emulsions on the physicochemical behavior, oil droplet morphology, and lipolysis in the small intestine. Emulsions were prepared by using as emulsifiers a milk protein concentrate (MPC), a calcium-depleted-MPC (to improve solubility and emulsifying capability), and sodium caseinate. To perform the *in vitro* gastric digestion, the authors applied a dynamic gastric digestion model (Human Gastric Simulator) according to Kong and Singh[26], measuring pH, droplet size change and microstructure, protein composition and hydrolysis, and oil

content in the emptied digesta. In the MPC-stabilized emulsion, for the action of the milk-clotting enzyme pepsin, closely knitted flocs of large size occurred after 10 min until the end of the gastric phase at pH > 6. This led to lower oil content in the emptied gastric digesta and a delay in the delivery of oil droplets to the small intestine. On the other hand, the emulsions stabilized by the calcium-depleted-MPC or the sodium caseinate formed flocs with relatively smaller sizes at later digestion times (40 min) and a lower pH (about pH 5). This resulted in higher oil content and faster delivery of oil droplets to the small intestine. The particle sizes of the gastric digesta followed the order: MPC emulsion > calcium-depleted-MPC emulsion > sodium caseinate emulsion. In contrast, the oil content followed the opposite order, thus suggesting that the emulsion flocculation behavior in the stomach leads to a difference in the amount of oil, and the size and structure of the oil droplets delivered to the small intestine, thus affecting the action of pancreatic lipase in the next intestinal phase. Indeed, in the last phase, the extent of lipid digestion followed the order: MPC emulsion < calcium-depleted-MPC emulsion ≤ sodium caseinate emulsion. The results obtained from this study show that the different behaviors of milk protein ingredients might be used to control lipid digestion. It is worth noting that the Human Gastric Simulator is a dynamic *in vitro* model that mimics the progressive acidification and emptying of GI, thus providing a better simulation of food digestion than the static protocols[26]. In contrast, it can't be used in routine evaluations, due to its complexity, thus semi-dynamic gastric models can be also used, which are simpler but physiologically relevant being able to simulate the gradual pH decrease and emptying and to provide the sequential addition of enzymes and gastric fluid[27].

Double emulsions

Compared to conventional O/W emulsions, double or multiple systems are great solution when preparing reduced-fat products; also, they have the capability of encapsulating, protecting, and controlling the release of both lipophilic and hydrophilic compounds. However, the major challenge for these types of emulsions is the stabilization, so Teixé-Roig et al.[28] studied a W/O/W emulsion for anthocyanins encapsulation and protection, comparing different outer emulsifiers: lecithin, Tween 20, and the combination of these two emulsifiers with a biopolymer, the sodium carboxymethylcellulose (CMC-Na). The lipid digestion profile of the emulsions was significantly different, according to the different droplet sizes obtained: a higher FFAs release (up to 42.68%) was obtained at the end of the duodenal digestion when Tween 20 was used; this emulsifier resulted indeed in the lower droplet size. The addition of CMC-Na to Tween 20 slowed down the FFAs release at first, but at the end of the intestinal phase no differences were found. Lecithin seemed to compete with bile salts and lipase at the oil-water interphase inhibiting lipid digestion and giving the lowest FFAs release (23.44%). Actually, lecithin also produced higher droplet sizes, affecting contact with digestive elements. When adding CMC-Na and lecithin, a lower FFAs initial digestion rate occurred, maybe related to the time needed for bile components to displace the biopolymer from the droplet surface and make the colipase to adsorb to the lipid interface in the presence of bile and then let lipase to bind to the co-lipase. The positive effect of CMC-Na addition to the lecithin-emulsion was observed also on the bioaccessibility of anthocyanin because the higher stability of the oil droplets during the GI simulation and the progressive FFAs release protected the bioactive compound avoiding its degradation. Indeed, anthocyanin is notoriously low stable in simulated intestinal fluid, hence it can be easily degraded and rapidly excreted in the digestive

process. Thanks to these results, the authors found an appropriate anthocyanin delivery system able to control its release.

Other authors[29] tested CMC-Na as a stabilizer for double emulsion intended as carrier for polyphenols contained in mango peel extract (MPE). The *in vitro* digestion evaluation was performed by applying the INFOGEST protocol with slight modifications. The results showed that the presence of CMC-Na caused bridging flocculation in the double emulsion, but then, after the gastric phase, the presence or the absence of the biopolymer did not affect the oil droplet size and microstructure compared to the undigested emulsions. On the contrary, after the intestinal phase, the emulsions had a substantial increase in the oil droplet size regardless of the CMC-Na presence, probably due to the bile salts or pancreatic lipase ability to destabilize the oil/water interface. Increasing concentrations of CMC-Na in the emulsions slowed down at first the lipid digestion rate and this might be related to a bile salt resistant interfacial network created by the biopolymer and to the observed flocculation phenomena. However, at the end of the intestinal phase no differences were observed in FFAs release with or without CMC-Na, possibly due to the desorption of the biopolymer from the interface of the droplets, letting bile salts and lipase to access the substrate and increase the lipid digestion. During the gastric phase, antioxidant release is triggered because of an osmotic imbalance between W_1 and W_2. However, the presence of CMC-Na slightly reduced the water diffusion between the two phases, thus maintaining a controlled release of the antioxidants during the small intestine phase.

The two studies about CMC-Na demonstrated that colloidal properties are very important to regulate the digestion behavior of double emulsions. By changing the physical properties of the inner water phase (W_1) through gelation and/or by using a structured oil phase it is possible to improve stability, encapsulation efficiency, and

release of the encapsulated bioactive compounds. For instance, Andrade et al.[30] studied the effect of W_1 gelation and the use of an oleogel as oil phase on the digestion stability and release of hydrophobic (phytosterols or Vitamin D3) and hydrophilic (Vitamin B12) molecules incorporated in double emulsions. The *in vitro* digestion simulation followed the INFOGEST protocol, without the initial oral phase. After 30 min of gastric phase, a complete digestion of the emulsifier (sodium caseinate) was observed and, therefore, the average oil droplet size decreased regardless the emulsion composition, due to the reduction of the interfacial layer and the release of the inner aqueous droplets. This release was confirmed by the decrease of encapsulated Vit. B12 concentration. The release was lower for emulsion with gelled inner phase, demonstrating the importance of physical properties in affecting the protection of hydrophilic compounds. During the duodenal phase, bile salts, phospholipids, and enzymes of the duodenal fluids caused significant aggregation and coalescence in all the emulsions studied and relatively few double emulsion droplets remained after 5 min. FFAs and hydrophobic compound releases were higher in emulsions prepared with oleogel rather than with the liquid oil, maybe due to the presence of the gelator trimyristin, which has a higher digestibility compared to long chain triacylglycerols. Moreover, a higher release was found for phytosterols compared to Vit. D3, possibly because of the different polarity of the two molecules, causing a different surface-to-core distribution within the lipid droplets. In particular, phytosterols seem to be closer to the interface, while Vit. D3 distribute towards the core of the lipid droplets.

Polysaccharides can act as stabilizers in double emulsions by increasing the viscosity of the outer water phase (W_2) and they can influence lipid digestion in the intestine. Three polysaccharides were studied by Teixé-Roig et al.[31], including Arabic gum, pectin, and sodium alginate for a low, intermediate, and high viscosity effect, respectively.

Different concentrations (from 0 to 2% w/w) were added in the W_2 phase with lecithin, and their effect on emulsion properties and on GI fate of encapsulated phycocyanin during *in vitro* digestion (INFOGEST) was evaluated. As expected, the viscosity increased with the increasing concentration of the polysaccharides, with higher value for 2% sodium alginate. All the double emulsions showed a gradual release of FFAs during the first 60 min of intestinal digestion, then only emulsions with a concentration of 2% pectin or higher than 1.5% sodium alginate continued to release FFAs. The authors suggested that this result might be due to the reduced intestinal enzyme diffusion process caused by the high viscosity and the polysaccharide adsorption at the O/W_2 interface. In addition, after the intestinal phase, they showed a higher digestion extent in emulsions with polysaccharides, except for the emulsions with Arabic gum, less than 1% sodium alginate or less than 1.5% pectin, which showed a similar final FFAs release to the emulsions without polysaccharides. In conclusion, sodium alginate at concentrations higher than 1.5% or pectin at 2% have a positive effect on lipid digestibility, while at low concentrations they had no effect, like Arabic gum. For the phycocyanin bioaccessibility, emulsions with 1.5 and 2% of sodium alginate had the lowest values and this might be related to the high viscosity and the presence of the polysaccharide at the interface O/W_2 reducing the diffusion through the oil film.

Emulsion-filled gels

Emulsion gels are considered the most suitable systems as fat mimetics, because of their semi-solid gel matrix that simulates the fat mouthfeel. This can lead to great advantages when designing a new food product intended to be used for reducing fats and calories in a diet, without losing physicochemical and sensory properties[32]. Moreover, these systems can be used as potential carriers of lipophilic bioactive compounds, providing

nutritional benefits and increased protection during the digestion process. A recent study highlighted the great potential of these delivery systems in co-encapsulating bioactive compounds with opposite chemical behavior[33]. Indeed, cold-set emulsion-filled gels of heat-denatured whey protein isolate (hWPI) were prepared with 0, 10, and 25% sunflower oil to deliver α-tocopherol (liposoluble, dissolved in the oil phase) and resveratrol (amphiphilic, previously dissolved in ethanol and then mixed with hWPI solution). The *in vitro* digestion evaluation found an inverse correlation between the percentage of oil and the amount of resveratrol and α-tocopherol released during both the gastric and intestinal phases. In particular, the release of bioactive compounds during the gastric phase was higher (about 90%) in gels without oil and with 10% oil for resveratrol and α-tocopherol, respectively. In the emulsion-filled gels with 25% oil, the bioactive compound release reached 64-80% during the gastric phase and 100% during the intestinal phase. After release, the stability of α-tocopherol was positively related to the released amount of resveratrol in the gastric environment, suggesting a synergistic behavior of the bioactive compounds to be considered when different molecules are encapsulated in the same delivery system. During the intestinal phase, resveratrol stability decreased because of the higher pH that degrades the molecule, especially without oil, whereas in 10 and 25%-oil gels the stability of resveratrol remained around 70-60%, while that of α-tocopherol decreased to 25%. Thus, this study showed that it is possible to deliver two bioactive compounds with different chemical properties with the same system and to manipulate the digestion process by changing the formulation of the delivery system. Finally, it also demonstrated that in co-encapsulation it is important to choose bioactive compounds able to promote a synergistic effect during digestion.

The importance of carefully evaluating the choice of delivery-system ingredients was highlighted also in a recent study by Lin et al.[34]. Alginate-based emulsion gels are

usually characterized using WPI as emulsifiers to improve the encapsulation efficiency during gelation and the stability of the encapsulated compounds during storage. However, the presence of these proteins may change the mechanical and behavioral properties of the emulsion gels during gelation and digestion, and so the ability to release the encapsulated compounds. By applying the INFOGEST protocol, the authors evaluated the mechanical properties and lycopene release of alginate-based emulsion beads with and without WPI and soy protein isolate (SPI). Even in the absence or in presence of the two proteins, gel rigidity increased at the end of oral and gastric digestion, probably because of the reaction between calcium ions in alginate-based gel beads and sodium bicarbonate in the simulated gastric fluids and the low pH causing a bead shrinkage. The lycopene and oil droplets were found to be released from alginate-based emulsion gels because of the bead structural degradation; interestingly, the two proteins stabilized the emulsion gel beads, delaying the structural degradation during the intestinal phase, and slowing down the release of encapsulated ingredient. A different delay extent was observed as a function of the protein used. This study reveals that it is possible to design emulsion gel beads with a controlled release of some hydrophobic bioactive compounds, by modulating the composition of the gel matrix. However, the authors underlined that to have a complete view of the role of proteins in emulsion gel properties, it is necessary to evaluate both the lycopene stability during storage and the bioavailability during the intestinal phase.

Another study demonstrating the possibility of delaying the emulsion breakdown due to bile salts and digestive enzymes modulating the gel rigidity was performed by Guo et al.[35]. They demonstrated that the salt concentration in a gel emulsion formulation plays an essential role in determining its firmness and the lipid digestion rate. The authors used an *in vitro* test methodology developed by themselves to evaluate the intestinal

lipolysis of canola oil in four heat-set WPI-stabilized emulsion gels prepared with NaCl and $CaCl_2$. In the case of a soft gel, with 7 mM of NaCl, no gel particles and few oil droplets remained just after 30 min of contact with the simulated intestinal fluid, which were completely lipolyzed after 60 and 120 min of intestinal digestion. By increasing the NaCl concentration to 70 mM, the core of the gel had only little changes compared to the surface where severe signs of oil droplet coalescence were observed at the end of digestion. Conversely, using 200 mM NaCl, no large particles were present after 120 min of intestinal digestion, with a rapid breakdown. Lastly, the addition of 25 mM of $CaCl_2$ during gel preparation improved the performance, particularly because the core particles were unaffected by digestion and the coalescence effect at the gel surface was very small. These results confirmed the ability of salts to generate a stronger gel network conferring significant protection to the oil droplets and so delaying lipid digestion. However, even though the results confirmed the gel rigidity influence on the digestion process, the authors performed only the intestinal step; it might be interesting to study the integrity of the gel even during the oral and gastric phases, to have a complete vision of the network behavior even after the gastric acid environment. Indeed, a similar study was made by Luo et al.[36] investigating the effect of two whey protein emulsion gel with different strengths, hard (obtained with 200 mM NaCl) and soft (obtained with 10 mM NaCl), on the digestion process and the bioaccessibility of capsaicinoids (CAP). The authors used a Human Gastric Simulator to conduct *in vitro* dynamic digestion[26] and demonstrated that, besides the gel characteristics, also the structure of the gastric digesta has an impact on the overall digestion process and, as presumable, on the CAP bioaccessibility. The buffering capacity during the gastric phase of both gels was comparable, but the oil droplet size of the emptied gastric digesta from the hard gel slightly increased after 210 min of digestion with very little

coalescence effect; on the opposite, the soft gel oil droplets underwent coalescence at 60 min. So, according to the study by Guo et al.[35], the soft gel matrix disintegrated more rapidly and released more oil droplets than the harder ones. As expected, the bioaccessibility of CAP from the food matrix was positively correlated with the extent of lipid digestion; additionally, the more lipolytic products are released from the food matrix and participate in micelle formation, the more CAP is solubilized in the aqueous phase, hence the higher is the bioaccessibility. In conclusion, the authors emphasized the importance of considering that the characteristic of the gastric digesta affect intestinal digestion and so the bioaccessibility of the bioactive compounds; in addition, the emptying of the small intestine is a dynamic process that leads to differences in the oil content, structure, and size of the digesta, affecting lipolysis during the intestinal phase. This aspect should be considered when static protocols are used. Actually, even if it is clear that the emulsion structure and composition may affect the digestion process, many studies apply different *in vitro* test methodologies, each with their pros and cons (e.g., not realistic peristaltic movements, not considering stomach morphology), which might have an impact on the results. Mella et al.[37] designed an *in vitro* mechanical gastric system (IMGS) that simulates the peristaltic movements of a J-shaped stomach in terms of frequency and force magnitude and reproduces the gastric pH curve and the emptying process. The IMGS was compared with the conventional INFOGEST protocol, performing the gastric phase in a beaker stirred at a constant speed (SBg); for both systems, the intestinal phase was conducted in a stirred beaker (SBi). The study was carried out using WPI-stabilized emulsion gels produced in a pH range from 4 to 7 to obtain different hardness and cohesiveness values. The percentage of FFAs released during the intestinal lipolysis was higher in the least hard and cohesive gels (obtained at pH 4), confirming the importance of the system structure in affecting

digestion behavior. Moreover, both digestion methods highlighted the importance of gastric motility and emptying when solid and semisolid food matrices are studied. Differences between the two *in vitro* methods were found in the intestinal proteolysis kinetic; when applying the IMGS-SBi methodology, the kinetic curve had a different shape according to the sample pH (i.e., the lag phase extent was found to be pH-dependent), and the proteolysis percentage was higher than that of the other method. On the contrary, when applying the SBg-SBi method, the kinetic was similar for all the samples and without a lag phase; the authors ascribed this result to the absence of the gastric-emptying process in this protocol, which brings an immediate hydrolyzation of the substrate. Finally, the higher percentage of proteolysis obtained with the IMGS-SBi method might indicate that digestion systems with agitated vessels may underestimate the intestinal proteolysis, and the authors infer that with the SBg-SBi system, the trypsin and/or chymotrypsin lose their activity due to the substrate or hydrolytic products accumulated in the system during digestion time. This study showed the multitude of factors that affect the *in vitro* digestion methodology and, consequently, the results. However, even though the IMGS-SBi methodology presented results closer to the *in vivo* physiological conditions thanks to the well-reproduced gastric-emptying simulation, the use of other *in vitro* methodologies requiring simpler and cheaper laboratory equipment, easily available for more researchers, must be considered and encouraged, possibly including structural changes occurring throughout the digestion.

Pickering emulsions

Reducing fat content may affect taste, texture, and overall quality of food products, thus, new strategies based on the control of lipid digestibility and bioavailability are under study. For instance, in Pickering emulsions the use of colloidal solid particles

instead of traditional emulsifiers can cause the formation of an irreversible film or network on the lipid droplet surface, limiting the exposure to digestive enzymes and GI fluids. To make emulsions more stable, food-grade nanoparticles have been used; however, the nanoparticles might affect the digestion and absorption processes, thus requiring further investigations. For this purpose, Zhou et al.[38] compared the impact of chitin nanocrystals and a non-ionic surfactant (i.e., Tween 80) in stabilizing a carotenoid-loaded O/W emulsion, by using the INFOGEST 2.0 method[19] and another static *in vitro* test with lower levels of lipase and higher levels of calcium[39]. The obtained results showed that, with both methods, nanochitin promoted the aggregation of the oil droplets under GI conditions, even during the oral phase, because neutral and low pH values caused nanochitin fibre precipitation, reducing the extent of lipid digestion in the small intestine. In addition, the bioaccessibility of carotenoids decreased with the increase in nanochitin concentration because of its ability to suppress lipid digestibility. Indeed, lipids were digested at 100% and 76% with 0% and 0.5% of nanochitin respectively, thus affecting the release of β-carotene from the oil droplets in the small intestine. The authors suggested that nanochitin might be interesting as a functional ingredient to slow down lipid digestion, however, it must be considered that it might also negatively impact the nutritional value of the food matrix in which it is incorporated, by reducing the bioavailability of lipophilic micronutrients. As for the two simulated digestion methods, the INFOGEST 2.0 protocol gave higher percentages of total released FFAs (99.6 *vs* 68.9%) and carotenoid bioaccessibility (55.2 *vs* 22.7%) with respect to the other procedure, possibly due to differences in the enzyme activities and calcium concentrations used in the two models; so, they were not comparable. As the authors remarked, being the INFOGEST 2.0 protocol validated with *in vivo* tests, it must be considered more reliable.

Nanocellulose can be used as solid particles for stabilizing Pickering emulsions and can be resistant to hydrolysis in the GI tract. However, nanocellulose has different crystalline allomorph and morphology, affecting the stabilization ability; a recent study by Li et al.[40] explored the influence of cellulose nanocrystals (CNCs-I and II) and nanofibers (CNFs) on *in vitro* digestion of emulsions, by simulating the oral, gastric, and intestinal phases. The evolution of droplet diameter after each step was observed; CNCs-I showed a higher hydrophobicity than CNCs-II, leading to smaller droplets. However, CNCs-I presented a higher Zeta potential, so the negative charge led to an electrostatic repulsion among nanocrystals, limiting the density and stability of the interface film. Bimodal diameter distribution curves were observed for both CNCs I and II nanocrystals and emulsions; conversely, CNFs showed a monomodal distribution. After oral digestion of the three emulsion samples, peaks of the particle size curves were slightly shifted to the right, but the volume-weighted mean diameter did not change, suggesting the stability of the emulsions during this phase. During gastric digestion, the droplet diameter values of the three emulsions increased from 2.7 - 4.6 μm to about 7 μm, remaining still relatively small and showing no differences among the samples. Only 0.5% volume droplets larger than 100 μm appeared in the CNCs I and II emulsions, but not in the CNFs one. After small intestine digestion, droplet diameter drastically increased with most of the droplets in the range of 10 - 100 μm. FFAs release during the small intestine phase was quick during the first 15 min for all the samples; for CNCs I and II emulsions the FFAs release after 120 min was above 45%, while being 28% for the CNFs sample. Since CNFs do not only absorb at the O/W interface but also connect droplets forming a network, the FFAs release is lower than that occurring in CNCs I and II samples, for the reduction of the surface area available for pancreatic lipase enzymatic hydrolysis. CNCs II-stabilized emulsion had particle

size and aspect ratio smaller than the sample stabilized by CNCs I, resulting in a denser film with a higher surface coverage, limiting the lipid digestion and giving a FFAs release of 48.67% compared with the 56.60% for CNCs I. This study showed how nanocellulose differences in crystalline allomorph and morphology have an impact on GIT digestibility and FFAs release. These results might help in controlling lipid digestion or the release of bioactive substances; however, it would be interesting to evaluate different concentrations of nanocellulose or a mixture of nanocrystals and nanofibers.

If opportunely extracted and treated, nanocellulose is non-toxic for humans, compatible for use with biological tissues, and biodegradable in the environment[41]. However, due to their small size and high surface area, nanoparticles can pass more easily through tissue and cell barriers, promoting biological damage and toxic reaction. Nowadays, only a few studies on nanocellulose[42, 43] evaluated the *in vitro* cytotoxic and genotoxic properties, indicating an absence of DNA damage and no effect on the inflammatory system in macrophages. There is still a knowledge gap about the benefits and possible risks of using nanoparticles in foods, which can limit their applications and consumer acceptance. Consequently, it will be important to establish the potential toxicity of these new ingredients, in view of establishing the levels at which they can be safely used in foods[44].

Other solid particles that can be applied in stabilizing Pickering emulsions are protein isolates obtained from different sources such as plants. Tang et al.[45] applied *Desmodium intortum* protein isolate (DIPI) to create a gel-like network structure with high stability intended as a carrier for β-carotene. The *in vitro* digestion simulation was conducted on both DIPI Pickering emulsions (DIPIPE) and Tween 20 emulsion (TE) as control. The authors evaluated FFAs release as an indicator of β-carotene bioavailability

because lipid degradation by lipase hydrolysis enables the release of β-carotene from emulsion droplets. After gastric digestion, flocculation was observed only in DIPIPE, due to the protein hydrolyzation into polypeptides with lower emulsifying properties. After the small intestine phase, tiny droplets were found in TE indicating higher lipid hydrolyzation, while DIPIPE showed smaller flocculated structures difficult to be reached by lipase, delaying fat hydrolysis. Actually, in the first 30 min of intestinal digestion, the FFAs release was rapid in both DIPIPE and TE, but faster in TE. Then the releasing rate significantly decreased until 60 min for both systems. Afterward, from 60 to 120 min, FFAs release did not change for TE, whereas for DIPIPE a slow increasing trend was observed, delaying fat degradation. The final FFAs release was about 48% for DIPIPE and 75% for TE. In the end, DIPIPE showed a gel network structure with a thicker interface layer inhibiting the lipase action and so the rate and extent of lipolysis; since the degree of lipolysis is positively correlated to micelle formation in which β-carotene dissolved, DIPIPE exhibited a lower bioactive compound bioaccessibility compared with TE. However, the authors highlighted that since plant proteins are natural and safe emulsifiers with positive health effects, DIPIPE could be a good carrier for β-carotene, modulating its release.

Another interesting work about the application of plant proteins for Pickering emulsion stabilization aimed to prepare pea protein isolate-chitosan (PPI-CS) nanoparticles to be used in emulsions systems for the delivery of eicosapentaenoic acid (EPA), possibly reducing the fishy smell[46]. Besides the comparison of the effect of different nanoparticle concentrations (0.5 – 4.0%) and corn oil fractions φ (0.4, 0.5, and 0.6) on physicochemical and rheological properties of PPI-CS Pickering emulsions, the authors evaluated EPA storage stability and digestion behavior by means of *in vitro* and *in vivo* methods. As for the EPA stability, the Pickering emulsions showed good properties,

with high retention rate and oxidation protection, even higher in emulsion with φ = 0.6 than in system with φ = 0.4. Similarly, the *in vitro* digestion method showed positive effects of the Pickering emulsions on the bioavailability of EPA, higher for emulsion with φ = 0.4, maybe due to the lower viscosity of the gel-like network with the lowest oil fraction, facilitating the movement of lipase to oil. As for the *in vivo* test, two groups of mice were fed with EPA encapsulated in the Pickering emulsion with φ = 0.6 (EPA-PE) or in the Tween 80 emulsion (EPA-Em). Intestinal content, small intestine wall, and blood samples were collected by a gavage, for 5 h. In the lipid fraction of the intestinal content, the concentration of EPA peaked at 1 h and then decreased in both the EPA-PE and EPA-Em groups. The same behavior was identified in the lipids of the small intestine wall but after 2 h. In both cases, the EPA concentration measured in the EPA-PE group was higher than that of EPA-Em group and this could be explained by the slower gastric emptying associated with PE, involving a longer time to release EPA. For the lipids of the blood serum, the EPA peak time was registered after 2 and 1 h for the EPA-PE and EPA-Em group respectively, because of the larger droplet size of PE, delaying the endocytosis speed and so the peak time. This work represents a promising starting point for the development of PPI-CS Pickering emulsions as delivery systems for EPA. Moreover, it underlines not only the importance of *in vitro* digestion tests for the evaluation of new delivery systems but also the need for *in vivo* digestion simulations, which can provide results closer to what really happens in the digestion process of Pickering emulsions. Ideally, a reliable and accurate *in vivo* study should be performed on humans. However, human clinical studies are typically complicated, expensive, time-consuming, and not always ethically feasible to perform. Therefore, researchers' interests are mainly focused on *in vitro* tests or animal models[47].

554 Dextrin can thick solutions depending on the molecular dimension and can affect
555 rheological properties, so defining the right concentration to completely saturate the
556 O/W interface without promoting depletion flocculation is crucial. This will presumably
557 influence the GI behavior, so Hu et al.[48] applied the INFOGEST procedure to simulate
558 the *in vitro* digestion of whey protein stabilized Pickering emulsions with different
559 concentrations of dextrin (e.g., 0, 7.5, and 15%) intended as a carrier for β-carotene.
560 The particle size distribution analysis was conducted for all the steps of digestion; at the
561 initial stage, all the emulsions showed a monomodal distribution with tiny fat droplets
562 except for the emulsions with dextrin which contained some large flocs. During the oral
563 phase, the absence of dextrin increased the particle size, oppositely to the ones with 7.5
564 and 15% dextrin in which no change in the microstructure occurred, suggesting that
565 dextrin would protect the protein-coated fat droplets from flocculation, inhibiting the
566 interaction with mucin. All the emulsions were highly unstable during the gastric phase,
567 probably because the anionic mucin molecules adsorbed onto the cationic protein-
568 coated fat droplets leading to neutralization. In addition, the whey protein layer on
569 droplets may be partially hydrolyzed by pepsin, promoting coalescence. During the
570 initial stage of the small intestine phase, the flocs formed during the gastric phase had a
571 breakdown, reducing the droplet size; however, the presence of dextrin might inhibit the
572 dissociation of the flocs or promote fat droplet coalescence, since the mean size of fat
573 droplets increased with progressing dextrin. This phenomenon would decrease the
574 available surface area for lipase adsorption, so retarding the lipid digestion process. At
575 the end of the small intestine phase, the samples incorporated with dextrin had an
576 increase in the droplet size. Most of the fat droplets in the Pickering emulsion without
577 dextrin were digested, oppositely to what happened in emulsions with dextrin. In the
578 end, increasing the dextrin amount decreased the β-carotene bioaccessibility from

14.1% without dextrin to 9.1% with 15.0% of dextrin; this might be related to the reduced lipid digestion with the increasing dextrin concentration. These results showed that dextrin might be applied to reduce fat intake since its presence inhibits the *in vitro* lipid digestion; the authors suggested that this might be due to the ability of dextrin to hinder the attachment of lipase to the fat droplet surface, to promote droplet flocculation and coalescence after the initial stage of the intestinal phase, and to increase the viscosity slowing the movement of lipase to the surface of the fat droplets. To better understand the dextrin role during emulsion digestion, a deeper study also considering dextrin digestibility as a function of the degree of hydrolysis (Dextrose Equivalence) would be desirable.

High Internal Phase Emulsions – HIPEs

Polymethoxyflavones (PMFs) have a low solubility in the aqueous phase, and they tend to easily crystallize in oil. The study by Wijaya et al.[49] presented a possible approach to overcome this issue, by loading HIPE with PMFs isolated from citrus peel powder, stabilized by a complex of WPI and low methoxy pectin (LMP), to improve the bioaccessibility and bioavailability of PMFs during digestion. During the lipid digestion, fatty acids are released, causing a pH decrease, so by using *in vitro* pH-stat methodology and an *in vitro* dynamic digestion model, HIPE complexes were compared with PMFs in medium-chain triacylglycerol (MCT) oil. *In vitro* lipolysis showed that almost all lipids in the HIPE complexes were digested within 10 min from the onset, reaching a plateau after 40 min. The improvement of lipid digestion of emulsion increased the bioaccessibility of two of the major PMFs of the citrus extract (i.e., tangerine and nobiletin). The authors highlighted that by applying a pH-stat lipolysis model some important factors involved in intestinal digestion are not considered, such

as dynamic bile salts and enzymes secretion, peristaltic movements, and adsorption of digestion fluids. The next step was to perform a computer programmed *in vitro* digestion simulation (TIM-1) to evaluate the bioaccessibility of PMFs released from HIPE complexes and MCT oil at each intestinal section as a function of time; the concentration of tangerine and nobiletin in HIPE systems gradually increased in the first 3 h at the major sites of absorption (i.e., jejunum and ileum), compared with the MCT oil that showed a quite low concentration. This was related to the previous results about the increased digestion of the oil phase of HIPE complexes, which increased the PMFs bioaccessibility in these systems. Besides the promising results, the authors suggested the need of applying an *in vivo* methodology to correlate these results and overcome the TIM-1 limitations (e.g., endothelial absorption, microorganism in the upper GI trait).

The increasing consumers' demand for "clean label" products has focused attention on the research of natural and sustainable ingredients. Lignin is the second most abundant biopolymer in nature, so Chen et al.[50] tested the effects of emulsifier concentration, lignin structure, and oil phase volume fraction on the microstructure, stability, and rheological properties of O/W HIPEs with β-carotene. Moreover, the bioaccessibility of the bioactive compound was determined by an *in vitro* digestion experiment. Enzymatic hydrolyzed lignin (EHL) from corncobs, alkali lignin (AL) from bamboo and organosolv lignin (OL) were tested as well as lignosulfonate solutions such as sulfonated lignin (SAL) from pine, sodium lignosulfonate (NaLS) and calcium lignosulfonate (CaLS). The retention of β-carotene in EHL and AL stabilized HIPEs were 87% to 95% after 7 days of storage at 55°C in the dark, or 30 days at room temperature and under light. In general, HIPEs exhibited good protection against photo- and thermal-oxidative degradation of β-carotene. At the initial stage of *in vitro* digestion simulation, FFAs were rapidly released from HIPEs with β-carotene; when increasing

the lignin concentration from 1 to 5%, the FFAs levels were respectively 40 and 80% when using AL, and 30 and 50% when using EHL. This was explained by the smaller droplet size increasing the interaction with lipase. Bioaccessibility of β-carotene was higher in AL-stabilized systems than in EHL-ones, and this is related to the smaller size and larger surface area, which are beneficial for oil lipolysis and the promotion of β-carotene micellization. Hydrophilic lignosulfonates such as SAL, NaLS, and CaLS and comparatively hydrophobic OL could not form stable emulsions.

Another interesting HIPE study[12] relates to the ability of soy protein isolate (SPI) nanoparticles to act as stabilizers so that it is possible to obtain a high internal phase Pickering emulsion (HIPPEs) with much greater stability than HIPEs. To create the colloidal particles needed to stabilize these emulsions, soy proteins were pretreated to make microgels (SPM), by forming a structured network in which oil and emulsion droplets are entrapped. The aqueous phase of HIPPEs was prepared with different concentrations of SPI (1.50, 1.75, and 2%), then heated and maintained at pH 7 for 30 min to fabricate the microgels. Different sunflower oil volume fractions were evaluated (0.78, 0.80, and 0.82) during the *in vitro* digestion analysis. A control emulsion was produced with 1.75% SPI and 0.10 oil volume fraction. The lowest FFAs release (19.8%) at the end of 120 min digestion was found in the emulsion with 2% SPI microgels, while the lower concentration of protein microgels gave the highest FFAs release (23.64%). The lipid digestion extent of HIPPEs was substantially less than that of oil, conventional emulsion, and Pickering emulsion, due to the protein effect resulting in a high rigidity and a good barrier property of the structured network, which was able to resist to the hydrolysis and action by the digestive enzymes. Thus, the study demonstrated good potentials of HIPEs in designing foods with specific physiological properties, such as regulating lipid uptake.

Real food applications: failure or success?

Table 2 summarized some studies that applied the different advanced emulsion systems in food matrices and characterized the final products. Hereby, these studies are presented with more details, commenting the major results.

Double emulsions

Sugar reduction can be achieved by different strategies, from the partial/total replacement to artificial sweetener inclusion; however, consumers may find the new products unpalatable and undesirable. Double emulsions enhance the taste perception of foods (e.g., sweetness, salty, and bitterness) thanks to the possibility of incorporating sugar or salt only into the outer phase, increasing the taste intensity and thus giving the possibility to lower salt and sugar concentrations compared with those used in single emulsions[51]. The study by Ilyasoglu Buyukkestelli and El[52] investigated the ability of a W/O/W double emulsion to give a sweet perception higher than a single emulsion (W/O) with the same sugar concentration. In detail, 15% sucrose was tested, in a double emulsion with 40:60 of W/O:W phases and a single emulsion with an equivalent ratio of W:O phases (24:76). For the double emulsion, sucrose was solubilized only in the outer water phase. Emulsions were evaluated by ten panelists from 25 to 60 years by applying the directional triangle test and serving them mixed with yoghurt (3:1 by weight). Although the sucrose concentration was the same in both samples, 75% of panelists assessed the double emulsion as sweeter than the control emulsion. These results confirm the hypothesis that taste receptors interact only with the outer phase of the double emulsion in a shorter time, thus affecting taste perception. This strategy can help to reduce sugar content in food without compromising the taste; however, also

679 technological quality parameters (e.g., viscosity, color, and stability) and shelf life must
680 be studied, to have a complete frame of the effect of sugar reduction.
681 Replacing or reducing an ingredient might affect food hardness or brittleness, and this is
682 the case for instance of reduced-fat bakery products in which the gluten network is more
683 developed than in the full-fat counterpart. The application of W/O/W double emulsions
684 as a way to reduce fat in biscuits is presented in the study by Moriano et al.[16]. A full-
685 fat soft-dough biscuit used as reference (STD) was compared with a reduced-fat
686 reference made with shortening (OPT) and a reduced-fat formulation with a gelled
687 double emulsion as shortening substitute (WOW). Fat reduction decreased dough
688 stiffness because of lower air incorporation compared to the STD formulation. The
689 reduced-fat samples showed a significantly higher moisture level because of the
690 presence of ingredients with a high-water retention capacity (i.e., polydextrose and
691 resistant starch), used as fat mimetics. As for biscuit texture, samples STD and OPT
692 showed comparable results for fracture strength, which was instead six-time higher in
693 WOW formulation due to a much more compact structure. This also affected the WOW
694 biscuits' milk absorption ability, porosity, and overall liking, which resulted
695 significantly lower than in STD and OPT. Thus, the use of a gelled double emulsion as
696 fat substitute in biscuits needs to be further investigated.
697 As presented in the numerous studies for *in vitro* simulated digestion, emulsions have
698 been widely used as a carrier for micronutrients, such as vitamins and/or minerals; in
699 the study by Kabakci et al.[53] a double emulsion was used for magnesium encapsulation
700 to prevent its chemical degradation and a consequent negative taste in food. The authors
701 evaluated the effect of magnesium addition on cake quality, comparing a cake without
702 magnesium, a cake with uncoated magnesium, and two cake samples with magnesium
703 included in a single or double emulsion. The double emulsion better protected

magnesium during baking compared with the single emulsion, while assuring comparable release percentages with the cakes containing uncoated magnesium both in simulated gastric and intestinal juices. Direct magnesium addition resulted in increased cake hardness because of the ability of salt to cause stronger bonds among proteins, and these results were confirmed also by the sensory analysis. As expected, cakes with direct addition of magnesium resulted in the lowest taste scores, while the cakes with the magnesium included in single or double emulsions showed no differences. This study demonstrated that double emulsions might be promising for mineral encapsulation and the production of functional foods.

Emulsion-filled gels

Meat and meat products present a high content of saturated fatty acids, so the need for reformulating is related to health concerns; some strategies include the use of vegetable and marine oils but the differences in the final texture and the susceptibility to oxidation of oils make their application challenging. Emulsion-filled gels are well known for their soft-solid texture that can mimic fat so the study by Paglarini et al.[54] had the aim of developing a functional emulsion gel with rheological and technological characteristics suitable to replace pork back fat in meat products. By using design of experiment techniques, the authors studied different emulsion ingredients (i.e., soy protein isolate, carrageenan, soy lecithin, inulin, pectin, sodium caseinate, and sodium tripolyphosphate) and they concluded that an emulsion gel prepared with soybean oil, soy protein isolate, carrageenan, and inulin can be effectively used to substitute pork back fat and obtain healthier meat products. In the study by Pintado et al.[55], emulsion gels based on extra-virgin olive oil, soy proteins, and alginate and enriched with polyphenols (from grape seeds and a mixture of grape seed and olives) were used as

729 animal fat replacers in frankfurters. Besides the reduction of fat content (about 50%
730 less) in frankfurters with emulsion gels, also a similar decrease in saturated fatty acid
731 was obtained. The total phenolic content in the enriched frankfurters was estimated to
732 be 414 mg/100 g, which is higher than other food matrices, and the authors highlighted
733 the difficulty to establish whether this quantity could offer positive health effects. This
734 aspect might be addressed in further studies to investigate by *in vitro* tests the
735 bioaccessibility and bioavailability of the polyphenols throughout all the digestion steps.
736 All the developed products resulted acceptable from a sensory point of view, thus
737 demonstrating that emulsion gels are appropriate delivery systems for phenols, which
738 may on the contrary result in unpleasant sensory characteristics when added directly.
739 Moreover, good thermal and storage stability were assessed for the frankfurters made
740 with the emulsion gels and the presence of phenolic compounds also improved
741 oxidative stability and safety during chilled storage of the reformulated products. Thus,
742 emulsion gels, eventually enriched with phenolic compounds, can be considered a
743 promising strategy for the replacement of animal fat in processed meats. Another study
744 confirmed that the use of emulsion gels is efficient in reformulating meat products[56]. In
745 this research, it is highlighted that meat industry generates several protein-rich by-
746 products like pork skin, which has a high collagen content known for its good gelling
747 and emulsifying properties. Thus, the authors used pork skin, canola oil, and dietary
748 fibers (i.e., inulin, α-cyclodextrin, polydextrose, and bamboo fiber) to develop emulsion
749 gels as pork back fat substitutes in emulsified meat products. By applying the design of
750 experiment techniques, pork skin, inulin, and bamboo fiber were selected for emulsion
751 gel optimization. The meat emulsion produced with the optimized emulsion gel
752 presented similar emulsion stability and pH to the one containing pork back fat, thus

753 demonstrating that emulsion gels made with dietary fibers can increase nutritional value
754 of meat products, while valorizing the use of a low-cost by-product such as pork skin.
755
756 ***Pickering emulsions***
757 Nowadays, the reduction of saturated and trans-fat content and the addition of dietary
758 fiber to improve the nutritional quality of baked goods has gained a lot of interest;
759 however, altering ingredients might be negative for the microstructure, taste, texture,
760 and appearance of the final food product. In the study by Xie et al.[57], O/W Pickering
761 emulsions to be used as a partial soybean oil substitute in biscuits were prepared using
762 dietary fiber of bamboo shoots as stabilizing particles. The introduction of dietary fiber
763 and the decrease in fat content led to the formation of an irregular network structure in
764 the biscuit dough, decreasing hardness but increasing springiness. The color of biscuits
765 did not change much when the fat replacement was less than 25%, while above this
766 value the brown color became lighter. Increasing dietary fiber content and reducing fat
767 content resulted in a change in the aspect of biscuits, which appeared rough and bubbly
768 the more the amount of fat was replaced. In addition, reducing fat content increased the
769 hardness of biscuits and the authors relate this result to the impact of dietary fibers on
770 the gluten matrix structure of the dough. Thus, further research is needed to improve the
771 fat-mimicking role of the Pickering emulsions based on bamboo fiber.
772 Pickering emulsions were also used in pound cake, which is a very popular dessert with
773 an excessive fat content (16-17%) and prone to microbial growth[58]. In particular,
774 different Pickering emulsions containing sunflower oil and stabilized with zein
775 nanoparticles were tested as partial butter replacers; they were also enriched with
776 cinnamon oil, which has demonstrated a wide spectrum of antimicrobial activity.
777 Pickering emulsion made with 20 g zein nanoparticle solution, 15 g sunflower oil, and 5

g cinnamon oil showed the best effect in replacing 20% butter in pound cakes, thus allowing a decrease in calories and an extent of the shelf-life without altering texture and color of the product.

Cellulose nanofibers (CNF) may be considered as a potential filler and fat replacer in meat products to reduce fat content, thanks to their excellent rheology characteristics and good Pickering emulsifying ability. The ability of CNF to stabilize O/W Pickering emulsion to be used as partial fat substitute of pork fat in emulsified sausages was evaluated in the study by Wang et al.[59]. A 30% pork back fat emulsified sausage was considered as control and in experimental sausages, 30 or 50% of pork fat was substituted with CNF-Pickering emulsions made with palm oil. Besides the obvious fat reduction, the pork fat substitution with Pickering emulsions resulted in a lower cooking loss, higher moisture content, higher lightness values, and improved viscoelastic and textural properties. Thus, the authors concluded that replacing pork fat with Pickering emulsions stabilized with CNF is a good strategy to increase the healthiness of meat products.

High Internal Phase Emulsions

Concerns about unbalanced consumption of saturated fats and environmental issues about the extensive use of palm oil and other tropical oils have raised the need for new alternatives to margarines and shortenings with similar technological properties, particularly important in manufacturing of laminated products (e.g., plasticity, melting temperature). For this purpose, a recent study[60] tried to convert soft-gelled emulsions made with monoglyceride, sunflower oil, and water into a solid fat material. To achieve the typical 20% water content and rheological properties of the roll-in margarine, the soft-gelled emulsion stabilized by the monoglyceride crystals was converted into a

HIPE by applying a drying step. Two samples at different monoglyceride concentrations (i.e., 4 and 9%) were compared with palm margarine. Gentle drying at 30°C for 16 h allowed to obtain emulsions with the right lipid content (80%), whereas longer treatment resulted in an emulsion breakdown. Additionally, the dried HIPEs showed a more solid-like appearance, and a morphological modification of oil droplets from spherical to partially coalesced in polyhedric elements. Based on the rheological behavior, the HIPE with 9% monoglycerides was selected for puff pastry preparation since it showed elastic modulus and critical stress values closer to those of margarine. Thanks to its plasticity, the HIPE was easily laminated between dough layers and did not melt. During baking, there was no oil leaking indicating a good retainment of the fat phase between the layers and allowing the typical leavening of puff pastry. Additionally, the puff pastry obtained with HIPE showed lighter color compared with the margarine puff pastry sample, but a comparable firmness and friability, confirmed also by the sensory analysis.

Conclusions

Several studies demonstrate that emulsions are promising systems to deliver bioactive compounds in food products. However, both digestion fate and real food applications need to be further investigated. *In vitro* digestion simulation is fundamental to understand if and how emulsion type, ingredients, and production technology might interfere with bioactive compound accessibility and availability. As reported in various papers, a delay of flocculation phenomena can help in modulating the release of the encapsulated compound, by modifying the emulsion integrity. The application of standardized *in vitro* digestion protocols should be encouraged to improve and share knowledge in a robust way, but also advancements in *in vivo* tests are desirable, to

verify the real effects in humans and contribute to the development of personalized nutrition. Further studies of emulsion-based delivery systems in real foods are important to evaluate the effects on the quality characteristics of final products, as well as to assess if bioactive compound activity and availability are retained in the processed matrix and during food digestion.

Disclosure statement: The authors report there are no competing interests to declare.

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Figure legend

Figure 1. Different types of emulsions: a) single oil-in-water emulsion; b) double water-in-oil-in-water emulsion; c) gel emulsion; d) Pickering emulsion.

Table 1. Composition and digestion fate of different emulsion types used as bioactive compound carriers.

Emulsion Type	Composition	*In vitro* methodology	Main Results	References
Single	O enrichment: α-carotene 75.6 μg/g oil β-carotene 204.6 μg/g oil Emulsion: 5% (w/w) enriched corn oil 0.5% (w/w) Tween 80 94.5% (w/w) Milli-Q water	INFOGEST[18]	Droplets < 1 μm: ↑ lipid digestion. Small and medium droplets: ↑ hydrolysis TAGs Conversion of MAGs to FFAs and glycerol: ↑ with small droplets. Carotenoid bioaccessibility: ↑ with small droplets	Salvia-Trujillo et al.[21]
	O enrichment: 0.1% (w/w) lycopene in soybean oil W: 5% (w/w) DBOS rice starch Emulsion: 10% (w/w) O 90% (w/w) W	Winuprasith et al.[23]	Lycopene bioaccessibility: ↑ in emulsions	Jain et al.[22]
	O: 1% (w/w) astaxanthin oleoresin in cod liver oil W: 0, 0.25 and 0.5% (w/w) XG 2-5% (w/w) WPI	Shrestha et al.[24]	XG stabilized emulsions: ↓ lipid digestibility ↓ astaxanthin released	Boonlao et al.[7]
	Emulsifier proteins: MPC, calcium-depleted-MPC, and sodium caseinate W: 5.0% (w/w) protein Milli-Q water Emulsion preparation:	Kong and Singh[26]	Flocculation rate in HGS: MPC > Calcium-depleted-MPC = sodium caseinate Particle sizes of gastric digesta: MPC > calcium-depleted-MPC > sodium caseinate	Wang et al.[25]

	4.0% (w/w) protein 20.0% (w/w) soybean oil		O delivery: Sodium caseinate > calcium-depleted-MPC > MPC Lipid digestion extent: Sodium caseinate ≥ calcium-depleted-MPC > MPC	
Double	W_1/O preparation: 22% (w/w) extract solution anthocyanins 3% (w/w) glycerol 70% (w/w) corn oil 5% (w/w) PGPR W_2 preparation: 1.5% (w/w) lecithin or Tween 20 CMC-Na Emulsion: W_1/O: W_2 25:75	INFOGEST[18]	FFAs release Tween 20 > Lecithin-stabilized emulsions with CMC-Na Lecithin-stabilized emulsions with CMC-Na: ↑ bioaccessibility of anthocyanin	Teixé-Roig et al.[28]
	W_1/O preparation: 70% (w/w) corn oil 22% (w/w) inner W_1 with/without MPE (1 mg/mL) in Milli-Q-NaCl 0.1 M 3% (w/w) glycerol 5% (w/w) PGPR W_2 preparation: Milli-Q-NaCl 0.1 M W_1/O/W_2 interface: 2% (w/w) Tween 20 0, 0.5, or 1% (w/w) CMC-Na	INFOGEST[18]	CMC-Na presence: Droplet flocculation ↓ lipid digestion = MPE antioxidant activity CMC-Na presence/absence: = FFAs release = MPE release	Velderrain-Rodríguez et al.[29]

Emulsion: W_1/O: W_2 25:73 W_1: 0.5% (w/w) NaCas 100 mM NaCl 100 mM $MgCl_2$ 1% (w/w) Vit. B12 O: 2% (w/w) PGPR 0.5% (w/w) phytosterols and Vit. D3 in soybean oil W_1/O preparation: 30% (w/w) W_1 70% (w/w) O Emulsion: W_1/O 10% (w/w) + 2% (w/w) NaCas solution in Milli-Q W_1/O/W_2: 3/7/90	INFOGEST[18]	Gelled inner phase: ↓ Vit. B12 release ↑ FFAs and phytosterols and Vit. D3 release	Andrade et al.[30]
W_1 preparation: 1.5% (w/w) PC extract 0.5% (w/w) NaCas 28% (w/w) solution of NaCl 0.1 M O: 2% (w/w) PGPR 68% (w/w) corn oil W_1/O preparation: 30% (w/w) W_1 70% (w/w) O W_2 preparation:	INFOGEST[18]	2% polysaccharides: ↓ PC release during gastric phase ↓ PC degradation during intestinal phase ↑ lipid digestibility 1.5-2% sodium alginate: ↓ PC bioaccessibility ↑ lipid digestibility Most suitable polysaccharide: 1.5% sodium alginate 2% pectin	Teixé-Roig et al.[31]

	2% (w/w) lecithin in NaCl 0.1 M pH 7 solution with 0, 0.5, 1, 1.5 and 2% of Arabic gum, pectin, or sodium alginate. $W_1/O/W_2$ preparation: 20% (w/w) W_1/O 80% (w/w) W_2			
Emulsion-filled gels	Gel: 5% (w/w) hWPI 0.0064% (w/w) resveratrol Emulsion: 5% (w/w) hWPI 0.0064% (w/w) resveratrol 10 or 25% (w/w) sunflower oil with 0.25% (w/w) α-tocopherol	Liang et al.[61] Remondetto, et al.[62]	Release of resveratrol and α-tocopherol after gastric digestion: Gels without oil and with 10% oil > emulsion filled-gel with 25% oil Release of resveratrol and α-tocopherol after intestinal digestion: Gels without oil and with 10% oil = emulsion filled-gel with 25% oil Stability after release: resveratrol and α-tocopherol synergic behavior ↑ gel rigidity at end of oral and gastric digestion	Bao et al.[33]
	Continuous phase: 4% (w/w) WPI or SPI in distilled water 0.4% (w/w) sodium alginate Lycopene-capsulated emulsions: 0.15% (w/w) lycopene 10% (w/w) sunflower oil Mixed with continuous phase 1:9 (w/w) Emulsion gel beads:	INFOGEST[18]	Lycopene and oil droplet release: alginate-based emulsions > protein-based emulsions	Lin et al.[34]

	Drop emulsion into 2% (w/w) $CaCl_2 \cdot 2H_2O$			
	WPI Emulsion: 10% (w/w) WPI 20% (w/w) canola oil Gel preparation: 50 mL WPI emulsion 7, 70 or 200 mM NaCl Or 200 mM NaCl + 25 mM $CaCl_2$	Internal procedure	↓ lipid digestion in firmer gels	Guo et al.[35]
	CAP loaded emulsion: 0.02% (w/w) CAP 19.98% (w/w) soybean oil 10% (w/w) WPI CAP loaded emulsion gel: Addition of NaCl for final concentration of 10 mM (soft gel) and 200 mM (hard gel)	Kong and Singh[26]	Disintegration gel particles: Hard gels < soft gels Hydrolysis of WPI: Hard gels < soft gels CAP bioaccessibility: Hard gels = soft gels	Luo et al.[36]
	W: 9.0% (w/w) WPI in phosphate-citrate buffer at pH 4 and 7 Emulsion gels: 70% (w/w) aqueous phase 30% (w/w) sunflower oil	Internal procedures and INFOGEST[18]	↑ FFAs release in softer gel Differences among *in vitro*/*in vivo* results	Mella et al.[37]
Pickering	W: 1.11% (w/w) Tween 80 in phosphate buffer solution (5 mM) O: 0.1% (w/w) β-carotene in corn oil	INFOGEST 2.0[19] Tan et al.[39]	Nanochitin crystal: ↓ lipid digestion ↓ bioaccessibility carotenoids Carotenoids bioaccessibility: 55.2% INFOGEST 2.0	Zhou et al.[38]

Emulsion: 90% (w/w) W 10% (w/w) O Nanochitin introduction: 0, 0.1, 0.3, 0.5 % (w/w) nanochitin suspensions added in emulsion in ratio 1:1 (v/v)		22.7% other methodology FFAs % release: 99.6% INFOGEST 2.0 68.9% other methodology	
1% (w/w) nanocellulose (CNCs-I, CNCs-II, and CNFs) in corn oil	Jiao et al.[63] Sarkar et al.[64] Sarkar et al.[65]	Nanocellulose presence: ↑ droplet diameter during gastric phase ↑↑ droplet diameter during intestinal phase FFAs release: CNCs-I > CNCs-II > CNFs	Li et al.[40]
W: 5% (w/v) DIPI solution in water O: 0.2% (w/v) β-carotene crystal powder in soybean oil DIPIPE: W/O: 3/7 Control group TE: 2% Tween 20 in ultrapure water O: 0.2% (w/v) β-carotene crystal powder in soybean oil W/O: 1/1	Lin et al.[66] Yi et al.[67]	Bioaccessibility β-carotene *in vitro* digestion: DIPIPE < TE	Tang et al.[45]
Nanoparticles solutions: 2.0% (w/v) PPI in water 0.5% (w/v) CS in acetic acid (1.0%, w/v)	Internal procedure and Han et al.[68]	EPA release during *in vitro* digestion: 60% < 40% emulsion EPA concentration in intestinal content in mice:	Ji et al.[46]

	PPI-CS suspensions: 0.5, 1.0, 2.0, 3.0, and 4.0% (w/w) in ultrapure water O: 10% (v/v) EPA in corn oil Final emulsions O concentrations: 40, 50 and 60% (w/w)		PE > single emulsion Blood serum EPA peak time: PE > single emulsion	
	O enrichment: 0.1% (w/w) β-carotene in corn oil Emulsions: WPI-stabilized with dextrin 0.0-15.0% (w/w) 95.0% (w/w) W 5.0% (w/w) O	INFOGEST[18]	Oral phase: ↑ particle size without dextrin Gastric phase: ↑↑ instability of all emulsions Small intestine phase: ↑ mean size of fat droplets with progressing dextrin concentration ↓ β-carotene bioaccessibility with progressing dextrin concentration	Hu et al.[48]
HIPEs	W: 10% (w/w) WPI and 5% (w/w) LMP in water O: 2% PMFs in MCT oil Emulsion: 20% W 80% O	Ting et al.[69] Ribnicky et al.[70]	*In vitro* lipolysis: Onset at 10 min, plateau after 40 min TIM-1 digestion model: ↑ Tangerine and nobiletin in the first 3h ↓ MCT oil ↑ PMFs bioaccessibility	Wijaya et al.[49]
	20% (w/w) lignin solution phase: EHL, AL, and OL in NaOH 1M SAL, NaLS and CaLS in distilled water. O:	Liang et al.[71] Tan et al.[72] Fan et al.[73]	HIPEs with AL: ↓ droplet size, ↓ surface areas FFAs release with lignin from 1.0 to 5.0%: AL > EHL	Chen at al.[50]

	0.5 mg/mL β-carotene in soybean oil Emulsions: 1.0, 3.0, 5.0, 8.0, 10.0% (w/w) lignin solutions 3.0, 3.3, 3.5% (w/w) APG 75.0, 78.0, 80.0, 82.0% (v/v) soybean oil		β-carotene bioaccessibility (with lignin 3.0% w/w): EHL < AL SAL, NaLS, CaLS, and OL → unstable emulsions	
	W: 1.50, 1.75 and 2.00% (w/w) SPI in deionized water O: sunflower oil Emulsions: 80% (v/v) of O	Internal procedure	↓ FFAs release with increasing SPI concentration	Wen et al.[12]

Abbreviations used: AL: alkali lignin; APG: alkyl polyglucoside; CaLS: calcium lignosulfonate; CAP: capsaicinoids; CMC-Na: sodium carboxymethylcellulose; CNCs-I and II: cellulose nanocrystals I and II; CNFs: cellulose nanofibers; CS: chitosan; DBOS: debranched and OSA-modified starch; DIPI: *Desmodium intortum* protein isolate; DIPIPE: *Desmodium intortum* protein isolate Pickering emulsion; EHL: enzymatic hydrolyzed lignin; EPA: eicosapentaenoic acid; FFAs: free fatty acids; HGS: Human Gastric Simulator; HIPPEs: high internal phase Pickering emulsions; hWPI: heat-denatured whey protein isolate; LMP: low methoxy pectin; MAGs: monoacylglycerols; MCT: medium-chain triacylglycerol; MPC: milk protein concentrate; MPE: mango peel extract; NaCas, sodium caseinate; NaLS: sodium lignosulfonate; O: oil phase; OL: organosolv lignin; PBS: phosphate buffer solution; PC: phycocyanin; PGPR: polyglycerol polyricinoleate; PMFs: Polymethoxyflavones; PPI: pea protein isolate; SAL: sulfonated lignin; SPI: soy protein isolate; TE: Tween 20 emulsion; TIM-1: computer programmed *in vitro* digestion simulation; W: aqueous phase; W_1 inner aqueous phase; W_2 outer aqueous phase; WPI: whey protein isolate; XG: xanthan gum.

Table 2. Food applications of different emulsion types.

Emulsion type	Food Application	Main Results	References
Double	Yoghurt	↑ perception of sweetness in double emulsion than single emulsion	Ilyasoglu Buyukkestelli and El[52]
	Biscuits	Closer network ↑ fracture strength ↓ dough structuration ↓ overall liking	Moriano et al.[16]
	Cake	= hardness cake = specific volume = weight loss = color	Kabakci et al.[53]
Emulsion-filled gels	Pork back fat replacer	Effective formulation for pork back fat substitution: Soybean oil, SPI, carrageenan, and inulin	Paglarini et al.[54]
	Frankfurters	↓ fat content ↓ saturated fatty acids Sensory acceptable Good thermal and storage stability	Pintado et al.[55]
	Meat emulsion	Optimized gel emulsion: Pork skin, inulin, and bamboo fiber. Stability and pH: meat emulsion with optimized emulsion = meat emulsion with pork back fat	Santos et al.[56]
Pickering	Biscuit	↑ overall appearance and texture. ↑ dough viscoelasticity ↑ dietary fiber ↓ fat energy	Xie et al.[57]

	Pound cake	↑ hardness = texture and color Best butter replace (20%): 25g ZNS + 15g sunflower oil + 5g EO	Feng et al.[58]
	Sausages	↓ fat reduction ↓ cooking loss ↑ moisture content ↑ lightness values ↑ viscoelastic textural properties	Wang et al.[59]
HIPEs	Puff pastry	No oil leaking Easy lamination Lighter color = firmness and friability	Calligaris et al.[60]

Abbreviation used: EO, cinnamon essential oil; SPI, soy protein isolate; ZNS, zein nanoparticle solution.