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3  
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- 21 **List of abbreviations**
- 22 **1-lysoPC** 1-lysophosphatidylcholine
- 23 **ALA**  $\alpha$ -linolenic acid
- 24 **ANSES** French National Agency for Food, Environmental and Work Health Safety
- 25 **AO** Antioxidant
- 26 **ARA** Arachidonic acid
- 27 **BSSL** Bile-salt simulated lipase
- 28 **CCK** Cholecystokinin
- 29 **CEH** Carboxyl ester hydrolase (pancreatic)
- 30 **CLSM** Confocal laser scanning microscopy
- 31 **CMC** Critical Micelle Concentration
- 32 **COB** Crude OB
- 33 **CRF** Chloroplast rich fraction
- 34 **DBE** Dissociating Bond Energy
- 35 **DGDG** Digalactosyldiacylglycerol
- 36 **DGMG** Digalactosylmonoacylglycerol
- 37 **DHA** Docosahexaenoic acid
- 38 **EPA** Eicosapentaenoic acid
- 39 **FA** Fatty acid
- 40 **FFA** Free Fatty acid
- 41 **GI** Gastrointestinal
- 42 **GL** Galactolipid
- 43 **GLP-1** Glucagon-like peptide 1
- 44 **GPLRP2** Guinea pig pancreatic lipase related protein 2
- 45 **HGL** Human gastric lipase
- 46 **HII** Inverse hexagonal phase
- 47 **HPL** Human pancreatic lipase
- 48 **HPLRP2** Human pancreatic lipase related protein 2
- 49 **LA** Linoleic acid
- 50 **L $\alpha$**  Lamellar phase
- 51 **LC-PUFA** Long chain polyunsaturated fatty acid

- 52 **MGDG** Monogalactosyldiacylglycerol
- 53 **MGMG** Monogalactosylmonoacylglycerol
- 54 **OB** Oil body
- 55 **PA** Phosphatidic acid
- 56 **PC** Phosphatidylcholine
- 57 **PE** Phosphatidylethanolamine
- 58 **PG** Phosphatidylglycerol
- 59 **PI** Phosphatidylinositol
- 60 **PL** Phospholipid
- 61 **PLA2** Phospholipase A2 (pancreatic)
- 62 **PLRP2** Pancreatic lipase related protein 2
- 63 **PO** Prooxidant
- 64 **PS** Phosphatidylserine
- 65 **PUFA** Polyunsaturated fatty acid
- 66 **PYY** Peptide YY
- 67 **ROS** Reactive oxygen specie
- 68 **SDA** Stearidonic acid
- 69 **SM** Sphingomyelin
- 70 **SQDG** Sulfoquinovosyldiacylglycerol
- 71 **TAG** Triacylglyceride
- 72 **TLC** Thin layer chromatography
- 73 **UV** Ultraviolet
- 74 **WOB** Washed OB
- 75 **WPI** Whey protein isolate

76 **ABSTRACT**

77 Most lipids in our diet come under the form of triacylglycerols, that are often redispersed and  
78 stabilized by surfactants in processed foods. In plant however, lipid assemblies constitute  
79 interesting sources of natural bioactive and functional ingredients. In most photosynthetic  
80 sources, polar lipids rich in  $\omega$ 3 fatty acids are concentrated. The objective of this review is to  
81 summarize all the knowledge about the physico-chemical composition, digestive behaviour and  
82 oxidative stability of plant polar lipid assemblies to emphasize their potential as functional  
83 ingredients in human diet and their potentialities to substitute artificial surfactants/antioxidants.  
84 The specific composition of plant membrane assemblies is detailed, including plasma  
85 membranes, oil bodies, and chloroplast; emphasizing its concentration in phospholipids,  
86 galactolipids, peculiar proteins and phenolic compounds. These molecular species are  
87 hydrolysed by specific digestive enzymes in the human gastrointestinal tract and reduced the  
88 hydrolysis of triacylglycerols and their subsequent absorption. Galactolipids specifically can  
89 activate ileal break and intrinsically present an antioxidant (AO) activity and metal chelating  
90 activity. In addition, their natural association with phenolic compounds and their physical state  
91 ( $L\alpha$  state of digalactosyldiacylglycerols) in membrane assemblies can enhance their stability to  
92 oxidation. All these elements make plant membrane molecules and assemblies very promising  
93 components with a wide range of potential applications to vectorise  $\omega$ 3 polyunsaturated fatty  
94 acids, and equilibrate human diet.

95

96 **Keywords:** plant membrane, polar lipids, digestive lipolysis, antioxidants, surfactants

## 97 **1 INTRODUCTION**

98 **Lipids are very important nutrients for human health but also for living 's growth**, playing  
99 several key roles in the homeostatic regulation of metabolism in both plants and animals, in  
100 particular through three main functions: i) Lipids can be used for energy storage in their neutral  
101 form (triacylglycerides, TAG); ii) lipids also play a crucial role in membrane structure. Indeed,  
102 polar lipids possess amphipathic structure and self-association properties, which allow them to  
103 define the cells and to segregate cellular compounds into organelles; iii) finally, many lipids are  
104 signalling molecules or at the basis of primary and secondary messengers such as eicosanoids  
105 or resolvins, which play a key role in signal transduction and molecular recognition processes.

106 **The digestion of lipids from the diet must provide two essential polyunsaturated fatty**  
107 **acids (PUFA):** linoleic (LA, C18:2,  $\omega$ 6) and  $\alpha$ -linolenic acid (ALA, C18:3,  $\omega$ 3). These two  
108 essential fatty acids (FA) are at the basis of an elongation and desaturation pathway, resulting  
109 in highly bioactive long chain (LC) PUFA such as arachidonic acid (ARA, C20:4,  $\omega$ 6),  
110 eicosapentaenoic acid (EPA, C20:5,  $\omega$ 3) and docosahexaenoic acid (DHA, C22:6,  $\omega$ 3).  
111 Nevertheless, the synthesis pathway of EPA and DHA from ALA exhibit a low conversion  
112 efficiency, thus it is also recommended to consume foods rich in its two essential FA. These  
113 LC-PUFA play indeed a crucial role in the homeostatic regulation of the body by producing  
114 signalling oxygenated lipids called eicosanoids and pro-resolvines [1].  $\omega$ 3 and  $\omega$ 6 FA exhibit  
115 opposite metabolic effects and compete for the synthesis of lipid mediators through their  
116 biosynthetic enzymes. Thus, a balanced consumption of  $\omega$ 3 and  $\omega$ 6, with an optimal  $\omega$ 6/  $\omega$ 3  
117 ratio of 4 and an adequate intake of each type of FA is recommended [2].

118 **Nevertheless, over the last three decades, the  $\omega$ 6/  $\omega$ 3 ratio has considerably increased in**  
119 **Western diets**, going from 1:1 up to 20:1 in very rich diets, due to a significant increase in the  
120 intake of  $\omega$ 6 and a concomitant decrease in the intake of  $\omega$ 3 [3]. Meanwhile, an increase in

121 overweight and obesity incidence has been observed. This increase is certainly multifactorial,  
122 but very likely linked to metabolic imbalances, including changes in the FA composition of  
123 diets. It is therefore essential to maintain our efforts and set up nutritional strategies to decrease  
124 the  $\omega_6/\omega_3$  ratio in our diets and recover normal homeostasis, in particular by increasing our  
125 intake of LC-PUFA  $\omega_3$ , such as EPA and DHA, while limiting the intake of total lipids [4].  
126 Such strategy can be achieved by favouring fish rather than meat in the diet, but also by  
127 increasing our  $\omega_3$  rich vegetable oils intake. More recently, it has also been proposed to enrich  
128 our diet with green vegetal-derived lipids, as an alternative source of essential PUFA [5].

129 **Indeed, some plant membrane assemblies or polar lipids blends are able to improve the**  
130 **assimilation of specific  $\omega_3$  PUFA**, that are better absorbed under the form of phospholipids  
131 (PL) than TAG [6]. However, this better absorption has been proved for long chain  $\omega_3$  PUFA  
132 but disproved for ALA [7]. In addition to PL, plant and algae membranes also include  
133 galactolipids (GL), associated to micronutrients, whose consumption leads to the production of  
134 essential  $\omega_3$  [8]. The great abundance of plants and algae on earth explains that these GL  
135 represent the major class of acylglycerol lipids in nature, and thus the principal source of FA  
136 (80% versus 20% in PL and vegetal TAG), including essential PUFA [9]. Furthermore, green  
137 vegetal and seeds such as walnuts or flax contain significant quantities of ALA (60-70% wt. of  
138 total fatty acids) [1] and are rich in phenolic compounds and other phytochemical derivatives.

139 **As a consequence, the consumption of PUFA-rich vegetal drinks has grown steadily in**  
140 **recent years.** This trend is boosted by new nutritional recommendations and an awareness of  
141 the beneficial environmental and health effect of plant derived fats and proteins. Vegetal drinks  
142 are generally under the form of emulsions, containing proteins, fibres but also oil bodies, which  
143 are lipoprotein assemblies composed of a TAG core encapsulated in a phospholipid-protein  
144 monolayer. The interest of these vegetal beverages is that they naturally contain surfactant

145 molecules that can stabilize these emulsions while presenting a good PUFA and global  
146 nutritional profile. If seed-based beverages have been developed, they are not very diversified  
147 in terms of botanical sources and the leafy parts of plants remain, to date, under-exploited in  
148 the field of human nutrition. In addition to their fibers and bioactive pigments contents, these  
149 leafy parts do contain lipids although it is most of the time ignored – many leafy juices are for  
150 instance wrongly labelled “lipid-free”. The digestibility of these specific plant lipids is also  
151 poorly known, in particular concerning GL, and despite their human consumption can reach  
152 200 mg by day [10] and could be boost up by using plant assemblies as functional ingredients.

153 However, in order to use properly plant polar lipids and lipoproteic assemblies, it is crucial to  
154 understand their digestive behaviour and their oxidation stability. It is thus the objective of the  
155 present review to summarize most of the recent data about plant lipid assemblies (membrane  
156 and OB), their physico-chemical composition and digestive behaviour. These elements will be  
157 detailed in three complementary paragraphs to unveil the potentials of these lipids as vectors of  
158 omega 3 PUFA and to modulate the digestion in given nutritional targets.

159

## 160 **2 PLANT MEMBRANE ASSEMBLIES' DESCRIPTION**

### 161 **2.1 Plasma membranes of plant cells**

162 The plasma membrane is the most complex plant cell membrane, containing lipids and proteins  
163 in proportions that vary according to the type of cell and plant species. The plasma membrane  
164 defines an interface between cells and their environment. Although many cellular functions are  
165 performed by protein compounds (e.g. signal recognition or transduction), lipids also play a  
166 crucial role in the membrane or in the signal transduction. Three main classes of lipids are  
167 present in the plasma membrane of plant cells, namely PL (28-50% wt.), sphingolipids (6-30%  
168 wt.), and sterols (23-52% wt.). The proportions and acyl chain lengths of these lipids influence  
169 the physical state and the properties of the formed lipid bilayer [11]. Compared to other cell  
170 membranes in the animal kingdom, plant plasma membranes are predominantly rich in  
171 phytosterols, with a sterol/PL ratio ranging from 0.6 to 1.5 wt.

172 The structural PL composing the plant plasma membrane are mainly phosphatidylcholine (PC)  
173 and phosphatidylethanolamine (PE) (68-80% wt.), as well as phosphatidylglycerol (PG),  
174 phosphatidylinositol (PI), phosphatidylserine (PS) and phosphatidic acid (PA); regarding FA  
175 composition, the membrane is mainly composed of palmitic acid (C16:0, 18-70% wt.) and LA  
176 (C18:2,  $\omega$ 6, 20-60% wt.), as well as ALA (C18:3,  $\omega$ 3, 7-26% wt.) [11]. A greater diversity of  
177 sphingolipids and sterols is observed in plants than in animals. These two compounds are  
178 essential structural compounds of membranes since they regulate the lipid packing and the  
179 phase transitions of the membrane bilayers [12].

180

## 181 **2.2 Chloroplast membranes**

182 Chloroplasts are flattened disc-shaped organelles of 2 to 10  $\mu\text{m}$  in diameter, present in the cell's  
183 cytoplasm of photosynthetic plants (Figure 1). They contain grana, which are characteristic  
184 cylindrical stacks composed of 400 nm diameter membrane discs, comprising between 5 and  
185 20 layers of thylakoid membranes [13]. The inner chloroplast envelope and the thylakoid  
186 membrane exhibit a similar lipid composition, in contrast to the outer chloroplast envelope,  
187 which exhibits a typical "eukaryotic cells" composition. Thylakoids are the photosynthetic  
188 membrane of chloroplasts and exhibit a fluid architecture, based on more than 100 different  
189 globular proteins (60-70% wt.) dispersed in a lipid matrix. This lipid matrix self-organizes as a  
190 stack of bilayers, composed mainly of PL and pigments (30-40% wt.). The main pigments are  
191 chlorophyll, the key element of photosynthesis, and carotenoids, which display an AO action,  
192 protecting the chlorophyll and the chloroplast membrane against oxidation [14]. Diverse  
193 phenolic compounds are also present in chloroplast and plant membranes, and contribute to the  
194 plant defence against external stresses. Thylakoids specificity is their unique lipid composition,  
195 that is very well preserved in higher plants and cyanobacteria. Thylakoids are made up of  
196 neutral GL in the form of monogalactosyldiacylglycerols (MGDG) (53% wt.) and  
197 digalactosyldiacylglycerols (DGDG) (27% wt.), which differentiates them from most other  
198 biological membranes. These GL corresponds to 80% of the total non-pigmented lipids of these  
199 membranes in higher plants and algae [15]. In addition to these two glycolipids, thylakoids  
200 contain the less abundant anionic glycerolipids sulfoquinovosyldiacylglycerol (SQDG) and PG,  
201 whose proportions vary according to the species.

202 This quartet of lipids shown in Figure 2 exhibits different phase behaviours, which govern the  
203 overall membrane behaviour and thus the thylakoids architecture. Indeed, DGDG, PG and  
204 SQDG form lamellar phases ( $L\alpha$ ), regardless of the membrane hydration rate and allow the  
205 formation of a bilayer; whereas the most abundant MGDG tends to form an inverse hexagonal

206 structure (HII) in aqueous solution. The MGDG/DGDG ratio, hydration and lipid profile  
207 therefore modulate the bilayers self-organization, including the coexistence and transition  
208 between L $\alpha$  and HII phases. In particular, the critical role of DGDG has been highlighted as  
209 one of the main factors in the membranes stacking by establishing hydrogen bonds between the  
210 polar heads of adjacent bilayers, leading to the dense packing of GL bilayers in thylakoids  
211 membrane. Additionally, the regularity between the stack induced by DGDG and the repulsive  
212 electrostatic forces triggered by the charged lipids PG and SQDG [16] allow a greater repulsion  
213 of water molecules. The lipids (SQDG being anionic) but also the proteins entangled in the  
214 membrane can strongly modulate the isoelectric point of thylakoids.

215 A distinction regarding the FA composition of GL exists between the so-called "eukaryotic" or  
216 "prokaryotic" biosynthetic pathway. Indeed, the distribution of C18 and C16 FA on the glycerol  
217 backbone depends on the site of synthesis (chloroplast or endoplasmic reticulum) of the diacyl  
218 precursors of GL. MGDG and DGDG resulting from the prokaryotic pathway (chloroplast) are  
219 mainly C18:3/C16:3 and C18:3/C16:0 respectively (as abundantly found in spinach for  
220 instance); while MGDG and DGDG from the "eukaryotic" pathway (endoplasmic reticulum)  
221 are mainly found with a composition C18:3/C18:3 [17, 18]. The pathways of synthesis of GL,  
222 especially DGDG and MGDG, are described in detail in the recent review of Sahaka et al. [8].

223 In comparison, the outer membrane of chloroplast is depleted in MGDG and contains other  
224 glycerophospholipids (PC, PE, PS, PI...), in addition to PG. In this sense, it is much more similar  
225 in functional and compositional terms to plasma membranes or non-photosynthetic organelles.

### 226 **2.3 Oil body assemblies and oil body membranes**

227 In seeds, nuts, and some other plant tissues, lipids can be found in the form of lipoprotein  
228 assemblies - called oleosomes or oil bodies (OB) -, ranging in size from 500 nm to 2.5  $\mu$ m [19],  
229 depending on growing conditions and environmental factors (Figure 3). These OB are very

230 promising functional food ingredients as recently underlined by Abdullah et al. [20]. The main  
231 physiological function of these entities is the storage of energy in the form of TAG, but they  
232 also play a role in many cellular processes, including the lipid transport, the membrane  
233 synthesis, and the protein storage and degradation [21, 22].

234 Representing up to 75% of the seed volumes, particularly in oilseeds and protein crops, OB are  
235 composed by a TAG core (94-98% wt.), covered by a monolayer of proteins (0.6-3% wt.)  
236 embedded in a PL monolayer (0.6-2% wt.) [23] (Figure 1 C). This membrane protects the OB  
237 from chemical or mechanical stress. The membrane of OB is composed by strong amphiphilic  
238 proteins (mainly oleosins), that confers natural emulsifying properties to OB and allows them  
239 to be located at the water/oil interface [24].

240 Oleosins are alkaline proteins ranging in size from 15 to 26 kDa depending on the seeds and  
241 isoforms [25]. Oleosins exhibit N- and C-terminal hydrophilic domains, presumably oriented  
242 horizontally or extended at the surface of the OB, that frame a highly hydrophobic core domain.  
243 It is assumed that this domain of 72 uncharged and uninterrupted residues fold into an  
244 hydrophobic hairpin with two branches of about 30 residues penetrating into the TAG core of  
245 OB [24]. The secondary and tertiary structures of this central domain remain however unknown,  
246 probably due to the fact that purified oleosins or oleosins present in artificial OB may not adopt  
247 their native conformation [26-28]. The presumed hairpin structure would extend over a length  
248 of 5 to 6 nm. Analyses performed on 1000 oleosins from various plants and green algae revealed  
249 no exceptions to this corresponding sequence  $X_{30}PX_5SPX_3PX_{30}$  (with X representing a large  
250 non-polar residue in  $X_5$  and  $X_3$  and a hydrophobic residue in  $X_{30}$ ) [29]. A transcribed gene  
251 coding for an oleosin-like protein has been observed in two species of primitive modern green  
252 algae, *Chlamydomonas* and *Volvox*. This protein could be the precursor of oleosin, which is  
253 believed to have evolved in green algae to give rise to universal oleosins, whose genes are

254 present in all evolved plant species. Despite these evolutionary diversifications, these  
255 specialized oleosins conserve the central hydrophobic hairpin domain.

256 The isoelectric point of OB oleosins determined on seven species by isoelectric focusing  
257 indicated a range of 5.7-6.6 (rapeseed 6.5, mustard 5.7, cotton 6.3, flax 6.0, maize 6.3, peanuts  
258 6.6, sesame 6.4) [30]. This acid pI contributes to the stability of OB by bringing negative  
259 charges at physiological pH to the membrane, which lead to electrostatic repulsion and steric  
260 hindrance between objects, thus avoiding aggregation and coalescence phenomena. Depending  
261 on the content of protein and PL, which vary according to the type of seed, there is an increase  
262 in the overall size of the OB when these percentages decrease. The size and mass composition  
263 of OB isolated from different seeds are listed in Table 2.

264 Many studies have analysed the lipid composition of different oilseeds but there is less chemical  
265 characterization data on OB from these seeds, as their composition are not exactly similar. The  
266 FA profile of polar lipids in OB is generally enriched in unsaturated FA compared to the TAG  
267 profile but keeps the same pattern in terms of predominant PUFA. The PL classes present in  
268 OB include five types: PC, PE, PI, PG and PS. PC is consistently in the largest proportion,  
269 representing between 40 and 65% wt. of PL. PE and PI are then present at 5-15% wt. of PL. PE  
270 is functionally very important due to its small polar head, that favours membrane curvatures  
271 [31]. PL distribution in different oilseed species is shown in Table 3.

272 OB extraction from plant seed by aqueous extraction results in a naturally emulsified product  
273 with a higher stability than emulsions prepared using surfactants such as Tween 20, or proteins  
274 such as caseinates or whey protein isolate (WPI) [32]. The natural stability of OB [33] has  
275 raised interest for their application in many areas, from cosmetic and pharmaceutical to food  
276 industry [34, 35], which explains why their use is quite widespread in the preparation of food  
277 emulsions such as creams, salad dressings, mayonnaise or liquors [36].

278 Plant polar lipids take part into the human diet, either under concentrated form (lecithin) or as  
279 naturally-assemblies (nut consumption, green vegetable consumption...), but are differently  
280 digested than TAG and can modulate TAG digestion.

281

## 282 **3 DIGESTIBILITY AND DIGESTION MODULATION PROPERTIES OF** 283 **PLANT MEMBRANE LIPIDS**

284 The digestion of lipids depends on their chemical nature, but also on their dispersion state and  
285 on the stability of this dispersion during digestion. Indeed, the hydrolases (lipases,  
286 phospholipases and galactolipases) that are involved in the digestion, act at lipid-water  
287 interfaces.

288 The molecular characteristics of these lipid molecules have an impact on their hydrolysis and  
289 adsorption kinetics but also on their metabolic fate. Lipids represent an extremely wide range  
290 of molecules with diverse structures and functions, whose behaviour, due to their different  
291 physico-chemical properties, is not the same in the digestive system [37]. The gastrointestinal  
292 behaviour of molecules, membrane fragments and assemblies, as well as their effect on  
293 digestion are presented in the schematic overview Figure 4.

### 294 **3.1 Digestibility of plant polar lipids: enzymes at work, influence of chemical and physical** 295 **properties**

#### 296 **3.1.1 Enzymes involved in plant polar lipid hydrolysis in Humans**

297 In humans, no lipolytic enzyme acting on polar lipids is produced in the upper part of the  
298 gastrointestinal tract (oral cavity, oesophagus and stomach). Hence, the lipolysis of these polar  
299 lipids starts in the small intestine. Several lipolytic enzymes are secreted by the exocrine  
300 pancreas into the duodenum, and work together to digest dietary lipids: colipase-dependent  
301 pancreatic lipase (HPL), pancreatic phospholipase A2 (PLA2), pancreatic carboxyl ester  
302 hydrolase (CEH) also called bile-salt simulated lipase (BSSL), and pancreatic lipase related  
303 protein 2 (PLRP2) [38]. Lipolytic enzymes exhibit a more or less extensive specificity for their  
304 substrate. Human PLA2 (14000 Da, 125 AA) is responsible for the intestinal hydrolysis of  
305 glycerophospholipids, including the conversion of PC into 1-lysophosphatidylcholine (1-

306 lysoPC). In adults, PLA2 represents only a small portion of the lipolytic enzymes, with HPL  
307 and CEH accounting for the largest part [39]. HPL is the main enzyme responsible for the  
308 efficient intestinal digestion of dietary TAG in humans. Its enzymatic activity is dependent on  
309 its specific cofactor colipase. It has been estimated that HPL is responsible for the release of  
310 56% of TAG acyl chains in the lumen, whereas human gastric lipase (HGL) only achieves 10  
311 to 25% in the stomach, depending on the type of ingested meal.

312 Galactolipase activity has also been found in human pancreatic juice and in duodenal content  
313 [40-42]. In contrast to HPL, PLRP2 (50 kDa) has been shown to efficiently hydrolyse GL in  
314 the human pancreatic juice, to a similar level to PLRP2 of rats *in vivo* where little or no tritium-  
315 labelled GL were found intact in the chyle [43]. De Caro et al. [41] characterized the specificity  
316 of human PLRP2 (HPLRP2), and demonstrated that HPLRP2 activity towards TAG and PL  
317 was very low, but was high on MGDG monomolecular films. PLRP2 with similar structure has  
318 also been found in the intestines of other species, with higher galactolipase activities in  
319 particular in monogastric herbivores, such as guinea pig (GPLRP2), whose diets contain a  
320 significant amount of GL due to their herbivore dietary habits. The structural features of  
321 enzymes with galactolipase activity in humans, monogastric herbivorous species but also in  
322 some fish, foliovorous insects and microorganisms have been reviewed in detail by Sahaka et  
323 al. [8].

324 CEH (72 kDa, 753 AA) has also been shown to possess galactolipase activity [44], but to a  
325 lesser extent than PLRP2. Like HPLRP2, this non-specific enzyme is able to hydrolyse various  
326 lipid substrates including TAG, PL, cholesterol and vitamin esters, in addition to GL. CEH is  
327 present in higher concentration than HPLRP2 in human pancreatic juice and duodenal content,  
328 which suggests a significant contribution to the digestion of GL [38, 45]. Like PLRP2, CEH is  
329 more active on its substrates in the presence of bile salts. CEH is also able to hydrolyse the three  
330 TAG positions without distinction, but has a strong preference for MAG, thus contributing to

331 the total hydrolysis of TAG in synergy with other lipases. This specificity distinguishes CEH  
332 from other digestive esterases and allows it to hydrolyse partial acylglycerols into final reaction  
333 products (free fatty acids (FFA) and glycerol) in new-borns [46].

334 **3.1.2 Digestibility of plant polar lipids: the influence of i) the structural**  
335 **organization of the substrate and ii) the enzyme specificity within the same substrate**  
336 **group**

337 Lipolysis is a complex interfacial process, where the bioaccessibility of lipids depends on  
338 several parameters, including the structure and the composition of the food matrix, the lipid  
339 physical states and the enzyme specificities. The lipase action in the duodenum depends on the  
340 structure of its substrate but also on the structural changes that the substrate undergoes during  
341 digestion.

342 In the diet, lipids are mostly found in the emulsified form, which is constitutively closest to  
343 their native structure. Recent studies in the field of nutrition have shown that the organization  
344 of lipids in an emulsified form would modulate the digestion and bioavailability of fatty acids  
345 [47]; these studies have highlighted the importance of the interfacial activation of enzymes  
346 involved in the digestion of emulsified lipid droplets [48, 49]. Indeed, some lipases are inactive  
347 in the bulk phase, the lid covering the active site being in a close conformation. When the  
348 lipolytic enzyme encounters an interface, the lid undergoes conformational changes, allowing  
349 the substrate access to the hydrophobic cavity surrounding the active site of the enzyme. One  
350 has to be cautious however with the assignment of interfacial activation to the opening of a lid  
351 controlling the access to the enzyme active site. PLA2 for instance shows interfacial activation  
352 on PL micelles although it has no lid. A more general definition of the interfacial activation of  
353 lipolytic enzymes is a much higher activity on their lipid substrates when forming a  
354 supramolecular assembly, *i.e.* emulsion for lipases and micelles or liposomes for  
355 phospholipases. PLA2 for instance is weakly active on monomeric medium-chain PL but its

356 activity is drastically increased when the substrate concentration exceeds the Critical Micelle  
357 Concentration (CMC), above which micelles are formed [38]. In addition, the hydrolytic  
358 activity of pancreatic PLA2 requires the presence of a calcium ion that interacts with the  
359 substrate and will activate the catalytic effect of the active site of the enzyme. This interfacial  
360 activation process is different for human pancreatic lipase (HPL), which does not require  
361 calcium and is active at the oil-water interface. The presence of a lid in lipases and its  
362 conformational changes are certainly associated with this drastic environmental change for a  
363 water-soluble protein. Changes in the lid conformation result in the exposure of a large  
364 hydrophobic surface that allows lipase adsorption at the oil-water interface. While HPL can  
365 bind to the lipid droplets and hydrolyse TAG on its own *in vitro*, it requires the presence of a  
366 colipase to access its substrate in the presence of bile salts. Indeed, it has been established that  
367 the action of HPL on TAG emulsions *in vitro* is inhibited by bile salts, which hinders the  
368 adsorption of lipase at the lipid-water interface. The presence of a colipase, a 10 kDa protein  
369 produced by the pancreas, allows the specific anchoring of the lipase through the formation of  
370 a 1:1 complex in the presence of bile. However, some gastrointestinal lipases do not need  
371 interfacial activation, such as HPLRP2, which, on the contrary to HPL, acts preferentially on  
372 substrates forming monomers or small aggregates dispersed in solution, such as MAG, PL and  
373 GL [50].

374 The physical state of the emulsified lipids is also of importance for the hydrolytic activity of  
375 the enzymes. It seems indeed quite intuitive that solid state lipids will not get hydrolysed due  
376 to the reduced molecular motions and diffusion rate in solid substrates. More importantly, a  
377 study on the action of pancreatic lipase on various water-soluble and water-insoluble substrates  
378 revealed that the hydrolysis activity only occurred when lipid saturation was exceeded, the rate  
379 of reaction depending on the surface concentration of the substrate per unit volume [38].  
380 Additionally, it is now well-established that the specific surface area of the emulsion is often

381 correlated with lipolytic activity. Nevertheless, the presence of surfactants - bile salts, PL,  
382 proteins - can modulate this correlation by promoting the reversibility of interfacial adsorption  
383 of lipase by forming a barrier or simply by competitive effects.

384 The enzymes specificity towards their substrates is also important and may not be equivalent  
385 within the same lipid group. Overall, several PLA2 have been shown to have a PL head group  
386 specificity and a preference for anionic substrates such as PG and PE, more than for zwitterionic  
387 PL such as PC [51]. The substrate specificity is slightly different in the case of pancreatic PLA2,  
388 whose physiological substrates in the small intestine are the dispersions of long-chain PC in  
389 bile salts. A study by [52] has investigated the aqueous long-chain PC/sodium cholate system  
390 as a substrate for PLA2. The results showed that PC was a good substrate for PLA2 in the form  
391 of large mixed micelles of PC and bile salts, with a PC/cholate molar ratio of approximately  
392 0.8. Similar results were obtained by [53], who showed an optimum activity of pancreatic PLA2  
393 on mixed micelles of bile salts and PC at a PC/bile salt ratio of 0.5.

394 [54] studied the *in vitro* HPLRP2 hydrolysis of natural long-chain MGDG and DGDG extracted  
395 from spinach leaves, compared to the hydrolysis of synthetic medium acyl chain MGDG and  
396 DGDG. A continuous pH-stat assay was established, based on the titration of FFA released  
397 from the hydrolysis of mixed micelles of GL and bile salts. The galactolipase activity of PLRP2  
398 was found to be weakly affected by the acyl chain length, in contrast to the bile salt/substrate  
399 ratio, which governs the micellar solubilization of DGDG and MGDG. This ratio is therefore  
400 an important parameter in determining the optimum conditions for measuring the activity of  
401 PLRP2. Overall, both the acyl chain length and size of the polar head have no major impact on  
402 the active site of PLRP2 compared to classical HPL [50]. The analysis of lipolysis product of  
403 MGDG and DGDG including the FA profile analysis also confirmed the specificity of HPLRP2  
404 for the hydrolysis of the ester bond at the *sn*-1 position of acylglycerolipids [54]. Since ALA is

405 the main FA (>60%) found at this position in MGDG and DGDG isolated from chloroplast, it  
406 is the main FA released upon the hydrolysis of GL by PLRP2.

### 407 **3.2 Digestibility of natural assemblies**

#### 408 **3.2.1 Chloroplasts**

409 The enzymatic hydrolysis of galactolipids from thylakoid membranes and their effects on the  
410 digestive system have been the subject of several studies. As previously mentioned, GL,  
411 including chloroplast rich fraction (CRF) and thus thylakoid membranes, are digested by  
412 enzymes in the pancreatic juice, including PLRP2 and CEH to a lesser extent [38]. Indeed, [42]  
413 have studied the *in vitro* digestion of CRF from pea vine field residue and spinach leaves by the  
414 human pancreatic juice, where MGDG and DGDG were rapidly hydrolysed in  
415 monogalactosylmonoacylglycerol (MGMG) and digalactosylmonoacylglycerol (DGMG). The  
416 impact of thylakoid supplementation upon digestion have been summarized in a recent review  
417 by [55]. Overall, the reviewed studies indicate that the thylakoid membranes have been found  
418 to reduce the digestion and absorption of TAG. Indeed, the thylakoid supplementation in  
419 humans induces an increase in the secretion of the satiety hormones cholecystinin (CCK) and  
420 glucagon-like peptide 1 (GLP-1) as well as the suppression of the hunger hormone ghrelin,  
421 allowing the blood glucose regulation through the increase of insulin secretion and the decrease  
422 of appetite. The reduced appetite due to the secretion of satiety hormones suggests a delayed  
423 absorption of the nutrients in the intestine, which causes the prolongation of lipid digestion, as  
424 demonstrated by *in vivo* studies on animal [56] but also on human cohorts [57]. The observed  
425 effects on the hepatic metabolism lead to a decrease in circulating LDL and total cholesterol,  
426 as well as in the plasma lipid and glucose levels. Also, a decrease in the secretion of leptin was  
427 observed, which regulates the storage of fat and glucose in the body, raising the interest of the  
428 probable anti-diabetes effect of thylakoids. A decrease in serum TAG levels as a consequence  
429 of thylakoid supplementation in mice was observed by [58]. The effects of spinach thylakoids

430 on the distribution of fat content in the body, the faeces and the liver were studied. The results  
431 showed in mice an increase in faecal fat content (23 mg/day compared with 10 mg/day in the  
432 control), a decrease in body fat deposition and a reduction of liver fat accumulation; this led to  
433 an overall weight loss in mice supplemented with thylakoids compared to the control. Reduced  
434 distribution of fat cells in these mice was also observed, ranging from 30-100  $\mu\text{m}$  compared to  
435 50-150  $\mu\text{m}$  in the control mice, suggesting that a thylakoid supplementation would stimulate  
436 lipid storage in smaller cells through precursor recruitment, thus avoiding inflammation of over-  
437 extended cells and alteration of metabolic homeostasis. A similar phenomenon was also  
438 reported by [59], who observed a decrease in the cholesterol absorption in the human subjects  
439 supplemented with milk PL and sphingomyelin (SM), but also a decrease in the degree of  
440 reabsorption of endogenous cholesterol from the intestinal mucosa. Indeed, 20 to 25% of the  
441 ingested SM had been found intact in the ileum, suggesting that their interactions by the  
442 formation of a complex with cholesterol could explain the significant cholesterol reflux in the  
443 ileum after meals (1510-1750 mg).

444 The hypothesis of the absence of polar lipid digestion must be excluded to explain the overall  
445 decrease in intestinal absorption of TAG induced by thylakoid membrane supplementation.  
446 Indeed, thylakoid membranes have been shown to have an inhibitory effect on the HPL complex  
447 *in vitro*, even in the presence of bile salts, which reduced the fat digestion by 80% [60]. The  
448 analyses performed on thylakoids treated with trypsin showed that the inhibitory effects on HPL  
449 were provided by intrinsic transmembrane protein complexes, and especially by the presence  
450 of several hydrophobic groups. One of the possible mechanisms put forward by [60] to explain  
451 the regulation of appetite was based on a competitive phenomenon at the lipid-water interface.  
452 The hypothesis was that thylakoid membranes can adsorb directly to the surface of the lipid  
453 substrate because of their tendency to be at a liquid-liquid interface, preventing access of the  
454 lipase-colipase complex by steric hindrance. Nevertheless, the hypothesis of the inhibition of

455 intestinal lipolysis by GL was based on *in vitro* digestion studies performed with classical  
456 pancreatic lipase only by and the possible hydrolysis of GL of the thylakoid membrane by  
457 PLRP2 and CEH was not considered. The observed satiety effects observed by [60] could thus  
458 have been generated by other thylakoids components (proteins and liposoluble compounds).

459 The intestinal absorption and the metabolic fate of GL in rats were studied by [43]. In these  
460 experiments, rats were fed with [<sup>3</sup>H]FA-labelled DGDG and [<sup>14</sup>C]-labelled DGDG mixed with  
461 soybean oil enriched or not in PC to determine whether the GL were completely hydrolysed  
462 and re-esterified to TAG and PL in the chylomicrons, or whether some could be absorbed as  
463 such. After the recovery of total lipid extracts and aqueous phases from blood and  
464 gastrointestinal organs, the individual lipid classes were separated by thin layer  
465 chromatography (TLC) and the radioactivity was measured in the different extracts. In most  
466 tissues the presence of [<sup>3</sup>H] radioactivity was associated with TAG and PL, indicating that  
467 DGDG given orally to rats were hydrolysed and their FA further re-esterified in TAG and PL  
468 synthesized in the enterocytes and subsequently found in the chyle and the plasma. These  
469 studies excluded a direct absorption of lyso-GL in the intestine nor their use for the resynthesis  
470 of GL in enterocytes.

471 Most of the answers concerning the digestion of GL were obtained on MGDG, DGDG and  
472 SQDG purified from leaf lipid extracts such as spinach. However, further studies on the  
473 mechanisms of GL digestion by direct action of pancreatic enzymes are still required on more  
474 varied and complex sources, such as not purified whole plant material, in order to elucidate  
475 complex mechanisms regarding the inhibition and synergistic effects. Studies performed with  
476 CRF represent one step forward in this direction.

### 477 **3.2.2 Oil bodies**

478 OB are not highly concentrated in plant polar lipids, but they form quasi-natural or at least  
479 minimally processed emulsions when OB have been extracted from their natural matrix and

480 redispersed in a water phase. For this reason, their digestion has attracted a lot of attention over  
481 the last decade.

482 Several data have been collected on the stability and the behaviour of OB during digestion [36].  
483 [61, 62] studied the behaviour of almond OB during their *in vitro* gastric and intestinal  
484 digestion. Acid aggregation was the most important phenomena observed under gastric  
485 conditions, reflecting that gastric pH brought OB close to their pI. A partial proteolysis of at  
486 least a part of the oleosins composing the membrane was also postulated. During the transit  
487 through the intestinal compartment, the transition to a neutral pH and the addition of bile salts  
488 led to the rupture of the aggregates formed in the gastric phase of digestion. The results  
489 indicated a lag phase before the onset of lipolysis, due to a delayed adsorption of HPL,  
490 suggesting that the preserved membrane structure of the OB could hinder the pancreatic lipase  
491 access to the oil/water (o/w) interface. Similar results had been obtained by [63], who studied  
492 almond OB as substrates for several purified lipases, including HPL and GPRLP2. These two  
493 lipases were found to be active on OB, but with a lower activity (18 to 38%) than that observed  
494 on artificial almond oil emulsions. The protein/PL layer covering the OB was found to slow  
495 down the hydrolysis by decreasing the specific activity of the enzyme, and a more or less long  
496 lag phase before the onset of hydrolysis was observed. This lag phase was quite characteristic  
497 of high packing lipid systems, such as chylomicrons, which have a relatively close structure to  
498 OB, as well as milk fat globules. GPLRP2 and the HPL/colipase complex had also been found  
499 to have phospholipase activity on PL of the PL/oleosin layer, despite the presence of the  
500 proteins. The phospholipase activity, even low, could be one of the mechanisms that could  
501 explain the action of lipases on OB TAG. Indeed, the introduction of defects in the PL layer  
502 would allow the lipase to penetrate the membrane and hydrolyse the TAG core. This hypothesis  
503 had been put forward by [64], who showed that the action of a phospholipase was required  
504 before the action of the plant lipase to access the core of TAG during the growth of cucumber

505 seeds. Another possible mechanism would be related to the TAG partitioning at the OB o/w  
506 interface, which would be hydrolysed by lipases bound to the interface without penetrating the  
507 membrane.

508 [65] studied the impact of exogenous proteins on the fate of OB from sunflower seeds in the  
509 gastrointestinal (GI) tract during their *in vitro* digestion. SDS-PAGE analyses of crude OB  
510 (COB) and washed OB (WOB) emulsions indicated a more or less extensive degradation of  
511 oleosins after the gastric digestion step in the presence of pepsin, followed by the intestinal  
512 digestion in the presence of bile salts and porcine pancreatic lipase. A qualitative analysis of  
513 the SDS-PAGE results concluded to a quasi-complete degradation of WOB proteins after two  
514 hours of digestion under gastric conditions, suggesting hydrolysis and/or removal of all oleosins  
515 by pepsin cleavage. In the case of WOB nevertheless, the SDS-PAGE results indicated the  
516 presence of a 6.7 kDa protein fragment after the gastric digestion phase, which may represent  
517 the central hydrophobic domain (as well as residual fragments of terminal hydrophilic domain)  
518 of oleosins, which remained anchored to the oil phase despite the pepsin action on the groups  
519 exposed to the aqueous phase. Additionally, data indicate the presence of oleosins that remained  
520 "intact" despite gastric digestion. The exogenous proteins present in sunflower seed could  
521 explain this only partial hydrolysis of oleosins on the surface of COB. Since sunflower OB are  
522 able to associate with exogenous proteins, the latter could have formed a protective layer  
523 covering the OB, thus affecting their surface potential as confirmed by  $\zeta$ -potential  
524 measurements, and partially shielding the oleosin domains exposed to digestion. This "apparent  
525 protection" from enzymatic digestion was not found at such level in the case of WOB enriched  
526 in WPI or caseinates, which raises the interest of using OB in their native non-isolated form.  
527 The different behaviour of OB during the gastric phase induced significant changes in the  
528 droplet morphology at the beginning of the duodenal phase. Although bile salts were dominant  
529 at the interface of all droplets, COB exhibited significantly smaller droplet sizes than WOB

530 emulsions, whether or not enriched with WPI or caseinates; this may have impacted the rate of  
531 digestion of OB. Finally, a net reduction of the protein band was observed in all types of  
532 emulsions (COB, WOB, WOB-WPI, WOB-caseinates) after the intestinal digestion phase *in*  
533 *vitro*, indicating the removal of the oleosins remaining on the surface of OB by the bile salts.

534 The formation of an exogenous protein layer on the surface of unwashed OB droplets may  
535 explain the similar protection by oleosins observed in the case of *in vitro* GI digestion of almond  
536 OB [61]. Indeed, SDS-PAGE analyses indicated the presence of amandine peptides, resistant  
537 to pepsin hydrolysis on OB surface even after 15 minutes under gastric conditions. Confocal  
538 laser scanning microscopy (CLSM) images supported this observation, showing that the surface  
539 of the almond OB was completely covered with proteins, with the almond proteins and their  
540 digestion peptides remaining attached to the surface of OB under gastric conditions. During the  
541 duodenal phase, despite the hydrolysis of some surface proteins into smaller fragments, a 2-  
542 minutes lag phase was observed before the release of the FFA, suggesting a slower penetration  
543 of pancreatic lipase at the droplet interface, probably hindered by the membrane assembly of  
544 the almond OB and the protective layer formed by exogenous proteins.

### 545 **3.3 Effect of the addition of isolated constituents from plant assembly on TAG digestion**

#### 546 **3.3.1 Effect of GL addition on TAG emulsion digestion**

547 The digestion behaviour of lecithin-stabilized olive oil emulsion droplets coated with various  
548 amounts of spinach GL was studied by [66], the objective of this work being to quantify the  
549 effects of MGDG and DGDG on the *in vitro* activity of porcine pancreatic lipase under duodenal  
550 conditions. The results indicated that DGDG adsorbed at the droplet interface interfere with the  
551 adsorption and penetration of the lipase-colipase complex, preventing close contact with the  
552 substrate, and thus may have an inhibitory effect on the intestinal lipolysis. Interface  
553 measurements indicated significant molecular interactions between the heads of the DGDG,  
554 resulting in a tighter molecular arrangement, slowing down the interfacial penetration of

555 pancreatic lipase. The inhibitory effect persists under physiological conditions, since DGDG  
556 are highly surfactant molecules, they are not entirely displaced by bile salts. As a result, it  
557 slows down the enzymatic hydrolysis of TAG by pancreatic lipase and may be used to trigger  
558 the secretion of satiety hormones by the delivery of undigested fat in the distal ileum,  
559 subsequently leading to a loss of appetite. Because of their ability to delay and slow down the  
560 lipolysis of lipid droplets, DGDG appear to be effective tools in the development of novel food  
561 emulsions to regulate the fat intake and in the treatment of metabolic disorders such as obesity.  
562 Such inhibition effects of GL on the pancreatic lipase activity are interesting but they should be  
563 tested under conditions involving all pancreatic lipolytic enzymes, including PLRP2 and CEH,  
564 to validate this approach. Indeed, [66] only tested these effects of GL on the HPL-colipase  
565 complex without taking into account the possible lipolysis of GL under normal physiological  
566 conditions.

### 567 **3.3.2 Effect of the addition of glycerol-PL as plant lecithin on TAG emulsion** 568 **digestion**

569 Plant lecithins are also believed to modulate the lipid digestion, thus influencing the lipid  
570 metabolism, as oil emulsification with lecithin has been shown to increase the bioavailability  
571 of FA during intestinal absorption [6]. Indeed, as previously mentioned, the amphiphilic nature  
572 of PL brings them natural emulsifiers properties, which results in the spatial arrangement of PL  
573 at the surface of lipid droplets, either in the course of the emulsion preparation or in the GI tract  
574 upon mixing of dietary lipids with that contains endogenous PC (95% wt.). By promoting the  
575 emulsification of dietary fat, PL allow increasing the specific area available for the lipase  
576 adsorption thereby increasing the rate of lipolysis and the subsequent intestinal adsorption. The  
577 direct dose-dependent impact of rapeseed lecithin just blended in bulk oil to increase the  
578 lymphatic lipids and the ALA bioavailability was also very recently reported in rat by [67].

579 Regarding the impact of various interfaces on lipid digestion, [68] showed that the *in vitro*  
580 gastric lipolysis of a linseed oil emulsion was increased by 30% when the lipid droplets were  
581 coated with soy lecithins instead of caseinate or Tween 80. These results were confirmed *in*  
582 *vivo*, with the faster intestinal absorption and bioavailability of ALA observed with soy lecithin,  
583 compared to other emulsifiers. Plant lecithins have also been shown to exhibit a potential  
584 beneficial impact on lipid absorption and a subsequent enhancement of lipid profile in plasma,  
585 liver and adipose tissues [6]. [69] have shown that the long-term supplementation of soy-  
586 derived PC in hyperlipidaemic mice prevented lipid accumulation by lowering the plasma TAG,  
587 cholesterol and leptin levels, thereby lowering the LDL/HDL ratio and alleviating obesity-  
588 related complications. These results suggest the potential of soy lecithins in the delivery of  
589 certain specific FA to certain tissues and in the prevention of obesity-related cardiometabolic  
590 disorders. However, further research is needed to determine whether these effects are found  
591 under normal human lipidemic conditions and to investigate the effects of lecithins from other  
592 sources with different lipid composition. To balance these potential beneficial effects, it is  
593 however worth noting that the use of lecithin as emulsifier may bypass the satiety mechanisms  
594 triggered in the distal part of the small intestine due to a faster gastrointestinal lipolysis and a  
595 subsequent fat absorption [68]. Liquid test meals containing a lecithin stabilized emulsion are  
596 digested at a much higher rate than normal solid-liquid meals with non-pre-emulsified fat [38].  
597 Overall the lipolysis measured at the Angle of Treitz (end of duodenum) can thus reach around  
598 60% with this type of liquid meal while it is only around 30% with a normal meal. Other studies  
599 in healthy volunteers and obese patients [70, 71] have shown a faster absorption of emulsified  
600 dairy fat compared to the same amount of non-emulsified fat. Altogether, these findings may  
601 lead to lower levels of undigested fat and FFA in the lower part of the small intestine and thus  
602 impair the release of satiety hormones like GLP-1 and peptide YY (PYY). Thus, a risk/benefit  
603 assessment should be performed before developing food product with additional plant lecithins.

604 Improving the fat digestion and absorption could be interesting to avoid malnutrition in  
605 premature infants and elderly for instance, while it could be an aggravating factor of obesity in  
606 other subjects.

### 607 **3.3.3 Effect of oleosin addition on TAG emulsions digestion**

608 To our knowledge, the effect of the addition of purified oleosins on the digestion of lipid  
609 emulsion droplets has not been documented to date. Nevertheless, [63] have investigated the  
610 susceptibility of almond OB to the *in vitro* lipolysis by various purified lipases. From the known  
611 behaviour of these lipases on PL-stabilized emulsions like Intralipid (soybean oil emulsified  
612 with egg lecithins), it is possible to deduce some effects of oleosins on lipolysis. Depending on  
613 the lipase, the specific activity measured on OB was found to range from 18 to 38% of the  
614 specific activity measured on almond oil emulsified by Arabic gum, indicating that oleosins  
615 combined with PL have an overall negative effect on lipolysis. For instance, human gastric  
616 lipase (HGL) shows a specific activity of  $71 \pm 3$  U/mg on almond OB versus  $202 \pm 21$  U/mg  
617 on Arabic gum-emulsified almond oil, and around 600 U/mg on Intralipid [72]. While PL  
618 favour the activity of HGL on soybean oil (Intralipid), their combination with oleosins in OB  
619 results in an 8-fold lower activity. These effects on HGL can be compared to those of casein  
620 that also reduces the activity of gastric lipase on rapeseed [73] and flaxseed [68] oils compared  
621 to lecithin alone. With human pancreatic lipase (HPL), colipase is required to measure the  
622 activity on almond OB in the absence of bile salts, while HPL alone is active on almond oil  
623 emulsified by Arabic gum. As seen with HGL, PL activity on OB ( $1079 \pm 98$  U/mg) is reduced  
624 compared to its activity on Arabic gum-emulsified almond oil ( $2865 \pm 269$  U/mg), but only 23-  
625 fold. This activity is characterized by a lag-time before steady state kinetics are observed. It is  
626 worth nothing that, on the contrary to HGL, HPL is not readily active on Intralipid and other  
627 emulsions presenting PL at their surface like milk fat globule [72]. HPL activity on these  
628 substrates usually needs to be triggered by the addition of FFA or the combined action of other

629 enzymes like HGL or PLA2. FFA favour the adsorption of HPL at the oil-water interface.  
630 Similarly, the combination of PL and oleosins in OB allows the binding and action of HPL  
631 associated with colipase. Therefore, oleosins may also have positive effects on lipolysis.

632 Oleosins are known to have a stabilizing role in OB, and this interfacial part very likely  
633 modulate their digestion. Indeed, as natural surfactant components of OB, they provide an  
634 electrostatic and steric stabilization of OB assembly at a physiological pH. Their degradation  
635 has been observed to decrease the OB emulsions stability by [74], which was illustrated by an  
636 increase in the polydispersity of the OB size, a decrease in the interfacial layers elasticity and  
637 alterations of interactions between droplets.

638 The behaviour at the air-water interface of purified oleosins extracted from maize germ OB was  
639 studied by [75]. It appeared that the behaviour of maize oleosins at the air-water interface was  
640 comparable to that of milk proteins and egg yolk alipoproteins, which are used to stabilise many  
641 food preparations. The high surface activity of these proteins is of interest to their use as  
642 emulsifiers to stabilize foods. Nevertheless, a review by [76] reported the allergenicity of  
643 peanut, hazelnut and sesame oleosins, identified and characterized as major lipophilic allergens  
644 in susceptible individuals. This explains why the use of oleosins as emulsifiers or encapsulants  
645 for protein or lipidic drug substances raises certain health issues, particularly for cosmetic,  
646 pharmaceutical or food applications. For example, [77] proposed that increasing the oleosin  
647 content in cocoa beans would promote a mechanism to enhance cocoa flavour by generating  
648 more hydrophilic peptides and hydrophobic amino acids, the precursors of key components of  
649 cocoa flavour. Depending on the application, the patents submitted raise the importance of  
650 removing contaminants such as other proteins from the OB during purification, with the aim of  
651 reducing allergenicity, colour and odour and specially to increase the stability.

652           **3.4. Potential of plant membranes to vectorize compounds of interest in food**  
653 **applications**

654           **3.4.1. GL composition and ability to vectorize essential  $\omega$ 3 PUFA**

655 Due to their abundant presence in nature, thylakoid lipid membranes represent an important  
656 source of essential fatty acids, especially thanks to the unique composition of GL, which are  
657 rich in PUFA and in particular ALA. Among the numerous botanical sources offered by vegetal  
658 biodiversity, spinach thylakoids have been the subject of many scientific studies [78-80]. The  
659 thylakoid lipids of spinach are composed of the quartet MGDG, DGDG, SQDG, and PG, in  
660 proportions presented in table 1, according to a review by [81]. In comparison, non-  
661 photosynthetic sources, such as seeds, possess a different lipid profile, with still GL, but in a  
662 lower proportion and different distribution and FA composition. For example, in wheat flour,  
663 DGDG and MGDG account for 77% wt. of total GL, but DGDG are present in higher amounts  
664 than MGDG, contrary to what is found in the leaves. GL are mainly found in amyloplasts,  
665 whose number and size thus influence the concentration of GL found in wheat flour . As a  
666 comparison, the GL composition of wheat flour in spring is provided in table 1, according to  
667 [82].

668           **3.4.2. Ability of plant lecithins to encapsulate molecules of interest**

669 In a recent study, Vegara et al. [83] have evidenced the ability of liposomes made from rapeseed  
670 PL and stigmasterol (ST) at low concentration to protect lactoferrin during gastrointestinal  
671 digestion *in vitro*. The stability, size and organization of the liposomal vesicles were studied.  
672 The good balance in the membrane composition of the formulated liposomes made it possible  
673 to delay their hydrolysis during the gastric and intestinal digestion phases, thus making it  
674 possible to obtain a significant quantity of intact lactoferrin in the intestine after 120 minutes  
675 of digestion. Overall, liposomes made with rapeseed PL, properly combined with other lipids,  
676 have shown their potential to be used as an oral delivery system for the molecules sensitive to

677 the digestive enzymes, such as lactoferrin, thanks to their protective capacity throughout the  
678 digestive process.

679

680       **4 OXIDATION OF MEMBRANE COMPOUNDS AND**  
681       **MODULATING EFFECTS ON THE DIFFERENT**  
682       **COMPARTMENTS OF THE DIGESTIVE SYSTEM**

683       **4.1 Susceptibility to oxidation of plant polar lipids**

684               **4.1.1 Lipid oxidation**

685       To increase the PUFA intake and especially dietary  $\omega$ 3, it is of the utmost importance that the  
686       plant membranes lipids are stable to oxidation. Reactive oxygen species (ROS) are generated  
687       in cell membranes and are responsible for the degradation of many cellular compounds,  
688       including lipids, proteins and prooxidant (PO) or antioxidant species such as chlorophyll,  
689       carotenoids and phenolic compounds. Treatments such as heating or exposure to light, or the  
690       presence of trace metals in raw materials also play a catalytic role in triggering the oxidation of  
691       lipids in food matrix during their storage [84].

692       Lipid oxidation is a phenomenon whose main mechanisms, kinetics, and factors of variation  
693       are well known today. This radical chain reaction affects the unsaturated fatty acids present in  
694       oil, fat or structural lipids and is generally schematized according to three main steps. i) The  
695       first step of initiation leads to the formation of a first alkyl radical by the abstraction of a  
696       hydrogen atom on methylene group either between two double bonds (at a bis-allylic position)  
697       or adjacent to a double bond (allylic hydrogen atom) by the ROS. This removal thus constitutes  
698       the limiting step of the reaction since it requires the availability of a hydrogen, which is only  
699       the case in lipids having one or more unsaturated chains. This explains the susceptibility of  
700       PUFA to oxidation, whose stability is inversely proportional to the number of unsaturation they  
701       contain. ii) During the second step of propagation, the alkyl radicals formed during the initiation  
702       step react with molecular oxygen, leading to the formation of a peroxy radical; which in turn  
703       react with other unsaturated FA to form hydroperoxides. The latter primary oxidation products

704 are highly unstable and can be converted into secondary oxidation products. iii) The final  
705 termination step then closes the reaction chain when two radical products react with each other  
706 to form a non-radical product.

707 The oxidation of lipids is a phenomenon that one seeks to avoid since it is responsible for 1)  
708 irreversible loss of essential PUFA, 2) early reactions that favour the faster propagation of  
709 oxidation, and 3) the organoleptic degradation of food products, through the formation of  
710 secondary oxidation products, sometimes toxic, that can enter the blood circulation through the  
711 diet and be further absorbed in body tissues, inducing the development of cancer,  
712 atherosclerosis and inflammatory diseases [85].

713 Delaying the onset of lipid oxidation is thus crucial, since it can cause the deterioration of food  
714 products and reduce their shelf life [86, 87].

#### 715 **4.1.2 The structural factors within the plant polar lipids that conditions their** 716 **sensitivity to oxidation**

717 The sensitivity of lipids to oxidation increases with their level of unsaturation whatever their  
718 form: homogeneous lipid medium (oil), biphasic system (o/w emulsion), dispersion (liposomes  
719 in aqueous phase). Nevertheless, many exceptions to this simple rule can be observed  
720 depending on the chemical structure of the lipid containing the unsaturation (FA, TAG or PL  
721 esters), its physical state and its environment (presence of PO metal ions, initial oxidation state,  
722 initial degree of hydrolysis, presence of other AO, partition coefficient in oil/water emulsion  
723 and proximity with interface...) [86, 87]. At a given unsaturation level and in a given  
724 environment, the susceptibility of the lipid molecule to oxidation will mainly depend on the  
725 Dissociating Bond Energy (DBE) necessary to break the carbon-hydrogen (C-H) bond of allylic  
726 or bi-allylic hydrogen atom. The lower this DBE, the faster the oxidation of the molecule.  
727 However, the access to this data for GL in comparison with phospholipids or TAG is not  
728 straightforward but can be inferred from bibliographic data obtained on molecules having

729 similar acyl chains. Within PL, PE, which is often the most unsaturated and plasmalogen-rich,  
730 is the most rapidly degraded. Conversely, PC is more resistant. The oxidative stability of GL  
731 has been less documented although their PUFA content makes them potentially very sensitive  
732 to oxidation [88, 89]. The comparisons of GL, PL and TAG reactivity have been conducted  
733 using sources with different unsaturation levels and they tend to indicate a higher stability of  
734 GL over PL, which are also more stable than TAG. In these experiments, the impact of natural  
735 PO or AO is limited by the fact that the extracts have been purified, but differences in the  
736 physical state of molecule can also modulate the oxidative reactivity. The physical state  
737 (solid/liquid) of TAG, PL or GL (either in hexagonal packing in micelles or in bilayers under  
738 condensed liquid, expanded liquid or gel) will determine the phase distributions and the local  
739 concentrations of substrates. PO or AO agents can also influence the oxidation kinetics, as well  
740 as the quality of the local environment around the molecule. For instance, hydrolysis reactions  
741 generating FFA promote oxidation via interaction phenomena with metal ions that boosts the  
742 initiation and propagation of the oxidation reaction. The control of all these factors is difficult  
743 and may explain some opposition in studies seeking to link the molecular form and the  
744 sensitivity to oxidation.

#### 745 **4.2 Reactivity of polar lipids in natural plant membrane assembly**

746 In the plant kingdom, membrane defence systems are set up to control the ROS concentration  
747 and thus protect the cells. The plants possess several types of AO but also innate enzymes that  
748 are able of neutralizing ROS or regenerating the active forms of AO species. This explains why  
749 a relative chemical stability to oxidation is observed in the different native forms of plant  
750 membrane assemblies.

751           **4.2.1 Chloroplasts**

752 Thylakoid GL are continuously exposed to oxidative stress due to their very high levels of  
753 PUFA as well as their light exposure during photosynthesis [90]. GL may have a different  
754 response to oxidative stress than other classes of lipids such as PL or TAG.

755 The composition of various AO molecules in different spinach extracts was recently  
756 investigated by [91], who studied the AO properties of thylakoids and their possible application  
757 in the cells protection against ultraviolet (UV) radiation. The examined thylakoid extracts  
758 contained phenolic compounds (*i.e.* flavonoids, tannins) as well as pigments (*i.e.* chlorophyll  
759 and carotenoids). All thylakoid extracts tested by the ORAC assay in this study demonstrated  
760 AO capacities that were maintained during storage at 4°C for 1 to 79 months, although these  
761 decreased over time. In addition to various AO molecules, the presence of AO enzymes was  
762 reported in spinach thylakoids, including superoxide dismutase, which regulates the level of  
763 damaging radicals by scavenging superoxide anions, and whose activity does not appear to have  
764 been affected by storage. The enzymatic activity and structural constituents of thylakoids are  
765 believed to be responsible for the potential antioxidant activity of chloroplast in their native  
766 form *in situ*.

767           **4.2.2 Oil bodies**

768 In addition to their significant physical stability, OB exhibit relatively high chemical stability  
769 to oxidation during storage, despite the presence of a significant amount of unsaturated fatty  
770 acids in the majority of oilseeds [92-95]. The presence of endogenous proteins in the mixed  
771 PL/protein membrane surrounding the TAG core, but also of exogenous storage proteins in the  
772 continuous phase in nut-derived juice for instance; partly explains the oxidative stability of  
773 unsaturated FA [23]. In addition to proteins and lipids, OB also constitute a rich source of AO  
774 bioactive compounds, including tocopherols and phytosterols, which have been shown to be  
775 associated with the surface of objects. In particular, [96] reported the presence of a high amount

776 of tocopherol in sunflower OB (0.57 mg/g lipid) extracted by a single wash cycle with sodium  
777 phosphate buffer. Further extraction of the organelles with a 9 M urea solution removed the OB  
778 exogenous proteins as well as some phenolic compounds less hydrophobic than tocopherols,  
779 resulting in a significant increase in the OB tocopherols concentration, supporting the  
780 hypothesis that the tocopherols are intrinsically bound to the structure of oilseed OB.

781 The presence of a high amounts of tocopherols and phenolic AO compounds has been found in  
782 several types of oilseeds, allowing the protection of OB PUFA from oxidation and thus  
783 contributing to their oxidative stability *in vivo* in seed grain.

#### 784 **4.2.3 Lecithins**

785 In addition to their multiple above-mentioned biological and biochemical properties, plant  
786 lecithins have shown AO properties regardless of the source, which is partly attributable to their  
787 high concentration of PL. Indeed, PL possess various AO properties, the mechanisms of which  
788 were clearly detailed in a recent review by [97] and also elucidated in terms of chemical  
789 mechanisms by [98]. In particular, PL exhibit an AO role by chelating the PO metal species and  
790 are able to displacing or regenerating the primary AO species. In addition, scientific studies  
791 have shown that the presence of phenols in plant lecithins, in synergy with PL, increased the  
792 AO capacity of lecithins [99]. For instance, the reactive polar head of PL (amine for PE and  
793 PC, reducing sugar for PI) have been shown to develop synergy with tocopherols, enhancing  
794 the capacity of free radical termination of this latter [100]. To explain such synergy, it was  
795 hypothesized that PL could facilitate the hydrogen or electron transfer to tocopherols and its  
796 regeneration. However, as for OB, these AO capacities are highly dependent on the quality and  
797 the structure of food matrix, and can thus be altered by processing.

#### 798 **4.3 Uses of plant polar lipids protection against oxidation reported in literature**

799 In emulsions, the molecules are distributed according to their polarity and surface activity  
800 within the different phases, which are the oil phase, the water phase and the interfacial region.

801 In these systems, lipid oxidation is a phenomenon that occurs at the droplet interface, where the  
802 presence of a large surface area facilitates interactions between lipids and water-soluble PO  
803 species. Many factors influence the rate of lipid oxidation in these emulsified systems,  
804 including the physico-chemical properties of the interface (thickness, charge, composition,  
805 rheology, permeability) and those of the continuous phase (oxygen concentration, AO and PO  
806 composition, pH, etc.). The current trend towards PUFA-rich, but also minimally-processed  
807 products limits the use of traditional oxidation control methods such as synthetic AO; therefore,  
808 the natural stable systems where lipid oxidation can be controlled, such as plant membrane,  
809 have raised interest over the last few years.

#### 810 **4.3.1 Chloroplasts membrane**

811 The emulsions stabilized by chloroplast thylakoid membrane have been proposed as functional  
812 food to promote satiety and were characterized in terms of stability, interfacial tension and  
813 droplet size but not in terms of stability to oxidation [101]. The stability to oxidation of  
814 chloroplast membrane polar lipids with different levels of unsaturation has been studied. [90]  
815 compared the oxidative stability of GL (MGDG, DGDG and SQDG) from spinach and algae to  
816 PC from salmon oil and to TAG from soybean and sardine oil. Data were obtained after  
817 purification of all lipids to avoid the influence of potentials AO and PO which are naturally  
818 present in these products, such as chlorophyll, carotenoids or tocopherols. The results of the  
819 analyses indicated that MGDG and DGDG of spinach and algae had an overall higher oxidation  
820 stability than that of TAG or PC from marine animal sources. MGDG and DGDG from spinach  
821 and algae showed the same oxidative stability as TAG from soybean oil, whereas the latter had  
822 a lower number of unsaturation numbers. SQDG from spinach and algae showed even a greater  
823 oxidative stability than MGDG and DGDG but presented on average a lower number of  
824 unsaturation than GL fraction of the same sources. Altogether, these results suggested that the  
825 galactosyl and sulfoquinovosyl residues of GL could protect the PUFA unsaturation by

826 enhancing the DBE of allylic hydrogen and/or through different intermolecular interactions  
827 which could modify the molecules packing and reactivity.

828 More recently, [88] have evaluated the oxidative stability of three types of lipids: MGDG and  
829 DGDG of spinach and algae, as well as TAG from linseed oil, previously purified from AO and  
830 PO impurities. The greater oxidative stability of DGDG and MGDG compared to TAG from  
831 linseed oil was evidenced, regardless of the source and despite higher unsaturation levels than  
832 in the TAG fraction. Within GL classes, a greater oxidative stability of DGDG from spinach  
833 and algae compared to MGDG from the same sources was evidenced. In addition to the  
834 modulation of allylic hydrogen BDE, differences in self-assembly structure and physical state  
835 between MGDG and DGDG cannot be excluded. Indeed, [102] established that MGDG form  
836 at the interface a less compact structure than DGDG, with galactosyl headgroup extended at the  
837 interface. On the other hand, DGDG could pack their galactosyl moieties at the interface,  
838 adopting a lamellar phase  $L\alpha$  with parallel orientation of the headgroups with respect to the  
839 plane of the bilayer. This organization of DGDG enhanced their compacity and steric hindrance  
840 at the interface in comparison with MGDG, possibly limiting the interaction with ROS.

#### 841 **4.3.2 Oil bodies**

842 Scientific studies have raised the interest of natural OB emulsions for the preparation of stable  
843 emulsions, since they have a remarkable chemical stability compared to synthetic OB emulsions  
844 prepared with other surfactants. In particular, the stability of OB extracted from *Echium*  
845 *Plantagineum* has been studied *ex vivo* by [95]. The interest of this plant is that its consumption  
846 has a potentially beneficial effect on health since *E. Plantagineum* contains a high concentration  
847 of PUFA, half of which is part of  $\omega$ 3 and includes a significant amount of stearidonic acid  
848 (SDA, C18:4,  $\omega$ 3) (14% wt.), which is converted to up to 30% in EPA in the human body.  
849 Despite the natural oxidative stability of oilseeds *in vivo* during storage, a high concentration  
850 of PUFA results in a susceptibility to oxidation after oil extraction from the seed. The oxidative

851 stability of emulsions of *E. Plantagineum* washed OB dispersed in an aqueous phase was  
852 compared to that of synthetic emulsions of *E. Plantagineum* oil stabilized by emulsifiers: SDS  
853 and Tween 20; as well as to that of bulk oil. After 7 days of incubation at 40°C, a significantly  
854 less marked oxidation was evidenced in the case of the natural *E. Plantagineum* OB emulsion.  
855 Different hypotheses have been proposed to explain this conservation of the oxidative stability  
856 of natural OB emulsions *ex vivo*: (i) the potential AO role of oleosins on the surface of OB by  
857 metal chelation or through sacrificial oxidation; (ii) the formation of a thin and compact layer  
858 by protein (oleosin) arrangement on the surface of OB, which stabilizes the droplet interface  
859 and provides a barrier to oxygen and reactive species ; (iii) the differences in surface  
860 area/volume ratio between the natural OB emulsion and the stabilized synthetic emulsions,  
861 which would reduce the effective surface concentration of tocopherols in the processed  
862 emulsions and explain the observed oxidation differences.

863 Similar results had been previously obtained by [103], who compared the oxidative stability of  
864 natural suspensions of washed sunflower OB with that of formulated sunflower oil emulsions  
865 stabilized with SDS and Tween 20. The results indicated higher concentrations of primary and  
866 secondary oxidation products in the formulated emulsions. The presence of a stable lipid-water  
867 interface was suggested as an argument for the oxidative stability of the natural sunflower OB  
868 emulsion, in addition to the presence of seed protein residues in the continuous phase, which  
869 are preferentially oxidized or chelated by free metal ions. However, it should be pointed out  
870 that the method of OB extraction may affect their physical integrity, and therefore their recovery  
871 method is crucial in maintaining the emulsion chemical stability [104].

872 OB therefore appear as a natural form of oil encapsulation, providing significant protection  
873 against oxidative stress. As developed in paragraph 4.2.2, the presence of tocopherols and  
874 phenolic compounds closely associated with OB in significant concentrations also raises the  
875 interest in the application of OB as a deliver agent for AO such as vitamin E.

876

### 4.3.3 Lecithins

877 Lecithins have been shown to be suitable for use as emulsifiers but also because of their AO  
878 activity, especially in bulk vegetal oils. Indeed, some PL present in plant lecithins showed the  
879 ability to act in synergy with tocopherols, thus improving AO activity [105]. This property  
880 would be related to the ability of PL to give a hydrogen atom of their amine function, allowing  
881 the regeneration of the oxidized tocopherol quinone in its initial form. The amine functions of  
882 PC, PS or PE, and the sugar residue of PI have also been shown to exhibit metal chelation  
883 properties.

884 [100] studied the AO effects of soybean lecithins on the oxidative stability of several vegetal  
885 bulk oils, as well as their synergistic effect with different forms of tocopherols. The best  
886 protective effects against oxidation were observed after incorporation of 5% wt. of lecithin  
887 containing high proportions of PC and PE, especially in oils rich in LA, such as rapeseed, walnut  
888 or soybean. Also, the combination of these lecithins with the  $\gamma$ - and  $\delta$ -tocopherol forms was  
889 shown to be more effective than with the  $\alpha$ -tocopherol or the compounds in their individual  
890 forms. This study showed in particular the potential use of lecithins in the protection of refined  
891 vegetal oils against oxidation. Nevertheless, PC was also shown to have no synergistic effect  
892 with tocopherols, and even in some cases to exhibit PO properties. Indeed, the study by [106]  
893 on the AO activity of PL on perilla oil had shown the AO efficacy of PE and PS in synergy with  
894 a mixture of tocopherols, whereas PC had shown little effect. Also, PE had been shown to  
895 increase the activity of  $\alpha$ -tocopherol, but not PC [107]. Due to its intermediate hydrophilic-  
896 lipophilic balance (HLB), PC exhibits the ability to form reverse micelles when its  
897 concentration is above the CMC, which themselves have been reported to have PO activity [97].

898 In emulsified fish oil systems, lecithins appear to promote the dispersion of other active AO,  
899 thereby limiting the free-radical propagation in the medium [108]. Nevertheless, studies are

900 needed on the synergistic effects of lecithins with phenolic compounds in emulsified vegetal  
901 oil systems, as data are, to our knowledge, still lacking.

#### 902 **4.4. Plant polar lipids oxidation through digestive system**

903 Oxidation of plant lipids can occur prior the ingestion, but can also takes place during the  
904 digestion [109]. During the oral phase, oxidation phenomenon is quite limited in time and the  
905 enzyme activity is supposed to be low. Conversely, the gastric digestion phase takes place at  
906 low pH in the presence of oxygen and catalysts found in food, and the oxidation phenomenon  
907 can thus be quite high and leads to vegetal oil peroxidation and oxidation of vitamin E, C and  
908 beta-carotene. Some AO of food such as polyphenols and glutathione peroxidases can help to  
909 reduce the hydroperoxides production in gastric phase. Gastrointestinal glutathione peroxidases  
910 are also important actors limiting gastric hydroperoxides. TAG monohydroxyperoxydes can be  
911 hydrolysed by HPL almost similarly to non-oxidized TAG but non-absorbable oxidized lipids  
912 can also reach the distal part of the intestine and may favour oxidation and inflammation. The  
913 peculiar oxidative behaviour and AO effect of PL from plant membrane in the gastrointestinal  
914 part is still unknown and should be further investigated. Main data and hypothesis of structural  
915 level that determine plant polar lipids susceptibility to oxidation are summarize in Table 4.

916        **5 CONCLUSION**

917    The plant membrane gathers a diversity of GL and phospholipids, selectively acylated with  
918    PUFA and are already organized as lamellar structure, embedded with proteins and phenolic  
919    compounds.

920    Among these polar lipids, GL concentrating ALA and phospholipids can be used as specific  
921    vector of PUFA. They are hydrolysed by specific intestinal enzymes (PLRP2, PLA2 and CEH)  
922    and allow the reduction of the TAG digestion and absorption. GL specifically can activate the  
923    ileal break. Concerning the potential inhibition activity on pancreatic lipase, *in vitro* studies  
924    involving all pancreatic enzymes are needed to determine the exact involved mechanisms.

925    These plant polar lipids also intrinsically present AO activity (higher stability to oxidation than  
926    TAG) and metal chelating activity. In addition, their natural association with the phenolic  
927    compounds in membrane assemblies and their physical state ( $L\alpha$  state of DGDG) can enhance  
928    their stability to oxidation.

929    Minimally-processed plant assemblies such as OB or chloroplasts which have acidic isoelectric  
930    point and are not hydrolysed by gastric enzyme can thus be preserved during this phase and be  
931    conveyed with minimal transformation to duodenum. Their stability to oxidation in gastric  
932    phase remains to be elucidated.

933    Altogether, all these elements make these plant membrane molecules and assemblies very  
934    promising components with a wide range of application to vectorise  $\omega$ 3 PUFA and equilibrate  
935    human diet.

936 **Author Contributions** – C. Bourlieu, V. Vié and J. Kergomard determined the outline and  
937 content of the review, designed the tables and figures, J. Kergomard wrote the review, all  
938 authors corrected and implemented the review.

939

940 **Conflicts of Interest** – none to declare.

941



943 **TABLES**944 **Table 1** Lipid composition (in percent by weight) of spinach leaves chloroplasts membrane and wheat  
945 flour

		MGDG	DGDG	SQDG	PG	Others
Chloroplast	Thylakoids <sup>a</sup>	53.0	27.0	7.0	7.0	6.0
	Inner	49.0	30.0	5.0	8.0	8.0
	membrane <sup>b</sup>					
	Outer	17.0	29.0	6.0	10.0	38.0
	membrane <sup>b</sup>					
Amyloplasts	Wheat flour <sup>c</sup>	0.4	1.0	-	-	0.4

<sup>a</sup> Isolated from spinach leaves [% wt.], [110]

<sup>b</sup> Isolated from spinach leaves [% wt.], [111]

<sup>c</sup> Spring wheat flour [% w.],[82]

Source: [81]

946

947

948 **Table 2** Size ( $\mu\text{m}$ ) and mass percentage composition of OB isolated from various seeds

Seed	Rapesee	Flaxsee	Mustar	Cotto	Corn	Peanu	Sesame	Soybea	Sunflower
	d	d	d	n		t		n	
Diameter ( $\mu\text{m}$ )	0.6 <sup>a</sup>	1.3 <sup>a</sup>	0.7 <sup>a</sup>	1.0 <sup>a</sup>	1.4 <sup>a</sup>	2.0 <sup>a</sup>	2.0 <sup>a</sup>	0.4 <sup>b</sup>	1.0-2.0 <sup>d</sup>
Neutral Lipids [% wt.]	94.2 <sup>a</sup>	97.6 <sup>a</sup>	94.6 <sup>a</sup>	97.0 <sup>a</sup>	97.6 <sup>a</sup>	98.2 <sup>a</sup>	97.4 <sup>a</sup>	85.9 <sup>c</sup>	96.0 <sup>d</sup>
Proteins [% wt.]	3.5 <sup>a</sup>	1.3 <sup>a</sup>	3.2 <sup>a</sup>	1.7 <sup>a</sup>	1.4 <sup>a</sup>	0.9 <sup>a</sup>	0.6 <sup>a</sup>	8.2 <sup>c</sup>	(/)
PL [% wt.]	2.0 <sup>a</sup>	0.9 <sup>a</sup>	1.6 <sup>a</sup>	1.2 <sup>a</sup>	0.9 <sup>a</sup>	0.8 <sup>a</sup>	0.6 <sup>a</sup>	5.8 <sup>c</sup>	2.5 <sup>d</sup>
FFA [% wt.]	0.4 <sup>a</sup>	0.1 <sup>a</sup>	0.2 <sup>a</sup>	0.1 <sup>a</sup>	0.1 <sup>a</sup>	0.1 <sup>a</sup>	0.1 <sup>a</sup>	(/)	(/)

<sup>a</sup> [30]

<sup>b</sup> [112]

<sup>c</sup> [113]

<sup>d</sup> [31]

950 **Table 3** Mass percentage composition of PL of OB isolated from various seeds

P	Rapesee	Flaxsee	Mustar	Cotto	Corn	Peanut	Sesame	Sunflow	Soybea
L	d	d	d	n				er	n
P	59.9 <sup>a</sup>	57.2 <sup>a</sup>	53.1 <sup>a</sup>	58.9 <sup>a</sup>	64.1 <sup>a</sup>	61.6 <sup>a</sup>	41.2 <sup>a</sup>	81.0 <sup>c</sup>	58.0 <sup>d</sup>
C						60.8 <sup>b</sup>			
P	5.9 <sup>a</sup>	2.8 <sup>a</sup>	15.5 <sup>a</sup>	4.6 <sup>a</sup>	8.1 <sup>a</sup>	5.0 <sup>a</sup>	15.8 <sup>a</sup>	13.0 <sup>c</sup>	15.0 <sup>d</sup>
E						5.1 <sup>b</sup>			
PI	14.0 <sup>a</sup>	6.9 <sup>a</sup>	13.1 <sup>a</sup>	18.1 <sup>a</sup>	7.6 <sup>a</sup>	8.4 <sup>a</sup>	20.9 <sup>a</sup>	5.0 <sup>c</sup>	14.0 <sup>d</sup>
						8.6 <sup>b</sup>			
P	(/)	(/)	(/)	(/)	(/)	(/)	(/)	(/)	(/)
G									
P	20.2 <sup>a</sup>	33.1 <sup>a</sup>	18.3 <sup>a</sup>	18.7 <sup>a</sup>	20.2 <sup>a</sup>	25.0 <sup>a</sup>	22.1 <sup>a</sup>	(/)	(/)
S						25.5 <sup>b</sup>			

<sup>a</sup> [30]

<sup>b</sup> [114]

<sup>c</sup> [31]

<sup>d</sup> [115]

951

952

953 **Table 4** Main data and hypothesis of structural level that determine plant PL susceptibility to oxidation

Compounds assembly	/ Molecular oxidation	stability to	Partition in O/W system	Chelating effect	Physical assembly	ref
Galactolipids	Galactolipids > TAG; SQGD > galactolipids	PC >				[90]
Galactolipids	DGDG > MGDG				Potential effect of L $\alpha$ packing of DGDG	[88]
Phospholipids	PL > TAG			Chelating pro-oxidant metals	Segregate PC in membrane	[97]
Phospholipids			Synergy with tocopherol: facilitate its regeneration through H transfer from amine functions of the PC, PS or PE, and the sugar residue of the PI			[100, 116]
OB	OB > artificial emulsion with same TAG core	reconstituted	Potential AO effect of oleosin at interface			[95, 103]

954

955

956

957 **FIGURES**

958 Figure captions.

959 **Figure 1** Vegetal assemblies' description: (A) Chloroplast, (B) Oil Body, (C) Plasma  
960 membrane - Micrograph adapted from [117].

961 **Figure 2** Structure of the main plant membrane lipids: A) Monogalactosyldiacylglycerol  
962 (MGDG), B) digalactosyldiacylglycerols (DGDG), C) sulfoquinovosyldiacylglycerol (SQDG),  
963 D) Phosphatidylglycerol (PG), E) Phosphatidylcholine (PC), F) Phosphatidylethanolamine  
964 (PE), G) Phosphatidylinositol (PI) and H) Phosphatidylserine (PS).

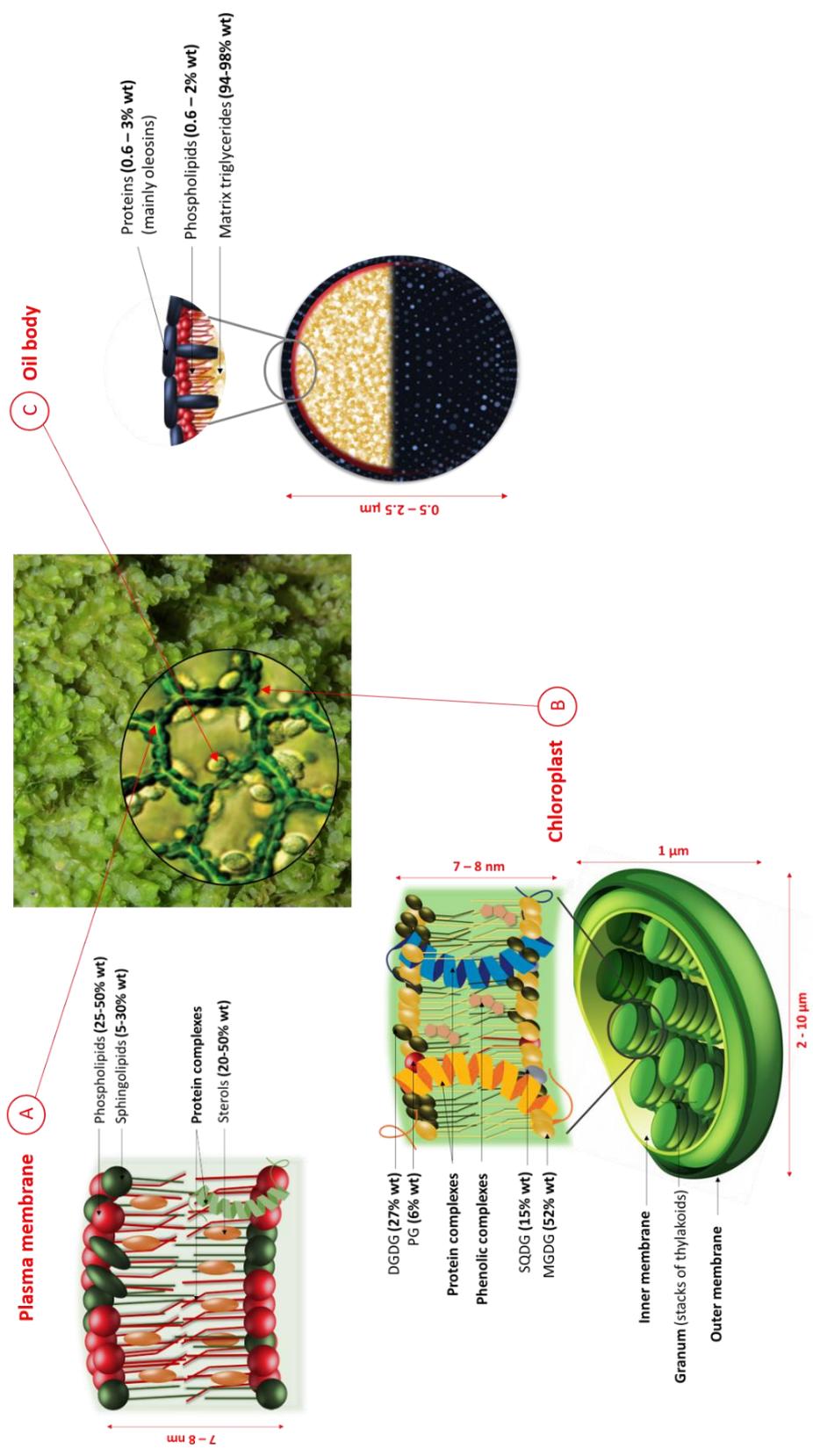
965 **Figure 3** Micrographs of vegetal tissues with embedded OB: A) Walnut OB (CLSM, red: PL,  
966 blue: proteins and green: TAG), unpublished results; B) Cashew Nut OB (CLSM, red: PL,  
967 blue: proteins and green: TAG), unpublished results; C) Almond OB (TEM), reproduced with  
968 authorization from [118]; D) Rice OB (TEM) · reproduced with authorization from [119].  
969 Abbreviations. CLSM, confocal laser scanning microscopy; TEM, transmission electron  
970 microscopy.

971 **Figure 4** Schematic overview of the gastrointestinal behaviour of plant membrane molecules,  
972 fragments and assemblies, and their modulating digestion effects.

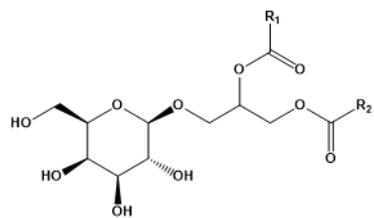
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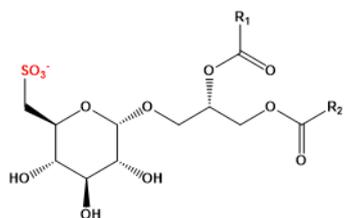
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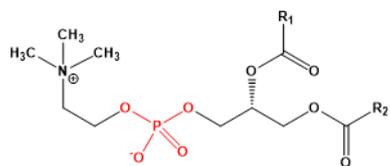
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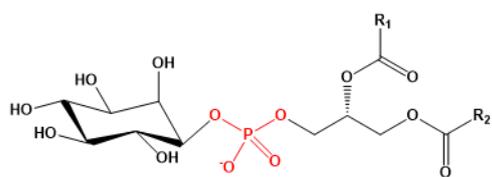
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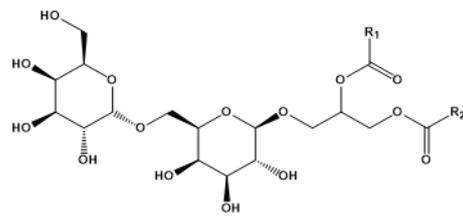
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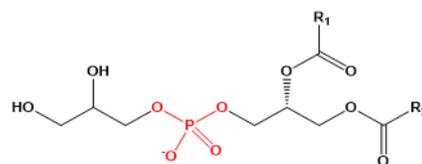
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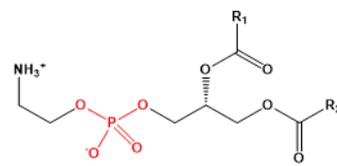
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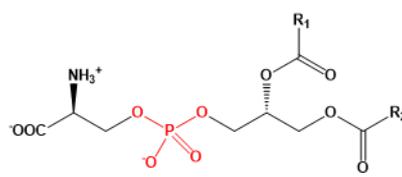
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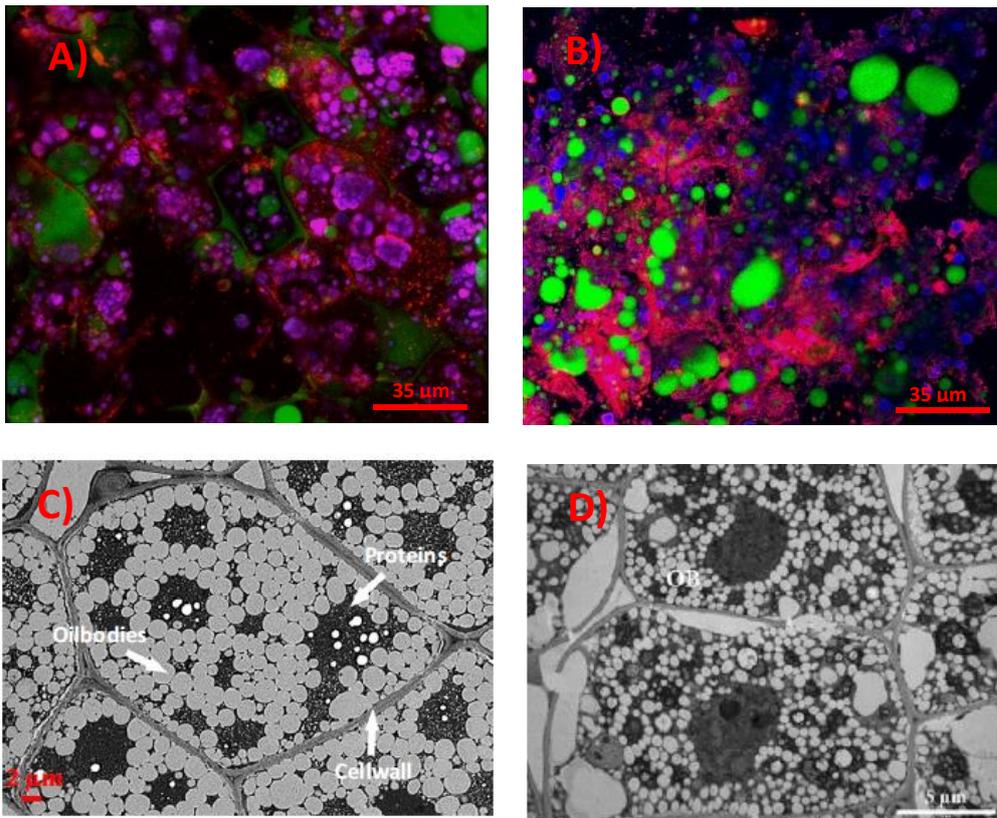


(F)



(H)

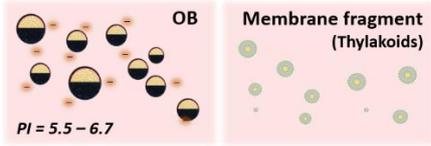




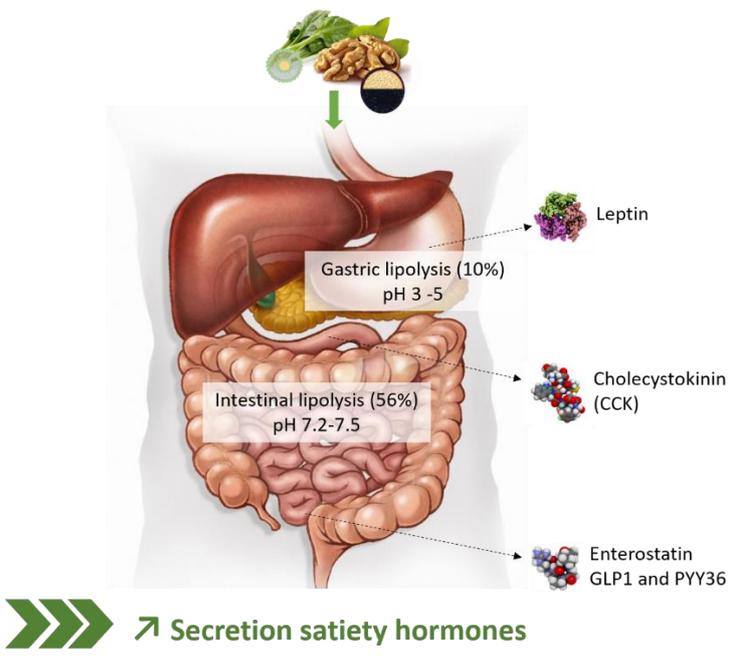
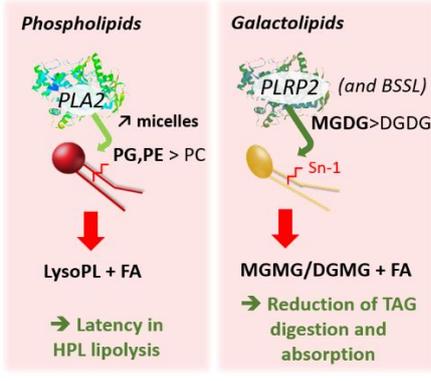
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**Gastric behavior:** aggregation but stability



**Intestinal behavior:** aggregation but stability



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