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REVIEW

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Relevance of various components present in plant protein ingredients for lipid oxidation in emulsions

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Abstract

Plant protein ingredients (isolates, concentrates) are increasingly used for food formulation due to their low environmental impact compared to animal-based proteins. A specific application is food emulsions, of which the physical and oxidative stability need to be supported. The emulsifying properties of diverse plant proteins have already been largely covered in literature, whereas only in a few studies the chemical stability of such emulsions was addressed, especially regarding lipid oxidation. In the few examples available mostly the effects caused by proteins were elaborated, whereas those caused by non-protein components have hardly been considered. Yet, plant protein ingredients are characterized by high compositional complexity, with notably a plethora of non-protein components. Topics covered in this review, therefore, include the composition of various types of plant protein ingredients (i.e., legumes, oil seeds) in relation to the fractionation processes used, and the potential effects on lipid oxidation in emulsions. The composition varies greatly among species and depends on the harvest conditions (i.e., year, location), and genetics. In addition, fractionation processes may lead to the accumulation or dilution of components, and induce chemical changes. Both protein and non-protein components can act as pro- or antioxidants contingent on their concentration and/or location in emulsions. Since the chemical composition of plant protein ingredients is often hardly reported, this makes a-priori prediction of an overall effect difficult, if not impossible. Standardizing the fractionation process and the starting material, as well as in-depth characterization of the resulting fractions, are highly recommended when aiming at rationally designing food emulsions.

KEYWORDS
emulsions, lipid oxidation, non-protein components, plant protein ingredients, plant protein modifications, protein fractionation

INTRODUCTION

Proteins are widely used to stabilize oil-in-water (O/W) food emulsions, not only because they are important macronutrients, but also because they are known to protect such systems against physical destabilization and lipid oxidation (Elias et al., 2008; Gumus et al., 2017; McClements, 2004). Plant proteins are nowadays of special interest due to the lower environmental impact compared to proteins of mammal origin (Aiking, 2011). We see a rapid increase in the number of studies pertaining to their application in emulsions, and their interfacial and emulsifying properties. Yet, lipid oxidation in such plant protein-based emulsions has been only scarcely investigated so far, and mostly reported without solid explanations for the underlying mechanisms.

Most of the researchers studying the oxidative stability of plant protein-stabilized emulsions have focussed on the effect of the protein pre-treatment, protein location, and the relevance of the emulsification method on lipid oxidation (Table 1). In dairy protein-stabilized emulsions, it is well-

TABLE 1 Overview of studies in which lipid oxidation in O/W emulsions stabilized by plant proteins was investigated. All researchers used high-pressure homogenization to prepare the emulsions. For further information on the preparation conditions, the reader is referred to the original articles.

	Protein processing				Emulsion preparation conditions[b]						
Protein material	Starting material	Method	Protein purity [%][a]	Non-protein [%][a]	Protein [%][a]	Oil [%]	Aqueous phase	Droplet [μm]	°C	Highlights pertaining to lipid oxidation	Paper
SPI (native or preheated)	Commercial defatted soybean meal	IEP precipitation (pH: 8/4.8/7.5)	91.5 (wet basis)	N.R.	0.5%–4% (w/v)	20% (v/v)	pH 7; ±300 mM NaCl	$d_{4,3}$: 0.8–37	50	High stability at conc. >1%; improved stability at increased ionic strength/heat	Shao & Tang (2014)
SPI	Commercial	N.R.	≥90 (dry basis)	<4% fat; <6% moisture; <5% ash	4% (w/v) (native or heated at 95°C, 15 min)	10% or 20% (v/v)	Deionized water	$d_{4,3}$: 0.6–3.7	37	Higher stability with higher oil content; prepared with 100 and 200 MPa	Fernandez-Avila and Trujillo (2016)
SPI (non-modified or dextran-glycated); WPI	Commercial ingredients	N.R.	SPI: 79; WPI: 88	N.R.	DUMAS (N: 5.71): 0.25% (w/w)	10% (w/w)	PB; pH 7; 10 mM	$d_{3,2}$: 0.1–0.2	40	Inhibition capacity: glycated SPI ≈ SPI/dextran mixture > SPI > WPI	Feng et al. (2021)
CAS, WPI, SPI	Commercial ingredients	N.R.	CAS: >99; WPI: 97.6; SPI: ≥90	SPI: ≤6% moisture, ≤1.5% lipids	0.2%–1.5%	5% (w/w)	Acetate-imidazole buffer; pH 3; 5 mM	$d_{3,2}$: 0.26–0.47	37	Stability: CAS > WPI > SPI	Hu et al. (2003)
CAS; WPI; SPI	Commercial ingredients	N.R.	WPI: 97.6; SPI: 86.0; CAS: 96.9	N.R.	WPI: 0.25%–1.5% (w/w); CAS/SPI: 0.05%/0.01%	10% (w/w)	Acetate/imidazole buffer; pH 7; 10 mM	N.R.: 0.4–0.6	20	Non-adsorbed proteins increase stability; effectiveness: SPI > CAS > WPI	Faraji et al. (2004)
Pea fractions, WPI	Commercial ingredients	N.R.	PPI: 70; WPI: 97–98.4	N.R.	DUMAS (factor: 5.7): 2% (w/v)	10% (w/w)	PB; pH 7; 10 mM; 0.2 mmol/L iron-EDTA	$d_{3,2}$: 3–4.6	25	Stability: full PPI > insoluble fraction PPI > WPI	Hinderink et al. (2021)
PPC; WPI	Commercial ingredients + SFPL + sesamol	N.R.	PPC: 55; WPI: 92	N.R.	2% (w/w)	10% (w/w)	PB; pH 7; 10 mM	$d_{3,2}$: 0.15–0.21	55	Sesamol improves stability in SFPL (65%) and protein based emulsions (85%).	Wang et al. (2021)
LPC; PPC & FBPC + whole fraction WPI	Commercial ingredients	Purification: precipitation (pH 7/4.5/7) + centrifugation & collection supernatant	Starting materials: LPC: 55; PPC: 55; FBC: 60; WPI: 94	Fat: 3.0/3.1; ash: 4.9/5.0; carbs: 27/33; water: 8/9	2% protein (w/v)	10% (w/w)	PB; pH 7; 10 mM; (+100 μM iron sulfate)	$d_{3,2}$: 0.13 (WPI); $d_{3,2}$: 0.38–0.41 (plant)	37	With iron: WPI > LP/FB > PP; no iron: PP/FB ≥ WPI >> LP + highest mitigation effect though non-adsorbed proteins	Gumus et al. (2017)
PPI (native and alkaline treated)	Milled pea seeds	IEP precipitation (pH: 8/4.5/7)	N.R.	N.R.	1% (w/v)	25% (v/v)	pH 7; N.R.	$d_{3,2}$: 1.53–2.3 to 4–11	37	Alkaline treated pea protein < native pea protein; due to improved interfacial properties and steric hindrance	Jiang et al. (2014)
Faba bean (FB) flour, SPI	SPI: commercial; FB: microwave (MW) or heated (CV)	FBP: milling, hydration flour, centrifugation & filtering supernatant	N.R.	N.R.	3% protein (w/v)	10% (w/v)	N.R.	N.R.	37	CV heat treatment increased stability for FB emulsions; stability CV > SPI and MW FB.	Gürbüz et al. (2018)
FBPI hydrolysates	FB flour	IEP precipitation (pH: 4.5/8)	89	N.R.	1% (w/v)	5% (w/v)	N.R.	$d_{3,2}$: 0.05–0.16	37	Low degree of hydrolysis (DH = 4%) showed highest stability	Liu et al. (2019)

Abbreviations: BRP, brown rice protein; CAS, sodium caseinate; conc, concentration; FBPC, faba bean protein concentrate; FBPI, Faba bean protein isolate; HP, hemp protein; HPH, high pressure homogenizer; IEP, isoelectric point; LPC, lentil protein concentrate; N.R., not reported; PB, phosphate buffer; PPC, pea protein concentrate; PPI, pea protein isolate; RSH, rotor-stator-homogenizer; SFP, sunflower protein; SFPL, sunflower phospholipids; SPI, soy protein isolate; WPI, whey protein isolate.

[a]Provided by the supplier, unless otherwise stated.

[b]For further details on the preparation conditions the reader is referred to the original article.

known that some emulsion formulation parameters (e.g., high pH leading to negatively charged proteins), and a small droplet size, tend to promote lipid oxidation (Berton-Carabin et al., 2014; McClements & Decker, 2000; Horn et al., 2012; Sørensen et al., 2007). Results from several studies support higher oxidative stability in the presence of excess dairy proteins (Berton et al., 2011; Haahr & Jacobsen, 2008; McClements & Decker, 2018; Osborn & Akoh, 2004). Conversely, only a few researchers have investigated the effect of plant proteins on lipid oxidation in emulsions (Table 1). In contrast to dairy protein ingredients such as whey protein isolate, plant protein isolates and concentrates have a lower purity (i.e., mass percentage of proteins on dry basis) and substantial amounts of various non-protein components accompany the proteins, which are often not analyzed, nor even considered to play a role in lipid oxidation.

The impact that plant protein ingredients may have on lipid oxidation in food emulsions is highlighted in this review. An overview is given of plant protein-stabilized emulsions and current work on lipid oxidation in such emulsions followed up by the examination of how fractionation processes and pre-treatments influence the physicochemical properties of plant protein ingredients, and how this is reported to affect lipid oxidation in food emulsions. Furthermore, a section on proteins and other components present in "plant protein ingredients," a term we used for powdered materials that have been enriched with proteins starting from plant seeds/grains is provided. The reported effects of protein and non-protein components on lipid oxidation in emulsions, and a short outlook and recommendations will complete the review.

PROTEIN-STABILIZED EMULSIONS AND LIPID OXIDATION: GENERAL ASPECTS

Many proteins are amphiphilic molecules, adsorbing at the oil–water interface and thereby lowering the interfacial tension. The adsorption process of proteins was previously described to occur in four stages (Damodaran, 2004; Wilde et al., 2004):

Diffusion from the continuous phase to the interface
Adsorption at the interface
Rearrangement and partial unfolding/denaturation of the proteins exposing buried regions
Establishment of a viscoelastic layer via the formation of cross-links and local phase separation

During homogenization, fast protein adsorption is important to prevent droplet recoalescence. The adsorption rate of proteins depends on their molecular characteristics such as size, flexibility, conformation, and interactions (intra- and intermolecular) with the environment (McClements, 2015). Compared to dairy-based proteins, plant proteins often have low solubility at neutral to slightly acidic pH, which makes them inherently less effective in stabilizing emulsions against coalescence and flocculation. In addition, even soluble plant proteins often have a native or process-induced quaternary structure as oligomers or very small aggregates (sometimes referred to as "soluble aggregates"), rendering their adsorption process slower and more complex as compared to dairy proteins.

Drusch et al. (2021) recently wrote an excellent review on the limitations in understanding the interfacial behavior of plant proteins. The adsorption behavior is not only influenced by solubility, but also by the change in size upon dissociation to monomers or aggregation (depending on the ionic strength and pH). This will increase or decrease the emulsifying capacity depending on the molecular characteristics and intramolecular interactions. Proteins with a very low solubility, such as aggregated plant proteins, may act more like colloidal particles, thereby stabilizing emulsions via a mechanism similar to that of Pickering particles (Drusch et al., 2021; Sarkar & Dickinson, 2020). Next to rapidly forming an interfacial film upon and immediately after homogenization, the suitability of a protein as emulsifier is contingent upon its ability to prevent emulsion destabilization in a later stage. Proteins with good emulsifying and stabilizing properties are associated with a low increase in droplet size over storage time, with a performance that is unaffected by environmental stresses (e.g., change in pH, ionic strength, or temperature) (McClements, 2007).

Next to the capacity of proteins to physically stabilize emulsions, their ability to protect the system against chemical degradation, and in particular, lipid oxidation, is an important criterion. There is a common consensus that in emulsions, lipid oxidation is initiated at the oil–water interface (Berton-Carabin et al., 2014; Laguerre et al., 2020; Tong et al., 2000), implying that the properties of the interfacial layer surrounding the oil droplets are relevant in that respect. Proteins are known to have antioxidant properties due to their ability to chelate metal ions and scavenge free radicals (Elias et al., 2008), and they are usually highly effective at preventing lipid oxidation when in the continuous phase, compared to interfacial proteins of which the role is more ambivalent when it comes to lipid oxidation (Berton et al., 2012; Faraji et al., 2004; Gumus et al., 2017). Faraji et al. (2004) showed that in the continuous phase of an O/W emulsion, soy proteins were more effective to counteract lipid oxidation compared to whey protein isolate (WPI) or casein, suggesting a good antioxidant effect of this plant protein source. Similarly, Hinderink et al. (2021) studied the oxidative stability of emulsions prepared with WPI or different fractions of pea protein isolate (PPI) and found the lowest oxidative stability for the WPI-based emulsions. These are first indications that some commercially available plant (in particular, legume) protein ingredients can be associated with a higher oxidative stability of emulsions, as compared to systems prepared with conventional sources of proteins.

The homogenization process is another factor that can be of importance for the subsequent oxidative stability of emulsions, as the forces applied within a homogenizer not only induce droplet break-up, but also increase the temperature of the emulsion. In addition, when used at large scale, high pressure homogenization may lead to cavitation which either directly influence lipid oxidation by generating free radicals, or indirectly through altering protein distribution between the emulsion's phases (Section Anti- or prooxidant properties of plant proteins), and droplet size (Horn et al., 2012; Sørensen et al., 2007). Smaller droplets have a higher interfacial area per oil volume for prooxidants and lipids to contact each other, which is believed to enhance lipid oxidation (Berton-Carabin et al., 2014; Li et al., 2020). The pH is also an important factor as it will determine the net surface charge of proteins, which will either repel metal cations (<isoelectric point) or attract (and sometimes chelate) them (>isoelectric point) and thereby reduce or enhance lipid oxidation (Section Protein composition analysis in plant protein ingredients) (McClements & Decker, 2000).

PLANT PROTEIN MATERIALS: SOURCES AND PROCESSES

Plant protein ingredients for food applications originate mainly from legumes (i.e., pulses, soybean), cereals (e.g., wheat, oat, rice), or oilseeds (i.e., sunflower, rapeseed). Recently, proteins from pseudo-cereals (e.g., amaranth, chia) and algae or tubers (e.g., potato) have also been reported (Loveday, 2020). In plant seeds, most of the proteins are organized as storage proteins possibly in the form of protein bodies (González-Pérez & Arellano, 2009; Herman & Larkins, 1999). Following Osborne (1909), the main classes of proteins in legumes and oilseeds are 2S water-soluble albumins, and 7S and 11S globulins which are soluble in low salt solutions. Prolamins, alcohol-soluble proteins, are the main storage proteins in most cereals with the exception of oat and rice where glutelins (extractable by weak alkaline and dilute detergent solutions) are mainly found together with globulins (González-Pérez & Arellano, 2009). The ratio between the protein classes differs between species, and between cultivars (Gueguen, 1983; Maplestone et al., 1985).

Sources for plant protein materials

Currently, the plant protein ingredients mostly used for stabilizing emulsions are from the *Leguminosae* or oilseed families (Lam & Nickerson, 2013). Table 2 shows typical chemical compositions of legumes and oilseeds reported in literature. The protein content in legumes is, in general, higher than in cereals (European Commission, 2018). Therefore, in the present review, the focus lies on legume protein ingredients, of which production is expected to increase in the coming years (European Commission, 2020). There are, to the best

TABLE 2 Chemical composition of various legume and oil seeds.[a]

Protein		Concentration [% dry matter]					Total phenol content [mg/g]	Article
Family	Type	Protein[b]	Carbohydrates	Lipids	Ash	Phytic acid		
Legume	Soy	41	7.6	19.6/25.4	5.3/5.5	1.0–1.47	2.3	Belitz et al., 2009; Cheryan, 1980; Han & Baik, 2008
	Pea	21.3–28.4	53.7–65.2	0.7–1.6	2.3–3.4	0.42–1.2	2.5	Belitz et al., 2009; Han & Baik, 2008; Wang, Hatcher, et al., 2010
	Chickpea	20.5–27	54.6/60.8	5–6.1	3–3.5	2.12	2.2	Adamidou et al., 2011; Belitz et al., 2009; Han & Baik, 2008
	Faba bean	26.7–31.4	44.1–64.5	1.1–2.3	3.6–4.3	3.2	1.28 (total tannins)	Adamidou et al., 2011; Belitz et al., 2009; Khalil & Mansour, 1995
	Lentil	26.5–28.6	57.6	0.8–1.6	3–3.6	N.R.	11.8–12.0	Belitz et al., 2009; Han & Baik, 2008
Oilseed	Sunflower	10–27.1	18–26[c]	34.6–48	2.0–3.8	1.7	1.1–4.5 (chlorogenic acid; %dm)	González-Pérez & Vereijken, 2007; Saeed & Cheryan, 1988
	Rapeseed	30.7–32.9	14.4–15.7	46.4–48.7	4	N.R.	N.R.	Farag et al., 1986

Abbreviation: N.R., not reported.
[a]Only measured values per dry matter are reported.
[b]$N = 6.25$.
[c]Method of determination not reported.

of our knowledge only a few studies about lipid oxidation in emulsions using other plant protein sources (Table 1) (Cheng et al., 2010; García-Moreno et al., 2020; Qiu et al., 2015; Zhang et al., 2020).

Protein fractionation processes

Plant protein ingredients are currently obtained by either dry or wet processing. Dry processing is used for protein concentrates (purity 50%–70%) and wet processing for protein isolates (purity >70%) (Boye et al., 2010; González-Pérez & Arellano, 2009). These processes are not standardized (yet) and the main aim is to obtain a high protein yield, neutral powder color, and minimal off-flavor, with only little focus on the physicochemical state of the protein and the final ingredient composition (in particular, non-protein components). Nicolai and Chassenieux (2019) reported that differences in heat-gelation properties of soy and pea protein are most probably a reflection of differences in ingredient production method, rather than in the protein's biological origin.

Plant protein ingredients mostly consist of storage proteins that have low aqueous solubility, which hampers their production with high protein purity (Derbyshire et al., 1976). Therefore, harsh processing conditions are usually applied to increase solubilization, and through that the protein yield. In the following sections, the general processing steps for wet and dry processing (Figure 1) are described briefly, and discussed regarding their influence on the state and composition of the final material (indicated with red boxes in Figure 1), which will then further be discussed in relation to the potential effects on lipid oxidation in Section Protein modifications and interactions with other components during fractionation processes. For further information on the wet and dry fractionation processes, the interested reader is referred to for example, the reviews of Fernando (2021), Boye et al. (2010), González-Pérez and Arellano (2009), and Schutyser et al. (2015).

Pre-processing

For plant protein fractionation, the seed size needs to be reduced through grinding/milling, and defatting may be needed (for oilseeds and oil-rich legumes) (Carré, 2021), as discussed below.

Defatting

The oil is separated by cold (<40°C) or hot (80–100°C) pressing and/or by solvent extraction (e.g., hexane) at temperatures around 107°C (Östbring et al., 2020). These high temperatures lead to physicochemical modification of proteins and lipids (González-Pérez & Arellano, 2009) (see Sections Protein modifications and interactions with other components during fractionation processes, Anti- or prooxidant properties of plant proteins, and Effect of low molecular weight components on lipid oxidation in plant protein-stabilized emulsions). Moreover, the choice of extraction solvent will not only influence the type of residual lipids (i.e., polar vs. non-polar lipids) but also the protein structure (Carré, 2021) (see Section Protein modifications and interactions with other components during fractionation processes). All these effects together contribute to the oxidative potential of the emulsion.

Dehulling

Seeds of oil-poor legumes (e.g., dry pea, lentil, faba bean) are generally first dehulled (and split) using hammer mills (loose seed coats) or abrasive mills (tightly adhering seed coats), which can be facilitated by either wet pre-treatment (e.g., soaking), or dry pre-treatment (pitting or scratching the surface by abrasion). In addition to fiber reduction, dehulling reduces polyphenols in the final protein ingredient (Fernando, 2021; Wood & Malcolmson, 2020) which can impact emulsion oxidation in different ways (either with a pro- or an antioxidant effect) (see Section Effects of non-protein component-son lipid oxidation in plant protein-stabilized emulsions).

Grinding

The seeds are ground, directly or after dehulling, with impact mills (e.g., pin-, hammer-, jet mills), to facilitate fractionation during subsequent wet or dry processing (see Sections Wet processing and Dry processing). During grinding, cells release not only protein bodies and starch granules, but also enzymes such as lipoxygenase, and lipids (Mizutani & Hashimoto, 2004), which may induce lipid oxidation during flour storage (González-Pérez & Arellano, 2009; Wood & Malcolmson, 2020). To mitigate this, heat treatments before ("roasting") or after milling are occasionally applied to inactivate enzymes (Wood & Malcolmson, 2020) and thereby limit oxidative reactions (see Section Effects of non-protein componentson lipid oxidation in plant protein-stabilized emulsions).

Wet processing

Wet processing is the most widely applied fractionation process for preparing commercial plant protein isolates, which are commonly used in studies on lipid oxidation in emulsions (Table 3). The process starts with fine flour, of which the particle size is generally not reported. Although a small particle size is needed to facilitate fractionation; for example, Russin et al. (2007) observed a higher protein yield and solid recovery for particle sizes <90 μm, it also relates to being more prone to lipid oxidation (see the aforementioned effect of grinding).

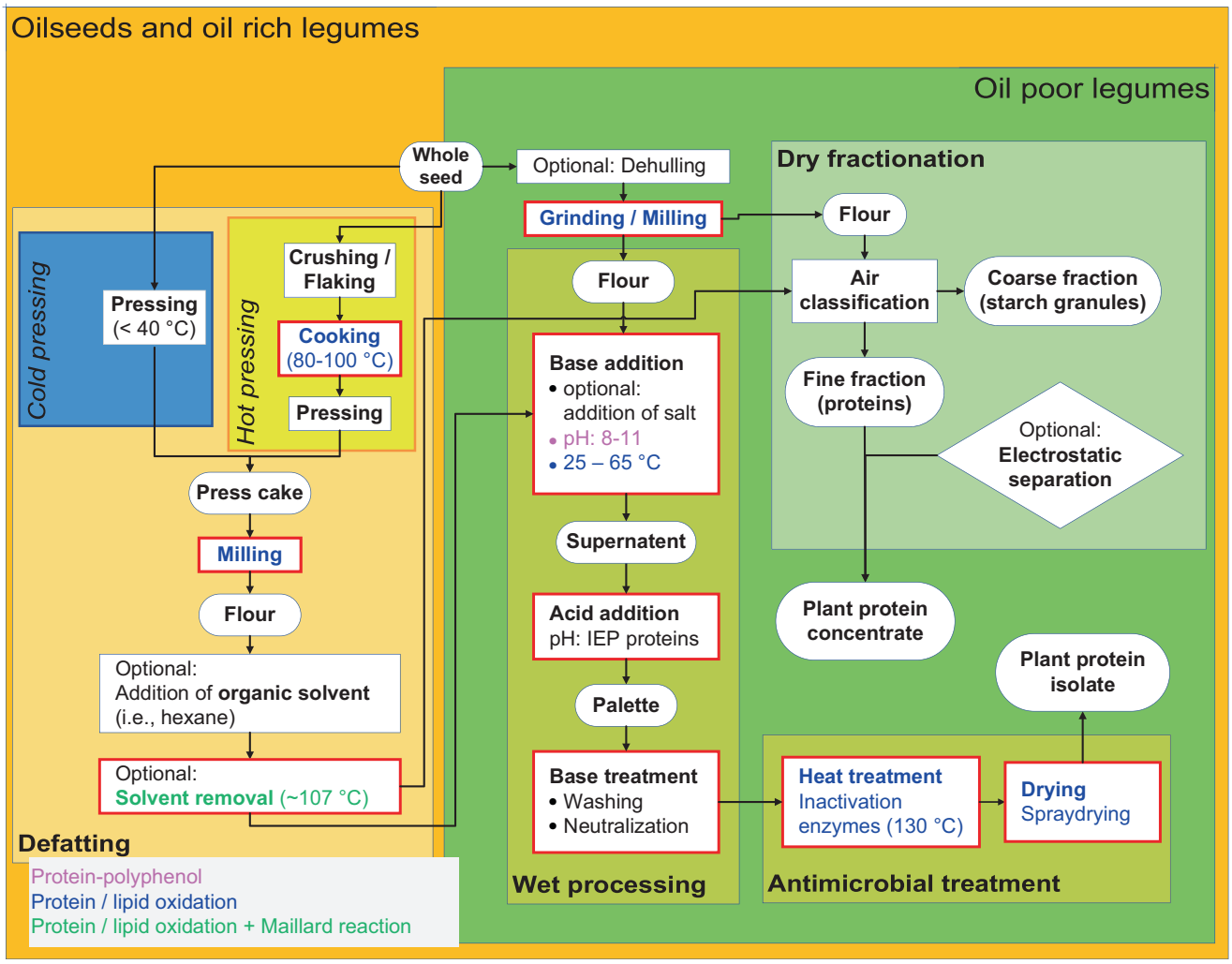

FIGURE 1 Schematic overview of the fractionation processes of plant proteins with the critical steps (marked in with red frames) for a change in state (protein-polyphenol interactions [purple], protein and lipid oxidation [blue] together with Maillard reaction [green]) and composition of the material. The reader is referred to the online version for the color codes.

To obtain protein isolates, the flour is suspended in water at high pH and temperatures often up to 65°C to increase protein solubility, which may be enhanced by the addition of salts. This leads to chemical modifications and interactions of proteins and enhances the phytic acid concentration in the protein material, which all have implications for the oxidative stability of emulsions (see Sections Protein modifications and interactions with other components during fractionation processes and Phytic acid).

To separate proteins from insoluble fibers and starch, the suspension is centrifuged and the pH of the "supernatant" is adjusted to the isoelectric point (IEP) to precipitate proteins and separate them from other soluble components; fibers, antinutritional factors (e.g., phytic acid, trypsin inhibitors). This modulates the phytic acid concentration in the final protein material (see Section Phytic acid). The chosen pH will determine the ratio of different plant proteins in the final ingredients, as the different plant protein types (i.e., 7S and 11S) differ slightly in IEP (Kimura et al., 2008), e.g., albumins have been reported to be removed (Tanger et al., 2020). Protein types differ in their antioxidant potential in the emulsions (see Section Anti- or prooxidant properties of plant proteins), and this may explain differences found between different preparation methods. The final plant protein isolates are obtained after a heat treatment (to inactivate microbes, and enzymes such as lipoxygenase Jiang et al., 2016) and a drying step (Figure 1). This can promote physicochemical changes in proteins (denaturation, aggregation, oxidation) and lipids (oxidation), and through that the subsequent propensity of emulsions prepared with those plant protein ingredients to oxidize (see Sections Protein modifications and interactions with other components during fractionation processes and Effect of low molecular weight components on lipid oxidation in plant protein-stabilized emulsions).

TABLE 3 Chemical composition of the five most common legume protein isolates obtained by experimental isoelectric point (IEP) precipitation or commercial IEP precipitation (%/dry matter [DM]) reported in literature.

Type	Source	Protein [%]	Carbohydrates [%]	Lipids [%]	Ash [%]	Article
Chickpea	IEP precipitation	78.0–88.1[a]	3.3–11.8	1.0–3.5	2.7–4.3	Paredes-López et al., 1991; Sánchez-Vioque et al., 1998
Faba bean	IEP precipitation	75.4–90.1[a,b]	n.d.	3.2–5.0	1.7–5.2	Vogelsang-O'Dwyer et al., 2020; Pedrosa et al., 2020; Gueguen, 1983
Lentil	IEP precipitation	86.2–89.5[a]	1.9–9.5	0.5–4.72	3.8–5.7	Can Karaca et al., 2011; Alonso-Miravalles et al., 2019
Pea	IEP precipitation	84.6–90.0[a,c]	3.4/n.d./n.d.	n.d./1.0–8.5	0.9–6.0	Cui et al., 2020; Gao et al., 2020; Gueguen, 1983; Kornet et al., 2020
	Commercial precipitation	73.6–80.7[c,d]	4.4/n.d.	n.d./8.9	1.5–6.5	Kornet et al., 2020; Moll et al., 2022
Soy	IEP precipitation	85.3–91.7[c]	5.47–10.3	0.65–2.3	2.1	Can Karaca et al., 2011; Peng et al., 2020
	Commercial precipitation	83.3[c]	13.3	0	3.4	Peng et al., 2020

Abbreviation: n.d., not determined.
[a]$N = 6.25$.
[b]$N = 5.45$.
[c]$N = 5.7$.
[d]$N = 5.36$.

Dry processing

The main method for dry processing is air classification, a process based on differences in the size and density of the particles present in the flour, which separates the starting material into a protein-rich and a protein-poor fraction still containing starch granules, fibers, and other components (Pelgrom, Wang, et al., 2015). The obtained purity is typically between 50% and 70% protein and lower compared to wet fractionation (Loveday, 2020; Schutyser et al., 2015). Therefore, the process is often chosen for protein concentrate production (Day, 2013). Because of the low protein content, some non-protein components (including fibers, ash, and anti-nutritional components) are higher in protein concentrates than in isolates which will impact the oxidative potential of emulsions in different ways (see Section Effects of non-protein componentson lipid oxidation in plant protein-stabilized emulsions).

Due to the mild process conditions involved in comparison with wet fractionation, dry fractionation leads to fewer protein modifications, as described in the next section, which is, among others, important for the techno-functional and nutritional properties of the plant protein ingredient. For instance, Vogelsang-O'Dwyer et al. (2020) demonstrated a higher solubility (pH 4–8) and the formation of stronger gels with faba bean concentrate than with isolate, which was due to the presence of starch and fibers forming complexes with the proteins. Similarly, higher solubility and foamability were observed for pea and lentil protein concentrates, compared to the corresponding isolates (Pelgrom et al., 2013; Pelgrom, Boom, et al., 2015). The latter effect was attributed to the presence of albumins that are removed during wet fractionation (Kornet et al., 2022; Tanger et al., 2020; Yang et al., 2022). The higher solubility allows proteins to adsorb faster, and their localization in the emulsion will determine anti- or prooxidant effects in emulsions as is described in Section Anti- or prooxidant properties of plant proteins.

Protein modifications and interactions with other components during fractionation processes

Protein denaturation

As pointed out previously, protein structure and thus techno-functionality are greatly influenced by the fractionation process (Alonso-Miravalles et al., 2019; Anandharamakrishnan et al., 2008; González-Pérez & Arellano, 2009; Tanger et al., 2020; Vogelsang-O'Dwyer et al., 2020). Protein denaturation results in almost irreversible changes in the secondary and tertiary structures, which can lead to protein aggregation (Croguennec et al., 2003;Delahaije et al., 2016; Wang, Nema, et al., 2010). Protein denaturation can be

induced by high temperatures (e.g., >60°C – depending also on the water activity Fujita & Noda, 1981), pH (Delahaije et al., 2016; Wang, Nema, et al., 2010), or by oxidative reactions (see next section, Protein oxidation), and enzymatic reactions (Creusot & Gruppen, 2007).

Solvent removal (defatting), wet processing at elevated temperature, and alkaline and acidic treatment (Carré, 2021; Hamm et al., 2013) (Figure 1; Section Wet processing) may induce protein denaturation and thereby decrease protein solubility, which can impair their emulsifying properties (Arrese et al., 1991; Gao et al., 2020), and modulate their antioxidant activity (Section Anti- or prooxidant properties of plant proteins) (Jiang et al., 2014).

Protein oxidation

Although less documented in food systems compared to lipid oxidation, protein oxidation can also be an important alteration in food matrices, especially when proteins and unsaturated lipids coexist (Lund et al., 2011). For detailed information on the chemical pathways of protein oxidation, the reader is referred to Davies (2016), Hellwig (2019), and Heinonen et al. (2021). Briefly, amino acid side chains (i.e., sulfur-containing methionine and cysteine, or aromatic amino acids) may oxidize to form a protein radical (Davies, 2005; Pattison & Davies, 2001). This radical is then either transferred to the protein backbone or between neighboring amino acids. In case of thiol- or tyrosine-containing amino acids, oxidation can lead to the formation of disulfide bridges or dityrosine, respectively, via intra- and intermolecular crosslinking, which may result in protein aggregation. For legume proteins such as vicilin and legumin, disulfide linkages are expected to be limited, because they are generally low in sulfur-containing amino acids (González-Pérez & Arellano, 2009). Yet, other covalent reactions may be involved (Duque-Estrada et al., 2020; Wu et al., 2009). For instance, dityrosine formation was observed in oxidized soy protein isolates under reportedly anaerobic conditions (Chen et al., 2013; Huang et al., 2006).

Protein oxidation is faster at high temperatures and under alkaline conditions (Davies, 2016; Heinonen et al., 2021); conditions found during defatting, wet processing (Sections Pre-processing and Wet processing), and antimicrobial treatments (Figure 1). Protein and lipid oxidation are often interrelated in biological and food matrices, although protein oxidation is often overlooked in the latter (Heinonen et al., 2021). Both reactions can be initiated by reactive oxygen species (hydroxyl radicals) formed by the Fenton reaction in the presence of iron or copper. In addition, proteins can react with lipid oxidation products (lipid radicals, or secondary products such as aldehydes) leading to protein oxidation (Elias et al., 2008; Heinonen et al., 2021). This broad range of oxidative reactions results in a wide variety of oxidation products that may help induce protein aggregation, amino acid side chain modification, and protein fragmentation (Lund, 2007).

Protein-polyphenol interactions

Proteins and polyphenols may form complexes non-covalently via hydrophobic, ionic interactions or hydrogen bonds, or covalently via conjugation of o-quinones (Keppler et al., 2020). Non-covalent complexations are described in the review of Le Bourvellec and Renard (2012). Under alkaline conditions in the presence of oxygen, o-quinones are formed that react with amino acid side groups of proteins or peptides containing primary amines (e.g., lysine) via Michael addition or as Schiff base and free thiol groups (i.e., cysteine). Less frequently quinones react with indole groups (i.e., tryptophan), amide groups (i.e., asparagine, glutamine), imidazole groups (i.e., histidine), and phenolic groups (i.e., tyrosine) (Hurrell & Finot, 1984; Kroll et al., 2003;Li et al., 2016; Pierpoint, 1969). The thiol groups are kinetically preferred and lead to the formation of colorless products unlike phenol-amine products (Li et al., 2016; Pierpoint, 1969). The reaction between o-quinones and amino acid residues is enhanced by alkaline pH, increased temperature (conditions found during wet processing; Section Wet processing), and more reaction sites in proteins (i.e., thiol, amines) and polyphenols (i.e., hydroxyl groups) (Hurrell & Finot, 1984; Kroll et al., 2003). The o-quinone-protein conjugate further oxidizes to cross-linked aggregates (Keppler et al., 2020; Kroll et al., 2003).

Covalent conjugation between proteins and polyphenols may result in a change in secondary and tertiary structure, leaving the proteins more disordered (Karefyllakis et al., 2018; Liu et al., 2016), and in blocking positively charged amino groups (Li, Pan, Li, et al., 2023; Wei et al., 2015). The effect of conjugation on the solubility and zeta potential (as a function of pH) compared to the pure protein differs in literature, ranging from no effect (Pham et al., 2019), to a shift in IEP to lower pH (Liu et al., 2016) or to a more negative surface charge for a wide pH range (6–12) with consequently higher solubility at high pH (Wei et al., 2015). In general, this was suggested to improve the physical stability compared to emulsions stabilized by proteins only, although it is good to point out that often no differentiation was made between flocculated and coalesced droplets. Only Santos et al. (2022) measured droplet size distribution over time in combination with macroscopic pictures, and higher physical (and chemical) stability was reported; the latter also by Pham et al. (2019). All emulsions were at pH > 6 and it is very likely that at acidic pH an emulsion stabilized by the conjugates would have had lower physical stability. This was

also pointed out in a review by Hong Quan et al. (2019) who concluded that the emulsifying effect of the covalent conjugation of proteins and polyphenols greatly depends on the system.

Wei et al. (2015) compared the behavior of covalently and non-covalently linked bovine milk protein-polyphenol interactions to that of pure bovine milk proteins, and found no effect on solubility; an increased denaturation temperature and antioxidant activity; and a decreased hydrophobicity compared to the controls. For covalently linked protein-polyphenol conjugates, the denaturation temperature, antioxidant activity, and hydrophobicity were reported to be higher, leading to emulsions being more stable to degradation of ß-carotene compared to those made with non-covalent complexes; which relates to emulsions being more stable against lipid oxidation. However, Li, Pan, Li, et al. (2023), Li, Pan, Lan, et al. (2023), Li et al. (2022) showed that the antioxidant capacity greatly depends on the ratio of protein-polyphenol conjugates and complexes, generally increasing with increasing polyphenol concentration (for conjugates) or at a specific ratio (for complexes). Although this protein modification route can improve their ability to physically and oxidatively stabilize emulsions, it is good to keep in mind that the color changes due to covalent protein-polyphenol conjugation will affect the consumer acceptance of food products.

IMPACT OF PLANT PROTEIN INGREDIENTS ON LIPID OXIDATION IN EMULSIONS

Table 1 summarizes the studies in which lipid oxidation in plant protein-based emulsions is addressed, focusing mainly on the influence of protein treatment (e.g., hydrolysis and pH-shift), pH and ionic strength. Very sparingly, different protein fractionation processes were used, and comparison with dairy proteins is often not considered. The emulsification conditions (pressure, type of equipment), composition (e.g., pH, droplet size), as well as storage conditions vary greatly among studies, and most importantly, details regarding the comprehensive composition of the plant protein ingredient are lacking. The composition not only differs between crop types, cultivars, and origins (Cui et al., 2020; Lam et al., 2018; Wang, Hatcher, et al., 2010), but also between batches of protein isolate or concentrate of the same biological origin. This is a major omission, since non-protein ingredients may affect the oxidative stability of emulsions greatly, as discussed in the following sections. In Figure 2, a typical impression is given of the diversity of components and their localization in plant protein-stabilized emulsions. The results of the studies summarized in Table 1 are the consequence of many effects playing a role at the same time. One of them is the composition, but also differences in droplet size (Section Protein-stabilized emulsions and lipid oxidation: General aspects), protein denaturation (Section Protein modifications and interactions with other components during fractionation processes) and in the concentration of aqueous phase proteins can also be involved (Section Anti- or prooxidant properties of plant proteins).

Protein composition analysis in plant protein ingredients

In Table 3, the reported chemical composition of plant protein isolates (chickpea, faba bean, lentil, pea, and soybean) obtained by commercial or small-scale isoelectric precipitation is shown. The protein contents range between 73 and 91 wt% dry matter, leaving 9–27 wt% for non-protein components, such as lipids (0.5–8.9 wt%) and ash (1.5–6.5 wt%). In general, the protein content was determined using the classic nitrogen-to-protein conversion factor (N-factor) of 6.25, which is actually an overestimation of the actual values for plant proteins (Krul, 2019), that are usually between 5.2 and 5.9 (Sosulski & Holt, 1980) and varies not only

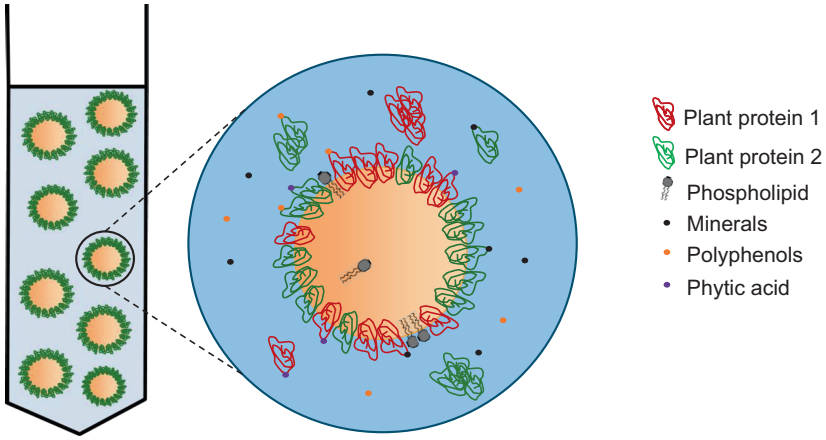

FIGURE 2 Schematic overview of the composition of an O/W emulsion stabilized by a plant protein ingredient (not to scale). For simplicity, only two types of proteins are schematized here, but many more are normally found in the protein composition of typical plant protein isolates and concentrates.

between crop type but also within one crop type. For example, for soy protein concentrates and isolates, the N-factor varies between 5.2 and 5.8 depending on the cultivar, growing conditions, and ingredient purity (Tomé et al., 2019). Using an N-factor of 6.25 results in an overestimation of the protein content between 5% and 20%, and thus an underestimation of non-protein content. For ash, this would typically then be from 1.6 to 7.8 wt%. The underestimation of non-protein components may affect the interpretation given to effects on oxidation. This is even more relevant for plant protein concentrates having a higher concentration of non-protein components, compared to isolates (Section Dry processing).

Anti- or prooxidant properties of plant proteins

Proteins may act as antioxidants via different mechanisms. In general, proteins can scavenge free radicals, react with lipid oxidation products, or chelate/repel metal ions depending on the pH (Elias et al., 2008). Scavenging and inactivating reactive compounds occur through the amino acids lysine, arginine, proline, cysteine, tyrosine, tryptophan, phenylalanine, histidine, and methionine (Elias et al., 2008; Guidea et al., 2020; Žilić et al., 2012). Radical scavenging mostly goes together with protein oxidation (see Section Protein modifications and interactions with other components during fractionation processes); with proteins acting as antioxidants if the formed radical is transferred to amino acids buried in the interior, as otherwise the radical can react with lipids (Elias et al., 2008), stressing the importance of the molecular environment. Protein denaturation (Section Protein modifications and interactions with other components during fractionation processes) results in modulated antioxidant properties, as the relevant amino acids get exposed or buried and thereby the scavenging capacity toward radicals will be altered.

Metal ions are chelated by amino acids with a carboxyl side group (asparagine, glutamine), an amino (histidine, lysine), thiol (cysteine) or hydroxyl (tyrosine) group (Guidea et al., 2020). Chelation mainly occurs at pH above the IEP when amino acid side groups have a negative charge; at low pH (positive charge) no metal complexation occurs. Every metallic cation needs at least two ligands to interact with (Baakdah & Tsopmo, 2016; Walters et al., 2018). This also stresses the relevance of protein tertiary structure as affected by pH, temperature, and/or protein oxidation (see Section-Protein modifications and interactions with other components during fractionation processes), as upon structural changes, ligands may become available or be hidden in the interior of the protein molecule.

Depending on the location in the emulsion, various effects will take place: (1) When adsorbed at the oil–water interface, free radicals scavenging can lead to protein oxidation, as described earlier, which can propagate lipid and protein oxidation. At pH > IEP, proteins will bind or chelate metal cations, bringing them in close proximity to the oil. Metal ions may indirectly (Fenton reaction leading to hydroxyl radicals) or directly induce the formation of protein radicals (Baron, 2013; Hellwig, 2019), which can, in turn, abstract a hydrogen atom to labile molecules in their vicinity such as polyunsaturated fatty acids, and thus initiate lipid oxidation (Giebauf et al., 1996; Østdal et al., 2002). At acidic pH, metals are repelled from the protein-covered interface (Elias et al., 2008; McClements & Decker, 2018), making emulsions oxidatively more stable. (2) When proteins are present in the aqueous phase, they may chelate metal ions, keeping them away from the interface thus enhancing oxidative stability (Faraji et al., 2004; Gumus et al., 2017). Furthermore, reactive oxygen species (ROS) can be inactivated, and water-soluble lipid oxidation products reduced (i.e., 4-hydroxyhex-2-enal, 4-hydroxynon-2-enal) (Elias et al., 2008; Faraji et al., 2004; Vandemoortele et al., 2020). This latter mechanism is expected to limit perceived lipid oxidation off-flavors (Elias et al., 2008), but its overall relevance for lipid oxidation progress is not clear. For instance, in a recent study, ten Klooster et al. (2022) showed that small aldehydes such as 4-hydroxy-2-nonenal have a tangible, yet limited prooxidant effect in O/W emulsions. Overall, the main antioxidant effect of proteins in emulsions can be ascribed to the proteins present in the aqueous phase binding prooxidant metal ions and thus keeping them away from the interface and thus from interacting with polyunsaturated fatty acids (Berton et al., 2011; Faraji et al., 2004; Gumus et al., 2017). Furthermore, proteins in the bulk aqueous phase may oxidize, but that would be without inducing lipid oxidation given their localization (Giebauf et al., 1996; Østdal et al., 2002).

All proteins have an antioxidant potential, but differ greatly in the extent of this potential when applied in O/W emulsions (Kiokias et al., 2006). In literature (Faraji et al., 2004; Feng et al., 2021; Gumus et al., 2017; Gürbüz et al., 2018; Hinderink et al., 2021; Hu et al., 2003; Wang et al., 2021) various plant protein ingredients (Table 1) have been evaluated and produce varying results, which also holds true for the dairy-based ingredients. Compared to whey protein isolate (WPI), plant proteins tend to produce emulsions with higher oxidative stability. For purified plant protein ingredients (isolates, or soluble fraction), a higher iron chelating capacity compared to WPI was reported (Feng et al., 2021; Gumus et al., 2017; Hinderink et al., 2021), but this capacity was lower than that of sodium caseinate (NaCas) (Faraji et al., 2004). Furthermore, the chelating capacity was demonstrated to be lower for the insoluble or full fraction of plant protein ingredients compared to WPI (Hinderink et al., 2021).

Lentil, pea and faba bean proteins have a similar iron binding capacity, but the oxidative stability of the corresponding emulsions varied substantially (pea ≈ faba bean >> lentil) (Gumus et al., 2017), indicating that the oxidative stability of protein-based emulsions is not related only to the metal chelating ability of the protein ingredients.

It is presumable that the protein fractionation process subsequently affects lipid oxidation in emulsions, because of protein denaturation, aggregation (Section Protein modifications and interactions with other components during fractionation processes), or oligomer dissociation, which impact the interfacial composition (Drusch et al., 2021; Gueguen et al., 1988). The prevalence of certain proteins in the interface (e.g., vicilin > legumin) may influence not only the physical but also the oxidative stability, and this may relate to the state of the protein as a result of the treatment received (e.g., protein denaturation, Section Protein modifications and interactions with other components during fractionation processes), the amino acid composition (i.e., amino acids with antioxidant properties) and their solubility, leading to changes in distribution over the aqueous phase and interface (Boye et al., 2010; Huu Thanh & Shibasaki, 1976; Kimura et al., 2008). Hinderink et al. (2021) showed that using only the insoluble part of pea protein isolate resulted in physically stable emulsions, yet with high lipid oxidation levels whereas using the full ingredient was beneficial to the oxidative stability of emulsions, even though they were then less stable to coalescence. This indicates that the different constituents of a complex plant protein ingredients (in composition and colloidal state) play various roles in the physical and oxidative stability of emulsions, which is notably driven by their prevalence at the droplet surface versus in the continuous phase.

During the steps in plant protein ingredient fractionation that involve high temperatures (e.g., defatting), Maillard reactions may occur (Hellwig & Henle, 2014; Hodge, 1953; Ludwig, 1979; Salazar-Villanea et al., 2016), which may have diverse consequences. Advanced Maillard reaction products such as melanoidins have been shown to improve the oxidative stability of lipids in food emulsions (Table 1) (Feng et al., 2021; Manzocco et al., 2000; Morales, 2005); however, some Maillard reaction products are carcinogenic (5-hydroxymethylfurfural, acrylamide) (O'Mahony et al., 2017; Poulsen et al., 2013; Tareke et al., 2002), although for most foods, the concentrations present are believed to be of low risk to health (Hellwig & Henle, 2014).

To be complete, proteolysis to form bioactive peptides can improve the antioxidant capacity of a given protein-containing material (Table 1) (Durand, Beaubier, Fine, et al., 2021; Durand, Beaubier, Ilic, et al., 2021; Liu et al., 2019; Torres-Fuentes et al., 2015, 2014; Yesiltas et al., 2022). This will mainly be relevant for protein ingredients processed in the presence of proteases (e.g., alcalase, prolyve) added on purpose, or natively present (Aiking, 2011), and is an important way to combat lipid oxidation in an effective, natural manner (Durand, Beaubier, Fine, et al., 2021).

Effects of non-protein components on lipid oxidation in plant protein-stabilized emulsions

Phytic acid

Phytic acid has the structure of myo-inositol with six phosphate esters attached to all carbons (IP6) (Graf & Eaton, 1990), which allows complexes being formed with multivalent cations and positively charged proteins (Oatway et al., 2001). The concentration of phytic acid typically varies between 1 and 5 wt% DM in cereal, legume, and oil seeds (Cheryan, 1980; Graf & Eaton, 1990), and occurs mainly as phytic acid-calcium (phytate) or calcium–magnesium (phytin) complex. The antioxidant effect of phytic acid is well documented (Campos-Vega et al., 2010; Canan et al., 2012; Empson et al., 1991; Graf et al., 1987; Graf & Eaton, 1990; Pei et al., 2020); the main capacity is complexation of multivalent cations such as iron and copper. Besides, phytic acid accelerates the transition of Fe^{2+} to Fe^{3+} and forms a complex with Fe^{3+}, making it catalytically inactive, and thereby preventing the formation of hydroxyl radicals (·OH) (Empson et al., 1991; Graf et al., 1987).

The protein solubility decreases after complexation with phytic acid (Cheryan, 1980; Graf & Eaton, 1990) which mainly occurs at pH below the IEP where proteins are overall positively charged. At higher pH (6–10), phytates and proteins mainly interact via cations forming phytate-cation-protein linkages (Cheryan, 1980), whereas at pH > 10 phytic acid is insoluble. At pH above the IEP, the zeta potential of protein-covered droplets was shown to remain negative, thus ensuring physical emulsion stability by electrostatic repulsion, and oxidative stability through metal complexation by proteins and phytic acid (Pei et al., 2020). Conversely, at pH below the IEP, complexation between proteins and phytic acid reduced the net charge of protein-covered droplets leading to flocculation. This was hypothesized to enhance transfer of lipid oxidation products between droplets (Pei et al., 2020), although for the latter effect, the mechanisms and conditions under which it substantially occurs still have to be further unraveled.

During wet fractionation processes (Section Wet processing), at pH 8–10, phytic acid-salt-protein linkages are formed and coextracted (especially upon addition of salt), leading to higher concentration of Cu^{2+} and Fe^{3+} in the final protein material which might act as prooxidants (Section Effect of low molecular weight components on lipid oxidation in plant protein-stabilized emulsions). Only at very high pH (>11),

phytic acid is removed during the first (centrifugation) step. Fuhrmeister and Meuser (2003) showed that the precipitation pH not only influenced the protein yield but also the phytic acid concentration in pea protein isolate; the concentration of both proteins and phytic acid increase at pH < IEP, with a maximum at pH 3.5 and a phytic acid content ~5% DM. For dry fractionation, phytic acid was shown to accumulate in the protein fraction, reaching a concentration of 2.2%–3.3% (DM) for faba bean concentrates and 1.8%–2% DM for pea protein concentrates (Coda et al., 2014; Saldanha do Carmo et al., 2022). The concentration of phytic acid in plant protein ingredients is relevant for lipid oxidation in plant protein-stabilized emulsions, yet rarely determined in literature. Depending on the concentration, the effect of phytic acid on the oxidative stability of emulsions may vary, especially at pH below the IEP as shown by Pei et al. (2020).

In food emulsions that generally have a pH between 3 and 7, phytic acid may bind to proteins either directly or via multivalent cations; thus the pH influences the location of reactive metal ions in the system (see Section Effect of low molecular weight components on lipid oxidation in plant protein-stabilized emulsions) and thereby lipid oxidation. It is good to keep in mind that phytic acid is an antinutritional compound that limits the bioavailability of minerals and other nutrients and should thus be limited in foods (Campos-Vega et al., 2010). This may be mitigated by crop varietal selection, and/or by for example, soaking the seeds prior to protein fractionation (Martín-Cabrejas, 2019).

Polyphenols

Polyphenols in legumes are mainly tannins, phenolic acids, and flavonoids. Their content varies largely with legume type, cultivar (1–7.5 mg gallic acid equivalent/g DM) (Padhi et al., 2017), and fractionation method (Xu & Chang, 2007). The majority of the polyphenols stay in the flour during pressing (Section Pre-processing) (Yang et al., 2014) as proteins and polyphenols interact non-covalently and covalently (see Section-Protein modifications and interactions with other components during fractionation processes).

Phenolic compounds have one or more phenyl rings with at least one hydroxyl group in their molecular structure (Robards et al., 1999). Phenolics can act as antioxidants via the formation of a phenoxy radical, and chelation of cations via two neighboring hydroxyl groups. Phenoxy radicals can be formed due to the low binding energy of the hydrogen in the hydroxyl group, which is relevant for alkyl and/or alkoxyl groups adjacent to the hydroxyl groups which decrease the binding energy of hydrogen. The phenoxy radical can be stabilized via intramolecular relocation of electrons, or via donation of an electron leading to negatively charged reactive species and a relatively stable positively charged phenol radical (Quideau et al., 2011).

There are, to the authors' knowledge, no published studies to date in which polyphenol content in plant protein ingredients is linked to the oxidative stability of emulsions prepared with such ingredients. Xu et al. (2018) extracted polyphenols from chickpea, lentil, and yellow pea at different germination time points; the total amount of extracted polyphenols increased with germination time for pea and chickpea, and decreased for lentil. Germination time and changes in polyphenol content were not clearly related to antioxidant activity, that is, radical scavenging activity (DPPH), oxygen radical absorbance capacity (ORAC), and iron binding capacity. The oxidative stability of Tween 20-stabilized emulsions with added polyphenols (200 μg gallic acid equivalence/g oil) increased with the addition of polyphenols for chickpea and pea, but not for lentil compared to the control emulsions without polyphenols (chickpea > yellow pea > lentil ≥ control). Bound polyphenols (esterified to macromolecules, e.g., proteins (Shahidi et al., 2016; Wang et al., 2020)) led to a better oxidative stability than free polyphenols in case of chickpea and pea. An increase in antioxidant activity for chickpea polyphenols (free and bound) and for free pea polyphenols in the emulsions was observed with increasing germination time of the samples (Xu et al., 2018).

It should be pointed out that at high concentrations, phenols may act as prooxidants (Quideau et al., 2011; Robards et al., 1999); quinones and their semiquinone radical anions may lead to reactive oxygen species via redox cycling, which promotes lipid oxidation (Schieber, 2018). In the presence of iron and at low pH, phenols can initiate the Fenton reaction by reducing Fe^{3+} to Fe^{2+} (Zhou & Elias, 2013), leading to hydroxyl radicals which, in turn, lead to the formation of lipid radicals. This is also relevant for plant protein-based emulsions, as via the plant protein ingredients also metal ions are introduced (Section Effect of low molecular weight components on lipid oxidation in plant protein-stabilized emulsions).

Effect of low molecular weight components on lipid oxidation in plant protein-stabilized emulsions

Lipids

The measured total lipid content in plant protein ingredients can vary a lot, not only because of differences in crop, cultivar, and fractionation process (type of process, presence of a defatting step or not) (Belitz et al., 2009; Deep Singh et al., 2008), but also in relation to the lipid extraction procedure used for the compositional analysis. Oilseeds, which contain between 34 and 55 wt% lipids/DM (González-Pérez & Vereijken, 2007; Rosa et al., 2009; Saeed &

Cheryan, 1988) lead to protein ingredients with higher oil contents of up to 20 wt% DM (Östbring et al., 2020), whereas for legume seeds, such as pea and lupine, the lipid content in the protein isolates can still be around 10 wt% DM (Keuleyan et al., 2023), unless a defatting step is applied. The presence of fat in plant protein ingredients may decrease the physical, and oxidative stability of emulsions, since legume seeds contain a high amount of (poly)unsaturated fatty acids that are prone to oxidation during protein fractionation (i.e. defatting, grinding, wet processing, antimicrobial treatment; Sections Pre-processing and Wet processing), possibly initiating oxidation and off-flavor production in emulsions (Gueguen, 1983; Rackis et al., 1970; Sánchez-Vioque et al., 1998) which might explain the results found by Hu et al. (2003) (Table 1). Especially in plant protein flours with high concentrations of lipoxygenase, this increases lipid, and protein oxidation (Huang et al., 2006), and thus protein solubility, and emulsifying capacity (see Section Protein modifications and interactions with other components during fractionation processes) (Carré, 2021; Sánchez-Vioque et al., 1998).

Most analytical methods to determine the total lipid content in dried ingredients use hexane, which allows for extracting neutral lipids (triacylglycerols, mono- and diacylglycerols) and fatty acids, but is not that suited for polar lipids (e.g., phospholipids) (Carré, 2021; Koc et al., 2011). This may lead to an underestimation of the total lipid content, and that of polar lipids obviously. It is important to point out that phospholipids can have oxidative implications through (1) regeneration of oxidized tocopherols (Samdani et al., 2018), (2) formation of Maillard reaction-like products (when the amino group of phosphatidylethanolamine reacts with the carbonyl group of lipid oxidation products) (Lu et al., 2017; Zamora & Hidalgo, 2005), or (3) chelation of metal ions. At the oil–water interface, the latter effect might promote lipid oxidation (Cui & Decker, 2016), especially with phospholipids being pre-oxidized prior to emulsion preparation (Pan et al., 2013), such as during ingredient preparation. Nevertheless, in intact plant seeds, the natural lipid storage droplets (called oleosomes or oil bodies) are surrounded by phospholipids and hydrophobic proteins, and are known to have a high oxidative stability. This may be due, at least in part, to the antioxidant activity of interfacial phospholipids (Fisk et al., 2008; Gray et al., 2010; Wijesundera & Shen, 2014), suggesting that in such conditions, antioxidant effects would prevail over potential prooxidant ones.

Saponins

Saponins are found in lentils, chickpeas, soybeans, beans, and peas, with soybeans having the highest concentration (5.6 wt% DM). They consist of a non-polar triterpene or steroid aglycone attached to polar mono- or oligosaccharides, which makes them amphiphilic and able to adsorb to the oil–water interface (Martín-Cabrejas, 2019). Most saponins form insoluble complexes with β-hydroxysteroids, and with iron, zinc, and calcium (Milgate & Roberts, 1995; Shi et al., 2004). Rickert et al. (2004) showed that up to 64% of the total saponins increasingly end up in the soluble fraction of soy protein isolates with pH (8.5 -> 10.5) and temperature (25 -> 60°C). The extracted amount of saponins increased (8.8 vs. 12.2 μmol/g; dry base) with DDMP (2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one) saponins becoming less prevalent as their non-DDMP counterparts. DDMP saponins inhibit protein and lipid oxidation by scavenging of free superoxide and hydroxyl radicals (Kerem et al., 2005; Tsujino et al., 1994; Yoshiki & Okubo, 1995). The conditions applied during protein fractionation facilitate conversion from DDMP to non-DDMP saponins, thus, the antioxidant properties may be substantially affected. When adsorbing at the interface, the DDMP form would generally limit lipid oxidation through radical scavenging properties whereas the non-DDMP would have no such effect. Therefore, for plant protein ingredients, the antioxidant effect of saponins may be relevant (Zhang et al., 2020), and would need to be determined through well-designed emulsion studies.

Minerals

Minerals such as iron and copper are naturally occurring in plant seeds (2–2000 mg/100 g crop) (Martín-Cabrejas, 2019; Sá et al., 2020), with concentations differing per cultivar, growing conditions, etc. (Hall et al., 2017). Transition metals such as iron and copper are known to decompose hydrogen peroxide (H_2O_2) via the Fenton reaction, forming hydroxyl radicals (Halliwell & Gutteridge, 1984; Knight & Voorhees, 1990), and to decompose lipid hydroperoxides to peroxyl or alkoxyl radicals (Schaich, 1992), thus promoting lipid oxidation. In general, a low concentration of soluble metals in plant protein ingredients would logically limit lipid oxidation; however, due to complexation with proteins (Section Anti- or prooxidant properties of plant proteins) (Manamperi et al., 2011), and possibly phytic acid (Section Phytic acid) during protein fractionation, the metal concentration in the final ingredient may be higher than in the starting raw material; thus, affecting oxidation in the emulsion depending on location and chelate type (Sections Anti- or prooxidant properties of plant proteins, Phytic acid, and Effect of low molecular weight components on lipid oxidation in plant protein-stabilized emulsions (phospholipids)).

When comparing different plant protein sources (Table 1), part of the differences observed for lipid oxidation in emulsions could be explained by metal content, although the latter was not reported. There is probably a trade-off, since biofortification of foods by selecting crop varieties rich in certain micronutrients is of interest to address nutritional deficiencies (e.g., iron, zinc,

and iodine), which is desirable (Jha & Warkentin, 2020). However, this may induce issues regarding the oxidative stability of the ingredients and the derived food matrices. Therefore, it is important to measure the physicochemical quality of plant protein sources; which is not common practice, to the best of the authors' knowledge.

Carotenoids/tocopherols

These components are naturally present in plant-based material, and are well-known lipophilic antioxidants (Martín-Cabrejas, 2019; Mudgil & Kamal-Eldin, 2020); through their conjugated double bonds, they are able to quench singlet molecular oxygen and scavenge free radicals (Frankel, 1991; Mudgil & Kamal-Eldin, 2020; Stahl & Sies, 2003). The tocopherol and carotenoid content differ greatly between legumes, and species of the same type (Boschin & Arnoldi, 2011). Due to their lipophilicity, carotenoids and tocopherols are mostly removed together with the oil when applying a hexane extraction step (Chen et al., 2011), but residual amounts may remain in the plant protein ingredient (Sicaire et al., 2015). Pea and other low lipid legumes that are not defatted, lead to protein materials with substantial amounts of tocopherols (>500 mg/kg endogenous lipids for pea protein isolate/concentrate) (Keuleyan et al., 2023), which may confer a good oxidative stability to O/W emulsions (Wang et al., 2022; Yi et al., 2018).

CONCLUSION AND PERSPECTIVES

Plant protein ingredients contain many other components than proteins, of which some possess pro- or antioxidant properties either individually or in conjunction with others. Their concentrations greatly vary with crop and cultivar, and the fractionation process used, and these aspects are not well documented, yet. This makes it rather impossible to attribute the susceptibility of emulsions to lipid oxidation to specific components present.

In addition, various aspects that we report here fall under several research fields (agriculture, genetics of plants/crops, protein functionality, protein processing, food technology, lipid chemistry), and it is clear that in order to achieve a complete understanding of the impacts of plant protein ingredients on the oxidative stability in emulsions prepared thereof, collaboration between experts from all fields is needed.

We suggest to actively seek collaborations, and report the composition of plant protein ingredients in much greater detail than the current common standards, so comparisons are made possible at a much higher level. Hereby, the documentation would need to include the categories presented in this review, and ideally also the colloidal state of proteins, and the presence of any oxidized species. If available, this is expectedly a crucial step toward the rational use of plant protein ingredients in food emulsion design. In this sense, also modeling efforts as recently done in our lab can help quantify differences in lipid oxidation in emulsion, thus helping in pinpointing which effects are actually taking place (Schroën & Berton-Carabin, 2022).

To adjust the composition of plant protein ingredients, the fractionation processes can be used as a tool to fit the functional properties to the application of the final plant protein material. For this, innovative mild technologies can be considered, such as those presented by Pelgrom, Boom, et al. (2015), Funke et al. (2021), Peng et al. (2020), Silventoinen et al. (2018). So far, these protein ingredients were tested for their ability to physically stabilize emulsions, but not yet for lipid oxidation stability, which we identify as an important field to develop.

AUTHOR CONTRIBUTIONS

All authors contributed to the writing and development of the manuscript, and ideation of the review. The final copy of the manuscript was edited and proofread by the two co-authors.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

ETHICS STATEMENT

No human or animal subjects were used in this research.

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