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# 1 *Pisum sativum* vs *Glycine max*, a comparative review of

# 2 nutritional, physicochemical, and sensory properties for food uses

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- 5

### 6 Highlights

- 7 Soybean and pea have similar protein structures, leading to similar functional properties.
- 8 Pea has fewer allergenic factors than soybean.
- 9 As regards nutritional aspects, pea could be an effective soybean substitute.
- Pea has more amino acids and more isoenzymes involved in off-flavour development than
  soybean.

### 12 Abstract

### 13 Background

14 Current issues surrounding meat consumption and changes in eating habits have motivated the 15 development of meat substitutes. Vegetable proteins, especially legume proteins, are of interest. 16 Soybean is the most widely used legume crop for food, but due to some negative aspects, it may be 17 relevant to develop soybean substitutes.

### 18 Scope and approach

Pea is a possible alternative to soybean and the latter was thus compared with pea to determine if pea would be a good substitute. This review provides a brief insight into the similarities and differences between soybean and pea regarding composition and nutrition as well as physicochemical and sensory aspects impacting their food uses.

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#### 23 Key findings and conclusions

As pea is less allergenic than soybean but has similar nutritional and functional properties, it could be a great alternative to soybean. Nevertheless, some particularities of the pea composition can lead to a stronger "beany" off-flavor and so a less acceptable product. In addition, the isoenzymes involved in the development of off-flavor are more varied in the pea. The pea off-flavor may thus be more complicated to control.

29

#### 30 Keywords

31 Pisum sativum, Glycine max, protein, functionality, enzymes, lipoxygenase

32

## 33 1 Introduction

34 Considering the current debate regarding the high level of animal protein in our diet, it is of interest to investigate meat substitutes. Today, it is known that animal proteins cause various problems. First, 35 from an agronomic point of view, animal protein production uses large agricultural areas, consumes a 36 37 lot of water and energy, produces a high quantity of methane and greenhouse gases, and depletes and pollutes agricultural areas, water, and air (Bittman, 2007; Harvard Chan, 2012). A diet rich in animal 38 39 protein is also related to health problems such as an increase in diabetes and cardiovascular diseases 40 (Daniel, Cross, Koebnick, & Sinha, 2011; Song, Manson, Buring, & Liu, 2004; Walker, Rhubart-Berg, 41 McKenzie, Kelling, & Lawrence, 2005). Moreover, people are now more concerned with animal 42 welfare and increasing numbers are becoming vegetarians, vegans, or flexitarians (Derbyshire, 2017; 43 Futuribles, 2018; Kersche-Risch, 2015). However, to achieve food security and protein intake for the 44 growing world population, meat consumption needs to be reduced and thus meat substitutes need to be 45 found (de Boer, Schösler, & Aiking, 2014; ONU, 2017). For all these reasons, it is important to 46 develop more meat substitutes such as in cultured meat, insects, mycoproteins and, most importantly, vegetable proteins (American Dietetic Association, 2009; Datar & Betti, 2010; Elzerman, Hoek, van
Boekel, & Luning, 2011; Hoek et al., 2011; Verbeke, 2015).

Until recently, vegetable proteins had been set aside because of their low protein content and a less favorable amino acid score compared to animal proteins (V. R. Young & Pellett, 1994). The total absorption and utilization of all the amino acids by the body is limited by the essential amino acid present in the lowest quantity, called the limiting amino acid. In addition, vegetable proteins are less digestible than animal proteins due to indigestible carbohydrate constituents, protein structures, and antinutritional factors (Gilani, Cockell, & Sepehr, 2005).

55 However, associating different sources of protein resolves the issue of limiting amino acids. For example, associating a protein with a low Lysine content and a protein with a high Lysine content 56 57 allows optimal absorption and utilization of the amino acid because the two proteins are 58 complementary (American Dietetic Association, 2009). There are three main sources of vegetable 59 protein: cereals, legumes, and oilseeds. Pulses are key to the development of vegetable protein as they are not major allergens and have numerous advantages (AFPRAL, 2016; American Dietetic 60 Association, 2009; V. R. Young & Pellett, 1994). Pulse crops are favorable for agricultural areas as 61 62 the symbiotic bacteria on the root nodules can fix the nitrogen then release it in the ground so the 63 plants do not require any additional nitrogenous fertilizer. This also helps the establishment of 64 subsequent crops in the case of crop rotation. Furthermore, they contain high levels of essential amino acids, in particular lysine. Considering these advantages, it is important to develop more legume areas 65 (Bordat, Lemanceau, Jegou, & Boidevezi, 2018; Terres Inovia, 2017a; Terres Univia, 2017). 66

Today, soybean (*Glycine max*) is the most widely used legume with 336 Mt produced annually worldwide and 140 000 t produced in France, mainly in the center and south (Terres Inovia, 2017b; Terres Univia, 2018). However, soybean has some negative aspects. Despite its advantages as a legume, most soybean production is intensive, which implies adding fertilizer and pesticides and using large amounts of water. In addition, due to the use of pesticides, 70% of soybean produced are plantpesticide GM crops, which can resist the addition of strong pesticides (Terres Inovia, 2017b; Terres Univia, 2018). 74 New legumes to substitute soybean are being investigated. A potentially great rival to soybean is the 75 field pea (*Pisum sativum*), belonging to the *Fabaceae* and *Papilionoïdeae* phylogenetic group like 76 soybean. Around 11 Mt of pea are produced every year in Europe and worldwide (Terres Univia, 77 2017). France is one major producer in European Union with 600 000 t/year (Terres Inovia, 2017a; 78 Terres Univia, 2017). Pea is widely used in crop rotation, which is highly beneficial for the soil as it 79 helps the establishment of subsequent crops without plowing thanks to its small amount of chaff and 80 early harvest that helps maintain the structure of the soil. Pea also increases the yield of the next crop 81 in the case of crop rotations (+ 6-12 q/ha for wheat after pea compared to a wheat after wheat), reduces 82 the environmental impact of the crop system by helping to control weeds and the biological processes 83 within the crop rotations, as well as bringing financial gain (Terres Inovia, 2017a, 2017b; Terres 84 Univia, 2017, 2018).

The aim of this study was, therefore, to compare different aspects of pea and soybean to determine if pea can substitute soybean. The first aspect is the composition and the impact on utilization, with a focus on functional properties. The second aspect is nutritional quality, with a focus on nutritional value and allergenicity. The third aspect is the composition and the impact on flavor, with a focus on substrates and enzymes responsible for flavor development.

## 90 2 Structure and physicochemical properties of soybean and pea

### 91 proteins

Different studies compare the functional properties of soybean and pea proteins, but contradictory
results have been found in the literature, possibly due to differences in protein purity, isolation
methods, and seed variety (Barac, Pesic, Stanojevic, Kostic, & Bivolarevic, 2015).

A comparison of three articles, each comparing the functional properties of soybean and pea protein isolates purified using the same method, is presented in **Table 1**. In each reference column, different functional properties of soybean and pea protein isolates are compared. As these articles did not use the same determination methods and thus do not have comparable results, a color code is used to distinguish the functional properties; the best are presented in green and the worst in red. As can be 100 seen, contradictory results are reported depending on the reference (Aluko, Mofolasayo, & Watts, 101 2009; Barac et al., 2015; Fernàndez-Quintela, Macarulla, Barrio, & Martìnez, 1997). As it is not 102 possible to compare the functional properties of soybean and pea based on these contradictory data, 103 comparing the protein structure, which is strongly related to the functional properties, may possibly 104 provide more clues regarding the similarity of soybean and pea proteins.

105 Indeed, if proteins have the same primary sequences and 3D structures, they are more likely to have 106 similar properties. In isolates or concentrates, the main proteins are globulins (7S and 11S). The 107 soybean globulins are glycinin (11S) and conglycinin (7S), and the pea globulins are legumin (11S), 108 vicilin, and convicilin (7S). The comparison of their 3D structures, as presented in **Table 2**, shows that 109 soybean and pea proteins are really similar. The comparison of the protein sequences using Uniprot 110 (UniProt, 2019) indicates a high percentage of identity, as shown in **Table 2**. The identity represents 111 the correspondence between the two sequences. With an identity superior to 30%, the proteins can be 112 considered as homologous (Chifolleau, 2018; Perrière, 2012). When the data is available, it is also 113 possible to compare the beta-sheet and alpha-helix positions between the proteins. The comparison 114 between Glycinin 1 and Legumin A shows that most of the beta-strands and alpha-helices are in the 115 same position, as shown in Figure 1.

116 Consequently, as the 3D structures and the sequences of the proteins are similar, they may have 117 essentially the same functional properties and thus may be used in the same ways. Soybean protein 118 functionality is currently well understood whereas pea proteins are still in development. However, 119 because of the similarities between the proteins, pea could potentially be used in the same way as 120 soybean.

The literature partially confirms the similarity in protein structure and, therefore, functionality. Based on the solubility, and emulsifying and foaming properties of native and heat-treated soybean and pea isolates, soybean and pea have really close functional properties (Barac et al., 2015). For example, the solubility of soybean and pea is very similar depending on the pH and the seed variety used. Furthermore, soybean and pea have similar patterns for the soluble protein composition. For the emulsifying properties, a strong impact of the genotype is observed. However, as the emulsifying property is strongly related to solubility, if the proteins have the same solubility the emulsifying property may also be similar. The similarity of the functional properties of the two proteins can also be seen with studies on products made with soybean and pea protein. For example, with yoghurt-like products made with soybean or pea protein, a similar texture is observed by sensorial analysis (Yousseef, 2017).

# 132 **3** Nutritional quality of soybean and pea proteins

#### 133 3.1 Nutritional value

To compare the nutritional aspects of soybean and pea, two indices can be compared: the DIAAS (lowest Digestible Indispensable Amino Acid Score) based on the amino acid reference ratios, and the PDCAAS index (Protein Digestibility Corrected Amino Acid Score). These indices are calculated as follows:

$$DIASS = 100 * \frac{mg \ digestible \ dietary \ indispensable \ amino \ acid \ in \ 1g \ of \ dietary \ protein}{mg \ of \ the \ same \ dietary \ indispensable \ amino \ acid \ in \ 1g \ of \ the \ reference \ protein}$$

$$PDCAAS = \frac{mg \ of \ limiting \ amino \ acid \ in \ the \ protein}{mg \ of \ limiting \ amino \ acid \ in \ a \ reference \ protein} * \% \ true \ fecal \ digestibility$$

The PDCAAS is still currently the most widely used index but has some limitations (Mathai, Liu, & Stein, 2017). For example, the true fecal digestibility is used in the calculation whereas the ileal digestibility is more accurate. Moreover, PDCAAS values generally underestimate or overestimate the quantity of amino acids. For these reasons, the FAO recommends using the DIAAS, calculated with the individual ileal digestibility of the different indispensable amino acids. These parameters, with the percentage digestibility of the proteins, are compared between different samples in **Table 3**.

According to these data, soybean has a PDCAAS of 0.71 to 1.09, a DIAAS of 0.43 to 1.05, and a digestibility of 92 to 98.5 %, and pea has a PDCAAS of 0.49 to 0.96, a DIAAS of 0.45 to 0.822, and a digestibility of 69 to 99.0 %. The values vary depending on the calculation method and the protein form (Hayes, 2018; Hughes, Ryan, Mukherjea, & Schasteen, 2011; Mathai et al., 2017; Rutherfurd, Fanning, Miller, & Moughan, 2015; UNJURY®, 2019). Even in the same protein form (isolate or concentrate), the indices vary over a wide range depending on the calculation and the method used (on rats or pigs). Soybean and pea nutritional values seem close, even if soybean seems to have higher values (closer to the reference protein). However, if we consider the average pea PDCAAS (0.78), it still has a higher value than beans, rice, peanut or wheat that have a PDCAAS of 0.63, 0.53, 0.52, and 0.40, respectively (Hughes et al., 2011). It is also interesting to note that combinations of pea and cereal proteins are giving more favourable nutritional scores.

155 These differences in indices between soybean and pea may be due to two factors: the limiting amino 156 acid and the digestibility factor. The amino acid content ratios of soybean and pea are close, as shown 157 in Table 4 (% of amino acid/total amino acid), but some essential amino acids are present in lower 158 quantities in pea than in soybean, which would explain some differences (Carrera et al., 2011; 159 Leterme, Monmart, & Baudart, 1990). The digestibility factor is also slightly different. As seen in 160 **Table 3**, the digestibility factors of soybean and pea seem to vary depending on the protein form 161 (isolate, concentrate) and protein content (globulins, albumins), with pea having one of the lowest digestibility factors (Hayes, 2018; Hughes et al., 2011; Mathai et al., 2017; Rutherfurd et al., 2015; 162 163 UNJURY®, 2019).

With a lower content of sulphur amino acids and less digestible protein, pea has a lower nutritional value than soybean. However, depending on the product form and content, pea protein can still be a good substitute as regards this aspect.

### 167 **3.2** <u>Allergenicity</u>

Besides its isoflavone content, whose impact on health is still under debate, soybean is sometimes set
aside because of its allergenicity. The EFSA classify plant allergens into four main families according
to sequence homology, 3D structure, and function: prolamins, cupins, profilins, and Bet v1 (EFSA
Panel on Dietetic Products, Nutrition and Allergies (NDA), 2014).

Soybean is a major allergen, with 30 IgE-binding proteins, leading to all stages of allergic reactions.
These 30 proteins include different families: cupins with 7S (vicilin – Gly m5), 11S (legumin – Gly m6), 15S, and 2S globulins; prolamin (hydrophobic protein Gly m1 and Gly m2); pollens peers;

oleosin; 2S albumin, and P34 protease. Some of these are major allergens: β-conglycinin (Gly m5),
glycinin (Gly m6), and P34 protease (Glym Bd 30 kDa) (Gomez-Andre, Deschildre, Bienvenu, & Just,
2012). Soybean is also one of the main allergens involved in FPIES (Food Protein-Induced
Enterocolitis Syndrome), a non-IgE allergic reaction characterized by vomiting and hemorrhagic
diarrhea (Bidat E., 2017). Nowadays, soybean allergies are more prevalent due to the increasing use of
soybean as an additive in foods.

The literature suggests pea is less allergenic than soybean, which may facilitate its food use. First of all, pea seems to be very rarely involved in FPIES (Bidat E., 2017) and is not a major allergen (AFPRAL, 2016). Pea contains less allergenic factors but its allergenicity, as in soybean, is caused by 7S globulin (vicilin and convicilin) (Sanchez-Monge et al., 2004), with the major allergen being convicilin (Dreyer, 2013).

According to the EFSA, the immunogenicity of an allergen is partially related to the structural features of the proteins and their biological activity. Soybean and pea proteins are really close, as discussed above, so their major allergens are supposedly the same: 7S and 11S globulins (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2014). Based on ANSES reports, pea can now be considered as an emergent allergen (Cérou, 2019). Although pea seems less allergenic than soybean, as the same proteins are involved in allergic reactions, pea could become as allergenic as soybean.

192 To obtain more clues as to the allergenic potential of pea, the number of soybean and pea allergens 193 identified in databases was compared. COMPARE (COMprehensive Protein Allergen Resource) 194 (COMPARE Allergen Database, 2019) and Base AllergenOnline (Base AllergenOnline, 2019) were 195 used. For pea (Pisum sativum), four allergens were found in COMPARE and seven in Base 196 AllergenOnline. For soybean (Glycine max), 41 allergens were found in COMPARE and 42 in Base 197 AllergenOnline. From the allergens identified, pea seems less allergenic than soybean. However, pea 198 allergenicity is still being investigated; some soybean allergens may be present in pea. To verify this 199 point, soybean allergens were firstly classified by "family": 2S albumins, glycinin, β-conglycinin 200 (alpha, alpha' and beta subunits), trypsin inhibitor, P34 protease, and profilin. Then, some key 201 allergens in each family were investigated in pea using a BLAST search of the allergen sequences

from COMPARE (COMPARE Allergen Database, 2019) (FASTA format) on Uniprot (UniProt, 2019)
(e threshold <1, BLOSUM62 matrix, gapped true).</li>

The 2S albumin, trypsin inhibitor, and profilin allergens were not found in pea and nor was one of the major allergens (*Major Gly 50kDa allergen*). However, the glycinin allergen (*glycinin*) was found in the pea legumin sequences, with 43.00 % to 55.90 % identity. The  $\beta$ -conglycinin subunit allergens were also found in the pea vicilin and convilin sequences with 40.20 % to 57.30 % identity.

Thus, according to current knowledge, pea seems to contain fewer allergens than soybean, with no 2S
albumin, trypsin inhibitor, or profilin allergens. Major pea allergens are vicilin and convicilin.

### 210 4 Development of the volatile fraction in pea and soybean

### 211 **proteins**

Raw soybean and pea are compared here. The composition of the matrix has an impact on flavor. The
typical legume off-flavor, known as "beany", "green" or "earthy/musty" (Murat, 2013; Vara-Ubol,
Chambers, & Chambers, 2004), is the combination of different volatile compounds belonging to
different chemical families such as aldehydes, ketones or alcohols.

Many of these compounds are typically found in legumes (Rackis, Sessa, & Honig, 1979) and are generated via different pathways. The enzymatic and non-enzymatic degradation of lipids, amino acids, and peptides are primarily responsible for off-flavor development (Angerosa, Alessandro, Basti, & Vito, 1998; Rackis et al., 1979). Thus, investigating the substrates in these reactions (lipids and amino acids) and the enzymes involved in the enzymatic pathways is of interest to estimate the "beany" flavor development potential. For example, if the matrix has more substrates or enzymes, the potential of developing a "beany" flavor or a stronger "beany" attribute is higher.

#### 223 4.1 Substrates

#### 224 **4.1.1** Lipids

Lipids are the main substrates in the production of different aldehydes and ketones that lead to different alcohols. The different lipids lead to different volatile compounds. The lipid content in Soybean is approximately 20 %, whereas the lipid content in pea is only 2 % (Eriksson, 1967; Murat,
2013; Triboi & Triboi-Blondel, 2002; G. Young & Mebrahtu, 1998). The different fatty acids present
in soybean and pea are presented in Table 5.

230 The most important fatty acids in soybean are linoleic acid, oleic acid, and linolenic acid (Grela & 231 Günter, 1995; Shoemaker & Hammond, 1988; Sultan, Dikshit, & Vaidya, 2015). The most important 232 fatty acids in pea are linoleic acid, oleic acid, palmitic acid, and linolenic acid (Eriksson, 1967; Grela & Günter, 1995; Murat, 2013; Murcia & Rincón, 1991). Among the fatty acids, the unsaturated ones 233 234 are of interest because they are the main substrates of volatile compounds. For example, linoleic acid 235 leads to the formation of hexanal, and oleic acid to the formation of octanal (Frankel, 1983; Gargouri, 236 Akacha, Kotti, & Rejeb, 2008; Murray, Shipton, Whitfield, Kennett, & Stanley, 1968; Sessa, 1979; 237 Snyder, Frankel, Selke, & Warner, 1988).

As can be seen in **Table 5**, the fatty acid profiles of soybean and pea are similar. However, the lipid content in soybean is higher than in pea, which can lead to more lipid degradation. Consequently, soybean may develop more volatile and off-flavor compounds.

241 However, enzymatic and non-enzymatic oxidation of lipids starts really early and rapidly after the 242 harvest. The off-flavor compounds formed are perceptible at very low levels (in the order of ppb and ppm). Moreover, the typical "beany" off-flavor is generally expressed when the compound is present 243 244 between 1 and 10 ppm in the matrix (Rackis et al., 1979; Vara-Ubol et al., 2004). Therefore, even the 245 smallest quantity of lipids is enough to develop the strong, characteristic off-flavor of legumes, as is 246 the case in pea (Angerosa et al., 1998; Rackis et al., 1979; Schindler et al., 2011, 2012). Thus, even the 247 2% of lipids in pea are enough to develop a strong off-flavor. Maturity and moisture content can also 248 have an impact. For example, the low moisture content in mature soybean limits oxygen diffusion in 249 the center of the soybean (cotyledon) and consequently lipid degradation, whereas the high moisture 250 content in fresh immature peas facilitates the reaction (Sessa, 1979).

#### **4.1.2 Amino acids**

Amino acids are the second main substrates of volatile compounds. The amino acid profiles of pea and soybean are presented in **Table 4 (g of amino acid/100g of protein)**.

254 Proteolysis directly contributes to off-flavor development by releasing peptides and amino acids (Marilley & Casey, 2004). Some amino acids are the substrates of some strong volatile flavor 255 256 compounds. For example, leucine, isoleucine, and valine are the main precursors of branched aldehydes such as 2- and 3-methylbutanal (Angerosa, Mostallino, & Basti, 2001; Lee, Lee, Choi, 257 Hurh, & Kim, 2013; Murray, Shipton, Whitfield, & Last, 1976; Rodríguez-Bernaldo De Quirós, 258 259 López-Hernández, González-Castro, De la Cruz-García, & Simal-Lozano, 2000). Sulfurous 260 compounds develop from the degradation of sulfurous amino acids, mainly methionine (Marilley & 261 Casey, 2004; Rodríguez-Bernaldo De Quirós et al., 2000). The breakdown of amino acids also leads to 262 the formation of pyrazines. The process involves different amino acids such as alanine, leucine, serine, 263 and threonine (Murray et al., 1976; Shu, 1999; Wang & Odell, 1973).

Unlike lipid degradation, amino acid degradation is slow and takes place throughout the product's life cycle. As shown in table 4 (g of amino acid/100g of protein), pea contains more amino acids involved in off-flavor development (leucine, serine, and threonine). For example, even if the ratios of leucine are similar in pea and soy, pea contains more leucine (6.6 g / 100 g instead of 3.5 g/100g in soybean). As a consequence, pea can lead to the development of more volatile compounds and thus a stronger off-flavor.

#### 270 **4.2** Enzymes

Enzymes involved in the enzymatic oxidation of lipids are of primary interest because they lead to different volatile compounds. Two main enzymes are investigated here: lipoxygenase and hydroperoxide lyase. Lipase breaks down triglycerides into free fatty acids. Then, lipoxygenase transforms the different free fatty acids into fatty acid hydroperoxide. Hydroperoxide lyase then cuts the fatty acid hydroperoxides into acids and volatile compounds such as aldehydes (Gargouri et al., 2008; Moreno & Peinado, 2012). Depending on the substrate specificity and the cleavage site of the 277 enzyme, different compounds are formed. There are, therefore, different isoenzymes of lipoxygenase 278 and hydroperoxide lyase.

279 4.2.1

#### Lipoxygenase

280 Lipoxygenase activity (EC 1.13.11.12) catalyzes fatty acid oxidation into fatty acid hydroperoxides 281 (HPOD) by adding a hydroperoxide functional group to the carbon chain. Depending on the 282 isoenzyme, lipoxygenase introduces the group at carbon 9, 10 or 13 to form the corresponding 9-, 10-283 or 13- fatty acid HPOD (Lumen, Stone, Kazeniac, & Forsythe, 1978; Pinsky, Grossman, & Trop, 284 1971; Rackis et al., 1979). A strong lipoxygenase is found in legumes, and particularly in Fabaceae, 285 and high levels of HPOD are formed even with a low lipid content (Fauconnier et al., 1995; Veldink, 286 Vliegenthart, & Boldingh, 1977).

287 Differences in the molecular masses and amino acid composition of soybean and pea lipoxygenase 288 (Eriksson & Svensson, 1970) indicate different isoenzymes.

According to the literature, soybean contains three or four isoenzymes (LOX-1, LOX-2, LOX-3, and 289 290 LOX-4) with different optimum pHs. By combining the various references, an overview of the 291 properties of lipoxygenase isoenzymes in soybean is proposed in **Table 6**.

292 LOX-1 has an optimum pH of nine to ten and LOX-2, LOX-3, and LOX-4 have an optimum pH of six 293 to seven (Eriksson & Svensson, 1970; Eskin, Grossman, Pinsky, & Whitaker, 1977; Fauconnier et al., 294 1995; Grosch & Laskawy, 1975; Kalua et al., 2007; Sessa, 1979; Veldink et al., 1977). More 13-295 HPOD is formed at pH 9, therefore, LOX-1 working at pH 9 should be a 13-LOX with an oxygen 296 being added to carbon 13 of the fatty acids (Fauconnier et al., 1995; Pinsky et al., 1971). Equivalent 297 levels of 13- and 9-HPOD are formed at pH 6, therefore, LOX-2 and LOX-3 are two different isoenzymes, 13-LOX and 9-LOX, respectively (Sessa, 1979). According to Matoba et al. (1985), 298 299 LOX-2 is mainly responsible for the formation of hexanal, a 13-HPOD product of linoleic acid and is 300 thus a 13-LOX. The remaining LOX-3 is, therefore, a 9-LOX. But, according to Grosch & Laskawy 301 (1975), LOX-3 produces hexenal, a 13-HPOD product of linolenic acid. Two hypotheses are possible 302 here: LOX-2 is a 13-LOX and LOX-3 is a 9-LOX, or LOX-2 and 3 are both 9- and 13-LOX possibly with different substrate specificities. According to the enzyme properties such as the impact of  $Ca^{2+}$  on enzyme activity (Sessa, 1979), it is more likely that LOX-2 and LOX-3 are different. LOX-4 is unidentified.

According to the literature, pea contains three, four or five isoenzymes (LOX-1, LOX-2, LOX-3, LOX-4, and LOX-5) (Eskin et al., 1977; Veldink et al., 1977). By combining the various references, an overview of the properties of isoenzymes in pea is proposed in **Table 6**.

309 Identifying the different properties of these isoenzymes is a bit harder in pea because less work has 310 been done on this topic. As 9- and 13-HPOD are found, pea contains 9-LOX and 13-LOX. Different 311 optimum pHs are found. At pH six to seven, 9- and 13-HPOD are found suggesting two isoenzymes working at this pH (9-LOX and 13-LOX). 13-HPOD is also found at a pH above eight, suggesting a 312 313 13-LOX working at a pH above eight (Matsui, Sasahara, Akakabe, & Kajiwara, 2003; Veldink et al., 314 1977). Therefore, it is proposed that LOX-1 is a 13-LOX at a pH above eight, LOX-2 is a 13-LOX at pH six to seven, and LOX-3 is a 9-LOX at pH six to seven, as in soybean. At least one of the enzymes 315 316 is heat-stable because activity is still observed after heat treatment (Rhee & Watts, 1966). It makes 317 sense to identify the heat-stable enzyme as LOX-1, as in soybean. The two isoenzymes identified by Sessa (1979) are inhibited by  $Ca^{2+}$  but, because five isoenzymes are identified here, it is not possible to 318 know which isoenzymes are inhibited by  $Ca^{2+}$ . 10-HPOD is also present in pea (Matsui et al., 2003). 319 320 As far as we know, no 10-LOX responsible for the formation of 10-HPOD has been identified in pea 321 but exists in other matrices (Assaf, Hadar, & Dosoretz, 1997; Badenhop & Wilkens, 1969; Husson, 322 Thomas, Kermasha, & Belin, 2002; Matsui et al., 2003; Wurzenberger & Grosch, 1984). Thus, it is 323 proposed that one of the remaining isoenzymes is a 10-LOX, called here LOX-5. 10-LOX has an 324 optimum pH of six, is inactive at pH eight, and is heat-labile (Assaf et al., 1997; Matsui et al., 2003; 325 Wurzenberger & Grosch, 1984). LOX-4 is unidentified.

The comparison of the lipoxygenase profiles of soybean and pea indicates that the catalytic activities of the isoenzymes are similar. However, because of the differences in the amino acid composition of some isoenzymes, the substrate specificity may not be the same. These conclusions regarding pea isoenzymes are in accordance with the first conclusions made (Yoon & Klein, 1979): two isoenzymes working at acidic-neutral pH, close to soybean LOX-2 and LOX-3, and another isoenzyme working at
alkaline pH, close to soybean LOX-1.

In addition to this comparison, differences between the various soybean and pea isoenzymes may also be observed regarding the substrate specificity of the isoenzymes. For example, all the isoenzymes do not exhibit activity on linoleic acid and methyl linoleate (Eskin et al., 1977; Sessa, 1979). Moreover, because isoenzymes forming mixtures of 9- and 13-HPOD also exist, one or more of the isoenzymes listed may be of this kind. This 9- and 13-LOX may be one of the isoenzymes identified but with a higher specificity for 9-HPOD or 13-HPOD or may be the last unidentified isoenzyme (Yoon & Klein, 1979).

Moreover, it seems that pea contains more isoenzymes than soybean and thus has a stronger enzymatic oxidation activity. For example, pea seems to contain a 10-LOX and soybean does not. 10-HPOD may be formed in soybean, but via autoxidation pathways more than enzymatic pathways and therefore more 10-HPOD products such as 1-octen-3-ol may be formed in pea (Husson et al., 2002).

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#### 4.2.2 <u>Hydroperoxide Lyase</u>

Fatty acid hydroperoxides are cleaved by hydroperoxide lyase to form a volatile compound and an acid. Hydroperoxide lyase catalyzes the cleavage of the bond between the hydroperoxide functional group and the adjacent E double-bond. Hydroperoxide lyases can be divided into two groups: heterolytic and homolytic and depending on their cleavage site. Thus, 9-HPL cleaves 9-HPOD, 13-HPL cleaves 13-HPOD but also 9 and 13-HPL cleave 9 and 13-HPOD (Gargouri, Akacha, & Legoy, 2004; Kondo, Hashidoko, & Mizutani, 1995).

In soybean, the 13- pathway is predominant. Products of 9-HPOD are only found in traces. Thus, 13-HPL is the main HPL present in soybean (Matoba et al., 1985). In pea, many HPOD degradation products are found, suggesting the presence of the two isoenzymes (9-HPL and 13-HPL) contrary to other legumes such as beans in which only 13-HPL is found. Moreover, more 13-HPOD products are found, indicating the presence of the strongest 13-HPL activity or the presence of a 9 and 13-HPL with a higher affinity for 13-HPOD (Hornostaj & Robinson, 2000; Kalua et al., 2007). In addition, with the presence of a lot of 10-HPOD degradation products, it is highly possible that pea also contains a 10-HPL.

The HPL activity in pea is optimal around pH six, but the 13- activity is less sensitive to an increase in pH than the 9- activity, which is reduced by half at a pH above seven. These pH characteristics may indicate, as with lipoxygenases, different 13-HPL: one with an optimum pH of six and one with an optimum pH above seven (Hornostaj & Robinson, 2000).

The comparison of the hydroperoxide lyase profiles of soybean and pea clearly shows that the isoenzymes are different. Only one isoenzyme - 13-HPL - is predominant in soybean whereas at least two - 9-HPL and 13-HPL - are found in pea. With this information, it is clear that more products resulting from the degradation of HPOD by HPL are produced in pea than in soybean and thus, a higher number of different volatile compounds are found in pea.

## 367 **5** Conclusion

368 Beyond its agronomic advantages, pea has some advantages because it is less allergenic than soybean. 369 The two legumes have many functional and nutritional similarities. However, pea has some 370 particularities that could lead to more off-flavor and thus, a less appreciated product. For example, pea 371 contains more amino acids involved in off-flavor development and more enzymes involved in lipid 372 oxidation that contribute to the "beany" attribute. As a result of these particularities, pea may contain particular aromatic compounds responsible for the "beany" flavor and also higher quantities of some 373 374 of these compounds. Moreover, some work is still needed to characterize the pea off-flavor and the 375 odorant compounds involved. Work must be conducted to control and to correct the pea off-flavor in 376 order to produce acceptable food products for consumers.

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### 646 **<u>Figure caption</u>**

- 647 **<u>Figure 1:</u>** Sequence alignment for glycinin 1 (GlyG1-SOY) and legumin A (LegA-PEA).
- 648 Representation of similarities and beta sheet and alpha helix positions (UniProt, 2019)Legend: beta
- 649 sheet, alpha helix, similarity

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650 Figure 1

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- Table 1: Comparison of functional properties of soybean and pea protein isolates purified using the same method (Aluko et al., 2009; Barac et al., 2015;
   Fernàndez-Quintela et al., 1997).
- 655 References [1] (Fernàndez-Quintela et al., 1997), [2] (Aluko et al., 2009), [3] (Barac et al., 2015); Units used for the functional properties may be different for
- 656 the three references. Details are given in the table. <sup>1</sup>: Oil droplet size- $d_{3,2}$  (µm).
- *Pea C = Calvedon Variety, Pea P = Provansalac Variety, Soybean B = Bosa Variety, Soybean O = Olga Variety, Soybean DB = Black Domestic Variety;*
- nd = not determined;
- *a,b,c* = letters represent the statistical differences based on the original article; for a given reference, samples with the same letters are not statistically
- *different;*
- *best functional properties, worst functional properties, medium or equal functional properties.*

Reference				[1]		[2]			[3]		
Protein			Pea	Soybean	Pea	Soybean	Pea C	Pea P	Soybean B	Soybean O	Soybean DB
Isolate prepara	ation		isoelectric point wet-milling precipitation of flour					oelectric poin precipitation			
Protein conten	t of solution (%)			1		1			0.1		
Absorption	Water (g/g pro	t)	1.7	1.3	nd	nd	nd	nd	nd	nd	nd
of	Oil (g/g prot)		1.2	1.1	nd	nd	nd	nd	nd	nd	nd
~		рН 3.0	20	20			47.16 a	51.82 b	53.19 bc	54.14 c	55.25 c
Solubility		pH 5.0	9	9	nd	nd	5.69 a	6.98 b	8.45 cd	9.32 d	7.69 bc
[1]: % [3]: %		pH 7.0	54	90	nd	nd	59.77 b	54.03 a	79.35 d	81.27 d	71.59 c
[3]. /0		pH 8.0	75	100	_		87.54 b	79.40 a	85.68 b	88.23 c	85.37 b
	Capacity [1], [3]: (%) [2] : (mL)	рН 3.0	- 15	22	15 b	5 a	333.80 b	316.8 a	900.0 e	650.0 d	400.1 c
		рН 5.0			15 b	14 a	283.3 a	316.0 b	483.4 e	333.0 c	400.0 d
		рН 7.0			21 b	16 a	333.3 b	350 c	333.3 b	350 c	316.6 a
Foaming		pH 8.0			nd	nd	366.7 c	350 b	350 b	316.6 a	366.6 c
properties	Stability (%)	pH 3.0	- 94	93	35 a	50 b	40.0 a	94.74 b	100 b	100 b	100 b
		рН 5.0			18 b	5 a	10.53 b	5.26 a	13.79 b	20 c	58.33 d
		pH 7.0			29 a	35 b	80.0 b	66.67 a	95.0 c	95.24 c	100 c
		pH 8.0			nd	nd	100 b	85.7 a	95.24 b	100 b	81.8 a
		pH 3.0			32 a	55 b	34.02 b	32.20 b	33.75 b	36.40 c	23.85 a
	Capacity	pH 5.0		1	20 a	22 ab	25.25 d	12.90 b	25.94 d	18.13 c	10.76 a
	[2]: $(\mu m)^1$ [3]: $(m^2/g)$	pH 7.0	nd	nd	19 a	40 b	32.52 c	24.98 a	27.98 b	49.01 e	38.62 d
Emulsion	[5]. (1178)	pH 8.0	_	-	nd	nd	43.11 b	46.50 c	28.62 a	51.51 d	57.63 e
properties		pH 3.0			82 a	84 b	36.17 b	64.05 e	19.57 a	53.11 d	46.55 c
	Stability	pH 5.0	. 1		100 a	100 a	91.29 e	40.44 d	27.43 b	15.95 a	29.15 c
	[2]: (%) [3]: (min)	рН 7.0	nd	nd	100 a	100 a	57.62 c	90.24 e	16.16 a	44.58 b	66.32 d
	[J]. (mm)	pH 8.0			nd	nd	29.60 b	92.81 e	20.63 a	64.73 d	36.39 c
Gelation (g/kg	()		180	160	nd	nd	nd	nd	nd	nd	nd

- 663 <u>Table 2:</u> Comparison of structure and primary sequence of soybean and pea 11S and 7S protein
  664 fractions.
- 665 3D models from SWISS-MODEL (Bienert et al., 2017; Guex, Peitsch, & Schwede, 2009; SWISS-
- 666 MODEL, 2019; Waterhouse et al., 2018). Percentage of identity obtained by sequence comparison
- 667 (SC) with the Uniprot Align function (UniProt, 2019).

	Soybean	Pea	% identity
	Glycinin 2	Legumin A2	
115			
SC	Glycinin 1 - 2 - 3	Legumin A - A2	53.2
SC	Glycinin5	LeguminJ	54.8
	β-conglycinin alpha1	Convicilin	
78			
SC	β-conglycinin alpha1 - alpha2	Convicilin	43.7
	β-conglycinin beta1	Vicilin	
78			43.7
SC	β-conglycinin beta1 - beta2	Vicilin	55.5

668 <u>**Table 3:**</u> DIAAS, PDCAAS, and digestibility factors of soybean and pea extracts (Cosucra, 2017; Hayes, 2018; Hughes et al., 2011; Mathai et al., 2017;

- 669Rutherfurd et al., 2015; UNJURY®, 2019)
- 670 Legend: calculation 1: for infant (0 to 6 months), 2: for child (6 months to 3 years), 3: for preschool children (2 to 5 years), 4: for children, adolescents, and
- 671 adults (> 3 years). Nd = not determined.

				Coloulated	DIAAS											
Protein			Reference	Calculated on	1	2	4	1	2	3	4	Digestibilit (%)				
	Isolate		Hayes (2018) & Cosucra (2017)	unknown		nd			0.96			98				
		Nutralys	Hayes (2018)	unknown		nd			0.93			97.3				
Pea	Concentrate		Hughes et al. (2011)	unknown nd						nd						
			Rutherfurd et al. (2015)	male rats	0.822					99.0						
			Mathai et al. (2017)	pigs	0.45	0.62	0.73	0.49	0.71	0.75	0.84	95				
	Flour		UNJURY® (2019)	unknown (2019)		nd			nd							
	Isolate		UNJURY® (2019)	unknown	nd				95							
			Isolate Hughes et al. (2011)		Hughes et al. (2011)	Hughes et al. (2011)	Hughes et al. (2011)	rats	rats nd			0.95 to 0.98				96-44 to 97.88
Soybean			Rutherfurd et al. (2015)	male rats	0.898 to 0.906					98.2 to 98.						
			Mathai et al. (2017)	pigs	0.43	0.84	0.98	0.71	0.86	0.93	1.02	94				
	Conc	entrate	Hughes et al. (2011)	rats		nd			1.00			96.97				
	Flour		Mathai et al. (2017)	pigs	0.73	0.89	1.05	0.72	0.93	0.98	1.09	92				

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**Table 4:** Amino acid composition of soybean and pea, in % of amino acid/total amino acid and in

675 g/100g, (Carrera et al., 2011; Leterme et al., 1990). Essential amino acids are in bold.

Amino		-	-		DI	-	m	<b>T</b> 7 <b>1</b>			. 1		G		CI	D	a	-
acids	Iso	Leu	Lys	Met	Phe	Thr	Trp	Val	Arg	His	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr
						%	of amir	no acid	/total a	imino	acid							
Soybean	4.7	8.3	5.7	1.4	5.4	3.9	1.4	4.6	7.0	2.5	4.3	10.6	1.9	19.4	4.6	5.3	5.9	3.2
Pea	3.7	7.4	7.7	1.2	4.7	4.0	1.1	4.4	7.7	2.8	4.8	12.0	1.7	19.0	4.8	4.2	5.4	3.5
						g	of ami	ino aci	d /100	g prote	ein							
Soybean	2.0	3.5	2.4	0.6	2.3	1.6	0.6	1.9	2.9	1.1	1.8	4.4	0.8	8.1	1.9	2.2	2.5	1.4
Pea	3.3	6.6	6.8	1.0	4.2	3.6	0.9	3.9	6.8	2.5	4.3	10.7	1.6	16.9	4.3	3.4	4.8	3.1

Fatty acids		(g/100g of fatty acids)					
		Soybean	Pea				
Palmitic acid	C16:0	5.9	17.1				
Stearic acid	C18:0	3.4	4.8				
Oleic acid	C18:1	26.9	19.1				
Linoleic acid	C18:2	53.3	31.2				
α-Linolenic acid	C18:3	6.4	7.1				
Other		3.2	20.3				

679 Other includes: C<sub>10:0</sub>, C<sub>12:0</sub>, C<sub>20:0</sub>, C<sub>20:1</sub>, C<sub>22:0</sub>, C<sub>22:1</sub> and unknown acids.

681	Table 6: Proposed overview of the different soybean and pea lipoxygenase isoenzymes (Assaf et al.,
682	1997; Eriksson & Svensson, 1970; Eskin et al., 1977; Fauconnier et al., 1995; Gargouri et al., 2008;
683	Grosch & Laskawy, 1975; Husson et al., 2002; Kalua et al., 2007; Lumen et al., 1978; Matoba et al.,
684	1985; Matsui et al., 2003; Moreno & Peinado, 2012; Pinsky et al., 1971; Rackis et al., 1979; Rhee &
685	Watts, 1966; Sessa, 1979; Veldink et al., 1977; Wurzenberger & Grosch, 1984; Yoon & Klein, 1979)

Lipoxygenase pH of activit		pI	Type HPOD		T°C sensitivity	Ca <sup>2+</sup> impact	
			SOY				
LOX-1	9	5.68	13-LOX	13-HPOD	Heat-stable	No impact	
LOX-2	6-7	6.25	13-LOX or 9/13-LOX	13-HPOD	Heat-labile	Stimulated	
LOX-3	6-7	6.15	9-LOX or 9/13-LOX	9-HPOD	Heat-labile	Inhibited	
LOX-4	6-7	unknown	unknown	unknown	unknown	unknown	
			PEA				
LOX-1	>8	5.82	13-LOX	13-HPOD	Heat-stable	unknown	
LOX-2	6-7	6.0-6.15	13-LOX	13-HPOD	Heat-labile	unknown	
LOX-3	6-7	6.80	9-LOX	9-HPOD	Heat-labile	unknown	
LOX-4	unknown	unknown	unknown	unknown	unknown	unknown	
LOX-5	6-7	unknown	10-LOX	10-HPOD	Heat-labile	unknown	