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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.
- 10 - Pea has more amino acids and more isoenzymes involved in off-flavour development than  
11 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*

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

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23 *Key findings and conclusions*

24 As pea is less allergenic than soybean but has similar nutritional and functional properties, it could be  
25 a great alternative to soybean. Nevertheless, some particularities of the pea composition can lead to a  
26 stronger “beany” off-flavor and so a less acceptable product. In addition, the isoenzymes involved in  
27 the development of off-flavor are more varied in the pea. The pea off-flavor may thus be more  
28 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  
35 investigate meat substitutes. Today, it is known that animal proteins cause various problems. First,  
36 from an agronomic point of view, animal protein production uses large agricultural areas, consumes a  
37 lot of water and energy, produces a high quantity of methane and greenhouse gases, and depletes and  
38 pollutes agricultural areas, water, and air (Bittman, 2007; Harvard Chan, 2012). A diet rich in animal  
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,

47 vegetable proteins (American Dietetic Association, 2009; Datar & Betti, 2010; Elzerman, Hoek, van  
48 Boekel, & Luning, 2011; Hoek et al., 2011; Verbeke, 2015).

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

55 However, associating different sources of protein resolves the issue of limiting amino acids. For  
56 example, associating a protein with a low Lysine content and a protein with a high Lysine content  
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  
60 are not major allergens and have numerous advantages (AFPRAL, 2016; American Dietetic  
61 Association, 2009; V. R. Young & Pellett, 1994). Pulse crops are favorable for agricultural areas as  
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  
65 acids, in particular lysine. Considering these advantages, it is important to develop more legume areas  
66 (Bordat, Lemanceau, Jegou, & Boidevezi, 2018; Terres Inovia, 2017a; Terres Univia, 2017).

67 Today, soybean (*Glycine max*) is the most widely used legume with 336 Mt produced annually  
68 worldwide and 140 000 t produced in France, mainly in the center and south (Terres Inovia, 2017b;  
69 Terres Univia, 2018). However, soybean has some negative aspects. Despite its advantages as a  
70 legume, most soybean production is intensive, which implies adding fertilizer and pesticides and using  
71 large amounts of water. In addition, due to the use of pesticides, 70% of soybean produced are plant-  
72 pesticide GM crops, which can resist the addition of strong pesticides (Terres Inovia, 2017b; Terres  
73 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 *Papilionoideae* 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).

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

## 90 **2 Structure and physicochemical properties of soybean and pea** 91 **proteins**

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

95 A comparison of three articles, each comparing the functional properties of soybean and pea protein  
96 isolates purified using the same method, is presented in **Table 1**. In each reference column, different  
97 functional properties of soybean and pea protein isolates are compared. As these articles did not use  
98 the same determination methods and thus do not have comparable results, a color code is used to  
99 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; Fernández-Quintela, Macarulla, Barrio, & Martínez, 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.

121 The literature partially confirms the similarity in protein structure and, therefore, functionality. Based  
122 on the solubility, and emulsifying and foaming properties of native and heat-treated soybean and pea  
123 isolates, soybean and pea have really close functional properties (Barac et al., 2015). For example, the  
124 solubility of soybean and pea is very similar depending on the pH and the seed variety used.  
125 Furthermore, soybean and pea have similar patterns for the soluble protein composition. For the  
126 emulsifying properties, a strong impact of the genotype is observed. However, as the emulsifying

127 property is strongly related to solubility, if the proteins have the same solubility the emulsifying  
128 property may also be similar. The similarity of the functional properties of the two proteins can also be  
129 seen with studies on products made with soybean and pea protein. For example, with yoghurt-like  
130 products made with soybean or pea protein, a similar texture is observed by sensorial analysis  
131 (Youssef, 2017).

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

#### 133 **3.1 Nutritional value**

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

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

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

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

144 According to these data, soybean has a PDCAAS of 0.71 to 1.09, a DIAAS of 0.43 to 1.05, and a  
145 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  
146 digestibility of 69 to 99.0 %. The values vary depending on the calculation method and the protein  
147 form (Hayes, 2018; Hughes, Ryan, Mukherjea, & Schasteen, 2011; Mathai et al., 2017; Rutherford,  
148 Fanning, Miller, & Moughan, 2015; UNJURY®, 2019). Even in the same protein form (isolate or

149 concentrate), the indices vary over a wide range depending on the calculation and the method used (on  
150 rats or pigs). Soybean and pea nutritional values seem close, even if soybean seems to have higher  
151 values (closer to the reference protein). However, if we consider the average pea PDCAAS (0.78), it  
152 still has a higher value than beans, rice, peanut or wheat that have a PDCAAS of 0.63, 0.53, 0.52, and  
153 0.40, respectively (Hughes et al., 2011). It is also interesting to note that combinations of pea and  
154 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  
162 digestibility factors (Hayes, 2018; Hughes et al., 2011; Mathai et al., 2017; Rutherfurd et al., 2015;  
163 UNJURY®, 2019).

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

### 167 **3.2 Allergenicity**

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

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



175 oleosin; 2S albumin, and P34 protease. Some of these are major allergens:  $\beta$ -conglycinin (Gly m5),  
176 glycinin (Gly m6), and P34 protease (Glym Bd 30 kDa) (Gomez-Andre, Deschildre, Bienvenu, & Just,  
177 2012). Soybean is also one of the main allergens involved in FPIES (Food Protein-Induced  
178 Enterocolitis Syndrome), a non-IgE allergic reaction characterized by vomiting and hemorrhagic  
179 diarrhea (Bidat E., 2017). Nowadays, soybean allergies are more prevalent due to the increasing use of  
180 soybean as an additive in foods.

181 The literature suggests pea is less allergenic than soybean, which may facilitate its food use. First of  
182 all, pea seems to be very rarely involved in FPIES (Bidat E., 2017) and is not a major allergen  
183 (AFPRAL, 2016). Pea contains less allergenic factors but its allergenicity, as in soybean, is caused by  
184 7S globulin (vicilin and convicilin) (Sanchez-Monge et al., 2004), with the major allergen being  
185 convicilin (Dreyer, 2013).

186 According to the EFSA, the immunogenicity of an allergen is partially related to the structural features  
187 of the proteins and their biological activity. Soybean and pea proteins are really close, as discussed  
188 above, so their major allergens are supposedly the same: 7S and 11S globulins (EFSA Panel on  
189 Dietetic Products, Nutrition and Allergies (NDA), 2014). Based on ANSES reports, pea can now be  
190 considered as an emergent allergen (C  rou, 2019). Although pea seems less allergenic than soybean,  
191 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,  $\beta$ -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

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

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

208 Thus, according to current knowledge, pea seems to contain fewer allergens than soybean, with no 2S  
209 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**

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

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

### 223 **4.1 Substrates**

#### 224 **4.1.1 Lipids**

225 Lipids are the main substrates in the production of different aldehydes and ketones that lead to  
226 different alcohols. The different lipids lead to different volatile compounds. The lipid content in

227 Soybean is approximately 20 %, whereas the lipid content in pea is only 2 % (Eriksson, 1967; Murat,  
228 2013; Triboi & Triboi-Blondel, 2002; G. Young & Mebrahtu, 1998). The different fatty acids present  
229 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  
233 & Günter, 1995; Murat, 2013; Murcia & Rincón, 1991). Among the fatty acids, the unsaturated ones  
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).

238 As can be seen in **Table 5**, the fatty acid profiles of soybean and pea are similar. However, the lipid  
239 content in soybean is higher than in pea, which can lead to more lipid degradation. Consequently,  
240 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  
243 ppm). Moreover, the typical “beany” off-flavor is generally expressed when the compound is present  
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).

#### 251 **4.1.2 Amino acids**

252 Amino acids are the second main substrates of volatile compounds. The amino acid profiles of pea and  
253 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  
255 (Marilley & Casey, 2004). Some amino acids are the substrates of some strong volatile flavor  
256 compounds. For example, leucine, isoleucine, and valine are the main precursors of branched  
257 aldehydes such as 2- and 3-methylbutanal (Angerosa, Mostallino, & Basti, 2001; Lee, Lee, Choi,  
258 Hurh, & Kim, 2013; Murray, Shipton, Whitfield, & Last, 1976; Rodríguez-Bernaldo De Quirós,  
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).

264 Unlike lipid degradation, amino acid degradation is slow and takes place throughout the product's life  
265 cycle. As shown in table 4 (g of amino acid/100g of protein), pea contains more amino acids  
266 involved in off-flavor development (leucine, serine, and threonine). For example, even if the ratios  
267 of leucine are similar in pea and soy, pea contains more leucine (6.6 g / 100g instead of 3.5 g  
268 /100g in soybean). As a consequence, pea can lead to the development of more volatile compounds  
269 and thus a stronger off-flavor.

#### 270 **4.2 Enzymes**

271 Enzymes involved in the enzymatic oxidation of lipids are of primary interest because they lead to  
272 different volatile compounds. Two main enzymes are investigated here: lipoxygenase and  
273 hydroperoxide lyase. Lipase breaks down triglycerides into free fatty acids. Then, lipoxygenase  
274 transforms the different free fatty acids into fatty acid hydroperoxide. Hydroperoxide lyase then cuts  
275 the fatty acid hydroperoxides into acids and volatile compounds such as aldehydes (Gargouri et al.,  
276 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, Kazeniak, & 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 Vliegthart, & Boldingh, 1977).

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

289 According to the literature, soybean contains three or four isoenzymes (LOX-1, LOX-2, LOX-3, and  
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  
298 isoenzymes, 13-LOX and 9-LOX, respectively (Sessa, 1979). According to Matoba et al. (1985),  
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

303 with different substrate specificities. According to the enzyme properties such as the impact of  $\text{Ca}^{2+}$  on  
304 enzyme activity (Sessa, 1979), it is more likely that LOX-2 and LOX-3 are different. LOX-4 is  
305 unidentified.

306 According to the literature, pea contains three, four or five isoenzymes (LOX-1, LOX-2, LOX-3,  
307 LOX-4, and LOX-5) (Eskin et al., 1977; Veldink et al., 1977). By combining the various references,  
308 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  
312 working at this pH (9-LOX and 13-LOX). 13-HPOD is also found at a pH above eight, suggesting a  
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  
315 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  
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  
318 Sessa (1979) are inhibited by  $\text{Ca}^{2+}$  but, because five isoenzymes are identified here, it is not possible to  
319 know which isoenzymes are inhibited by  $\text{Ca}^{2+}$ . 10-HPOD is also present in pea (Matsui et al., 2003).  
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.

326 The comparison of the lipoxygenase profiles of soybean and pea indicates that the catalytic activities  
327 of the isoenzymes are similar. However, because of the differences in the amino acid composition of  
328 some isoenzymes, the substrate specificity may not be the same. These conclusions regarding pea  
329 isoenzymes are in accordance with the first conclusions made (Yoon & Klein, 1979): two isoenzymes

330 working at acidic-neutral pH, close to soybean LOX-2 and LOX-3, and another isoenzyme working at  
331 alkaline pH, close to soybean LOX-1.

332 In addition to this comparison, differences between the various soybean and pea isoenzymes may also  
333 be observed regarding the substrate specificity of the isoenzymes. For example, all the isoenzymes do  
334 not exhibit activity on linoleic acid and methyl linoleate (Eskin et al., 1977; Sessa, 1979). Moreover,  
335 because isoenzymes forming mixtures of 9- and 13-HPOD also exist, one or more of the isoenzymes  
336 listed may be of this kind. This 9- and 13-LOX may be one of the isoenzymes identified but with a  
337 higher specificity for 9-HPOD or 13-HPOD or may be the last unidentified isoenzyme (Yoon & Klein,  
338 1979).

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

#### 343 **4.2.2 Hydroperoxide Lyase**

344 Fatty acid hydroperoxides are cleaved by hydroperoxide lyase to form a volatile compound and an  
345 acid. Hydroperoxide lyase catalyzes the cleavage of the bond between the hydroperoxide functional  
346 group and the adjacent E double-bond. Hydroperoxide lyases can be divided into two groups:  
347 heterolytic and homolytic and depending on their cleavage site. Thus, 9-HPL cleaves 9-HPOD, 13-  
348 HPL cleaves 13-HPOD but also 9 and 13-HPL cleave 9 and 13-HPOD (Gargouri, Akacha, & Legoy,  
349 2004; Kondo, Hashidoko, & Mizutani, 1995).

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

356 presence of a lot of 10-HPOD degradation products, it is highly possible that pea also contains a 10-  
357 HPL.

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

362 The comparison of the hydroperoxide lyase profiles of soybean and pea clearly shows that the  
363 isoenzymes are different. Only one isoenzyme - 13-HPL - is predominant in soybean whereas at least  
364 two - 9-HPL and 13-HPL - are found in pea. With this information, it is clear that more products  
365 resulting from the degradation of HPOD by HPL are produced in pea than in soybean and thus, a  
366 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  
373 particular aromatic compounds responsible for the “beany” flavor and also higher quantities of some  
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.

377



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379

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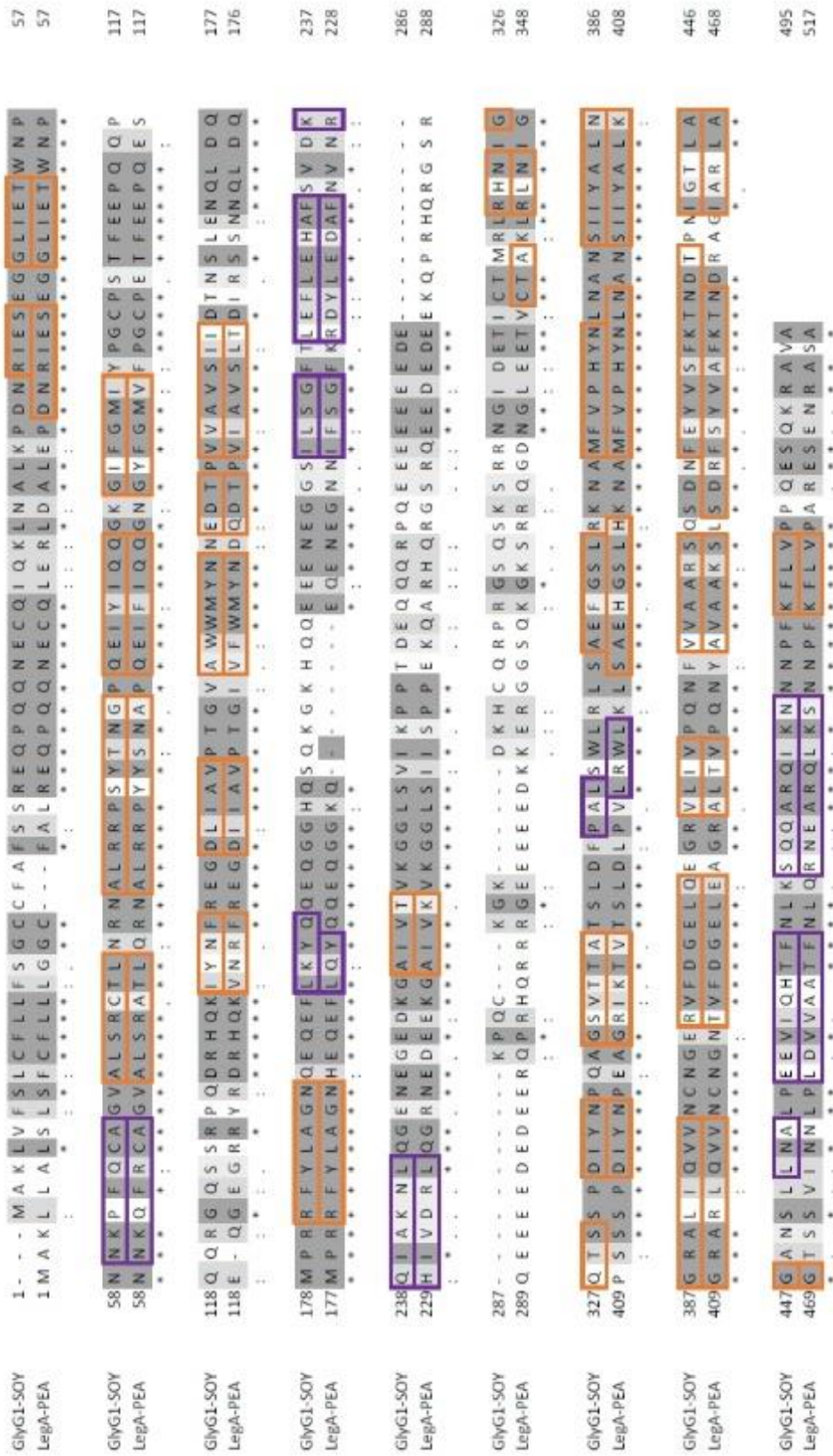
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646 **Figure caption**

647 **Figure 1:** 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*



653 **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;  
654 Fernández-Quintela et al., 1997).

655 *References [1] (Fernández-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- $d_{3,2}$  ( $\mu\text{m}$ ).*

657 *Pea C = Calvedon Variety, Pea P = Provansalac Variety, Soybean B = Bosa Variety, Soybean O = Olga Variety, Soybean DB = Black Domestic Variety;*  
658 *nd = not determined;*

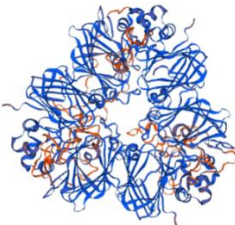
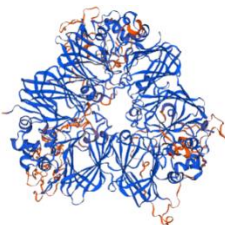
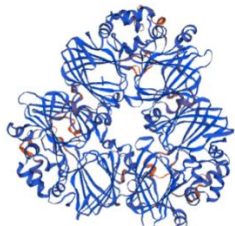
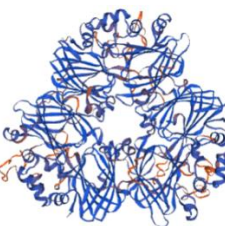
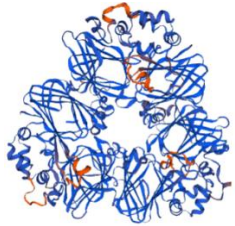
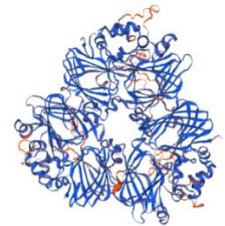
659 *a,b,c = letters represent the statistical differences based on the original article; for a given reference, samples with the same letters are not statistically*  
660 *different;*

661 *best functional properties, worst functional properties, medium or equal functional properties.*

662

Reference		[1]		[2]		[3]					
Protein		Pea	Soybean	Pea	Soybean	Pea C	Pea P	Soybean B	Soybean O	Soybean DB	
Isolate preparation		isoelectric point precipitation		wet-milling of flour		isoelectric point precipitation					
Protein content of solution (%)		1		1		0.1					
Absorption of	Water (g/g prot)	1.7	1.3	nd	nd	nd	nd	nd	nd	nd	
	Oil (g/g prot)	1.2	1.1	nd	nd	nd	nd	nd	nd	nd	
Solubility [1]: % [3]: %	pH 3.0	20	20	nd	nd	47.16 a	51.82 b	53.19 bc	54.14 c	55.25 c	
	pH 5.0	9	9			5.69 a	6.98 b	8.45 cd	9.32 d	7.69 bc	
	pH 7.0	54	90			59.77 b	54.03 a	79.35 d	81.27 d	71.59 c	
	pH 8.0	75	100			87.54 b	79.40 a	85.68 b	88.23 c	85.37 b	
Foaming properties	Capacity [1], [3]: (%) [2] : (mL)	pH 3.0	15	22	15 b	5 a	333.80 b	316.8 a	900.0 e	650.0 d	400.1 c
		pH 5.0			15 b	14 a	283.3 a	316.0 b	483.4 e	333.0 c	400.0 d
		pH 7.0			21 b	16 a	333.3 b	350 c	333.3 b	350 c	316.6 a
		pH 8.0			nd	nd	366.7 c	350 b	350 b	316.6 a	366.6 c
	Stability (%)	pH 3.0	94	93	35 a	50 b	40.0 a	94.74 b	100 b	100 b	100 b
		pH 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
Emulsion properties	Capacity [2]: (µm) <sup>1</sup> [3]: (m <sup>2</sup> /g)	pH 3.0	nd	nd	32 a	55 b	34.02 b	32.20 b	33.75 b	36.40 c	23.85 a
		pH 5.0			20 a	22 ab	25.25 d	12.90 b	25.94 d	18.13 c	10.76 a
		pH 7.0			19 a	40 b	32.52 c	24.98 a	27.98 b	49.01 e	38.62 d
		pH 8.0			nd	nd	43.11 b	46.50 c	28.62 a	51.51 d	57.63 e
	Stability [2]: (%) [3]: (min)	pH 3.0	nd	nd	82 a	84 b	36.17 b	64.05 e	19.57 a	53.11 d	46.55 c
		pH 5.0			100 a	100 a	91.29 e	40.44 d	27.43 b	15.95 a	29.15 c
		pH 7.0			100 a	100 a	57.62 c	90.24 e	16.16 a	44.58 b	66.32 d
		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 **Table 2:** 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
11S	Glycinin 2	Legumin A2	
			
SC	Glycinin 1 - 2 - 3	Legumin A - A2	53.2
	Glycinin5	LeguminJ	54.8
7S	$\beta$ -conglycinin alpha1	Convicilin	
			
SC	$\beta$ -conglycinin alpha1 - alpha2	Convicilin	43.7
7S	$\beta$ -conglycinin beta1	Vicilin	
			43.7
SC	$\beta$ -conglycinin beta1 - beta2	Vicilin	55.5



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

Protein	Reference	Calculated on	DIAAS			PDCAAS				Digestibility (%)		
			1	2	4	1	2	3	4			
Pea	Isolate	Pisane	Hayes (2018) & Cosucra (2017)	unknown	nd			0.96		98		
		Nutralys	Hayes (2018)	unknown	nd			0.93		97.3		
			Hughes et al. (2011)	unknown	nd			0.73		nd		
		Concentrate	Rutherford et al. (2015)	male rats		0.822			0.860		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		nd			nd		69	
Soybean			UNJURY® (2019)	unknown				nd		95		
		Isolate	Hughes et al. (2011)	rats		nd			0.95 to 0.98		96-44 to 97.88	
			Rutherford et al. (2015)	male rats		0.898 to 0.906			0.898 to 0.974		98.2 to 98.5	
			Mathai et al. (2017)	pigs	0.43	0.84	0.98	0.71	0.86	0.93	1.02	94
		Concentrate	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

672

673

674 **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 acids	<b>Iso</b>	<b>Leu</b>	<b>Lys</b>	<b>Met</b>	<b>Phe</b>	<b>Thr</b>	<b>Trp</b>	<b>Val</b>	Arg	His	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr
% of amino acid/total amino 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 amino acid /100g protein																		
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

676

677

678 **Table 5:** Soybean and pea fatty acids (Grela & Günter, 1995)

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
$\alpha$ -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.

680

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 activity	pI	Type	HPOD	T°C sensitivity	Ca <sup>2+</sup> impact
<b>SOY</b>						
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
<b>PEA</b>						
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

686

687