

1 **Lyophilised legume sprouts as a functional ingredient for diamine oxidase enzyme**  
2 **supplementation in histamine intolerance**

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18 **Abstract**

19 Diamine oxidase (DAO) is one of the key enzymes involved in the degradation of dietary  
20 histamine. An imbalance of histamine scavenging systems leads to histamine intolerance, a  
21 diet-related disorder that may be tackled by following a low-histamine diet. Recently, the  
22 supplementation with exogenous DAO enzyme of animal origin has received the green light  
23 as a novel food to enhance intestinal degradation of histamine. This work performed a  
24 screening for histamine-degrading capacity of *Leguminosae* species in order to explore its  
25 potential suitability as plant-derived active ingredient of enzymatic supplements. *In vitro* DAO  
26 activity was determined both in raw pulses and lyophilised sprouts by an enzymatic assay  
27 coupled to UHPLC-FLD and several germination and storage conditions were assessed. The  
28 sprouts of edible legumes showed an *in vitro* histamine-degrading capacity ranging from 36.0  
29 to 408.3 mU g<sup>-1</sup>, much higher than that found for the non-germinated seeds (0.14 - 1.95 mU  
30 g<sup>-1</sup>). The germination of legume seeds for 6 days in darkness provided the maximum DAO  
31 activity. Only the freezing storage of the lyophilized sprouts kept the enzymatic activity intact  
32 for at least 12 months. These results demonstrate that certain edible legumes could be  
33 suitable for the formulation of DAO supplements for the treatment of histamine intolerance.

34

35 **Keywords:** histamine; histamine intolerance; diamine oxidase (DAO) enzyme; legumes; food  
36 supplement.

## 37 **1. Introduction**

38 Histamine intolerance is a diet-related disorder that has been drawing the attention of the  
39 scientific community for the past two decades, although its awareness by both researchers  
40 and consumers has experienced a sharp increase during the last five years. Unlike in the well-  
41 known histamine intoxications, where the causative agent is clearly identified as unusually  
42 high amounts of histamine ingested through food, in histamine intolerance the interindividual  
43 functionality of the intestinal histamine degradation system plays a key role (EFSA, 2011;  
44 Sánchez-Pérez et al., 2018). Specifically, histamine intolerance, also referred-to as food  
45 histaminosis or food histamine sensitivity, is a disorder in the homeostasis of histamine  
46 caused by an imbalance in the degradation of dietary histamine that entails the onset of  
47 allergy-like symptoms, occurring even after the ingestion of small amounts of this amine  
48 (Maintz & Novak, 2007; Comas-Basté, Latorre-Moratalla, Bernacchia, Veciana-Nogués, &  
49 Vidal-Carou, 2017; Tuck & Biesiekierski, 2019).

50 Diamine oxidase (DAO) and histamine-N-methyltransferase (HNMT) are the two enzymes in  
51 charge of the histamine scavenging system in humans (Kaur et al., 2019). Due to its intestinal  
52 location, DAO is the key enzyme in the degradation of ingested histamine and its deficit has  
53 been suggested to be the main cause of histamine intolerance (Kovakova-Hanuszkova, Buday,  
54 Gavliakova, & Plevkova, 2015; Tuck & Biesiekierski, 2019; Kaur et al., 2019). Impaired DAO  
55 activity may be of genetic or acquired origin, with several causes capable to permanently or  
56 punctually compromise either the expression or the activity of DAO (Table 1) (Maintz, 2011;  
57 García-Martín, 2015; Kaur et al., 2019; Wagner, Buczyłko, Zielińska-Bliźniewska, & Wagner,  
58 2019).

59 Histamine intolerance is characterized by a wide variety of nonspecific gastrointestinal and  
60 extraintestinal symptoms owing to the distribution of the four histamine receptors along the  
61 different tissues and systems of the organism (Table 1) (Kovakova-Hanusikova et al., 2015;  
62 Tuck & Biesiekierski, 2019; Schnedl, 2019a). Gastrointestinal complaints have been found to  
63 be the most prevalent symptoms and according to a retrospective analysis performed by  
64 Schnedl et al. (2019a) the appearance of complex symptom combinations with more than  
65 three manifestations was recorded in 97% of the histamine intolerant individuals.  
66 Undoubtedly, the low specificity of histamine intolerance symptoms may account for the  
67 current challenges of its diagnosis (Kovakova-Hanusikova et al., 2015). Nowadays, the  
68 diagnosis is made after excluding IgE-mediated food allergies or underlying systemic  
69 mastocytosis, and by the presentation of two or more typical symptoms and their  
70 improvement after following a low-histamine diet (Kovakova-Hanusikova et al., 2015; Schnedl,  
71 2019a; Schnedl, 2019b). Nevertheless, the determination of serum DAO activity levels and the  
72 identification of SNPs or non-invasive biomarkers are currently being assayed in the search of  
73 evidence-based support to establish a clear diagnostic criterion (Comas-Basté et al., 2017;  
74 Izquierdo-Casas et al., 2018; Tuck & Biesiekierski, 2019).

75 The most advised strategy to prevent the onset of symptoms is following a low-histamine diet  
76 (Maintz & Novak, 2007; San Mauro Martín, Brachero, & Garicano Vilar, 2016; Sánchez-Pérez  
77 et al., 2018; Tuck & Biesiekierski, 2019). Several clinical studies are gathering increasing  
78 evidence on the efficacy of histamine exclusion on the improvement or remissions of  
79 symptoms (Guida et al., 2000; Hoffmann, Gruber, Deutschmann, Janel, & Hauer, 2013;  
80 Siebenhaar et al., 2016; Wagner et al., 2017; Son, Chung, Kim, & Park, 2018). In general, low-  
81 histamine diets exclude those foods susceptible to contain histamine due to bacterial spoilage  
82 (i.e. fish and preserved fishery products) or by fermentative processes (i.e. cheeses, sausages,

83 wine, beer, sauerkraut and fermented soy derivatives) (Comas-Basté, Latorre-Moratalla,  
84 Sánchez-Pérez, Veciana-Nogués, & Vidal-Carou, 2019a; Sánchez-Pérez et al., 2018). However,  
85 the wide and variable occurrence of this amine in foods leads to highly restrictive diets and  
86 makes it difficult to generate well-founded dietary recommendations (Sánchez-Pérez et al.,  
87 2018; Schnedl, 2019b; San Mauro Martin et al., 2016). Orally supplemented DAO enzyme has  
88 been proposed as a complementary treatment strategy that aims to improve histamine  
89 intolerants quality of life by enhancing their intestinal degradation of histamine (Comas-  
90 Basté, Latorre-Moratalla, Sánchez-Pérez, Veciana-Nogués, & Vidal-Carou, 2019b). Recently,  
91 the European Commission has granted the authorization as a novel food to porcine kidney  
92 protein extract as a food supplement in the form of enteric coated capsules (EU 2018/1023).  
93 The few available clinical studies are reporting variable yet promising efficacy rates for DAO  
94 oral supplementation in the remission of gastrointestinal, dermatological or neurological  
95 complaints associated to histamine intolerance (Komericki et al., 2011; Manzotti, Breda,  
96 Bioacchino, & Burastero, 2016; Yacoub et al., 2018; Izquierdo-Casas et al., 2019; Schnedl,  
97 2019b). However, until now little importance has been given to investigate for an alternative  
98 plant-derived source to formulate this enzymatic supplement. Some plant-derived amine  
99 oxidase enzymes have been used to design biosensors for the bio-recognition of biogenic  
100 amines as indicators of freshness in foods (Kivirand & Rinke, 2011). Recently, a published  
101 work from our research group has demonstrated a high *in vitro* histamine-degrading activity  
102 in lyophilised pea sprouts, thus indicating the potential nutraceutical role of legumes for the  
103 supplementation of DAO activity (Comas-Basté et al., 2019b). In this sense, a vegetal source  
104 of DAO enzyme could widen the target population of this enzymatic supplements while  
105 promoting more sustainable production practices.

106 Therefore, the aim of this work was to perform a screening for *in vitro* histamine-degrading  
107 capacity of frequently consumed Leguminosae species in the form of raw pulses and sprouts  
108 in order to evaluate its potential suitability as functional ingredient of enzymatic supplements  
109 for histamine intolerance. In addition, different sprouting and storage conditions were  
110 assayed in order to establish optimal settings to ensure maximum enzymatic activity of this  
111 plant-origin food matrix.

112

## 113 **2. Material and methods**

### 114 2.1. Reagents and chemicals

115 Histamine dihydrochloride and purified DAO from porcine kidney were purchased from  
116 Sigma-Aldrich (St. Louis, MO, USA). UHPLC-grade methanol and acetonitrile, hydrochloric acid  
117 0.1M, perchloric acid 70%, sodium di-hydrogen phosphate anhydrous and di-sodium  
118 hydrogen phosphate anhydrous were obtained from PanReac Química (Castellar del Vallès,  
119 Spain). Acetic acid, boric acid, 1-octanesulfonic acid sodium salt, phthaldialdehyde (OPA) and  
120 brij® L23 solution were acquired from Sigma-Aldrich (St. Louis, MO, USA); sodium acetate  
121 anhydrous, potassium hydroxide and 2-mercaptoethanol from Merck (Darmstadt, Germany).  
122 A LaboStar System from Evoqua Water Technologies (Warrendale, PA, USA) was used to  
123 produce ultrapure water (18.2 MΩcm).

124

### 125 2.2. Legume species

126 The following ten different species of the Leguminosae or Fabaceae plant family were  
127 considered in this study: alfalfa (*Medicago sativa* L.), broad bean (*Vicia faba* L.), chickpea

128 (*Cicer arietinum* L.), common bean (*Phaseolus vulgaris* L.), grass pea (*Lathyrus sativus* L.), lentil  
129 (*Lens culinaris* Medik.), mung bean (*Vigna radiata* (L.) R. Wilczek), green pea (*Pisum sativum*  
130 L.), soybean (*Glycine max* (L.) Merr.) and white lupin (*Lupinus albus* L.). The edible seeds of  
131 these frequently consumed legumes were acquired from local supermarkets and analyzed for  
132 its DAO enzymatic activity both as raw pulses and as lyophilised sprouts.

133

### 134 2.3. Obtention of lyophilised legume sprouts

135 For the obtention of the lyophilised legume sprouts, also known as legume epicotyls or  
136 seedlings, the seeds were soaked in distilled water overnight, strained and placed in an  
137 incubator (Memmert®, Memmert GmbH + Co. KG, Schwabach, Germany) over inert-surface  
138 trays for its germination (27°C, 70% RH) (Figure 1A). Different germination period length (3, 6  
139 and 9 days) and luminosity (absence and presence of light provided by cool white fluorescent  
140 lamps) combinations were performed in order to establish the optimal growing conditions that  
141 provided maximum DAO capacity of the sprouts. Water was sprayed every 12 h for seed  
142 germination. After germination, fresh legume sprouts were harvested, rinsed with distilled  
143 water and stored frozen in an ultra-low temperature freezer (-80°C). Prior to analysis, samples  
144 were freeze-dried (Cryodos-50, Telstar, Terrassa, Spain) and grinded in a mortar to provide a  
145 concentrated and homogenized lyophilised product.

146

### 147 2.4. Determination of DAO activity

148 DAO activity was determined both in lyophilised legume sprouts and in grinded raw pulses  
149 (Figure 1B). The determination of the *in vitro* histamine-degrading activity of the samples was  
150 performed following a procedure previously described by the authors (Comas-Basté et al.,

151 2019b). The detailed analytical protocol, the validation outcomes (i.e. linearity, sensitivity,  
152 precision and recovery) and the method attributes in terms of specificity for the substrate  
153 and the lack of matrix interferences are duly described in Comas-Basté et al. (2019b). Overall,  
154 this method is based on the monitoring of histamine degradation over the oxidative  
155 deamination reaction by DAO enzyme through an enzymatic assay coupled to ion-pair  
156 reverse-phase UHPLC and fluorescence detection. A shaker incubator (Ivymen® 100-D, JP  
157 SELECTA S.A., Abrera, Spain) was used for the enzymatic reaction and the chromatographic  
158 separation was achieved by an Acquity™ UHPLC apparatus (Waters Corp., Milford, MA, USA).  
159 In brief, the enzymatic reaction is promoted throughout the addition of 45 µmol/L histamine  
160 dihydrochloride to the leguminous sample homogenized in a 0.05 mol/L phosphate buffer  
161 solution (pH 7.2). The subsequent chromatographic analysis of aliquots along the enzymatic  
162 reaction allows to monitor histamine reduction and to estimate DAO activity, expressed as  
163 nmol of degraded histamine per minute/g of plant-tissue (mU g<sup>-1</sup>). Figure 2 contains four  
164 overlapping chromatograms where the degradation of histamine over the reaction time by a  
165 sample of lyophilised lentil sprouts may be seen. Purified DAO enzyme from porcine kidney  
166 was used as positive control.

167

168 2.5. Assessment of the stability of the enzymatic capacity of lyophilised legume sprouts during  
169 storage

170 The stability of the DAO activity of the lyophilised product was assessed over 12-month  
171 storage under three different temperatures: freezing (-20°C), refrigeration (4°C) and room  
172 temperature (20°C). Lentil and chickpea lyophilised sprouts were preserved in sealed tubes



173 protected from light and humidity at selected storage conditions and the enzymatic activity  
174 was re-evaluated after 2, 4, 6 and 12 months as previously described in section 2.3.

175

## 176 2.6. Statistical analysis

177 Statistical analysis of data was performed using IBM SPSS Statistics 23.0 statistical software  
178 package (IBM Corporation, Armonk, NY, USA). All results are presented as mean values  $\pm$  their  
179 standard deviation (mean  $\pm$  SD) of at least three independent experiments performed in  
180 duplicate. The Student's t test was applied to investigate the statistical significance of changes  
181 in the enzymatic activity among the different studied conditions. Differences with  $p < 0.05$   
182 were considered statistically significant.

183

## 184 3. Results and discussion

### 185 3.1. DAO activity of pulses and lyophilised legume sprouts

186 Table 2 shows the *in vitro* histamine-degrading capacity of the raw pulses and the lyophilised  
187 sprouts of ten different species of Leguminosae. The raw pulses that showed histamine-  
188 degrading activity were alfalfa, broad bean, common bean, lentil and white lupin. Among  
189 them, the highest enzymatic activity was detected in the dry seeds of lentils ( $1.95 \pm 0.05$  mU  
190  $g^{-1}$ ) ( $p < 0.05$ ). The other DAO-positive raw pulses showed much lower activity values, ranging  
191 from 0.14 to 0.55 mU  $g^{-1}$ . These results demonstrate the histamine-degrading ability of certain  
192 ungerminated legume seeds, in contrast with previously published works that dismissed the  
193 enzymatic activity of raw pulses to degrade amine substrates (Torrrigiani, Serafini-Fracassini,  
194 & Fara, 1989; Joseph & Srivastava 1995). The good detection ability of the currently used

195 method (i.e. detection limit of 0.025 mU) provided enough sensitivity to measure the DAO  
196 activity found in certain ungerminated legumes (Comas-Basté et al., 2019b).

197 Regarding the lyophilised legume sprouts, practically all analysed samples showed *in vitro*  
198 histamine-degrading capacity, although with a great variability among different Leguminosae  
199 species. The sprouts of beans and mung beans did not show this enzymatic activity. In detail,  
200 lyophilized green pea ( $408.3 \pm 16.4$  mU g<sup>-1</sup>) and grass pea sprouts ( $398.1 \pm 26.6$  mU g<sup>-1</sup>)  
201 showed the greatest DAO activity, closely followed by the seedlings of lentils, soybeans and  
202 chickpeas (301.0 – 322.0 mU g<sup>-1</sup>). On the other hand, lyophilized alfalfa, broad bean and white  
203 lupin sprouts showed the lowest enzymatic capacities, with mean activity values from 36.0 to  
204 142.9 mU g<sup>-1</sup>. Table 2 shows the statistical significance of differences in the DAO activity  
205 among species.

206 In view of these results, it has been demonstrated that the germination of the legume seeds  
207 enhances its ability to degrade histamine. Specifically, the DAO activity of the germinated  
208 seeds of alfalfa, broad bean, lentil and white lupin showed a marked increase ( $p < 0.05$ ), with  
209 enzymatic activities from 164 to 285-fold of that in non-germinated seeds. In this sense, Yang  
210 et al. (2011) also reported an important rise in the ability of broad beans to degrade  
211 putrescine, reporting an enzymatic activity increase of about 123-fold after the germination  
212 of this seed. The germination of dry seeds is a physiological process that starts with the  
213 imbibition of the pulses, activating enzymatic reactions and promoting variations in the  
214 chemical composition of the seed (Verni, Coda, & Rizzello, 2019). Therefore, it may be stated  
215 that among other multiple metabolic pathways, the sprouting of the legume seeds activates  
216 its DAO enzymatic activity. The higher occurrence of amine oxidase enzymes seems to be  
217 linked to developmental events and may be explained by the crucial role of the hydrogen

218 peroxide produced during deamination reactions, which is used by peroxidases in cell wall  
219 architecture, lignification, seed reserves mobilization and in response to pathogen attack  
220 (Torrrigiani et al., 1989; Joseph & Srivastava 1995; Laurenzi et al., 2001; Tavladoraki, Cona, &  
221 Angelini, 2016).

222 Overall, legume sprouts may be considered an interesting source of DAO that could be used  
223 as active ingredient in DAO supplements, since its enzymatic activity is comparable or higher  
224 than that reported for porcine kidney protein extract ( $230.8 \pm 11.9$  mU g<sup>-1</sup>), depending on the  
225 legume specie (Comas-Basté et al., 2019b). In fact, higher catalytic turnover rate for plant-  
226 origin amine oxidases was also reported in some works when compared with animal-derived  
227 enzymes (Masini et al., 2007; Comas-Basté et al., 2019b). On the other hand, in the case of  
228 raw pulses, despite the great easiness in its obtention, they showed very low enzymatic  
229 activity for a supplementation purpose.

230

### 231 3.2. Influence of germination conditions on DAO activity

232 The effect of luminosity as presence or absence of light during germination and three  
233 different sprouting period lengths (3, 6 and 9 days) were studied in order to stablish optimal  
234 growing and harvesting conditions to ensure maximum DAO capacity of this plant-origin  
235 matrix. Lentils and chickpeas were selected to test the influence of these two key factors  
236 related to the germination process for their great *in vitro* DAO capacity combined with the  
237 high germination rate of their seeds. In general, it may be stated that a very similar evolution  
238 profile of the DAO activity was observed for both species along the germination period (Figure  
239 2).

240 As it may be seen in figure 2, the highest enzymatic capacity was observed after 6 days of  
241 sprouting for both legumes ( $p < 0.05$ ). Moreover, a significant drop of the histamine-degrading  
242 activity was observed in chickpea sprouts from day 6 to day 9 of germination ( $p < 0.05$ ).  
243 Kivirand and Rincken (2007) studied the evolution of the specific activity of amine oxidase  
244 enzyme purified from *Pisum sativum* L. seedlings towards cadaverine during a 17-days  
245 germination process and reported a steadily increase of the enzymatic activity along the first  
246 eight days and a drop after this period. Likewise, Yang et al. (2011) reported a significant  
247 increase of the capacity to degrade putrescine by *Vicia faba* L. seeds along germination,  
248 reaching its maximum level at day seven.

249 Regarding the effect of luminosity, the highest DAO activity was reached in etiolated legume  
250 sprouts (i.e. grown in darkness) rather than in sprouts grown in the presence of light,  
251 regardless of the time of germination and the legume specie ( $p < 0.05$ ). Previously published  
252 works also confirm that etiolated seedlings possess significantly higher amine-degrading  
253 activity than those growing under the light (Federico, Angelini, Cesta, & Pini, 1985;  
254 Maccarrone, Rossi, Avigliano, & Finazzi Agro, 1991; Joseph & Srivastava 1995; Federico,  
255 Choudhary & Singh, 2000; Laurenzi et al., 2001; Yang et al., 2011). The absence of light may  
256 act as an adverse environmental condition, stimulating the expression of DAO activity, among  
257 other metabolic pathways (Yang et al., 2011). It has been suggested that the different  
258 behavior of DAO depending on the luminosity may be a response mediated by phytochrome,  
259 a vegetal protein that acts as a photoreceptor in charge of promoting several reactions of the  
260 plant in front of environmental stimulus (Joseph & Srivastava 1995; Yang et al., 2011).  
261 Similarly, other authors have reported a major expression of putrescine catabolic enzymes  
262 and a rise of  $\gamma$ -aminobutyric acid (GABA) production, metabolite derived from putrescine, in

263 seeds of legumes exposed to other types of stress, such as salt concentration, hypoxia or heat  
264 (Xing, Jun, Hau, & Liang, 2007; Fait, Fromm, Walter, Galili, & Fernie, 2008).

265 Overall, obtained results indicate that the most suitable combination for both species was  
266 found to be 6 days of germination of the seeds in darkness. Nevertheless, other  
267 environmental stress factors could also have an impact on the specific-histamine degrading  
268 capacity of this food matrices.

269

### 270 3.3. Storage stability of the enzymatic capacity of lyophilized legume sprouts

271 Lyophilization or freeze-drying is a low temperature dehydration process widely used to  
272 preserve pharmaceutical proteins (Czyż, Dembczyński, Marecik, & Pniewski, 2016). However,  
273 long-stability of the dried products is not easily ensured, as it may be affected by a variety of  
274 critical destabilization factors, such as oxidative processes and water activity (Czyż, et al.,  
275 2016). In this work, an assessment of 12-month storage stability of the DAO activity of  
276 lyophilised lentils and chickpea sprouts was performed at three different conditions: freezing,  
277 refrigeration and room temperature. As it may be seen in figure 3, both lyophilised legume  
278 sprouts showed a similar trend on the stability of its enzymatic activity for all studied storage  
279 conditions. Only the freezing storage of the lyophilized sprouts kept the enzymatic activity  
280 intact for at least 12 months. On the contrary, the storage in refrigerator or at room  
281 temperature supposed a marked decrease in the enzymatic capacity early from the first  
282 months. Concretely, one year of refrigerated storage entailed a mean loss of 55% ( $\pm 0.002\%$ )  
283 and 62% ( $\pm 0.001\%$ ) in chickpea and lentils sprouts, respectively. The highest loss of enzymatic  
284 activity was observed in all cases at room temperature, especially in lyophilised lentils  
285 sprouts, where 83% ( $\pm 0.002\%$ ) of reduction was achieved after 12 months.

286 In view of these results, product stability seems to be limited to freeze storage of the  
287 lyophilised legume sprouts. As storage was carried out protected from light, humidity and  
288 oxygen, a possible explanation for the loss of enzymatic activity could be the interaction  
289 among matrix components. Keeping in mind the main goal of obtaining a ready-to formulate  
290 source of DAO enzyme for the formulation of a potential new enzymatic supplement, shelf  
291 life stability should be ensured at least for refrigerated storage. Doubtlessly, future efforts  
292 should be made to select optimal formulation excipients to facilitate successful long-term  
293 stability while maintaining the high enzymatic activity of this matrix. Nevertheless, taking into  
294 account the specific stability of the enzymatic activity observed in the early 2-4 months of  
295 storage under refrigeration or at room temperature, other potential applications could be  
296 hypothesized for this plant-derived product. In fact, the use of lyophilised legume sprouts as  
297 ingredient to formulate functional foods of refrigerated shelf-life could be possible, as well as  
298 its addition in certain food formulations as a strategy to minimize the accumulation of  
299 histamine and other biogenic amines throughout the agri-food chain.

300

#### 301 **4. Conclusions**

302 Certain edible legumes have demonstrated to be a plant-origin source of DAO enzyme, thus  
303 confirming the potential suitability of this food for the formulation of DAO supplements for  
304 the treatment of histamine intolerance. Specifically, the germination of legume seeds for 6  
305 days in darkness has proven to provide the vegetal matrix with maximum histamine-  
306 degrading capacity. Although the lyophilization of the legume seedlings may be considered a  
307 suitable process to obtain a ready-to-formulate plant tissue, the storage stability of its  
308 enzymatic activity remains limited to the freezing storage of the product. Accordingly, further

309 studies are needed to select optimal formulation of the final product to guarantee the shelf-  
310 life stability of its DAO activity. Finally, it is of high importance to motivate the development  
311 of more clinical studies involving DAO enzymatic supplement to generate data on its potential  
312 clinical efficacy.

313

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317

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319

## 320 **References**

321 Choudhary, A., & Singh, R. P. (2000). Cadmium-induced Changes in Diamine Oxidase Activity  
322 and Polyamine Levels in *Vigna radiata* Wilczek Seedlings. *Journal of Plant Physiology*, *156*,  
323 704-710. [https://doi.org/10.1016/S0176-1617\(00\)80235-7](https://doi.org/10.1016/S0176-1617(00)80235-7).

324 Comas-Basté, O., Latorre-Moratalla, M. L., Bernacchia, R.; Veciana-Nogués, M. T., & Vidal-  
325 Carou, M. C. (2017). New approach for the diagnosis of histamine intolerance based on the  
326 determination of histamine and methylhistamine in urine. *Journal of Pharmaceutical and*  
327 *Biomedical Analysis*, *145*, 379-385. <https://doi.org/10.1016/j.jpba.2017.06.029>.

328 Comas-Basté, O., Latorre-Moratalla, M. L., Sánchez-Pérez, S., Veciana-Nogués, M. T., & Vidal-  
329 Carou, M. C. (2019a). Histamine and Other Biogenic Amines in Food. From Scombroid

330 Poisoning to Histamine Intolerance. In C. Proestos (Ed.), *Biogenic Amines*. London:  
331 IntechOpen. <https://doi.org/10.5772/intechopen.84333>.

332 Comas-Basté, O., Latorre-Moratalla, M. L., Sánchez-Pérez, S., Veciana-Nogués, M. T., & Vidal-  
333 Carou, M. C. (2019b). *In vitro* determination of diamine oxidase activity in food matrices by  
334 an enzymatic assay coupled to UHPLC-FL. *Analytical and Bioanalytical Chemistry*, *411*, 7595-  
335 7602. <https://doi.org/10.1007/s00216-019-02178-2>.

336 Commission Implementing Regulation (EU) 2018/1023 of 23 July 2018 correcting  
337 Implementing Regulation (EU) 2017/2470 establishing the Union list of novel foods. *Official*  
338 *Journal of the European Union*, *L 187*, 1-133.

339 Czyż, M., Dembczyński, R., Marecik, R., & Pniewski, T. (2016). Stability of S-HBsAg in long-term  
340 stored lyophilised plant tissue. *Biologicals*, *44*, 69-72.  
341 <https://doi.org/doi:10.1016/j.biologicals.2015.12.001>.

342 EFSA Panel on Biological Hazards (BIOHAZ) (2011). Scientific Opinion on risk based control of  
343 biogenic amine formation in fermented foods. *EFSA Journal*, *9*, 2393.  
344 <https://doi.org/10.2903/j.efsa.2011.2393>.

345 Fait, A., Fromm, H., Walter, D., Galili, G., & Fernie, A. R. (2008). Highway or byway: The  
346 metabolic role of the GABA shunt in plants. *Trends in Plant Science*, *13*, 14-19.  
347 <https://doi.org/10.1016/j.tplants.2007.10.005>.

348 Federico, R., Angelini, R., Cesta, A., & Pini, C. (1985). Determination of Diamine Oxidase in  
349 Lentil Seedlings by Enzymic Activity and Immunoreactivity. *Plant Physiology*, *79*, 62-64.  
350 <https://doi.org/10.1104/pp.79.1.62>.



351 García-Martín, E., Martínez, C., Serrador, M., Alonso-Navarro, H., Ayuso, P., Navacerrada, F.,  
352 Agúndez, J. A., & Jiménez-Jiménez, F. J. (2015). Diamine oxidase rs10156191 and rs2052129  
353 variants are associated with the risk for migraine. *Headache*, *55*, 276-286.  
354 <https://doi.org/10.1111/head.12493>.

355 Guida, B., De Martino, C., De Martino, S., Tritto, G., Patella, V., Trio, R., D'Agostino, C.,  
356 Pecoraro, P., & D'Agostino, L. (2000). Histamine plasma levels and elimination diet in chronic  
357 idiopathic urticaria. *European Journal of Clinical Nutrition*, *54*, 155-158.  
358 <https://doi.org/10.1038/sj.ejcn.1600911>.

359 Hoffmann, M., Gruber, E., Deutschmann, A., Jahnel, J., & Hauer, A. (2013). Histamine  
360 intolerance in children with chronic abdominal pain. *Archives of Disease in Childhood*, *98*, 832-  
361 833. <http://doi.org/10.1136/archdischild-2013-305024>.

362 Izquierdo-Casas, J., Comas-Basté, O., Latorre-Moratalla, M. L., Lorente-Gascón, M., Duelo, A.,  
363 Soler-Singla, L., & Vidal-Carou, M. C. (2019). Diamine oxidase (DAO) supplement reduces  
364 headache in episodic migraine patients with DAO deficiency: A randomized double-blind trial.  
365 *Clinical Nutrition*, *38*, 152-158. <https://doi.org/10.1016/j.clnu.2018.01.013>.

366 Izquierdo-Casas, J., Comas-Basté, O., Latorre-Moratalla, M. L., Lorente-Gascón, M., Duelo, A.,  
367 Vidal-Carou, M. C., Soler-Singla, L. (2018). Low serum diamine oxidase (DAO) activity levels in  
368 patients with migraine. *Journal of Physiology and Biochemistry*, *74*, 93-99.  
369 <https://doi.org/10.1007/s13105-017-0571-3>.

370 Joseph, P., & Srivastava, S. K. (1995). Photoregulation of Diamine Oxidase from Pea Seedlings.  
371 *Journal of Plant Physiology*, *146*, 108-114. [https://doi.org/10.1016/S0176-1617\(11\)81975-9](https://doi.org/10.1016/S0176-1617(11)81975-9).

372 Kaur, S., Ali, A., Siahbalaeei, Y., Ahmad, U., Nargis, F., Pandey, A. K., & Singh, B. (In Press).  
373 Association of Diamine oxidase (DAO) variants with the risk for migraine from North Indian  
374 population. *Meta Gene*. <https://doi.org/10.1016/j.mgene.2019.100619>.

375 Kivirand, K., & Rincken, T. (2007). Purification and properties of amine oxidase from pea  
376 seedlings. *Proceedings of the Estonian Academy of Sciences. Chemistry*, *56*, 164-171.

377 Kivirand, K., & Rincken, T. (2011). Biosensors for Biogenic Amines: The Present State of Art  
378 Mini-Review. *Analytical Letters*, *44*, 2821-2833.  
379 <https://doi.org/10.1080/00032719.2011.565445>.

380 Komericki, P., Klein, G., Reider, N., Hawranek, T., Strimitzer, T., Lang, R., Kranzelbinder, B., &  
381 Aberer, W. (2011). Histamine intolerance: lack of reproducibility of single symptoms by oral  
382 provocation with histamine: a randomised, double-blind, placebo-controlled cross-over  
383 study. *Wiener klinische Wochenschrift*, *123*, 15-20. [https://doi.org/10.1007/s00508-010-](https://doi.org/10.1007/s00508-010-1506-y)  
384 [1506-y](https://doi.org/10.1007/s00508-010-1506-y).

385 Kovacova-Hanusikova, E., Buday, T., Gavliakova, S., & Plevkova, J. (2015). Histamine, histamine  
386 intoxication and intolerance. *Allergologia et Immunopathologia*, *43*, 498-506.  
387 <https://doi.org/10.1016/j.aller.2015.05.001>.

388 Laurenzi, M., Tipping, A. J., Marcus, S. E., Knox, J. P., Federico, R., Angelini, R., & McPherson,  
389 M. J. (2001). Analysis of the distribution of copper amine oxidase in cell walls of legume  
390 seedlings. *Planta*, *214*, 37-45. <https://doi.org/10.1007/s004250100600>.

391 Maccarrone, M., Rossi, A., Avigliano, L., & Finazzi Agro, A. (1991). Activity and expression of  
392 diamine oxidase in lentil seedling under different growth conditions. *Plant Science*, *79*, 51-55.  
393 [https://doi.org/10.1016/0168-9452\(91\)90068-J](https://doi.org/10.1016/0168-9452(91)90068-J).

394 Maintz, L., & Novak, N. (2007). Histamine and histamine intolerance. *The American Journal of*  
395 *Clinical Nutrition*, *85*, 1185-1196. <https://doi.org/10.1093/ajcn/85.5.1185>.

396 Maintz, L., Yu, C. F., Rodríguez, E., Baurecht, H., Bieber, T., Illig, T., Weidinger, S., & Novak, N.  
397 (2011). Association of single nucleotide polymorphisms in the diamine oxidase gene with  
398 diamine oxidase serum activities. *Allergy*, *66*, 893-902. [https://doi.org/10.1111/j.1398-](https://doi.org/10.1111/j.1398-9995.2011.02548.x)  
399 [9995.2011.02548.x](https://doi.org/10.1111/j.1398-9995.2011.02548.x).

400 Manzotti, G., Breda, D., Di Gioacchino, M., & Burastero, S. E. (2016). Serum diamine oxidase  
401 activity in patients with histamine intolerance. *International Journal of Immunopathology and*  
402 *Pharmacology*, *29*, 105-111. <http://doi.org/10.1177/0394632015617170>.

403 Masini, E., Bani, D., Marzocca, C., Mateescu, M. A., Mannaioni, P. F., Federico, R., & Mondovì,  
404 B. (2007). Pea seedling histaminase as a novel therapeutic approach to anaphylactic and  
405 inflammatory disorders. A plant histaminase in allergic asthma and ischemic shock. *The*  
406 *Scientific World Journal*, *7*, 888-902. <https://doi.org/10.1100/tsw.2007.139>.

407 San Mauro Martin, I., Brachero, S., & Garicano Vilar, E. (2016). Histamine intolerance and  
408 dietary management: A complete review. *Allergologia et Immunopathologia*, *44*, 475-483.  
409 <https://doi.org/10.1016/j.aller.2016.04.015>.

410 Sánchez-Pérez, J., Comas-Basté, O., Rabell-González, J., Veciana-Nogués, M. T., Latorre-  
411 Moratalla, M. L., & Vidal-Carou, M. C. (2018). Biogenic Amines in Plant-Origin Foods: Are They  
412 Frequently Underestimated in Low-Histamine Diets? *Foods*, *7*, 205.  
413 <https://doi.org/10.3390/foods7120205>.

414 Schnedl, W. J., Lackner, S., Enko, D., Schenk, M., Holasek, S. J., & Mangge, H. (2019a).  
415 Evaluation of symptoms and symptom combinations in histamine intolerance. *Intestinal*  
416 *Research*, *17*, 427-433. <https://doi.org/10.5217/ir.2018.00152>.

417 Schnedl, W. J., Schenk, M., Lackner, S., Enko, D., Mangge, H., & Forster, F. (2019b). Diamine  
418 oxidase supplementation improves symptoms in patients with histamine intolerance. *Food*  
419 *Science and Biotechnology*, 28, 1779-1784. <https://doi.org/10.1007/s10068-019-00627-3>.

420 Siebenhaar, F., Melde, A., Magerl, T., Zuberier, T. M., Church, K., & Maurer, M. (2016).  
421 Histamine intolerance in patients with chronic spontaneous urticaria. *Journal of the European*  
422 *Academy of Dermatology and Venerology*, 30, 1774-1777. <http://doi.org/10.1111/jdv.13778>.

423 Son, J. H., Chung, B. Y., Kim, H. O., & Park, C. W. (2018). A histamine-free diet is helpful for  
424 treatment of adult patients with chronic spontaneous urticaria. *Annals of Dermatology*, 30,  
425 164-172. <http://doi.org/10.5021/ad.2018.30.2.164>.

426 Tavladoraki, P., Cona, A., & Angelini, R. (2016). Copper-Containing Amine Oxidases and FAD-  
427 Dependent Polyamine Oxidases Are Key Players in Plant Tissue Differentiation and Organ  
428 Development. *Frontiers in Plant Science*, 7, 824. <https://doi.org/10.3389/fpls.2016.00824>.

429 Torrigiani, P., Serafini-Fracassini, D., & Fara, A. (1989). Diamine Oxidase Activity in Different  
430 Physiological Stages of *Helianthus tuberosus* Tuber. *Plant Physiology*, 89, 69-73.  
431 <https://doi.org/10.1104/pp.89.1.69>.

432 Tuck, C. J., Biesiekierski, J. R., Schmid-Grendelmeier, P., & Pohl, D. (2019). Food Intolerances.  
433 *Nutrients*, 11, 1684. <https://doi.org/10.3390/nu11071684>.

434 Verni, M., Coda, R., & Rizzello, C. G. (2019). The Use of Faba Bean Flour to Improve the  
435 Nutritional and Functional Features of Cereal-Based Foods: Perspectives and Future  
436 Strategies. In V. R. Preedy, & R. R. Watson (Eds.), *Flour and breads and their fortification in*  
437 *health and disease prevention*, (pp. 465-475) 2nd ed., Cambridge: Academic Press.  
438 <https://doi.org/10.1016/B978-0-12-814639-2.00037-X>.

439 Wagner, A., Buczyłko, K., Zielińska-Bliźniewska, H., & Wagner, W. (2019). Impaired resolution  
440 of wheals in the skin prick test and low diamine oxidase blood level in allergic patients.  
441 *Advances in Dermatology and Allergology*, 36, 538-543.  
442 <https://doi.org/10.5114/ada.2019.89504>.

443 Wagner, N., Dirk, D., Peveling-Oberhag, A., Reese, I., Rady-Pizarro, U., Mitzel, H., & Staubach,  
444 P. (2017). Popular myth—Low-histamine diet improves chronic spontaneous urticaria—Fact  
445 or fiction? *Journal of the European Academy of Dermatology and Venerology*, 31, 650-655.  
446 <http://doi.org/10.1111/jdv.13966>.

447 Xing, S. G., Jun, Y. B., Hau, Z. W., & Liang, L. Y. (2007). Higher accumulation of gamma-  
448 aminobutyric acid induced by salt stress through stimulating the activity of diamine oxidases  
449 in *Glycine max* (L.) Merr. roots. *Plant Physiology and Biochemistry*, 45, 560-566.  
450 <https://doi.org/10.1016/j.plaphy.2007.05.007>.

451 Yacoub, M. R., Ramirez, G. A., Berti, A., Mercurio, G., Breda, D., Saporiti, N., Burastero, S.,  
452 Dagna, L., & Colombo, G. (2018). Diamine Oxidase Supplementation in Chronic Spontaneous  
453 Urticaria: A Randomized, Double-Blind Placebo-Controlled Study. *International Archives of*  
454 *Allergy and Immunology*, 176, 268-271. <https://doi.org/10.1159/000488142>.

455 Yang, R., Chen, H., & Gu, Z. (2011). Factors Influencing Diamine Oxidase Activity and  $\gamma$ -  
456 Aminobutyric Acid Content of Fava Bean (*Vicia faba* L.) during Germination. *Journal of*  
457 *Agricultural and Food Chemistry*, 59, 11616-11620. <https://doi.org/10.1021/jf202645p>.

458 **Figure captions**

459 **Figure 1.** Overall experimental procedure for the *in vitro* determination of DAO activity of  
460 lyophilised legume sprouts (a) and raw pulses (b). \*Germination conditions of 3/6/9 days and  
461 presence/absence of light were tested.

462 **Figure 2.** Overlapping chromatograms of histamine at the starting point (black) and after 1 h  
463 (blue), 2 h (red) and 4 h (green) of reaction for a sample of lyophilised lentil sprouts.

464 **Figure 3.** DAO activity of 3, 6 and 9 days-old lentil (A) and chickpea (B) sprouts grown in  
465 darkness (black) or light-exposed (white). Different letters and numbers denote statically  
466 significant differences ( $p < 0.05$ ) between luminosity and growing days, respectively.

467 **Figure 4.** Evolution of the *in vitro* DAO activity of lyophilised lentil (A) and chickpea (B) sprouts  
468 over 12 months of storage.











Table 1. Etiology and main symptoms of histamine intolerance.

Etiology	
Congenital	Genetic mutations in DAO gene (chromosome 7q34-q36) resulting in an altered expression or activity of the enzyme
Pathological	Inflammatory or degenerative intestinal disorders that compromise the secretion of DAO
Drug-induced	Enzymatic blockade by certain drugs (e.g. acetylcysteine, cimetidine, clavulanic acid and verapamil)
Symptoms	
Gastrointestinal tract	Bloating, flatulence, postprandial fullness, diarrhea, abdominal pain, constipation, nausea, emesis
Skin	Pruritus, flush, urticaria, eczema, swelling
Circulatory system	Tachycardia, hypotonia, collapse
Nervous system	Headache, dizziness
Respiratory apparatus	Rhinorrhea, rhinitis, nasal congestion, sneezing, dyspnea

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- 1 **Table 2.** DAO activity (mean  $\pm$  SD) of raw pulses and lyophilised sprouts of different species  
 2 of *Leguminosae* germinated during 6 days in darkness\*.

Legume		DAO activity (mU g <sup>-1</sup> )	
Scientific name	Common name	Raw pulse	Lyophilised sprout
<i>Medicago sativa</i> L.	Alfalfa	0.50 $\pm$ 0.03 <sup>a</sup>	142.9 $\pm$ 1.5 <sup>a</sup>
<i>Vicia faba</i> L.	Broad bean	0.36 $\pm$ 0.04 <sup>a</sup>	90.1 $\pm$ 6.9 <sup>a,b</sup>
<i>Cicer arietinum</i> L.	Chickpea	-	301.0 $\pm$ 9.3 <sup>c</sup>
<i>Phaseolus vulgaris</i> L.	Common bean	0.55 $\pm$ 0.03 <sup>a</sup>	-
<i>Lathyrus sativus</i> L.	Grass pea	-	398.1 $\pm$ 26.6 <sup>d</sup>
<i>Pisum sativum</i> L.	Green pea	-	408.3 $\pm$ 16.4 <sup>d</sup>
<i>Lens culinaris</i> Medik.	Lentil	1.95 $\pm$ 0.05 <sup>b</sup>	322.0 $\pm$ 18.1 <sup>c</sup>
<i>Vigna radiata</i> (L.) R. Wilczek	Mung bean	-	-
<i>Glycine max</i> (L.) Merr.	Soybean	-	305.9 $\pm$ 8.8 <sup>c</sup>
<i>Lupinus albus</i> L.	White lupin	0.14 $\pm$ 0.08 <sup>a</sup>	36.0 $\pm$ 6.9 <sup>b</sup>

- 3 \* Mean values in the same column followed by different letters are significantly different  
 4 (p<0.05).