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

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Abstract

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

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

1. Introduction

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

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

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

The most advised strategy to prevent the onset of symptoms is following a low-histamine diet (Maintz & Novak, 2007; San Mauro Martín, Brachero, & Garicano Vilar, 2016; Sánchez-Pérez et al., 2018; Tuck & Biesiekierski, 2019). Several clinical studies are gathering increasing evidence on the efficacy of histamine exclusion on the improvement or remissions of symptoms (Guida et al., 2000; Hoffmann, Gruber, Deutschmann, Jahnel, & Hauer, 2013; Siebenhaar et al., 2016; Wagner et al., 2017; Son, Chung, Kim, & Park, 2018). In general, low-histamine diets exclude those foods susceptible to contain histamine due to bacterial spoilage (i.e. fish and preserved fishery products) or by fermentative processes (i.e. cheeses, sausages, wine, beer, sauerkraut and fermented soy derivatives) (Comas-Basté, Latorre-Moratalla, Sánchez-Pérez, Veciana-Nogués, & Vidal-Carou, 2019a; Sánchez-Pérez et al., 2018). However, the wide and variable occurrence of this amine in foods leads to highly restrictive diets and makes it difficult to generate well-founded dietary recommendations (Sánchez-Pérez et al., 2018; Schnedl, 2019b; San Mauro Martin et al., 2016). Orally supplemented DAO enzyme has been proposed as a complementary treatment strategy that aims to improve histamine intolerants quality of life by enhancing their intestinal degradation of histamine (Comas-Basté, Latorre-Moratalla, Sánchez-Pérez, Veciana-Nogués, & Vidal-Carou, 2019b).Recently, the European Commission has granted the authorization as a novel food to porcine kidney protein extract as a food supplement in the form of enteric coated capsules (EU 2018/1023). The few available clinical studies are reporting variable yet promising efficacy rates for DAO oral supplementation in the remission of gastrointestinal, dermatological or neurological complaints associated to histamine intolerance (Komericki et al., 2011; Manzotti, Breda, Bioacchino, & Burastero, 2016; Yacoub et al., 2018; Izquierdo-Casas et al., 2019; Schnedl, 2019b). However, until now little importance has been given to investigate for an alternative plant-derived source to formulate this enzymatic supplement. Some plant-derived amine oxidase enzymes have been used to design biosensors for the bio-recognition of biogenic amines as indicators of freshness in foods (Kivirand & Rinken, 2011). Recently, a published work from our research group has demonstrated a high *in vitro* histamine-degrading activity in lyophilised pea sprouts, thus indicating the potential nutraceutical role of legumes for the supplementation of DAO activity (Comas-Basté et al., 2019b). In this sense, a vegetal source of DAO enzyme could widen the target population of this enzymatic supplements while promoting more sustainable production practices.

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

2. Material and methods

2.1. Reagents and chemicals

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

2.2. Legume species

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

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

2.3. Obtention of lyophilised legume sprouts

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

2.4. Determination of DAO activity

DAO activity was determined both in lyophilised legume sprouts and in grinded raw pulses (Figure 1B). The determination of the *in vitro* histamine-degrading activity of the samples was performed following a procedure previously described by the authors (Comas-Basté et al., 2019b). The detailed analytical protocol, the validation outcomes (i.e. linearity, sensitivity, precision and recovery) and the method attributes in terms of specificity for the substrate and the lack of matrix interferences are duly described in Comas-Basté et al. (2019b). Overall, this method is based on the monitoring of histamine degradation over the oxidative deamination reaction by DAO enzyme through an enzymatic assay coupled to ion-pair reverse-phase UHPLC and fluorescence detection. A shaker incubator (Ivymen® 100-D, JP SELECTA S.A., Abrera, Spain) was used for the enzymatic reaction and the chromatographic separation was achieved by an Acquity™ UHPLC apparatus (Waters Corp., Milford, MA, USA). In brief, the enzymatic reaction is promoted throughout the addition of 45 µmol/L histamine dihydrochloride to the leguminous sample homogenized in a 0.05 mol/L phosphate buffer solution (pH 7.2). The subsequent chromatographic analysis of aliquots along the enzymatic reaction allows to monitor histamine reduction and to estimate DAO activity, expressed as nmol of degraded histamine per minute/g of plant-tissue (mU g^{-1}). Figure 2 contains four overlapping chromatograms where the degradation of histamine over the reaction time by a sample of lyophilised lentil sprouts may be seen. Purified DAO enzyme from porcine kidney was used as positive control.

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

The stability of the DAO activity of the lyophilised product was assessed over 12-month storage under three different temperatures: freezing (-20°C), refrigeration (4°C) and room temperature (20°C). Lentil and chickpea lyophilised sprouts were preserved in sealed tubes protected from light and humidity at selected storage conditions and the enzymatic activity was re-evaluated after 2, 4, 6 and 12 months as previously described in section 2.3.

2.6. Statistical analysis

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

3. Results and discussion

3.1. DAO activity of pulses and lyophilised legume sprouts

Table 2 shows the *in vitro* histamine-degrading capacity of the raw pulses and the lyophilised sprouts of ten different species of Leguminosae. The raw pulses that showed histamine-degrading activity were alfalfa, broad bean, common bean, lentil and white lupin. Among them, the highest enzymatic activity was detected in the dry seeds of lentils (1.95 ± 0.05 mU g^{-1}) ($p<0.05$). The other DAO-positive raw pulses showed much lower activity values, ranging from 0.14 to 0.55 mU g^{-1}. These results demonstrate the histamine-degrading ability of certain ungerminated legume seeds, in contrast with previously published works that dismissed the enzymatic activity of raw pulses to degrade amine substrates (Torrrigiani, Serafini-Fracassini, & Fara, 1989; Joseph & Srivastava 1995). The good detection ability of the currently used method (i.e. detection limit of 0.025 mU) provided enough sensitivity to measure the DAO activity found in certain ungerminated legumes (Comas-Basté et al., 2019b).

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

In view of these results, it has been demonstrated that the germination of the legume seeds enhances its ability to degrade histamine. Specifically, the DAO activity of the germinated seeds of alfalfa, broad bean, lentil and white lupin showed a marked increase ($p<0.05$), with enzymatic activities from 164 to 285-fold of that in non-germinated seeds. In this sense, Yang et al. (2011) also reported an important rise in the ability of broad beans to degrade putrescine, reporting an enzymatic activity increase of about 123-fold after the germination of this seed. The germination of dry seeds is a physiological process that starts with the imbibition of the pulses, activating enzymatic reactions and promoting variations in the chemical composition of the seed (Verni, Coda, & Rizzello, 2019). Therefore, it may be stated that among other multiple metabolic pathways, the sprouting of the legume seeds activates its DAO enzymatic activity. The higher occurrence of amine oxidase enzymes seems to be linked to developmental events and may be explained by the crucial role of the hydrogen peroxide produced during deamination reactions, which is used by peroxidases in cell wall architecture, lignification, seed reserves mobilization and in response to pathogen attack (Torrrigiani et al., 1989; Joseph & Srivastava 1995; Laurenzi et al., 2001; Tavladoraki, Cona, & Angelini, 2016).

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

3.2. Influence of germination conditions on DAO activity

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

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

Regarding the effect of luminosity, the highest DAO activity was reached in etiolated legume sprouts (i.e. grown in darkness) rather than in sprouts grown in the presence of light, regardless of the time of germination and the legume specie ($p<0.05$). Previously published works also confirm that etiolated seedlings possess significantly higher amine-degrading activity than those growing under the light (Federico, Angelini, Cesta, & Pini, 1985; Maccarrone, Rossi, Avigliano, & Finazzi Agro, 1991; Joseph & Srivastava 1995; Federico, Choudhary & Singh, 2000; Laurenzi et al., 2001; Yang et al., 2011). The absence of light may act as an adverse environmental condition, stimulating the expression of DAO activity, among other metabolic pathways (Yang et al., 2011). It has been suggested that the different behavior of DAO depending on the luminosity may be a response mediated by phytochrome, a vegetal protein that acts as a photoreceptor in charge of promoting several reactions of the plant in front of environmental stimulus (Joseph & Srivastava 1995; Yang et al., 2011). Similarly, other authors have reported a major expression of putrescine catabolic enzymes and a rise of γ-aminobutyric acid (GABA) production, metabolite derived from putrescine, in seeds of legumes exposed to other types of stress, such as salt concentration, hypoxia or heat (Xing, Jun, Hau, & Liang, 2007; Fait, Fromm, Walter, Galili, & Fernie, 2008).

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

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

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

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

4. Conclusions

Certain edible legumes have demonstrated to be a plant-origin source of DAO enzyme, thus confirming the potential suitability of this food for the formulation of DAO supplements for the treatment of histamine intolerance. Specifically, the germination of legume seeds for 6 days in darkness has proven to provide the vegetal matrix with maximum histamine-degrading capacity. Although the lyophilization of the legume seedlings may be considered a suitable process to obtain a ready-to-formulate plant tissue, the storage stability of its enzymatic activity remains limited to the freezing storage of the product. Accordingly, further studies are needed to select optimal formulation of the final product to guarantee the shelf-life stability of its DAO activity. Finally, it is of high importance to motivate the development of more clinical studies involving DAO enzymatic supplement to generate data on its potential clinical efficacy.

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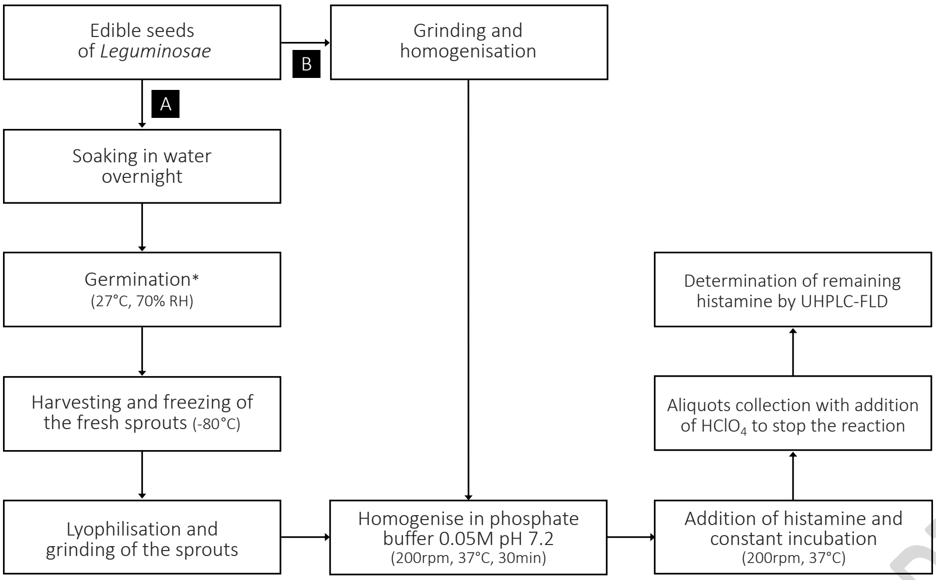
Figure captions

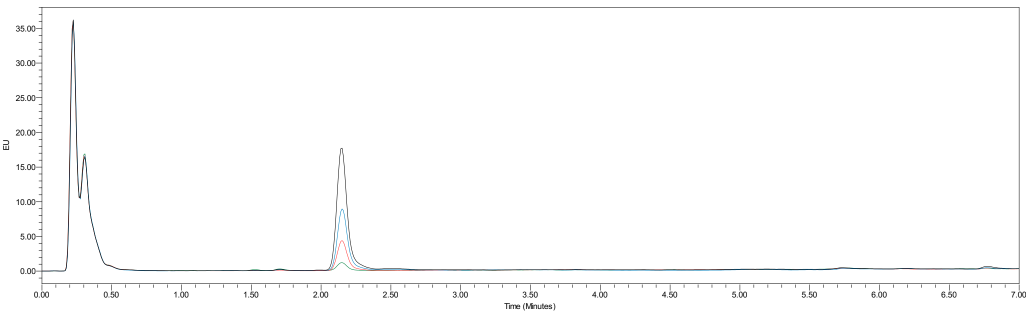
Figure 1. Overall experimental procedure for the *in vitro* determination of DAO activity of lyophilised legume sprouts (a) and raw pulses (b). *Germination conditions of 3/6/9 days and presence/absence of light were tested.

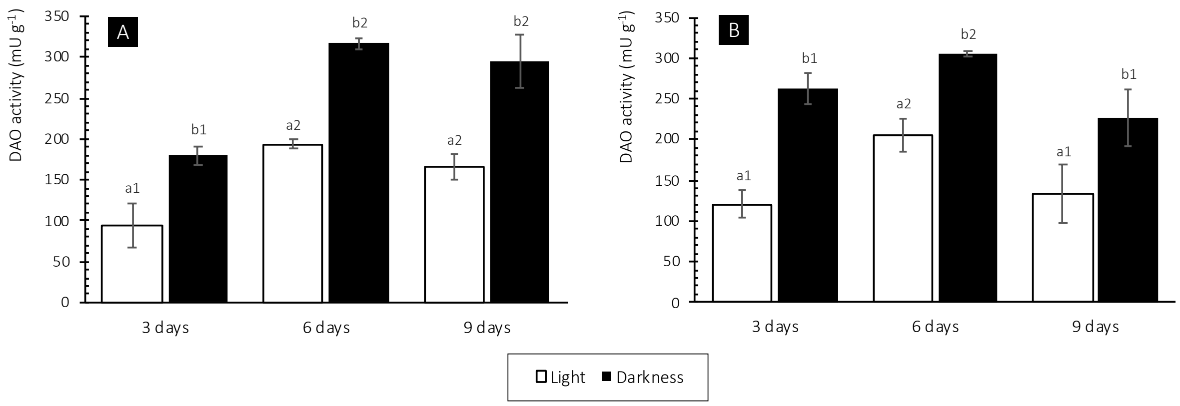
Figure 2. Overlapping chromatograms of histamine at the starting point (black) and after 1 h (blue), 2 h (red) and 4 h (green) of reaction for a sample of lyophilised lentil sprouts.

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

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


Edible seeds
of *Leguminosae*
A
B
Grinding and
homogenisation
Soaking in water
overnight
Germination*
(27°C, 70% RH)
Harvesting and freezing of
the fresh sprouts (-80°C)
Lyophilisation and
grinding of the sprouts
Homogenise in phosphate
buffer 0.05M pH 7.2
(200rpm, 37°C, 30min)
Addition of histamine and
constant incubation
(200rpm, 37°C)
Aliquots collection with addition
of $HClO_4$ to stop the reaction
Determination of remaining
histamine by UHPLC-FLD


EU
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
0.00 0.50 1.00 1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00 5.50 6.00 6.50 7.00
Time (Minutes)


A
B
DAO activity (mU g-1)
350
300
250
200
150
100
50
0
a1
b1
a2
b2
3 days
6 days
9 days
Light
Darkness

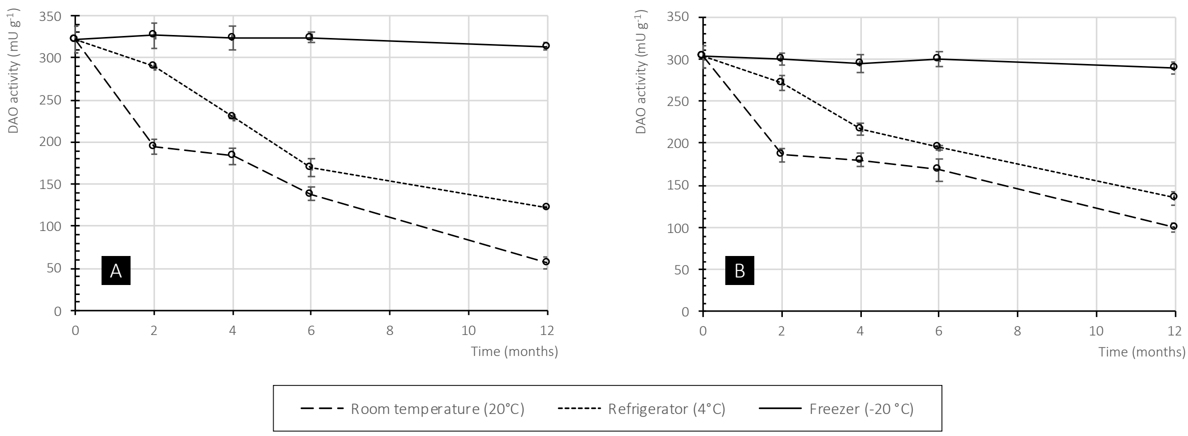

DAO activity (mU g-1)
350
300
250
200
150
100
50
0
A
0
2
4
6
8
10
12
Time (months)
B
Room temperature (20°C)
Refrigerator (4°C)
Freezer (-20 °C)

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

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

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

* Mean values in the same column followed by different letters are significantly different ($p<0.05$).