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Impact of technological factors on diamine oxidase (DAO) activity in porcine kidney extracts as active ingredient for the dietary management of histamine intolerance

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ABSTRACT

Histamine intolerance is associated with impaired histamine metabolism due to diamine-oxidase (DAO) enzyme deficiency at intestinal level. The recommended strategy to alleviate the symptomatology is a low-histamine diet supplemented with DAO. Different sources of DAO enzyme have been characterized in the literature (animal, vegetal and microbial), although only the porcine kidney protein extract has been approved as food supplement. Despite optimistic clinical results of DAO supplementation, variability in enzymatic activity of this type of active ingredient is reported, potentially linked to manufacturing processes. The aim of this work was to evaluate the impact of the raw material and the technological process of obtaining the porcine kidney protein extract on DAO activity, and its evolution during storage at room temperature (25 °C) and refrigeration (4 °C). Twenty batches of DAO-containing extract were analyzed and found to have a consistent histamine-degrading activity. The powder extract was prepared by mincing and homogenizing porcine kidneys, defatting with acetone, and drying at 40 °C for 6 h. The inclusion of a biocidal step before acetone extraction was evaluated on DAO activity. The use of sodium hypochlorite and irradiation (8kGy) did not have a negative impact on DAO activity. Moreover, the inclusion of an initial freeze-drying step of the raw material yielded an extract with higher DAO activity. On the other hand, the effect of applying higher temperatures (70 °C, 80 °C, and 90 °C) during the extract drying process for 3 and 6 h was evaluated, reporting a significant decrease of the enzymatic activity, with losses of 10–20 % for every 10 °C increase in temperature. Refrigeration was the only storage method capable of preserving enzymatic activity for at least 24 months. The manufacturing and storage of porcine kidney extract are crucial steps in the formulation of DAO supplements suitable for the treatment of histamine intolerance.

1. Introduction

The enzyme diamine oxidase (DAO, EC 1.4.3.22) catalyzes the oxidative deamination of the primary amino group of histamine and other diamines to yield the corresponding aldehyde, as well as stoichiometric amounts of ammonia and hydrogen peroxide (Bouvrette et al., 1997; Buffoni, 1966; Schwelberger & Bodner, 1997). This enzyme, which can be found in microorganisms, plants, and animals, is a homodimeric protein belonging to the category of copper-dependent amino oxidases (Boehm et al., 2017; Schwelberger et al., 2013). In humans, the major sites of DAO expression are placenta, kidney and intestine, being the latter which plays a key role in the degradation of dietary histamine (Comas-Basté et al., 2020b; Kovacova-Hanuskova et al., 2015). In the last decade, multiple studies have identified a population subgroup who suffer adverse effects after the ingestion of normal or even low levels of histamine due to deficient DAO activity (Zhao et al., 2022). Symptoms of this clinical condition, known as histamine intolerance, commonly affect the gastrointestinal, dermal and/or

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respiratory systems (Bodmer et al., 1999; Komericki et al., 2011; Schnedl & Enko, 2021). Some researchers have estimated that histamine intolerance may affect 1–3 % of the population, although prevalence data are still very limited. This percentage could potentially rise as understanding and diagnostic methods for histamine intolerance continue to advance (Maintz & Novak, 2007).

The main strategy used to avoid or lessen the symptoms of histamine intolerance is to follow a low-histamine diet (Lackner et al., 2019; Maintz & Novak, 2007; Wagner et al., 2017). As indicated by the European Food Safety Authority (EFSA), only foods with low histamine levels (below the detection limit) can be considered safe for individuals who suffer from this food intolerance (EFSA, 2011). However, as histamine and other biogenic amines are widely distributed in different food categories with highly variable concentrations (Gardini et al., 2016; Sánchez-Pérez et al., 2018), such diets are extremely restrictive and complex, and adherence is difficult (Cucca et al., 2022; Kovacova-Hanuskova et al., 2015; Schnedl et al., 2019). Moreover, the current food-related regulations do not consider the declaration of the absence or specific content of histamine in food labelling, which could help histamine intolerant individuals to make safer and informed choices (Sánchez-Pérez et al., 2021).

Recently, similar to the treatment recommended for lactose intolerance, it has been proposed that a low-histamine diet is accompanied by oral supplementation with exogenous DAO to promote the intestinal degradation of dietary histamine. The aim of this strategy is to allow patients to follow a less restrictive diet and improve their quality of life (Maintz & Novak, 2007). In 2017, the European Commission authorized the marketing of DAO supplements as a novel food for the dietary management of histamine intolerance, both as food supplement and as food for special medical purposes (Commission Implementing Regulation (EU) 2017/2470). The supplements are based on a protein extract from porcine kidney encapsulated in an enteric coating for protection during passage through the gastric environment before reaching the intestines, where histamine catabolism by DAO takes place. The occurrence of DAO in porcine kidney has long been verified and an extraction process was patented in the United States of America in 1942, involving a three-steps extraction treatment with acetone (approximately 7 L of acetone per kilogram of porcine kidney) and subsequent drying at 25 °C under vacuum to provide a protein concentrate with approximately 7 % DAO (William & Paul, 1942). Several DAO supplements are already available on the market, all of them formulated with a unit dose of 4.2 mg of porcine kidney extract (*i.e.* 0.3 mg of DAO enzyme per capsule) (Commission Implementing Regulation (EU) 2017/2470).

The few studies in the literature that have evaluated the effectiveness of DAO supplements indicate a positive effect on histamine intolerance symptoms, although the conclusions are limited by small study populations or short interventions (Cucca et al., 2022; Izquierdo-Casas et al., 2019; Komericki et al., 2011; Manzotti et al., 2016; Schnedl et al., 2019; Yacoub et al., 2018). Despite the optimistic results regarding the clinical efficacy of DAO supplements, considerable variability in the enzymatic activity of the active ingredient (powder porcine kidney protein extract) or the food supplement has been reported (Comas-Basté et al., 2019; Izquierdo-Casas et al., 2019; Kettner et al., 2020). Comas-Basté et al. (2019) analyzed the histamine-degrading activity of various supplements marketed in different countries, all of them formulated with porcine kidney extracts, finding values ranging between 0.04 and 0.20 mU/mg. In contrast, Kettner et al. (2020) reported the absence of enzymatic activity in a DAO supplement marketed in Germany. Both studies suggest that factors related either to the protein extraction from porcine kidney or to enzymatic instability along the storage could explain the variable DAO activity of the food supplements. For this reason, the aim of the present work was to evaluate the impact of the raw material and the technological process of obtaining the powder porcine kidney protein extract on histamine-degrading activity. Furthermore, the evolution of DAO activity during storage of the active ingredient was studied.

2. Material and methods

2.1. Samples preparation

The samples of protein extract from porcine kidney evaluated in this study were provided by a biomedical company located in Barcelona (Spain) and specialized in the manufacturing of active ingredients for nutraceutical products. Briefly, one experimental batch of powder protein kidney extract was obtained by mincing and homogenizing 1 kg of porcine kidneys with a PS-22 commercial meat mincer (Sammic, Azkoitia, Spain), followed by the addition of 8 L of acetone (PanReac Química, Castellar del Vallès, Spain) to defat and dehydrate the homogenized pig kidneys. The mixture of the porcine kidney batter with acetone was performed in a Nutsche Filter-Dryer (Büchi AG, Uster, Switzerland) at room temperature. Next, drying was carried out in a laboratory vacuum oven dryer (40 °C) for 6 h, with the paste evenly spread on metal trays, until a water content of <8–10 % was obtained. The effect of the time/temperature binomial on DAO activity was analyzed within this drying process by also subjecting the same batch of pig kidney extract to higher heat treatments (70 °C, 80 °C and 90 °C applied for 3 and 6 h). Finally, the resulting 150 g of active ingredient were sieved to ensure a granulometry of <1 mm.

The variability of the DAO activity of the protein extract from different batches of the raw material (porcine kidney) was evaluated. Thus, protein extracts from 20 batches of porcine kidney were analyzed, all of them obtained from a Spanish slaughterhouse over the course of a year.

In addition, DAO activity was studied after the application of different chemical and physical processes aimed at maintaining the biological safety of the final product. The biocidal treatments comprised the addition of 0.1 % sodium hypochlorite (20 mL of an aqueous solution at 5 %) or 0.2 % thymol (2-isopropyl-5-methylphenol) (40 mL of an alcoholic solution at 5 %) to the homogenized porcine kidney batter (both products were obtained from PanReac Química, Castellar del Vallès, Spain). The homogenized mixture was kept at room temperature for at least 15 min, prior to the acetone washing step. In the case of irradiation, it was performed at the premises of Aragogamma S.L. (Les Franqueses del Vallés, Barcelona, Spain). Gamma irradiation was applied by exposing the homogenized meat batter to a cobalt-60 irradiator at 15 °C and a dose rate of 10 KGy/h to achieve three different doses (8, 15 and 25 kGy).

The impact of the inclusion of a freeze-drying step of the raw material prior to the acetone extraction was also evaluated, its aim being to reduce the aqueous content of the product and thus minimize possible losses of DAO due to leaching. For this purpose, 10 different batches of porcine kidney were assessed. For each batch, 1 kg of porcine kidneys was grinded (as previously mentioned) and subsequently spread uniformly on metal trays (29.5 cm x 26.5 cm) in a single thin layer. The resulting paste was frozen overnight at −80 °C (NU-99728 J ultralow temperature freezer, NuAire, Plymouth, Minnesota, United States) and subjected to a freeze-drying process using a laboratory-scale freeze dryer (Cryodos-50, Telstar, Terrassa, Spain). The lyophilization procedure was performed with a chamber pressure of 0.22 mbar, increasing the temperature from −85 °C to 22 °C for 72 h. After lyophilization, the obtained dried paste was grinded using a domestic mill to obtain a homogenized powder.

Finally, the stability of the DAO activity of the porcine kidney extract was determined over 24 months of storage at room temperature (25 °C) or refrigeration (4 °C). The protein extract was maintained in sealed tubes protected from light and humidity and the enzymatic activity was measured at 2, 4, 6, 9, 12, 18 and 24 months.

All samples from different stages of the study were produced between 2021 - 2022 and sent to the Food and Nutrition Campus of the University of Barcelona for the analysis of DAO activity.

2.2. Determination of DAO activity in vitro

The experimental procedure used to determine DAO activity *in vitro* was that described by Comas-Basté et al. (2019). Briefly, 10 mg of powder porcine kidney protein extract was homogenized in 20 ml of 0.05 M phosphate buffer solution (pH 7.2) at 200 rpm and 37 °C for 30 min in a shaker incubator (NB-T205, N-BIOTEK, INC, Korea). Subsequently, a 45 μM histamine standard solution (Sigma-Aldrich, St. Louis, MO, USA) was added, the moment at which the enzymatic reaction begins ($t = 0$). The mixture was kept in constant incubation at 37 °C and 200 rpm and 500 μL aliquots of sample were removed at different reaction times. At each sampling point, 15 μL of 2 N perchloric acid solution was added to stop the enzymatic reaction. After a centrifugation step (4 °C, 5 min, 17,608 g), the supernatant was filtered through a 0.22 μm GHP filter (Waters Corp., Milford, MA, USA) and stored at 4 °C until analysis by ultra-high-performance liquid chromatography (UHPLC) (Latorre-Moratalla et al., 2009). The chromatographic determination of the remaining histamine was accomplished by online post-column derivatization at room temperature with 0.01 % (p/v) *o*-phthalaldehyde (Sigma-Aldrich, St. Louis, MO, USA) and fluorescence detection (λ_{ex}:340 nm and λ_{em}:445 nm). The results are expressed in nmoles of histamine degraded per minute per mg of porcine kidney extract (mU/mg).

2.3. Statistical analysis

Statistical analysis of data was performed with the SPSS Statistics 27.0 statistical software package (IBM Corporation, Armonk, NY, USA). For each different production condition, results are presented as mean values ± the standard deviation (mean ± SD). Normality was assessed using Q-Q plots and Shapiro Wilk test. Differences in DAO activity values among the different batches and treatment conditions were analyzed by non-parametric Kruskal-Wallis Test. Values of $p < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Influence of raw material and technological factors of the obtaining process of the porcine kidney protein extract on its DAO activity

One of the primary challenges in the formulation of food supplements from ingredients of biological origin is the variability that may exist between different batches of the raw material (Liu et al., 2022). Accordingly, DAO activity was measured in protein extracts from twenty different batches of porcine kidney produced over a year. As shown in Fig. 1, variations were found to be minimal, without significant differences among batches ($p > 0.05$), and an average value of 0.24 ± 0.02 mU/mg. The low variability in DAO activity found in porcine kidney protein extracts could be explained by the highly standardized techniques and conditions of animal rearing and slaughter within a specific slaughterhouse. This is relevant from a production point of view, as it ensures the product specifications remain constant between batches. To our knowledge, this is the first time that the effect of the raw material (in this case porcine kidney) on DAO activity in the resulting product has been evaluated. It remains pending to assess whether the raw material coming from different production slaughterhouse would also maintain a low variability in the DAO enzymatic activity. Two recent studies that analyzed protein extracts from porcine kidneys, which clearly proceeded from different countries and production slaughterhouses, reported DAO activity levels similar to those observed in the present study (Comas-Basté et al., 2019; Kettner et al., 2020). In these two cases, apart from the different origins of the raw material, the use of the same extraction procedure leading to a very similar degree of purification for the active ingredient could explain the low variability between products.

The process generally used to obtain protein extracts from porcine kidney includes drying in a vacuum oven, where the paste obtained after washing with acetone is exposed to a temperature of 40 °C for 6 h to remove any acetone residue and to reduce its water content (considered as the control sample for this experiment). Fig. 2 shows the DAO activity of powder porcine kidney protein extracts subjected to drying with different combinations of time and temperature. It was observed that applying more heat in the extraction process significantly reduced the enzymatic activity ($p < 0.05$), with a loss of approximately 10 - 20 % for each 10 °C increase in temperature. Exposing the extract to 70 °C resulted in up to 30 % loss of DAO activity compared to the control sample (40 °C, 6 h), which increased to >60 % at 90 °C ($p < 0.05$). On the other hand, the time of drying had less influence on the enzymatic activity. Thus, increasing heat exposure from 3 to 6 h resulted in only approximately 5 % loss of DAO activity. These results obtained in an unpurified porcine kidney extract coincide with those of Mondovi et al. (1992), who reported the stability of the activity of a purified DAO enzyme from porcine origin at temperatures below 60 °C, above which it decreased considerably due to thermal denaturation. Furthermore, according to information from a study by Matsuda and Suzuki (1981), the activity of DAO purified from *Vicia faba L.* leaves remains intact when subjected to temperatures below 60 °C for 10 min but decreases progressively at above 70 °C.

It may be necessary to apply sanitizing strategies to guarantee the biological safety of raw materials and/or the finished product. To the best of our knowledge, there is no information in the literature on the effect of biocide methods on the DAO activity of porcine kidney extract. The chemical and physical strategies tested in this study are widely used in the food field due to their ability to control biological hazards, including viral agents (Feliziani et al., 2016). As shown in Fig. 3, the addition of a 0.1 % aqueous solution of sodium hypochlorite did not influence DAO activity, resulting in no significant differences compared to the untreated control sample ($p > 0.05$). Although the influence of

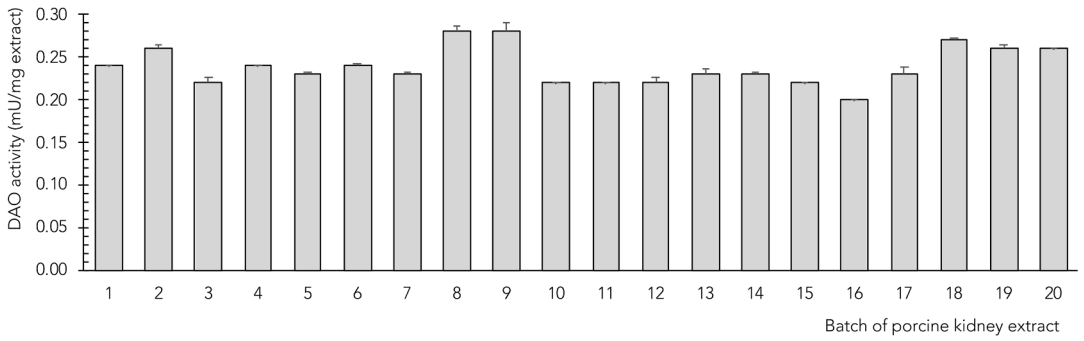

Fig. 1. *In vitro* diamine oxidase (DAO) activity (mU/mg) of protein extract prepared from 20 batches of porcine kidney. Results are presented as mean values ± the standard deviation (mean ± SD) of three analytical replicates.

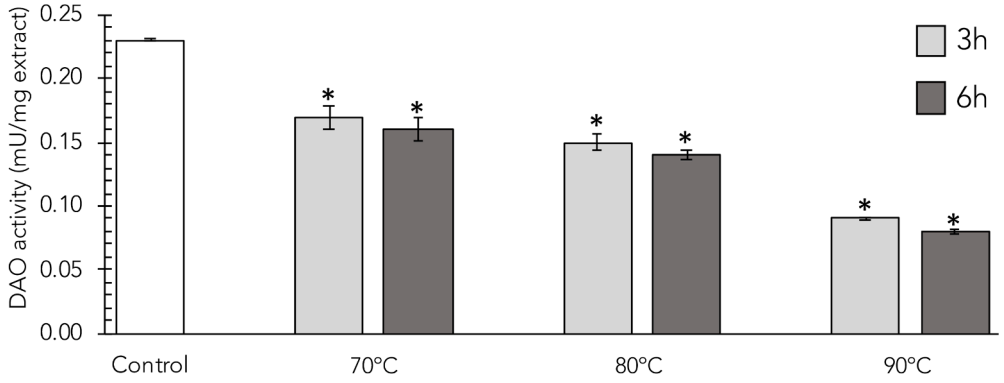

Fig. 2. Influence of different combinations of time and temperature during the drying process on the diamine oxidase (DAO) activity (mU/mg) of porcine kidney protein extract in comparison with the control sample (40 °C, 6 h). Results are presented as mean values ± the standard deviation (mean ± SD) from three different batches of powder porcine kidney protein extract obtained for each experimental condition. The asterisk indicates statistically significant differences compared to the control sample ($p < 0.05$).

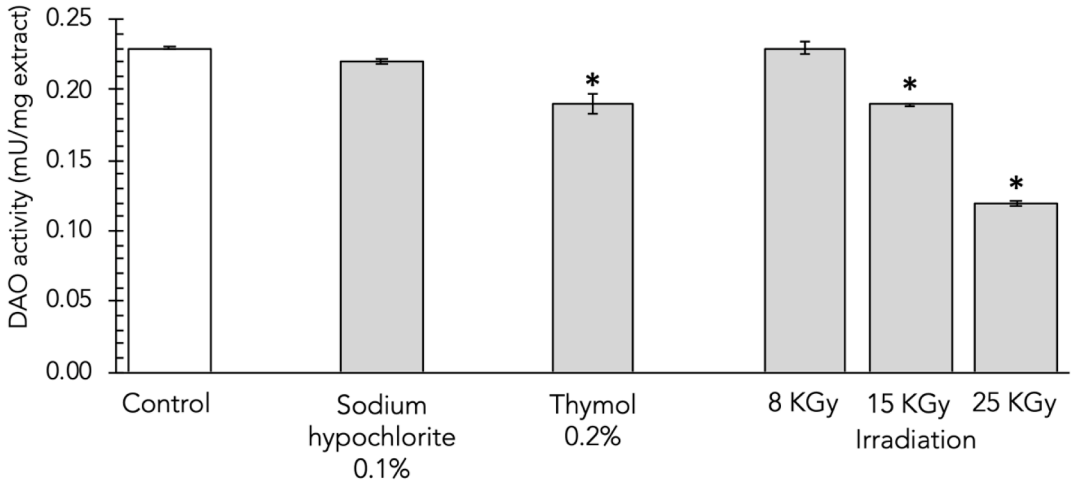

Fig. 3. Influence of biocidal treatments on the diamine oxidase (DAO) activity (mU/mg) of porcine kidney protein extract in comparison with the control sample (untreated). Sodium hypochlorite and thymol were applied at room temperature for 15 min, whereas irradiation was performed at 15 °C with a dose rate of 10 KGy/h. Results are presented as mean values ± the standard deviation (mean ± SD) from three different batches of powder porcine kidney protein extract obtained for each experimental condition. The asterisk indicates statistically significant differences compared to the control sample ($p < 0.05$).

this biocide treatment on DAO activity has not been studied previously, its impact on the catalytic capacity of other enzymes has been investigated. For example, Pequeño-Granado et al. (2015) reported that the application of sodium hypochlorite on leaves and petioles of *Jatropha curcas* did not affect the activity of polyphenol oxidase, even when used in concentrations of >3 %.

Due to the well-known antimicrobial properties of thymol extracts (Tapia-Rodriguez et al., 2023), the addition of 0.2 % thymol solution on this raw material was also tested. The obtained results showed that the application of this biocide solution caused a slight but statistically significant reduction in the DAO activity of the porcine kidney protein extract ($p < 0.05$). It is not clear the mechanism by which thymol could be responsible for this small loss in DAO enzymatic activity. The alcoholic solution that carries it could be another explanation, although the very low concentrations to which it was exposed in this experiment (4 % ethanol) could hardly exert a negative effect. Some authors have reported that alcohol concentrations higher than 24 % could begin to exert a damage on the intestinal DAO activity (Neree et al., 2018). However, the most studied hypothesis about the possible effect of alcohol on histamine toxicity is based on the fact that alcohol and its metabolite acetaldehyde can compete for the aldehyde dehydrogenase enzyme, which is involved in the metabolism of both alcohol and histamine, resulting in an accumulation of histamine and the enhancement of its toxicity.

Regarding irradiation, exposing the homogenized porcine kidneys at a dose of 8 kGy did not influence DAO activity of the obtained powder extract ($p > 0.05$), while irradiation at higher doses (15 and 25 kGy) provoked significant losses in this enzymatic activity ($p < 0.05$). The use of ionizing radiation has been authorized by the European Parliament since 1999 to reduce biological contamination of different foods for human consumption (Lista de, 2006). European regulations limit the irradiation dose to a maximum of 10 kGy, which has been shown to protect food against insects, parasites and non-sporulating pathogenic bacteria (Real Decreto 348/2001). The safety of applying radiation to food, without negative effects on nutritional and organoleptic quality, has been demonstrated, but there is still little information on how this type of sanitizing treatment may affect the activity of enzymes in food matrices. Hou et al. (2018) demonstrated that the application of irradiation at low doses (0.2 –1 kGy) in *Volvariella volvacea*, one of the most prized edible mushrooms in Asian cuisine, did not have any effect on the catalase and peroxide dismutase activity of this food. On the other hand, Wang et al. (2019) reported a significant inhibition of different enzymes involved in the lignification and enzymatic browning process in bamboo shoots irradiated with doses of 1–5 KGy. According to these reports and the results obtained in the present study, it seems that each enzyme responds in a different way to ionizing radiation treatment and needs to

be studied separately for each irradiation dose applied.

Finally, the effect of freeze-drying or lyophilization was also evaluated, a nonthermal drying method that could be applied in order to minimize loss of DAO activity through enzyme leaching during the processing. This operation allows the dehydration of the sample at relatively low temperatures and has a less negative effect on enzymes than a conventional thermal process, which facilitates partial or total inactivation depending on the intensity of the treatment (Liu et al., 2022). In the present study, the inclusion of an initial freeze-drying step of the raw material before protein extraction resulted in a significantly higher DAO activity of the powder porcine kidney protein extract ($p < 0.05$), practically doubling it (0.41 ± 0.02 mU/mg) compared to the control sample produced without this prior dehydration step (0.24 ± 0.02 mU/). To the best of our knowledge, there is no information in the literature about the effect of drying methods on the activity of DAO in animal-derived matrices. The freeze-drying process has also been studied for its influence on other enzymes in plant tissues, such as xanthine oxidase, polyphenol oxidase, peroxidase, and lactate dehydrogenase (Kawai & Suzuki, 2007; Loh & Lim, 2018; Srirangsan et al., 2010). For example, and in agreement with results of the current study, Loh and Lim (2018) demonstrated that freeze-drying was superior to conventional thermal methods in maintaining or even increasing polyphenol oxidase and peroxidase activities in avocado leaves.

3.2. Stability of the enzymatic activity of porcine kidney protein extract during storage

The stability of the DAO activity of powder porcine kidney protein extract stored under refrigeration (4 °C) and at room temperature (25 °C) for 24 months was determined. This active ingredient, that would be used in the formulation of DAO supplements, presented a low water activity, ranging from 0.10 to 0.12. As shown in Fig. 4, under refrigerated storage DAO activity remained intact for at least 24 months. In contrast, when the product was kept at room temperature, its ability to degrade histamine *in vitro* decreased progressively over time. It should be noted that the reduction of DAO activity during storage at room temperature occurred quite constantly and at a relatively slow rate, with a loss of 25 % (±0.01 %) after 12 months and 49 % (±0.02 %) after 24 months.

Available information on the stability of DAO activity in matrices of porcine origin is scarce and outdated. Bouvrette et al. (1997) studied the stability of DAO isolated from porcine kidney after several stages of purification by dialysis. The enzymatic activity remained stable for five months in a frozen solution (−80 °C), whereas losses of 20–30 % were observed after two months under refrigeration (4 °C). However, DAO catalytic stability increased if glycerol or sucrose were added as cryoprotective agents. More recently, Comas-Basté et al. (2020a) evaluated the activity of lyophilized sprouts from lentils (*Lens culinaris* Medik.) and chickpeas (*Cicer arietinum* L.), finding that the histamine-degrading activity of these plant matrices remained completely stable in frozen conditions (−20 °C) for at least 12 months, but significant losses occurred under refrigeration or at room temperature even in early storage. According to the available data, DAO catalytic activity seems more stable in protein extracts from porcine kidney compared to matrices of plant origin. However, regardless of the source, strategies are required to improve the maintenance of enzymatic activity during storage, especially at room temperature. Thus, the addition of disaccharides or glycerol as cryoprotectants, both during the enzyme extraction process and in formulations of DAO food supplements, should be explored for its potential benefits. In a study by Leonida et al. (2019), the activity of porcine DAO encapsulated in nanoparticles of a polysaccharide derived from chitin/chitosan was preserved when frozen (−20 °C) for five months. However, as freezing a food supplement is not ideal for its distribution, marketing, or conservation in domestic settings, more studies are needed to evaluate DAO stability under other storage conditions.

4. Conclusions

The findings of this study show that the DAO activity in porcine kidney protein extracts is scarcely influenced by variability of the raw material, resulting in an active ingredient with a consistent ability to degrade histamine. Among the different technological factors of the obtaining process, temperature control during the drying stage is key to guarantee maximum DAO activity of the product. Additionally, certain biocidal strategies, such as the addition of a 0.1 % aqueous solution of sodium hypochlorite and irradiation at a dose of 8 kGy, may be used to guarantee the biological safety of the extract without a negative effect on enzymatic activity. Moreover, the introduction of a freeze-drying or lyophilization step as a controlled technique for the dehydration of the raw material significantly increased the DAO activity of the porcine kidney extract. Finally, it has been confirmed that refrigerated storage preserves the histamine-degrading activity of the powder porcine kidney protein extract intact for at least 24 months, whereas its conservation at room temperature leads to a gradual decrease in the catalytic capacity of the active ingredient.

In view of these results, it is important to consider the effect of the evaluated technological processes on the enzymatic capacity of the active ingredient and to investigate in more detail the mechanisms responsible for the losses in DAO activity. These would include the

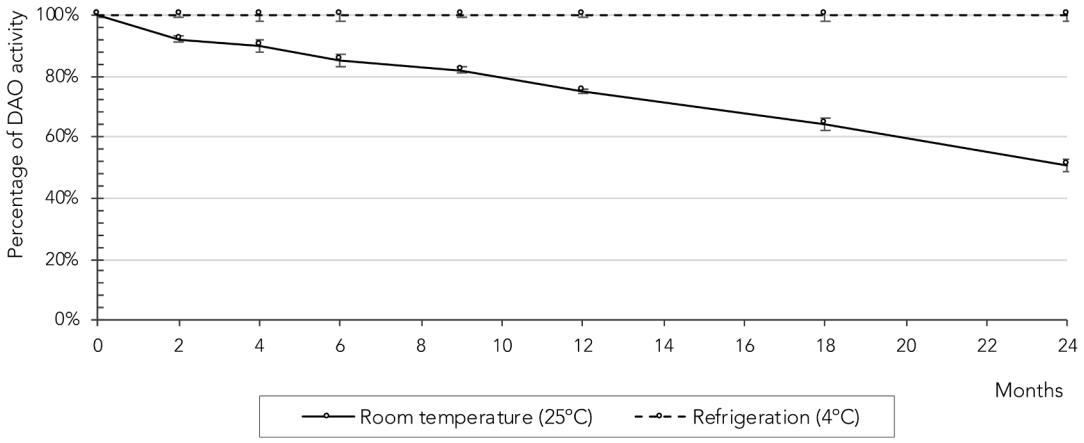

Fig. 4. Evolution of diamine oxidase (DAO) activity (mU/mg) of powder porcine kidney protein extract over 24 months of storage at room temperature (25 °C) and in refrigeration (4 °C). Results are presented as mean values ± the standard deviation (mean ± SD) from three different batches of powder porcine kidney protein extract obtained for each storage condition.

selection of the more suitable technological strategies to preserve histamine-degrading activity, as well as the design of new galenic formulations that optimize the shelf-life stability of this enzymatic activity.

Ethics statement

This research did not involve humans or animals.

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CRediT authorship contribution statement

Judit Costa-Catala: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Salvador Pellicer-Roca:** Writing – original draft, Investigation. **Irache Iduriaga-Platero:** Writing – original draft, Investigation. **Sònia Sánchez-Pérez:** Writing – original draft, Investigation. **M. Teresa Veciana-Nogués:** Writing – review & editing, Formal analysis. **M. Luz Latorre-Moratalla:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **M. Carmen Vidal-Carou:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Oriol Comas-Basté:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

Bodmer, S., Imark, C., & Kneubühl, M. (1999). Biogenic amines in foods: Histamine and food processing. *Inflammation Research, 48*(6), 296–300. https://doi.org/10.1007/s000110050463

Boehm, T., Pils, S., Gludovacz, E., Szoelloesi, H., Petroczi, K., Majdic, O., et al. (2017). Quantification of human diamine oxidase. *Clinical Biochemistry, 50*(7), 444–451. https://doi.org/10.1016/j.clinbiochem.2016.12.011

Bouvrette, P., Male, K. B., Luong, J. H. T., & Gibbs, B. F. (1997). Amperometric biosensor for diamine using diamine oxidase purified from porcine kidney. *Enzyme and Microbial Technology, 20*(1), 32–38. https://doi.org/10.1016/S0141-0229(96)00064-6

Buffoni, F. (1966). Histaminase and Related Amine Oxidases. *Pharmacological Reviews, 18*(4), 1163–1199.

Comas-Basté, O., Latorre-Moratalla, M. L., Sánchez-Pérez, S., Veciana-Nogués, M. T., & Vidal-Carou, M. C. (2019). *In vitro* determination of diamine oxidase activity in food matrices by an enzymatic assay coupled to UHPLC-FL. *Analytical and Bioanalytical Chemistry, 411*(28), 7595–7602. https://doi.org/10.1007/s00216-019-02178-2. Scopus.

Comas-Basté, O., Latorre-Moratalla, M. L., Rabell-González, J., Veciana-Nogués, M. T., & Vidal-Carou, M. C. (2020a). Lyophilised legume sprouts as a functional ingredient for diamine oxidase enzyme supplementation in histamine intolerance. *LWT, 125*, Article 109201. https://doi.org/10.1016/j.lwt.2020.109201

Comas-Basté, O., Sánchez-Pérez, S., Veciana-Nogués, M. T., Latorre-Moratalla, M., Vidal-Carou, M., & del, C. (2020b). Histamine intolerance: The current state of the art. *Biomolecules, 10*(8), 8. https://doi.org/10.3390/biom10081181

Commission Implementing Regulation (EU) 2017/2470 of 20 December 2017 establishing the union list of novel foods in accordance with regulation (EU) 2015/2283 of the European parliament and of the council on novel foods, 351 OJ L (2017). http://data.europa.eu/eli/reg_impl/2017/2470/oj/eng.

Cucca, V., Ramirez, G. A., Pignatti, P., Asperti, C., Russo, M., Della-Torre, E., et al. (2022). Basal serum diamine oxidase levels as a biomarker of histamine intolerance: A retrospective cohort study. *Nutrients, 14*(7), 7. https://doi.org/10.3390/nu14071513

EFSA Panel on. (2011). *Biological Hazards (BIOHAZ). Scientific Opinion on risk based control of biogenic amine formation in fermented foods. EFSA J, 9*, 1–93. https://www.efsa.europa.eu/en/efsajournal/pub/2393.

Feliziani, E., Lichter, A., Smilanick, J. L., & Ippolito, A. (2016). Disinfecting agents for controlling fruit and vegetable diseases after harvest. *Postharvest Biology and Technology, 122*, 53–69. https://doi.org/10.1016/j.postharvbio.2016.04.016

Gardini, F., Özogul, Y., Suzzi, G., Tabanelli, G., & Özogul, F. (2016). Technological factors affecting biogenic amine content in foods: A review. *Frontiers in Microbiology, 7*. https://www.frontiersin.org/articles/10.3389/fmicb.2016.01218.

Hou, L., Lin, J., Ma, L., Qu, S., Li, H., & Jiang, N. (2018). Effect of 60 Co gamma irradiation on postharvest quality and selected enzyme activities of Volvariella volvacea. *Scientia Horticulturae, 235*, 382–390. https://doi.org/10.1016/j.scienta.2018.02.074

Izquierdo-Casas, J., Comas-Basté, O., Latorre-Moratalla, M. L., Lorente-Gascón, M., Duelo, A., Soler-Singla, L., et al. (2019). Diamine oxidase (DAO) supplement reduces headache in episodic migraine patients with DAO deficiency: A randomized double-blind trial. *Clinical Nutrition, 38*(1), 152–158. https://doi.org/10.1016/j.clnu.2018.01.013

Kawai, K., & Suzuki, T. (2007). Stabilizing effect of four types of disaccharide on the enzymatic activity of freeze-dried lactate dehydrogenase: Step by step evaluation from freezing to storage. *Pharmaceutical Research, 24*(10), 1883–1890. https://doi.org/10.1007/s11095-007-9312-6

Kettner, L., Seitl, I., & Fischer, L. (2020). Evaluation of porcine diamine oxidase for the conversion of histamine in food-relevant amounts. *Journal of Food Science, 85*(3), 843–852. https://doi.org/10.1111/1750-3841.15069

Komericki, P., Klein, G., Reider, N., Hawranek, T., Strimitzer, T., Lang, R., et al. (2011). Histamine intolerance: Lack of reproducibility of single symptoms by oral provocation with histamine: A randomised, double-blind, placebo-controlled cross-over study. *Wiener Klinische Wochenschrift, 123*(1–2), 15–20. https://doi.org/10.1007/s00508-010-1506-y

Kovacova-Hanuskova, E., Buday, T., Gavliakova, S., & Plevkova, J. (2015). Histamine, histamine intoxication and intolerance. *Allergologia et Immunopathologia, 43*(5), 498–506. https://doi.org/10.1016/j.aller.2015.05.001

Lackner, S., Malcher, V., Enko, D., Mangge, H., Holasek, S. J., & Schnedl, W. J. (2019). Histamine-reduced diet and increase of serum diamine oxidase correlating to diet compliance in histamine intolerance. *European Journal of Clinical Nutrition, 73*(1), 102–104. https://doi.org/10.1038/s41430-018-0260-5

Latorre-Moratalla, M. L., Bosch-Fusté, J., Lavizzari, T., Bover-Cid, S., Veciana-Nogués, M. T., & Vidal-Carou, M. C. (2009). Validation of an ultra high pressure liquid chromatographic method for the determination of biologically active amines in food. *Journal of Chromatography A, 1216*(45), 7715–7720. https://doi.org/10.1016/j.chroma.2009.08.072

Leonida, M., Belbekhouche, S., Adams, F., Bijja, U. K., Choudhary, D. A., & Kumar, I. (2019). Enzyme nanovehicles: Histaminase and catalase delivered in nanoparticulate chitosan. *International Journal of Pharmaceutics, 557*, 145–153. https://doi.org/10.1016/j.ijpharm.2018.12.050

Lista de los alimentos o ingredientes alimentarios que los Estados miembros autorizan a tratar con radiación ionizante (De conformidad con el artículo 4, apartado 6, de la Directiva 1999/2/CE del Parlamento Europeo y del Consejo, relativa a la aproximación de las legislaciones de los Estados miembros sobre alimentos e ingredientes alimentarios tratados con radiaciones ionizantes) (El presente texto anula y sustituye al publicado en el Diario Oficial C 112 de 12 de mayo de 2006, p. 6) (2009). https://eur-lex.europa.eu/legal-content/ES/TXT/?uri=CELEX%3A52009XC1124%2802%29&qid=1730721658426.

Liu, Y., Zhang, Z., & Hu, L. (2022). High efficient freeze-drying technology in food industry. *Critical Reviews in Food Science and Nutrition, 62*(12), 3370–3388. https://doi.org/10.1080/10408398.2020.1865261

Loh, Z. H., & Lim, Y. Y. (2018). Drying effects on antioxidant activity, enzyme activity, and phytochemicals of avocado (Persea americana) leaves. *Journal of Food Processing and Preservation, 42*(10), e13667. https://doi.org/10.1111/jfpp.13667

Maintz, L., & Novak, N. (2007). Histamine and histamine intolerance. *The American Journal of Clinical Nutrition, 85*(5), 1185–1196. https://doi.org/10.1093/ajcn/85.5.1185

Manzotti, G., Breda, D., Di Gioacchino, M., & Burastero, S. (2016). Serum diamine oxidase activity in patients with histamine intolerance. *International Journal of Immunopathology and Pharmacology, 29*(1), 105–111. https://doi.org/10.1177/0394632015617170

Matsuda, H., & Suzuki, Y. (1981). Purification and properties of the diamine oxidase from vicia faba leaves. *Plant and Cell Physiology, 22*(5), 737–746. https://doi.org/10.1093/oxfordjournals.pcp.a076219

Mondovi, B., Befani, O., Gerosa, P., & Mateescu, M. A. (1992). Specific temperature dependence of diamine oxidase activity and its thermal stability in the presence of Polyvinylalcohol. *Agents and Actions, 37*(3–4), 220–226. https://doi.org/10.1007/BF02028112

Neree, A. T., Pietrangeli, P., Szabo, P. I., Mateescu, M. A., & Marcocci, L. (2018). Stability of Vegetal Diamine Oxidase in Simulated Intestinal Media: Protective Role of Cholic Acids. *Journal of Agricultural and Food Chemistry, 66*(48), 12657–12665. https://doi.org/10.1021/acs.jafc.8b04005

Pequeño-Granado I., Martínez-Ávila G., Aguirre-Arzola V., Iracheta L., Mojica-Marín V., & Rodríguez-Pérez G.. (2015). Efecto del NaClO sobre la actividad de la polifenol oxidasa en explantes de hoja Y PECIOLO.

Real Decreto 348/2001, de 4 de abril, por el que se regula la elaboración, comercialización e importación de productos alimenticios e ingredientes alimentarios tratados con radiaciones ionizantes, Pub. L. No. Real Decreto 348/2001, BOE-A-2001-6699 12825 (2001). https://www.boe.es/eli/es/rd/2001/04/04/348.

Sánchez-Pérez, S., Comas-Basté, O., Rabell-González, J., Veciana-Nogués, M. T., Latorre-Moratalla, M. L., & Vidal-Carou, M. C. (2018). Biogenic amines in plant-origin foods: Are they frequently underestimated in low-histamine diets? *Foods, 7*(12), 12. https://doi.org/10.3390/foods7120205 (Basel, Switzerland).

Sánchez-Pérez, S., Comas-Basté, O., Veciana-Nogués, M. T., Latorre-Moratalla, M. L., & Vidal-Carou, M. C. (2021). Low-histamine diets: Is the exclusion of foods justified by their histamine content? *Nutrients, 13*(5), 1395. https://doi.org/10.3390/nu13051395

Schnedl, W. J., & Enko, D. (2021). Histamine intolerance originates in the gut. *Nutrients, 13*(4), 1262. https://doi.org/10.3390/nu13041262

Schnedl, W. J., Schenk, M., Lackner, S., Enko, D., Mangge, H., & Forster, F. (2019). Diamine oxidase supplementation improves symptoms in patients with histamine intolerance. *Food Science and Biotechnology, 28*(6), 1779–1784. https://doi.org/10.1007/s10068-019-00627-3

Schwelberger, H. G., & Bodner, E. (1997). Purification and characterization of diamine oxidase from porcine kidney and intestine. *Biochimica et Biophysica Acta (BBA) Protein Structure and Molecular Enzymology, 1340*(1), 152–164. https://doi.org/10.1016/S0167-4838(97)00039-3

Schwelberger, H. G., Feurle, J., & Houen, G. (2013). New tools for studying old questions: Antibodies for human diamine oxidase. *Journal of Neural Transmission, 120*(6), 1019–1026. https://doi.org/10.1007/s00702-012-0936-2, 1996.

Srirangsan, P., Kawai, K., Hamada-Sato, N., Watanabe, M., & Suzuki, T. (2010). Improvement in the remaining activity of freeze-dried xanthine oxidase with the addition of a disaccharide–polymer mixture. *Food Chemistry, 119*(1), 209–213. https://doi.org/10.1016/j.foodchem.2009.06.016

Tapia-Rodriguez, M. R., Cantu-Soto, E. U., Vazquez-Armenta, F. J., Bernal-Mercado, A. T., & Ayala-Zavala, J. F. (2023). Inhibition of acinetobacter baumannii biofilm formation by terpenes from oregano (Lippia graveolens) essential oil. *Antibiotics, 12*(10), 10. https://doi.org/10.3390/antibiotics12101539

Wagner, N., Dirk, D., Peveling-Oberhag, A., Reese, I., Rady-Pizarro, U., Mitzel, H., et al. (2017). A Popular myth—Low-histamine diet improves chronic spontaneous urticaria—Fact or fiction? *Journal of the European Academy of Dermatology and Venereology, 31*(4), 650–655. https://doi.org/10.1111/jdv.13966

Wang, J., Jiang, J., Wang, J., Wang, Z., Yang, X., & Jia, L. (2019). The influence of gamma irradiation on the storage quality of bamboo shoots. *Radiation Physics and Chemistry, 159*, 124–130. https://doi.org/10.1016/j.radphyschem.2019.02.021

William H., & Paul S. (1942). Process of making histaminase preparations (United States Patent US2289194A). https://patents.google.com/patent/US2289194A/en.

Yacoub, M. R., Ramirez, G. A., Berti, A., Mercurio, G., Breda, D., Saporiti, N., et al. (2018). Diamine oxidase supplementation in chronic spontaneous urticaria: A randomized, double-blind placebo-controlled study. *International Archives of Allergy and Immunology, 176*(3–4), 268–271. https://doi.org/10.1159/000488142

Zhao, Y., Zhang, X., Jin, H., Chen, L., Ji, J., & Zhang, Z. (2022). Histamine intolerance—A kind of pseudoallergic reaction. *Biomolecules, 12*(3), 454. https://doi.org/10.3390/biom12030454