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Research Article

Plant Diamine Oxidase (DAO) Obtained from Pisum Sativum: Biochemical and Technological Characterization and Safety Profile for Its Application in Food Supplements

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Abstract

The histamine present in fermented foods, cheeses, fish and sausages can cause adverse reactions in people with deficiency or low activity of the enzyme diamine oxidase (DAO), responsible for their degradation. Plant-derived DAO supplementation, specifically Pisum sativum extract, represents a natural and safe alternative to restore this function in individuals sensitive to dietary histamine. This study examines the biochemical and technological characterization of plant DAO, as well as its safety profile supported by preclinical, clinical, and regulatory data. DAO is an amine oxidase containing copper, with copper-dependent catalytic activity. Its structure is made up of a dimer of identical subunits, each with an approximate molecular weight of 85 kDa and a copper atom essential for its function. The enzymatic activity, calibrated by specific spectrophotometric methods, remains stable and reproducible in commercial batches. The analysis by SDS-PAGE electrophoresis and UV-vis spectroscopy showed a pure enzyme, structurally intact and with reversible dependent activity of copper, confirming the identity and functionality of the ingredient. The production process of the Plant DAO includes rigorous stages of receiving, conditioning, sieving, mixing, forming tablets or capsules, and thorough quality controls at each stage. Only batches that meet all quality criteria are released for marketing, thus guaranteeing the safety and efficacy of the final product. Toxicological studies in animal models indicated no adverse effects at doses much higher than those recommended for humans. Clinical trials in healthy volunteers confirmed a good tolerance and safety profile for oral intake of DAO. The wide commercialization and use in several countries have not reported significant adverse events, highlighting its safety in the general population. This plant enzyme, certified according to international standards and approved by regulatory bodies, represents an effective option for the management of histamine intolerance, contributing to the improvement of associated symptoms and the quality of life of patients.

Keywords

Diamine Oxidase, Histamine, Intolerance, Plant, Safety, DAO, Supplementation

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1. Introduction

Dietary histamine, a bioactive compound present in fermented foods, cheeses, fish and sausages, can cause adverse reactions in individuals with deficiency or low activity of diamine oxidase (DAO), the key enzyme in its degradation. Supplementation with plant DAO derived from Pisum sativum offers a natural and safe pathway to restore metabolic capacity and relieve symptoms related to histamine accumulation. This work delves not only into the biochemical and technological characterization of the ingredient, but also into its safety profile, supported by regulatory, preclinical and clinical data.

Diamine oxidase (DAO) is an amine oxidase that contains copper and catalyzes the oxidative deamination of primary amines, such as histamine, cadaverine, and putrescine. Purified plant DAO occurs as a homodimer composed of two identical subunits with an approximate molecular weight of 170,000 Da (gel filtration) or 145,000 Da (ultracentrifugation), and each subunit contains one copper atom essential for enzymatic activity, totaling two copper atoms per dimer [1].

This enzyme is a glycoprotein with approximately 20% carbohydrate content by weight, rich in acidic amino acids such as aspartic and glutamic acid, with a low isoelectric point (~5.3) reflecting its net acidic character. In addition, it contains significant levels of lysine, arginine, leucine, and phenylalanine, contributing to its structural stability and functionality [2, 3]. The specific activity reaches up to 1.86 μmoles of oxidized substrate per minute and per mg of protein. Its spectrophotometric profile shows a maximum of visible absorption at 500 nm, characteristic of copper-containing enzymes and the possible involvement of pyridoxal phosphate [4].

2. Identity and Structure of Plant DAO

The identity and structure of plant DAO are confirmed by SDS-PAGE electrophoresis, showing an approximate subunit weight between 85–86 kDa, and sedimentation rate and diffusion studies that show a symmetrical behavior and molecular homogeneity. Atomic absorption spectroscopy verifies a copper content of around 0.074%, compatible with two copper atoms per enzyme dimer [5].

Characterization of diamine oxidase (DAO) by SDS-PAGE electrophoresis and UV-visible spectroscopy confirms the structural integrity of the enzyme and its copper-dependent catalytic mechanism. First, the electrophoretic analysis revealed a single predominant band close to 85 kDa, corresponding to the expected molecular weight for monomeric DAO derived from plant without detecting other proteins or degraded fragments, which shows high purity and structural homogeneity.

The UV-visible absorption profile showed a strong peak around 280 nm, typical of proteins, accompanied by a shoulder in the 350–370 nm region, indicative of the presence of the cofactor topaquione, a copper-mediated modification in a tyrosine residue of the active site, characteristic of copper-containing amine oxidase.

To assess the functional need for copper, enzyme activity assays were performed in the presence and absence of metal chelators such as EDTA. Activity decreased by more than 90% after chelating treatment, while the reintroduction of Cu^{2+} ions fully restored catalytic function, confirming that copper is an indispensable and reversible cofactor for the deamination that catalyzes DAO.

Table 1. *SDS-PAGE and Spectrophotometric Characterization of vegetable DAO.*

Parameter	Method	Result	Interpretation
Molecular weight	SDS-PAGE (12% gel)	Major band at ~85 kDa	Consistent with monomeric porcine DAO
Purity	SDS-PAGE	No additional bands visible	High purity confirmed; no detectable protein contaminants
Absorbance at 280 nm	UV-Vis Spectrophotometry	Strong peak	Indicates typical protein profile
Cofactor absorbance	UV-Vis (350–370 nm)	Shoulder peak observed	Suggests presence of topaquinone (copper-derived cofactor)
Activity without Cu^{2+}	Enzymatic assay + EDTA	<10% of initial activity	Enzyme activity is copper-dependent
Restored activity with Cu^{2+}	Enzymatic assay + $CuCl_2$ (post-EDTA)	>90% activity recovered	Confirms reversible copper requirement
Copper-dependence conclusion	Activity modulation by chelation/addition	Confirmed	DAO requires copper ions as essential cofactors

Together, these analytical data support that the enzymatic material used corresponds to a monomeric DAO of ~85 kDa, with structural and functional properties compatible with a copper-dependent biogenic amine oxidase, ensuring the identity and enzymatic specificity of the formulated ingredient.

3. Plant DAO Production

Plant DAO is obtained through a germination and extraction process that isolates and purifies fractions with significant enzymatic activity.

The manufacturing process begins with the initial receipt and conditioning of raw materials, ensuring that they meet the required quality and traceability standards. After passing rigorous controls, raw materials are properly stored until they are used in production. They are then screened to remove impurities and ensure that the particle size is homogeneous, followed by careful mixing to evenly distribute the active ingredients and excipients.

The resulting mixture is processed either by compression to form tablets or by encapsulation to obtain capsules, during which intermediate quality controls are carried out to ensure that the specifications are met. Subsequently, the tablets or capsules are subjected to a primary coating or packaging process, with a new quality check before moving forward.

The product then goes to secondary dosing and packaging, where it is placed in its final packaging, such as blister packs or jars, carrying out additional controls to ensure the integrity, correct presentation and protection of the product. Finally, the finished products are grouped into boxes, palletized and, if necessary, protected with strapping or shrink film to facilitate their safe transport and storage.

Only those batches that pass all the controls and meet the established quality criteria are released for distribution and sale. At any point in the process, if a non-conformity is detected, the affected material is removed and sent to a quarantine area for evaluation, and it is decided whether it can be reprocessed or discarded, thus ensuring the safety and quality of the final product.

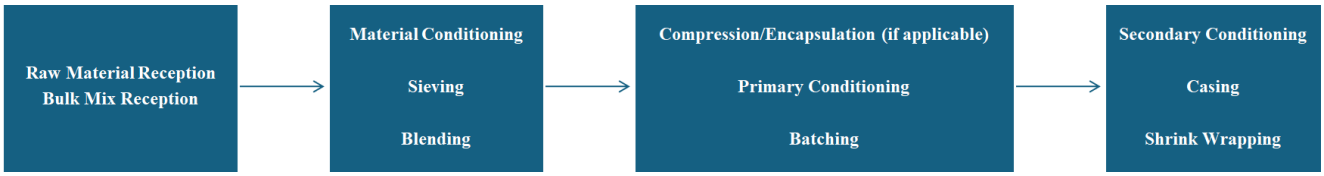

Figure 1. *Flow chart (summarized) of the plant-based DAO manufacturing process.*

The process described ensures that each batch of vegetable DAO meets the highest standards of quality, safety and traceability before reaching the consumer. The manufacturer is certified by international bodies such as ISO 22000, FDA and complies with strict Good Manufacturing Practices (GMP), backed by independent audits and a specialized team that keeps the quality systems under constant review and improvement.

From receipt, each raw material is carefully analyzed to ensure that it meets all internal and regulatory specifications, and only approved materials are used in production. Physical, microbiological and organoleptic parameters are controlled, and any non-standard material is blocked and managed according to protocols. During manufacturing, continuous inspections of weight, appearance, labeling and other characteristics are carried out in both compression and primary and secondary packaging, maintaining rigorous control at each stage.

In addition, samples are taken at different times of the process to analyze moisture, pH, hardness, friability, and other critical aspects, recording and preserving reference samples for each batch. Only those batches that pass all these controls are released for sale, and if any deviation arises, the batch is blocked and immediate corrections are applied.

In the event that a product needs to be recalled, there is a well-structured plan with a multidisciplinary team that coordinates the process, from the identification of the cause to the communication to the authorities and customers, the recovery of the product and its final disposal, all duly documented. Periodic drills and annual audits are carried out to ensure the effectiveness and continuous improvement of the system, thus guaranteeing total consumer protection and the company's reputation.

This comprehensive and careful system ensures that any quality or safety-related incidents are handled quickly and efficiently, maintaining confidence in both the product and the company.

DAO-specific activity is standardized in international units (U/mg), although for regulatory purposes it is expressed in milligrams per tablet.

The method used to measure this activity is colorimetric spectrophotometric (APP–DCHBS–HRP), based on the following principle: DAO oxidizes putrescine, which produces hydrogen peroxide (H_2O_2). It reacts with AAP and DCHBS in the presence of the enzyme peroxidase (HRP), forming a colored compound detectable at 515 nm by absorbance, which is calculated using an extinction coefficient of 2.6×10^4 M^{-1} cm^{-1}.

The results obtained by this method in four batches show the enzymatic activity of the ingredient, reflecting its quality and consistency.

Activity values expressed in U/mg indicate the ability of DAO to catalyze specific amine oxidation reactions. Each batch had a mean activity ranging from 293.66 to 311.23 U/mg, with standard deviations between 5.21 and 13.66 U/mg. These results reflect a high reproducibility of the extraction

and standardization process, evidencing an effective control of the quality of the ingredient. The slight variability observed is common in biological processes and remains within acceptable ranges for pharmaceutical ingredients and nutraceuticals [6].

In addition, protein concentration assays are performed using Bradford and Kjeldahl, and techniques such as SDS-PAGE are used to verify purity and structural integrity, as well as copper dependence detection to confirm enzymatic functionality.

The microbiological purity has been verified by culture methods, specific PCR and viral analysis, guaranteeing the absence of pathogens. Uniformity in the distribution of the active ingredient and excipients is ensured by precise sieving and mixing, which is essential for dose homogeneity in food supplements.

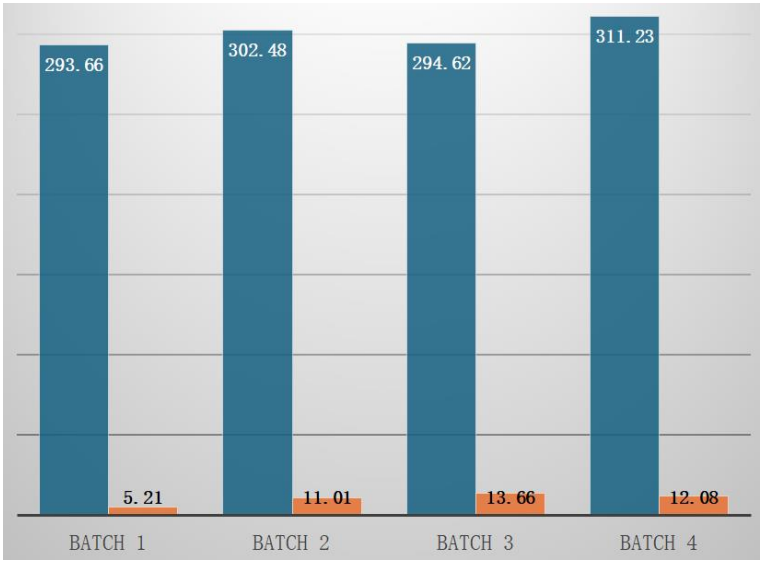

Figure 2. *Results of 4 batches of vegetable DAO by the spectrophotometric (colorimetric) method (Mean and SD in U/mg).*

4. Plant DAO Safety Profile

4.1. Preclinical Studies

Preclinical studies conducted to evaluate the safety of plant-based diamine oxidase (DAO) involved acute and subchronic toxicity assays in animal models. These studies demonstrated a level of NOAEL (Level No Adverse Effects Observed) significantly higher than the doses intended for human consumption, with no record of clinical, biochemical or histopathological alterations attributable to the ingredient. The results ensure that the consumption of DAO in the recommended doses does not present toxicological risks [7, 8].

4.2. Human Clinical Studies

In humans, phase I clinical trials were conducted with single ascending doses where DAO showed excellent tolerability and safety, even at doses much higher than those usually prescribed. No significant adverse effects related to acute oral intake of DAO were reported in healthy volunteers [7]. This is of vital importance to confirm the safety of DAO when administered as a dietary supplement.

4.3. Evidence of Safety and Efficacy in Actual Use

The extensive commercial experience of plant DAO, with more than one million units distributed since 2018 in markets such as Europe, the United States, Canada and Japan, does not register serious side effects. In addition, clinical trials in adults with histamine intolerance have demonstrated significant clinical improvements and absence of adverse reactions during use [9, 10]. These data confirm the favorable long-term safety profile and therapeutic efficacy of the ingredient.

4.4. Regulatory Compliance

Plant-based DAO is accredited by recognized regulatory bases, including Health Canada's Natural Health Product Ingredients Database (NHPID) [11] and is included in official lists of approved ingredients in the European Union [12]. Its manufacture is carried out under strict Good Manufacturing Practices (GMP) regulations, documented and controlled by independent entities, guaranteeing the quality, purity and safety of the product. Rigorous controls are applied throughout the production process, ensuring reproducibility and compliance with international standards.

5. Clinical Use of Plant DAO

As for the recommended daily use, as little as 0.30 mg of pure DAO per dose is sufficient to demonstrate safety and efficacy in individuals with histamine intolerance and it is suggested to administer one capsule/tablet per meal, up to a maximum of three daily. Supplementation is especially aimed at people with histamine intolerance, characterized by a reduced ability to metabolize it, a situation that can lead to symptoms such as migraines, gastrointestinal discomfort, skin flushing and similar allergic reactions. It is especially indicated for individuals with low endogenous DAO activity associated with genetic polymorphisms, gastrointestinal disorders, or other physiological factors that compromise histamine degradation [5].

The safety profile of DAO is favorable, having demonstrated its good tolerance in healthy adults between 18 and 50 years of age, without serious adverse events or significant alterations in vital or laboratory parameters in phase I clinical trials with doses of up to 210 mg of extract. Although DAO is naturally elevated during pregnancy and may contribute to the physiological regulation of histamine at this stage, there are no specific clinical studies yet in pregnant or lactating women, so caution is advised in these populations [7].

There are no specific contraindications other than known hypersensitivity to the components of the formula. As a precaution, its use in the pediatric population without medical supervision is not recommended due to limited clinical evidence [13]. In summary, DAO supplementation is indicated for adults with histamine deficiency or intolerance, including those who consume diets rich in this amine, and has been shown to be safe and well tolerated even at doses higher than those usually required.

6. Conclusions

Diamine oxidase is the main enzyme responsible for the degradation of dietary histamine, and decreasing its deficiency can reduce symptoms related to food intolerances [14]. The extraction of DAO from Pisum sativum, standardized in enzymatic activity and subjected to rigorous quality controls, presents a reliable and natural alternative to animal or synthetic sources. The stability of enzyme activity between batches is crucial to ensure clinical reproducibility and optimization of functional effect. In addition, applying homogenization and sieving techniques contributes to obtaining a final product of high sensory and pharmaceutical quality [15].

The activity and process data described support the technological and functional viability of plant DAO for its incorporation into food supplements and nutraceutical products, expanding its field of application in functional nutrition and digestive health.

In summary, the plant DAO extracted from Pisum sativum shows a high and homogeneous enzymatic activity in different batches, validating the extraction and purification process. Rigorous quality control and technological processing ensure a stable functional ingredient, suitable for use in food supplements aimed at dietary histamine metabolism. These results position plant DAO as a promising ingredient in nutrition and functional health.

Abbreviations

DAO	Diamine Oxidase
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
UV	Ultraviolet

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Author Contributions

Javier Moran: Conceptualization, Formal Analysis, Methodology, Supervision, Writing – original draft

Gerard Moles: Data curation, Funding acquisition, Investigation, Project administration, Resources, Software, Validation, Visualization, Writing – review & editing

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Data Availability Statement

The data supporting the outcome of this research work has been reported in this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Wolvekamp MC, de Bruin RW. Diamine oxidase: an overview of historical, biochemical and functional aspects. Dig Dis. 1994 Jan-Feb; 12(1): 2-14. https://doi.org/10.1159/000171432

[2] Elmore BO, Bollinger JA, Dooley DM. Human kidney diamine oxidase: heterologous expression, purification, and characterization. J Biol Inorg Chem. 2002 Jun; 7(6): 565-79. https://doi.org/10.1007/s00775-001-0331-1

[3] Steinebach V, Groen BW, Wijmenga SS, Jongejan JA, Duine JA. Identification of topaquinone, as illustrated for pig kidney diamine oxidase and Escherichia coli amine oxidase. Anal Biochem. 1995 Sep 1; 230(1): 159-66. https://doi.org/10.1006/abio.1995.1451

[4] Rinaldi A, Floris G, Finazzi-Agro A. Purification and properties of diamine oxidase from Euphorbia latex. Eur J Biochem. 1982 Oct; 127(2): 417-22. https://doi.org/10.1111/j.1432-1033.1982.tb06888.x

[5] Petersen J, Drasche A, Raithel M, Schwelberger HG. Analysis of genetic polymorphisms of enzymes involved in histamine metabolism. Inflamm Res. 2003 Apr; 52 Suppl 1: S69-70. https://doi.org/10.1007/s000110300059

[6] Bolton S, Bon C. Pharmaceutical Quality Control. Int J Pharm Sci. 2010; 12(3): 45-56.

[7] Molina Perello P, Puntes Rodriguez M, Coimbra Hurtado J, et al. Evaluation of the safety and tolerability of three single ascending doses of diamine oxidase (DAO) in healthy volunteers. medRxiv. 2024 Dec https://doi.org/10.1101/2024.12.05.24318468

[8] Amin K, Hallihan WJ, Brown PJ, et al. New Dietary Ingredient Notification for Diamine Oxidase (DAO). Marlyn Nutraceuticals; 2008.

[9] Izquierdo-Casas J, Comas-Baste O, Latorre-Morata M, et al. Diamine oxidase (DAO) supplement reduces headache in episodic migraine patients with DAO deficiency: a randomized double-blind trial. Clin Nutr. 2019 Feb; 38(1): 152-8. https://doi.org/10.1016/j.clnu.2018.01.013

[10] Schnedl WJ, Schenk M, Lackner S, et al. Diamine oxidase supplementation improves symptoms in patients with histamine intolerance. Food Sci Biotechnol. 2019 May; 28(6): 1779-84. https://doi.org/10.1007/s10068-019-00627-3

[11] Health Canada. Natural Health Product Ingredients Database. Available from: https://webprod.hc-sc.gc.ca/nhpid-bdipsn

[12] Commission Implementing Regulation (EU) 2017/2470 of 20 December 2017 establishing the Union list of novel foods in accordance with Regulation (EU) 2015/2283. Official Journal of the European Union. 2017 Dec 21.

[13] Ladero V, Calles-Enriquez M, Fernandez M, Alvarez MA. Toxicological effects of dietary biogenic amines. Curr Nutr Food Sci. 2010; 6(2): 145-156.

[14] Maintz L, Novak N. Histamine and histamine intolerance. Am J Clin Nutr. 2007 Nov; 85(5): 1185-96. https://doi.org/10.1093/ajcn/85.5.1185

[15] Sachdeva V, Roy A, Bharadwaj N. Current prospects of nutraceuticals. Curr Pharm Biotechnol. 2020; 21(10): 884-96. https://doi.org/10.2174/1389201021666200130113441

Biography

Javier Moran is a professor of Food & Regulatory Innovation at Catholic University of Murcia (UCAM). He is also a visiting professor at several universities (USIL, ISALUD, IFFE). He has participated in multiple international research collaboration projects in recent years. He currently serves on the Editorial Boards of numerous publications and has been invited as a Keynote Speaker, Technical Committee Member, Session Chair, and Judge at international conferences.

Research Field

Javier Moran: Food innovation, Food regulation, Functional foods, Nutraceuticals.