

Review

Waste to Value: L-Asparaginase Production from Agro-Industrial Residues

Enzo Corvello ¹, Bruno C. Gambarato ² , Nathalia V. P. Veríssimo ³, Thiago Q. J. Rodrigues ⁴ , Alice D. R. Pesconi ⁴, Ana K. F. Carvalho ⁴  and Heitor B. S. Bento ^{1,*} 

¹ School of Pharmaceutical Sciences, São Paulo State University (UNESP), Araraquara 14800-903, SP, Brazil; enzo.corvello@unesp.br

² Department of Material Science and Technology, University Center of Volta Redonda (UniFOA), Volta Redonda 27240-560, RJ, Brazil; bruno.gambarato@foa.org.br

³ School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto 14040-903, SP, Brazil; nathaliavieira@usp.br

⁴ Engineering School of Lorena, University of São Paulo (USP), Lorena 12602-810, SP, Brazil; thiago_queiroz@usp.br (T.Q.J.R.); arturpesconi@usp.br (A.D.R.P.); anacarvalho@usp.br (A.K.F.C.)

* Correspondence: heitor.bento@unesp.br

Abstract

The agro-industrial sector is a key pillar of the global economy, playing a central role in the supply of food, energy, and industrial inputs. However, its production chain generates significant amounts of residues and by-products, which, if not properly managed, may cause considerable environmental impacts. In this context, the search for alternatives to reuse these materials is essential, particularly when they can be converted into high-value products. One promising application is their use as a nutrient source for microorganisms in high-value biotechnological processes, such as the production of L-Asparaginase, an important enzyme used both in mitigating acrylamide formation in foods and as a biopharmaceutical in Acute Lymphoblastic Leukemia therapy. This approach offers a sustainable and competitive pathway, combining robust, scalable, and economical enzyme production with waste valorization and circular economy benefits. Although interest in developing more sustainable processes is growing, supported by international agreements and strategies for the valorization of agricultural residues, important challenges remain. The variability and impurity of residues pose significant challenges for producing biological products for the pharmaceutical and food industries. In addition, meeting regulatory requirements is essential to ensure product safety and traceability, while achieving high yields is crucial to maintain production viability compared to conventional media. Overcoming these barriers is critical to enable industrial-scale application of this approach. This review provides a residue-centered revision of the most relevant agro-industrial by-products used as substrates for L-asparaginase production, systematically comparing their compositional characteristics, fermentation strategies, and reported yields. Additionally, we present a novel SWOT (Strengths, Weaknesses, Opportunities, Threats) analysis that critically examines the technical, regulatory, and economic challenges of implementing residue-based processes on an industrial scale.

Keywords: residues; nutrient sources; biotechnological processes; high-value products; sustainability



Academic Editor: Sueli Rodrigues

Received: 29 August 2025

Revised: 22 September 2025

Accepted: 25 September 2025

Published: 26 September 2025

Citation: Corvello, E.; Gambarato, B.C.; Veríssimo, N.V.P.; Rodrigues, T.Q.J.; Pesconi, A.D.R.; Carvalho, A.K.F.; Bento, H.B.S. Waste to Value: L-Asparaginase Production from Agro-Industrial Residues. *Processes* **2025**, *13*, 3088. <https://doi.org/10.3390/pr13103088>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

L-Asparaginase (L-asparaginase, l-asparagine amidohydrolase, EC 3.5.1.1) is an enzyme that catalyzes the hydrolysis of asparagine into aspartic acid and ammonia, which underpins its wide application in both the pharmaceutical and food industries. In healthcare, it is primarily used as a biopharmaceutical in the acute lymphoblastic leukemia therapy, where it depletes extracellular asparagine, leading tumor cells to death due to their inability to synthesize this amino acid through endogenous mechanisms [1–3]. In the food industry, its application is associated with the reduction in acrylamide formation, a carcinogenic compound generated during the Maillard reaction, particularly in starchy foods, through the interaction of reducing sugars and asparagine. Thus, this enzyme reduces the concentration of one of the reaction substrates and, consequently, acrylamide formation, representing a safe alternative for use in various food products such as potatoes, coffee and bread [4–6].

The L-asparaginase production occurs mainly through bioprocesses employing microorganisms such as *Escherichia coli*, *Erwinia chrysanthemi*, *Aspergillus oryzae* and *Aspergillus niger*. Bacteria are predominantly used for the production of this protein for pharmaceutical purposes, while fungal L-asparaginases are more commonly employed for applications as food supplements [1,6,7]. The use of these microorganisms is largely due to their ability to produce several complex biomolecules that can be applied in different industrial sectors, produced in high concentrations and with a great benefit–cost ratio. However, several factors, including the type of carbon and nitrogen source, pH, time and temperature, directly affect process yield [7,8].

Waste generation is one of the major challenges faced by the agro-industrial industry, with more than 2 billion tons produced annually worldwide, causing significant environmental impacts [9–12]. Addressing this issue requires alternatives that not only contribute to environmental sustainability but also enhance process feasibility. In this context, the biorefinery concept has gained prominence, as it enables the conversion of low-value by-products into high-value-added products throughout the production chain [9,10]. Beyond mitigating environmental impacts, this strategy also reduces production costs, thus combining economic feasibility with a waste-to-wealth approach [4,5].

As these residues are generally rich in sugars and other nutrients, microorganisms are able to metabolize them, making them viable alternatives for use in production processes. They represent low-cost and easily accessible substrates, which can help reduce overall process costs, although in some cases a pretreatment step is required [11–13]. Therefore, the use of such residues aligns with the concept of a circular economy, promoting the conversion of environmental liabilities into high-value-added products [12,14].

Various microorganisms, including bacteria, fungi and yeasts, have already been applied in the fermentation of agro-industrial residues to produce commercially relevant biocompounds, such as enzymes (e.g., chitinase, amylase, phytase), biosurfactants, and proteins [10,11]. This strategy can also be extended to the production of biopharmaceuticals, such as L-asparaginase, a high-value-added therapeutic protein. This approach can enhance production feasibility, which is often limited by high costs, while maximizing biomass utilization, reducing energy consumption [10]. Such a production model promotes sustainability and industrial process diversification, strengthening the bioeconomy and creating new technological opportunities [10,15]. Hence, the use of biorefineries for obtaining enzymes and other bioactive compounds establishes a bridge between technological innovation, circular economy, and public health, reinforcing the role of biotechnology in building a more sustainable future [11,12,16].

Given the potential of agro-industrial residues to produce L-asparaginase and reduce production costs, this study explored the available alternatives. The findings indicate

that production is feasible, yet several challenges remain. Addressing these challenges is essential to achieving industrial-scale implementation. The general concept can be seen in Figure 1.

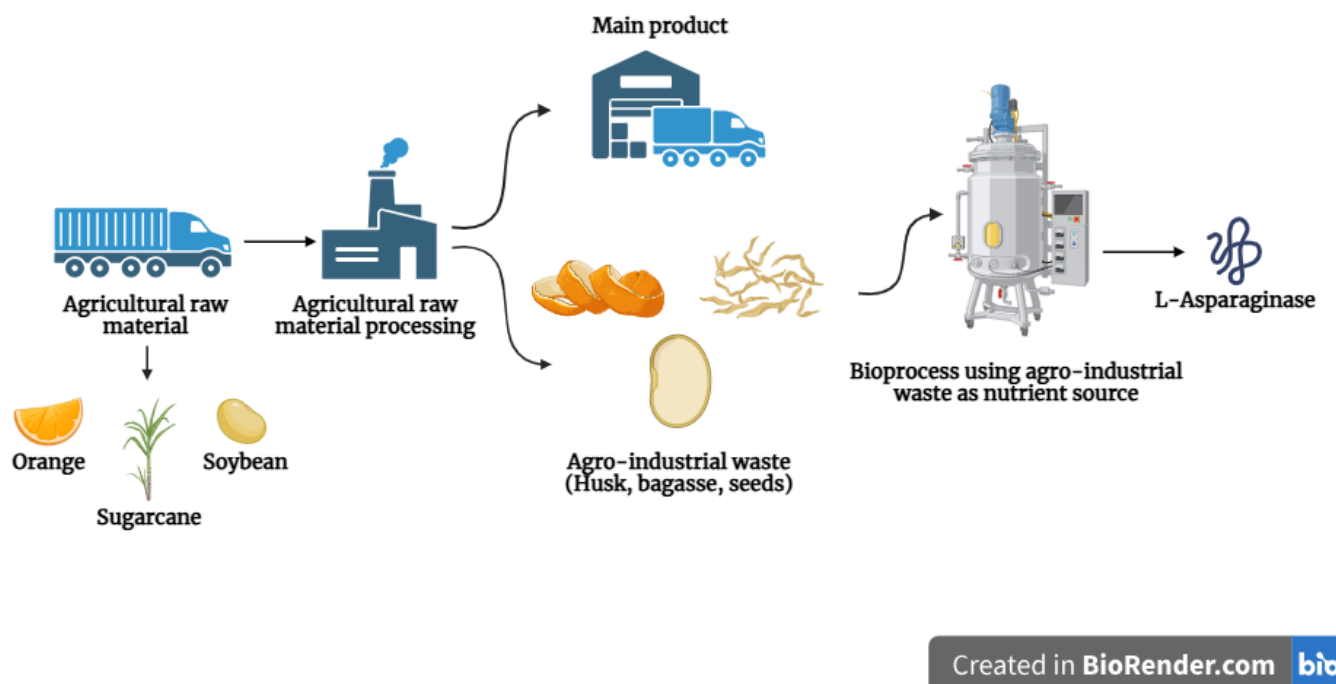


Figure 1. Overview of the use of agro-industrial waste for L-Asparaginase production.

2. Agro-Industrial Residues as Substrate

Agro-industrial residues have emerged as practical, low-cost feedstocks for microbial L-asparaginase production because they supply fermentable carbon, organic nitrogen, trace minerals, and, in some cases, endogenous asparagine while simultaneously valorizing waste streams. Recent syntheses emphasize that waste-based substrates can reduce medium costs, improve process sustainability, and align bioprocessing with circular-economy goals in both therapeutic and food applications of L-asparaginase [17–19]. Reviews focused on food safety also frame L-asparaginase as a mature mitigation tool for acrylamide formation, thereby motivating scalable, economical production routes that privilege cheap feedstocks. Together, these perspectives justify a residue-centric strategy for strain cultivation and process design [17–22].

Table 1 summarizes the agro-industrial residues explored for L-asparaginase production, indicating the producing microorganism, fermentation mode, pre-treatments, and reported activities.

In the context of asparaginase production, recent studies show that the spectrum of substrates is notably broad: wheat bran and legume brans were used as sole or mixed supports; oilseed cakes and corn gluten appear as nitrogenous co-substrates; and fruit/vegetable peels (papaya, carrot, onion, garlic, green pea) were assessed as basal media. Less conventional matrices include cactus cladode flours (*Opuntia/Nopalea*), a fruit-processing by-product (pitaya peel waste), a protein-rich insect biomass (*Tenebrio molitor*), and even agri-food by-product carbon sources such as cane molasses and glycerol pulses in photo-heterotrophic microalgal cultures where intracellular L-asparaginase activity is monitored. This diversity highlights how composition (carbohydrate/protein) and physical form (particulate solids vs. soluble syrups) jointly influence process choice (Solid State Fermentation vs. Submerged Fermentation, SSF vs. SmF) and performance [20–25].

Table 1. Agro-industrial residues applied as substrates for L-asparaginase production.

Residue	Microorganism	SSF/SmF	Pretreatment/ Supplementation	Best Results	Ref.
Wheat, arhar, kulthi brans	<i>Purpureocillium lilacinum</i>	SSF	RSM optimization	248.234 U/gds (optimized); 190.439 U/gds (main effects)	[21]
Soybean meal, corn gluten, groundnut de-oiled cake	<i>Aspergillus niger</i>	SSF	Ternary mixture; ANN optimization	141.45 ± 5.24 IU/gds	[20]
Pitaya peel waste	<i>Aspergillus niger</i>	SmF (induction)	Moisture optimization ~60%	≤0.6712 IU/mL	[23]
Papaya peel	<i>Aspergillus quadrilineatus</i>	SmF (screening)	Initial screening	≈2.99 U/mL	[22]
Carrot peel	<i>Aspergillus quadrilineatus</i>	SmF	Selected after screening for nutritional profile	Best in mass culture (not specified)	[22]
Defatted Tenebrio molitor biomass (insect)	<i>Penicillium</i> sp. LAMAI-505	SSF	Direct use of defatted biomass	≈2.75 U/g	[24]
Cane molasses/Glycerol	<i>Haematococcus pluvialis</i>	SmF/photo-heterotrophic culture	Carbon pulses (molasses or glycerol)	Intracellular qualitative changes	[25]
Cactus cladode flours (Opuntia/Nopalea)	<i>Aspergillus oryzae</i>	SSF	Low flour concentration (0.2% w/v), acidic pH	Not quantified	[26]

SSF: Solid-State Fermentation | SmF: Submerged Fermentation | RSM: Response Surface Methodology | ANN: Artificial Neural Network | gds: gram of dry substrate.

Nutritional characteristics of these residues underpin the observed yields. In SSF with *Aspergillus oryzae*, a two-level factorial design using Opuntia/Nopalea cladode flours showed that relatively low added flour (0.2% w/v), acidic pH, and modest inoculum supported L-asparaginase formation, consistent with these flours' modest nitrogen and carbon contents and amino-acid profiles (e.g., glutamate dominance), which collectively tune Carbon/Nitrogen (C/N) and buffering behavior [26]. In microalgal cultivation of *Haematococcus pluvialis*, pulses of cane molasses or glycerol (both agro-by-products) altered intracellular protein content and L-asparaginase activity during nitrogen starvation vs. enrichment, illustrating how carbon source type and C/N reprogram enzyme metabolism even outside classic fungal SSF [25]. Complementarily, Carbon–Nitrogen–Hydrogen–Sulfur (CHNS) characterization in SSF with *Aspergillus niger* linked lower medium C/N and higher protein supply (from soybean meal, corn gluten meal, groundnut de-oiled cake) to enhanced production, reinforcing nitrogen sufficiency as a primary lever on L-asparaginase biosynthesis [20].

Regarding fermentation mode, a pragmatic split is evident: SSF remains prevalent for particulate residues because it leverages the natural solid matrix and reduces free-water demand, while SmF is often chosen for soluble or pulped peels and for downstream enzyme purification. Reviews further note that, although industry frequently employs SmF for large-scale enzyme manufacture, L-asparaginase studies on agro-residues continue to exploit SSF to capitalize on mass-transfer advantages and the structural role of brans/peels as both support and substrate [17,19,26].

Operationally, moisture content and pH exert first-order effects in SSF, with inoculum size and sterilization practice as secondary drivers. An artificial neural network (ANN)-

guided optimization for SSF using a ternary blend of soybean meal, corn gluten meal, and groundnut de-oiled cake identified moisture as the most sensitive factor; the trained model projected an optimum of 70% moisture, $\text{pH} \approx 6.0$, and ~ 30 min autoclaving, which collectively maximized predicted L-asparaginase [20]. In a cactus-flour SSF, a factorial design converged on $\text{pH} 4.0$, 1% (v/v) inoculum, and 0.2% (w/v) flour as favorable, illustrating that optimal regimes can be highly matrix-specific and not necessarily neutral in pH [26]. Moisture targeting around 60% was likewise implicated in a pitaya-waste system with *A. niger*, underscoring moisture's recurrent centrality to oxygen diffusion and water activity in SSF beds [23].

Yield comparisons across studies show how substrate composition and process tailoring interact. In *A. niger* SSF driven by an ANN-assisted optimization, the model projected $\sim 141.45 \pm 5.24 \text{ IU gds}^{-1}$ at the optimum; sensitivity analysis ranked moisture > inoculum > pH , with model accuracy $R^2 \approx 0.99$ for training/validation [20]. Earlier SSF work with *Purpureocillium lilacinum* (formerly *Paecilomyces lilacinus*) on wheat, arhar, and kulthi brans reached higher magnitudes after response-surface refinement, achieving $\sim 248.234 \text{ U gds}^{-1}$, while main-effect studies recorded $\sim 190.439 \text{ U gds}^{-1}$ —benchmarks that remain competitive among waste-based systems [21]. In contrast, a pitaya-peel system used as an inducer with *A. niger* under optimized conditions yielded $\leq 0.6712 \text{ IU mL}^{-1}$, reflecting distinct titer units and a submerged/induction context rather than classic granular SSF [23].

Vegetable peels illustrate how screening choice guides downstream process mode. In submerged screening of multiple peels and agro-wastes for *Aspergillus quadrilineatus*, papaya peel originally gave the highest measured activity ($\approx 2.99 \text{ U mL}^{-1}$), yet the authors subsequently selected carrot peel for mass culture and reported it as best under their SmF conditions, citing nutritional profile, availability, and cost as deciding factors. This underscores that the “best” substrate can be context-dependent, changing between exploratory screening and scaled purification trials [22].

Protein-rich non-plant matrices offer a complementary route. An SSF employing *Penicillium* sp. LAMAI-505 on defatted *Tenebrio molitor* biomass (a circular by-product from insect farming) co-produced a biosurfactant and L-asparaginase, with a reported maximum of $\sim 2.75 \text{ U g}^{-1}$ for the enzyme—proof-of-concept that emergent agro-food side streams can function as nitrogenous scaffolds for enzyme production [24].

Case studies of mixed substrates further support synergy between carbon and nitrogen sources. In SSF, combinations of cereal brans and oilseed meals frequently outperform single substrates, because blends improve asparagine availability, moisture retention, and porosity. Reviews synthesize examples where ternary mixtures (e.g., soybean meal–cottonseed meal–wheat bran) outperform any single component, and where passion-fruit peel flour drives particularly high activities with *A. niger*—both trends that argue for mixture-design approaches in residue selection [17,19,22].

From a process-engineering perspective, some studies highlight that optimization methodology matters as much as substrate choice. Data-driven tools (ANNs) captured nonlinear moisture–biomass–activity interactions on mixed meal substrates; classical factorial/RSM designs delineated pH and inoculum windows for cactus flours; and knowledge syntheses call attention to algorithmic and AI-assisted strategies to shorten optimization cycles for waste-based L-asparaginase bioprocesses [17,20,26].

Finally, when positioning residue-based processes for food applications (e.g., acrylamide mitigation), the literature converges on two themes: (i) the enzyme's efficacy is established across diverse foods and (ii) industrial adoption will benefit from robust, scalable, and economical production—precisely what agro-residue routes target. Reports compile conventional mitigation tactics alongside enzymatic strategies, reinforcing the unique breadth of L-asparaginase and the need for supply at cost. The combination of

demonstrated efficacy in food matrices and the environmental co-benefits of waste valorization provides a coherent rationale to prioritize residue-based media in L-asparaginase bioprocess design going forward [17,18].

3. Microbial Sources for L-Asparaginase Production

Currently, the primary microorganisms associated with the commercial production of L-asparaginase are *Escherichia coli* and *Erwinia chrysanthemi*, which are recognized for their high potential in medical treatments, such as first-line therapies [27]. However, various other microbial sources, including filamentous fungi, yeasts, archaea, bacteria and actinomycetes, have also been reported as promising for enzyme production. This is due to their ease of handling, maintenance, rapid production and recovery, and high scalability [6,28]. Table 2 presents examples of L-asparaginase-producing microorganisms, induced by L-asparagine, along with their respective enzymatic activities and optimal operating conditions.

At present, 95.5% of the L-asparaginase sequences deposited in the National Center for Biotechnology Information (NCBI) are attributed to bacteria. The remaining organisms represent a smaller fraction, distributed among fungi (1.68%), animals (1.25%), plants (0.24%), archaea (0.88%), and viruses (<0.01%). Nevertheless, the enzymes secreted by these groups exhibit distinct and potentially attractive characteristics for various applications [29]. The activity data and optimal temperature and pH conditions for the L-asparaginases listed in Table 2 highlight this, showing significant heterogeneity in these properties, both among microorganisms from different taxonomic groups and among species of the same genus.

Other characteristics such as molecular mass, oligomerization state, and thermal stability are also associated with the microbial species of origin. In this context, the screening for new microorganisms and the characterization of their enzymes are fundamental steps for the development of new and improved applications, whether in medicine, biotechnology, or the food industry [30,31]. Understanding the characteristics of already available enzymes is the first step.

Table 2 illustrates the remarkable heterogeneity of reported L-asparaginase activities across microbial groups, with values ranging from less than 1 U/mL in some screening studies to more than 1000 U/mL or U/mg under optimized bacterial and fungal systems. This broad variation underscores how enzyme performance is strongly dependent not only on the microbial source but also on cultivation conditions and measurement units, which complicates direct comparisons. Nevertheless, Table 2 highlights clear benchmarks—such as the high titers achieved by optimized *Aspergillus* strains and certain bacterial isolates—that can serve as reference points for future process development. At the same time, the modest activities observed in less conventional microorganisms indicate opportunities for improvement through bioprospecting, strain engineering, or tailored fermentation strategies.

Although a wide range of microorganisms has been reported as potential sources of L-asparaginase, their suitability for large-scale or biorefinery applications is not uniform. Archaeal enzymes are valued for thermostability but may require specific operational conditions that limit broader use. Actinomycetes show high diversity, yet only part of the strains effectively produce the enzyme. Bacteria remain the most explored, with *E. coli* and *Erwinia chrysanthemi* being commercial references, but their application is restricted by immunological reactions. Fungi and yeasts offer advantages such as extracellular secretion and growth on low-cost substrates, though they depend on defined cultivation conditions and may face variability when agro-industrial residues are used. These aspects, along with further constraints and suitability issues, will be discussed in more detail in the following Sections 3.1–3.5, where specific references and examples are provided.

Table 2. Examples of L-asparaginase-producing microorganisms.

Class	Microorganism	Reactor	Optimal Conditions	Activity/ Specific Activity	Ref.
Archaea	<i>Thermococcus sibiricus</i> *	Shaker	90 °C and pH 9.0	83.361 U/mL	[32]
	<i>Pyrococcus</i> * <i>furiosus</i>	Shaker	50 °C and pH 8.0	12,321.4 U/mL	[33]
	<i>Pyrococcus abyssi</i> *	Shaker	80 °C and pH 8.0	1175 U/mg	[34]
Actinomycetes	<i>Streptomyces koyangensis</i> (SK4)	Shaker	-	136 U/mL	[35]
	<i>Streptomyces gulbargensis</i>	Shaker	40 °C and pH 9.0	3.23 U/mL	[30]
Bacteria	<i>Bacillus paralicheniformis</i> (AUMC B-516)	Shaker	35 °C and pH 8.0	116.4 U/mL	[36]
	<i>Bacillus licheniformis</i> (ASN51)	Shaker	37 °C and pH 8.0	499 U/mg	[37]
	<i>Escherichia coli</i> (MF-107)	Shaker	35 °C and pH 7.5–8.0	9.16 U/mg	[38]
	<i>Pectobacterium carotovorum</i>	Shaker	37 °C and pH 8.7	20 U/mL	[39]
	<i>Pseudomonas aeruginosa</i>	SSF	37 °C and pH 7.4	1900 IU/mg	[40]
	<i>Sphingomonas leidy</i> (VN01)	Shaker	37 °C	156 IU/mg	[41]
	<i>Zymomonas mobilis</i> (CP4)	Shaker	30 °C	16.55 IU/L	[42]
Yeast	<i>Trichoderma viride</i>	Shaker	37 °C and pH 7.5	71.3 U/mL	[43]
	<i>Meyerozyma guilliermondii</i>	Shaker	37 °C and pH 7.0	26.01 U/mL	[44]
	<i>Candida utilis</i> (ATCC 9950)	Fermenter (Batch)		245.6 U/mL	[45]
	<i>Saccharomyces cerevisiae</i> *	Shaker	40 °C and pH 8.6	196.2 U/mg	[46]
	<i>Leucosporidium scottii</i> (L115)	Shaker	-	178.1 U/gdcw ⁻¹	[47]
	<i>Lachancea thermotolerans</i>	Shaker	37 °C and pH 8.6	313.8 U/mg	[48]
	<i>Cyberlindnera subsufficiens</i> (GULAMMS8)	Shaker	-	57.54 U/mL	[49]
Fungi	<i>Meyerozyma guilliermondii</i>	Shaker	37 °C and pH 7.0	26.01 U/mL	[44]
	<i>Aspergillus oryzae</i> (IOC 3999)	Shaker	60 °C and pH 5.0	1443.57 U/mL	[50]
	<i>Aspergillus niger</i> (INCQS 40018)	Shaker	40 °C and pH 5.0	0.6712 U/mL	[23]
	<i>Penicillium brevicompactum</i> (NRC 829)	Shaker	37 °C and pH 8.0	132.4 U/mg	[51]
	<i>Cladosporium</i> sp.	Shaker	-	255–428 U/mL	[52]
	<i>Aspergillus caespitosus</i> ; <i>Aspergillus oryzae</i>	Shaker	-	0.0249 and 0.0139 U/mL	[18]
	<i>Fusarium equiseti</i> (AHMF4)	Shaker	-	40.78 U/mL	[53]
	<i>Penicillium sizovae</i> (2DSST1) and <i>Fusarium proliferatum</i> (DCFS10)	Shaker	-	3.68 and 1.86 U/mL	[54]
	<i>Aspergillus sydowii</i> and <i>Fusarium oxysporum</i>	Shaker	-	146 and 143 U/mL	[55]
	<i>Aspergillus niger</i>	SSF	50 °C and pH 9.0	187.19 U/mg	[56]
	<i>Fereydownia khargensis</i> (IBRC-M 30116)	Shaker	-	61.3 U/mL	[57]
	<i>Aspergillus oryzae</i>	SSF	-	16.122 U/g	[58]
	<i>Aspergillus caespitosus</i> (CCDCA 11593)	SSF	-	2.75 U/mL	[59]

* Recombinant. Expressed in *E. coli*. | SSF: Solid-State Fermentation.

Figure 2 summarizes the pros and cons of each microorganism class.

	Pros	Cons
Archaea	• May exhibit thermostability and tolerance to extreme conditions	• Potential cytotoxicity • Require extreme cultivation conditions • Little industrial experience and regulatory framework
Actinomycetes	• Generally extracellular enzyme • Many reported glutaminase-free L-Asparaginase	• Cytotoxicity risk
Bacteria	• Ease of scaling up • Relatively easier genetic engineering	• Hypersensitivity and immune inactivation • Intracellular enzyme • Possible endotoxin contamination
Fungi and Yeasts	• Expect to cause less immunogenicity • Can grow in a wide variety of agricultural by-products	• Scale-up can be challenging • Variability in production when using agro-industrial residues

Figure 2. Pros and cons of each microorganism class producer of L-Asparaginase.

3.1. Archaea

Archaeal organisms stand out for the wide diversity of proteins they produce and for being safe, as there are no records of pathogenic species in this group. Among those capable of synthesizing L-asparaginase, the genera *Pyrococcus* and *Thermococcus* are noteworthy [60]. Most available studies to date indicate that L-asparaginases from archaea can be divided into five different families (Asp1, Asp2, IaaA, Asp2like1 and Asplike2), with the first three similar to those found in bacteria [61]. The asparaginases found in *P. horikoshii*, *P. furiosus* and *T. kodakarensis* did not show lateral glutaminase activity, a desirable characteristic in clinical applications and a differentiator from other microbial sources. Furthermore, many enzymes produced by archaea exhibit thermostability and tolerance to extreme conditions, making them particularly suitable for biotechnological processes, especially in the food industry [62].

3.2. Actinomycetes

Actinomycetes are still underexplored regarding the production of L-asparaginases (ASNases), with the genus *Streptomyces* as the main exception. The first description of this enzymatic activity for the group occurred in the 1970s, with its identification in *S. griseus*. Since then, various species of the genus have been investigated, including *S. gulbargensis* [30], *S. albidoflavus* [63], *S. rochei* [64], and *S. koyangensis* [31]. Despite extensive investigation, not all *Streptomyces* species are capable of producing the enzyme. Rath et al. (2023) [65] evaluated 28 strains and observed ASNase activity in only 12 of them. Other genera of producing actinomycetes include *Actinomyces* sp., *Thermoactinomyces* sp., and *Nocardia* sp. [7].

3.3. Bacteria

Bacteria remain the main sources, in terms of quantity, of ASNases and are also the most extensively studied. It is now known that both Gram-positive and Gram-negative bacteria are capable of synthesizing the enzyme. While Gram-negative bacteria have received more attention, producing type I (cytosolic) L-asparaginases, Gram-positive bacteria have the advantage of secreting the enzyme, facilitating its application in industrial processes

and reducing purification costs [66,67]. The family of greatest interest is *Enterobacteriaceae*, represented by *Escherichia coli* and *Erwinia chrysanthemi*, which are currently used for commercial purposes [68]. However, these enzymes are often associated with immunological reactions [69]. Bioprospecting studies continue to reveal novel bacterial sources with high potential, such as *Sphingomonas leidyi*, which demonstrated a high enzymatic activity of 156 IU/mg [41].

3.4. Fungi and Yeasts

Fungi represent the second-largest group of L-asparaginase producers and are considered potential substitutes for bacteria due to high production yields, the ability to grow on low-cost substrates, and the production of extracellular enzymes. Furthermore, several fungal species are considered safe (GRAS), presenting little to no immunogenicity. Currently, the industrial production of L-asparaginase by fungi is mainly carried out through submerged fermentation; however, solid-state fermentation offers advantages such as the use of agro-industrial by-products and the generation of smaller volumes of waste [59,70]. Among the most common producing microorganisms are the genera *Aspergillus* sp. and *Penicillium* sp., whose optimal pH and temperature conditions range from 6.0 to 9.5 and from 30 to 50 °C, respectively. As for yeasts, various genera including *Saccharomyces* sp., *Candida* sp., *Pichia* sp., *Rhodotorula* sp., *Rhodospiridium* sp., and *Trichoderma* sp. also exhibit ASNase activity [71].

3.5. Advances in Protein Engineering and Bioprospecting

Given the growing demand for L-asparaginase and the limited yields of conventional methodologies, microbial protein engineering is emerging as a promising strategy, enabling industrial-scale production with increased efficiency and reduced costs. Recent studies demonstrate that both the optimization of cultivation conditions [72] and the genetic improvement of microorganisms result in significant production gains, reaching up to thousands of units per milliliter. Furthermore, the use of combined methodologies of rational design and directed evolution has allowed the development of L-asparaginase variants with greater thermal stability, enhanced specificity, and lower immunogenicity, which are essential for industrial applications [5,73].

4. Perspectives on L-Asparaginase Production Using Agro-Industrial Residues

This final section will examine the main trends and perspectives that may influence the future of L-asparaginase production from residues from the agro-industry. The focus is on how technological advances, environmental considerations, and regulatory factors intersect to create both opportunities and obstacles. To frame this discussion, we will first present an overview of emerging technologies and prospects in this field, followed by a SWOT analysis that critically assesses the strengths, weaknesses, opportunities, and threats associated with this strategy.

4.1. Technological and Sustainability Trends

In the context of biopharmaceutical production from renewable resources, it is essential to recognize recent progress and anticipate the directions that may guide future research and industrial implementation. Current developments indicate a movement toward sustainable practices, the adoption of emerging bioprocess technologies, and the expansion of application niches, while significant regulatory and technical hurdles persist. Table 3 summarizes these trends and perspectives, providing a concise overview of the opportunities and barriers that are likely to influence the next steps in L-asparaginase production from agro-industrial residues.

Table 3. Trends and perspectives in L-asparaginase production using agro-industrial residues, highlighting sustainability, technological advances, applications, and regulatory challenges.

Category	Trends and Perspectives	Potential Impacts	Ref.
Sustainability and green bioprocesses	Development of processes with low energy consumption, sustainable solvents, and reuse of inputs.	Cost reduction, lower environmental impact, and contribution to the circular economy. Increased industrial feasibility and compliance with environmental regulations.	[17,18,23,74]
Funding and public policies	Incentives for bioeconomy projects and financing of clean technologies at national and international levels.	Greater investment attractiveness and acceleration of industrial implementation.	[75]
Emerging technologies	Integration with advanced bioreactors (e.g., continuous), automation, and omics-based tools.	Better process control, improved scalability, and real-time optimization.	[67,76]
Metabolic engineering and bioprospection	Use of novel bioprospected or genetically modified microorganisms and robust recombinant systems to enhance enzyme yield from residues.	Higher productivity and improved efficiency in the utilization of heterogeneous substrates.	[8,66,77–79]
AI and digitalization	Application of AI, machine learning, big data, and digital twins to optimize media formulation, predict yields, and control processes.	Reduced R&D time and costs, more robust processes, and faster scale-up.	[77,80,81]
Integration into biorefineries	Combined production of L-asparaginase, bioenergy, enzymes, and biopolymers within multiproduct platforms.	Product diversification and greater economic valorization of residues.	[79,82,83]
Stability and formulation	Use of biocompatible additives, encapsulation, and stabilization technologies for residue-derived L-asparaginase.	Improved stability, longer shelf life, and expanded possibilities for industrial use.	[4,77,84–86]
Expanded applications	Extension of applications beyond pharmaceuticals, including the food industry (acrylamide reduction) and biotechnology.	Market expansion and broader commercial potential for the enzyme.	[29,87,88]
Regulation and safety	Increasing demand for traceability and validation in the use of residues as substrates.	Significant regulatory barriers, but opportunities for green and sustainable certification.	[75,89]

The use of agro-industrial residues as feedstocks for L-asparaginase production aligns with current efforts to promote sustainable bioprocesses and the principles of the circular economy [17,23,74]. For instance, production costs can be reduced by employing materials such as sugarcane bagasse, rice husks, or soybean cakes while simultaneously addressing waste disposal and environmental concerns [18]. This approach not only supports global sustainability agendas but also benefits from public policies and funding initiatives that encourage the development of bioeconomy projects, particularly in countries with strong agricultural sectors [75].

Technological progress has also played a decisive role in making this strategy more feasible. The adoption of energy-efficient operations and solvent recycling contributes to greener processes, while advances in bioreactor design, automation, and omics-based monitoring improve control and scalability [67,76,77,90]. For example, researchers have

been bioprospecting or engineering novel microorganisms with higher resistance and productivity to overcome the variability of complex substrates [8,66,78,79]. In parallel, new formulations of L-asparaginase, such as PEGylated variants, additives, encapsulated systems, and nano-based carriers, have been developed to improve stability, reduce immunogenicity, and extend therapeutic half-life [4,77,84]. Moreover, it is possible to predict yields and optimize conditions more effectively with artificial intelligence (AI) and other digital tools. Together, these developments point toward more robust and integrated production systems [77,80,81].

4.2. Regulatory Challenges and SWOT Analysis

L-asparaginase is beginning to find uses that go beyond its established role in oncology. One of the most promising areas is the food industry, where it can reduce acrylamide levels in baked or fried products, responding to demands for healthier and safer foods [29,87]. There is also growing interest in industrial biotechnology applications, such as in the biosynthesis of fine chemicals, which expands the potential market [88]. In parallel, its integration into multiproduct biorefineries provides opportunities to generate L-asparaginase alongside bioenergy, enzymes, and biopolymers, improving the economic return of agro-residue utilization [79,82,83].

Despite these advances, regulatory hurdles persist. Agricultural residues are heterogeneous in origin and composition, raising concerns about traceability, contaminants, and product consistency. These are critical issues for biopharmaceutical and food production, as they also involve the possibility of irritation, allergenicity, or other adverse effects, demanding rigorous assessment and validation prior to approval [89]. On the other hand, sustainability-oriented policies create new opportunities. Renewable energy programs, national waste management strategies and international agreements encourage the valorization of agricultural residues. In this context, assigning producers responsibility for the entire life cycle of their products and public–private partnerships can foster the adoption of green biorefineries, supporting eco-labeling and certifications that mitigate regulatory barriers, add market value, and enhance global acceptance of residue-derived enzymes [75].

Given the growing interest in sustainable biopharmaceutical production and the potential of agro-industrial residues as low-cost substrates, it becomes essential that we critically assess the advantages and limitations of this approach. Hence, Figure 3 summarizes this evaluation through a SWOT analysis of L-asparaginase production using agro-industrial residues, outlining the variables that influence the feasibility of its industrial application.

The strengths demonstrate the economic and environmental appeal of using agro-industrial residues. Their low cost and wide availability help to reduce raw material expenses, while their valorization supports sustainability and circular economy goals [18,91,92]. This advantage is particularly evident in countries with strong agricultural industries. In Brazil, for instance, sugarcane bagasse and soybean cakes are produced in vast amounts [92,93], while, in India and Southeast Asia, rice husks, wheat bran and palm oil residues represent major by-products of staple crops [94,95]. China also generates substantial volumes of maize stover and wheat residues, whereas, in Europe, wine-producing countries such as France, Italy, and Spain yield large amounts of grape pomace [94,96]. These diverse feedstocks allow multiple opportunities for integration into local bioprocesses, and in many regions, regulatory and social initiatives support their use to promote bioeconomy and waste valorization.

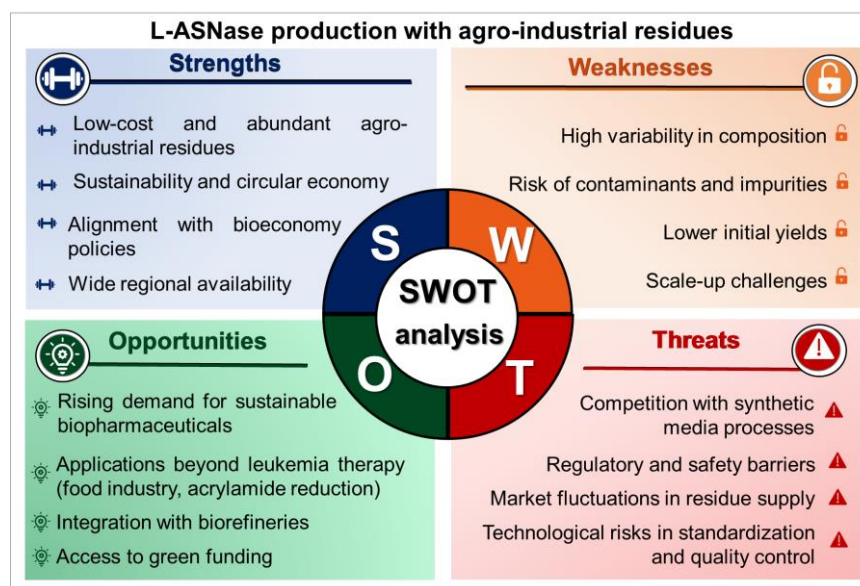


Figure 3. SWOT analysis of L-asparaginase production from agro-industrial residues, showing key strengths, weaknesses, opportunities, and threats of this sustainable strategy.

Weaknesses, however, reveal the complexity of translating these advantages into consistent industrial practice [94,97,98]. Residues vary considerably in chemical composition depending on crop, harvest, and processing methods, which complicates process reproducibility [94]. Contaminants such as pesticide residues or mycotoxins present additional risks, potentially reducing microbial growth efficiency or compromising enzyme safety. Productivity tends to be lower when compared to synthetic media optimized for yield, often requiring pretreatments, supplementation, and careful process engineering to achieve competitive results. Scaling up adds another layer of difficulty, as ensuring homogeneity, sterilization, and a steady industrial supply chain for heterogeneous residues is far from trivial [94,97].

Opportunities arise from broader global trends. The rising demand for sustainable and environmentally responsible biopharmaceuticals strengthens the case for processes based on renewable substrates [99,100]. L-asparaginase, beyond its central role in leukemia treatment, has a growing potential in the food industry, particularly in reducing acrylamide levels during baking and frying [101]. Integration into biorefineries represents another promising path, where residues can be processed simultaneously for the production of bioenergy, biopolymers, and enzymes, creating more resilient and efficient value chains [79,82]. Moreover, the availability of green funding and sustainability-oriented innovation programs, particularly in the European Union, North America, and parts of Asia, may provide financial and policy incentives that accelerate industrial uptake [75].

At the same time, threats remain significant. Conventional production in synthetic media is already well established, offering stable yields and predictable quality [94,97]. Regulatory and safety challenges may also create barriers since the use of heterogeneous residues in biopharmaceutical production raises strict requirements for traceability and control [89]. Market fluctuations in residue supply (linked to agricultural cycles, climate variability, or trade dynamics) pose additional risks to process stability. Finally, technological bottlenecks, particularly the need for robust methods to scale up production, standardize raw materials, and ensure consistent enzyme quality, may delay industrial translation [94,97].

Thus, this SWOT analysis suggests that, while agro-industrial residues offer a promising and sustainable pathway for L-asparaginase production, their successful adoption

depends on overcoming variability, safety concerns, and regulatory hurdles. Building reliable supply chains and developing scalable, standardized technologies will be crucial for this strategy to compete with conventional processes on a global scale.

Overall, the trends and perspectives discussed here suggest that producing L-asparaginase from agro-industrial residues offers real potential but also significant hurdles. Technological advances and new applications add momentum, while sustainability policies and funding opportunities create favorable conditions for development. At the same time, variability of raw materials, process control, and regulatory approval remain decisive for scaling up.

5. Conclusions

L-asparaginase production from agro-industrial residues has advanced from isolated trials to consistent demonstrations of feasibility, showing that low-cost substrates such as brans, oilseed cakes, fruit peels, and even insect biomass can sustain enzyme yields competitive with synthetic media. These studies confirm that residue-based processes not only reduce production costs but also align with global sustainability agendas, integrating waste valorization with the development of high-value therapeutic and industrial enzymes.

Emerging approaches are reshaping this field by expanding both the microbial spectrum and the technological tools available. Novel fungal and bacterial isolates, rationally engineered strains, and protein variants with enhanced stability and reduced immunogenicity are being combined with process innovations such as solid-state fermentation optimization, machine learning-guided design, and biorefinery integration. In parallel, applications of L-asparaginase are diversifying: while its established role in leukemia therapy still drives most research, its validated capacity to mitigate acrylamide in foods is creating a strong incentive for industrial-scale production from renewable substrates.

Despite these advances, significant challenges remain. Variability in residue composition, risks of contaminants, and difficulties in scaling up bioprocesses complicate reproducibility and regulatory approval. For pharmaceutical use, rigorous standards of purity, safety, and traceability must be met; for food and biotechnology applications, consistent performance across heterogeneous feedstocks is essential. Addressing these hurdles through standardized pretreatments, harmonized regulations, and robust supply chains will be decisive for transforming L-asparaginase production from agro-industrial residues into a competitive industrial reality.

Author Contributions: Conceptualization, H.B.S.B.; writing—original draft preparation, E.C., B.C.G., N.V.P.V., T.Q.J.R., A.D.R.P., A.K.F.C. and H.B.S.B.; writing—review and editing, E.C., B.C.G., N.V.P.V., T.Q.J.R., A.D.R.P., A.K.F.C. and H.B.S.B.; supervision, H.B.S.B. All authors have read and agreed to the published version of the manuscript.

Funding: Nathalia thanks the PRPI USP (process 20.1.09345.01.2). Enzo thanks FAPESP (2023/14606-0 and 2024/17115-0), and Ana Karine thanks CNPq (process n° 420432/2023-0).

Data Availability Statement: No new data were created or analyzed in this study.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

gds	Grams of dry substrate
gdcw	Grams of dry cell weight
SSF	Solid State Fermentation
SmF	Submerged Fermentation

References

1. Maese, L.; Rau, R.E. Current Use of Asparaginase in Acute Lymphoblastic Leukemia/Lymphoblastic Lymphoma. *Front. Pediatr.* **2022**, *10*, 902117. [\[CrossRef\]](#) [\[PubMed\]](#)
2. Darvishi, F.; Jahanafrooz, Z.; Mokhtarzadeh, A. Microbial L-asparaginase as a promising enzyme for treatment of various cancers. *App. Microb. Biotech.* **2022**, *106*, 5335–5347. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Pérez, M.T.; Belén, L.H.; Leteller, P.; Calle, Y.; Pessoa, A.; Fariás, J.G. L-Asparaginase as the gold standard in the treatment of acute lymphoblastic leukemia: A comprehensive review. *Med. Oncol.* **2023**, *40*, 150. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Jana, A.; Biswas, S.; Ghosh, R.; Modak, R. Recent advances in L-asparaginase enzyme production and formulation development for acrylamide reduction during food processing. *Food Chem. X* **2024**, *25*, 102055. [\[CrossRef\]](#)
5. Shishparenok, A.N.; Gladilina, Y.A.; Zhdanov, D.D. Engineering and Expression Strategies for Optimization of L-asparaginase Development and Production. *Int. J. Mol. Sci.* **2023**, *24*, 15220. [\[CrossRef\]](#)
6. Suresh, S.A.; Ethiraj, S.; Rajnish, K.N. A systematic review of recent trends in research on therapeutically significant l-asparaginase and acute lymphoblastic leukemia. *Mol. Biol. Rep.* **2022**, *49*, 11281–11287. [\[CrossRef\]](#)
7. Hosseini, K.; Zivari-Ghader, T.; Akbarzadehlaleh, P.; Ebrahimi, V.; Sharafabad, B.E.; Dilmaghanit, A. A Comprehensive Review of L-asparaginase: Production, Applications and Therapeutic Potential in Cancer Treatment. *Appl. Biochem. Microbiol.* **2024**, *60*, 599–613. [\[CrossRef\]](#)
8. Castro, D.; Marques, A.S.C.; Almeida, M.R.; Paiva, G.B.; Bento, H.B.S.; Pedrolli, D.B.; Freire, M.G.; Tavares, A.P.M.; Santos-Ebinuma, V.C. L-asparaginase production review: Bioprocess design and biochemical characteristics. *Appl. Microbiol. Biotechnol.* **2021**, *105*, 4515–4534. [\[CrossRef\]](#)
9. Lemes, A.C.; Egea, M.B.; Filho, J.G.O.; Gautério, G.V.; Ribeiro, B.D.; Coelho, M.A.Z. Biological Approaches for Extraction of Bioactive Compounds From Agro-industrial By-products: A Review. *Front. Bioeng. Biotechnol.* **2022**, *9*, 802543. [\[CrossRef\]](#)
10. Singh, R.; Das, R.; Sangwan, S.; Rohatgi, B.; Khanam, R.; Peera, S.K.P.G.; Das, S.; Lyngdoh, Y.A.; Langyan, S.; Shukla, A.; et al. Utilisation of agro-industrial waste for sustainable green production: A review. *Environ. Sustain.* **2021**, *4*, 619–636. [\[CrossRef\]](#)
11. Vućurović, D.; Bajić, B.; Trivunović, Z.; Dodić, J.; Zeljko, M.; Jevtić-Mučibabić, R.; Dodić, S. Biotechnological Utilization of Agro-Industrial Residues and By-Products—Sustainable Production of Biosurfactants. *Foods* **2024**, *13*, 711. [\[CrossRef\]](#)
12. Silva, S.O.; Mafra, A.K.C.; Pelissari, F.M.; de Lemos, L.R.; Molina, G. Biotechnology in Agro-Industry: Valorization of Agricultural Wastes, By-Products and Sustainable Practices. *Microorganisms* **2025**, *13*, 1789. [\[CrossRef\]](#)
13. Bala, S.; Garg, D.; Sridhar, K.; Inbaraj, B.S.; Singh, R.; Kamma, S.; Tripathi, M.; Sharma, M. Transformation of Agro-Waste into Value-Added Bioproducts and Bioactive Compounds: Micro/Nano Formulations and Application in the Agri-Food-Pharma Sector. *Bioengineering* **2023**, *10*, 152. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Martău, G.-A.; Odocheanu, R.; Dudu, A.I.; Dulf, F.V.; Toşa, M.I.; Vodnar, D.C. Towards circular economy: Agro-industrial by-products enabling in situ enzyme production for food and pharmaceutical applications. *Sustain. Chem. Pharm.* **2025**, *44*, 101974. [\[CrossRef\]](#)
15. Liu, T.; He, M.; Shi, R.; Yin, H.; Luo, W. Biofuel–Pharmaceutical Co-Production in Integrated Biorefineries: Strategies, Challenges, and Sustainability. *Fermentation* **2025**, *11*, 312. [\[CrossRef\]](#)
16. Nadar, C.G.; Fletcher, A.; de Almeida Moreira, B.R.; Hine, D.; Yadav, S. Waste to protein: A systematic review of a century of advancement in microbial fermentation of agro-industrial byproducts. *Compr. Rev. Food Sci. Food Saf.* **2024**, *23*, e13375. [\[CrossRef\]](#)
17. Mudaliar, S.; Kumar, V.; Verma, P. Bioprospecting of microbial L-asparaginase: Sustainable production from waste and its application for acrylamide reduction in food industry. *Process. Saf. Environ. Prot.* **2025**, *195*, 106737. [\[CrossRef\]](#)
18. Fernandes, M.L.P.; Veríssimo, L.A.A.; de Souza, A.C.; Schwan, R.F.; Dias, D.R. Low-Cost Agro-Industrial Sources as a Substrate for the Production of L-asparaginase Using Filamentous Fungi. *Biocatal. Agric. Biotechnol.* **2021**, *34*, 102037. [\[CrossRef\]](#)
19. Raina, D.; Kumar, V.; Saran, S. A critical review on exploitation of agro-industrial biomass as substrates for the therapeutic microbial enzymes production and implemented protein purification techniques. *Chemosphere* **2022**, *294*, 133712. [\[CrossRef\]](#)
20. Sharma, D.; Mishra, A. Synergistic effects of ternary mixture formulation and process parameters optimization in a sequential approach for enhanced L-asparaginase production using agro-industrial wastes. *Environ. Sci. Pollut. Res.* **2023**, *31*, 17858–17873. [\[CrossRef\]](#)
21. Vimal, A.; Kumar, A. Optimized Production of Medically Significant Enzyme L-Asparaginase Under Submerged and Solid-State Fermentation From Agricultural Wastes. *Curr. Microbiol.* **2022**, *79*, 394. [\[CrossRef\]](#)
22. Acharya, R.; Kanungo, T.; Gupta, N. Screening and Purification of L-asparaginase Production by *Aspergillus quadrilineatus* Using Agro Wastes and Vegetable Peels. *Curr. Trends Biotechnol. Pharm.* **2025**, *19*, 2322–2329. [\[CrossRef\]](#)
23. Honfoga, J.N.B.; Nascimento, P.A.; Ferreira, A.N.; da Silva, E.C.; Bauer, L.C.; Teixeira, J.M.; Santana, N.B.; Bonomo, R.C.F. Production and characterization of L-asparaginase by *Aspergillus niger*: A sustainable use of pitaya (*Hylocereus* spp.) by-products. *Sustain. Chem. Environ.* **2025**, *10*, 100255. [\[CrossRef\]](#)

24. Correa, H.T.; Vieira, W.F.; Pessoa, A., Jr.; Cardoso, V.L.; Coutinho Filho, U. Production of Biosurfactant and L-Asparaginase Using Dry *Tenebrio molitor* Larvae as Innovative Biomass for Fermentation and Liquid Extractive Process. *Waste Biomass Valorization* **2023**, *14*, 4061–4069. [CrossRef]
25. De Moraes, L.B.S.; Mota, G.C.P.; Molina-Miras, A.; Sánchez-Mirón, A.; del Carmen Cerón-García, M.; Gálvez, A.O.; de Souza Bezerra, R.; García-Camacho, F. Enhancing high-value metabolite production and asparaginase activity in *Haematococcus pluvialis*: Impact of different organic carbon sources and feeding strategies. *Biomass Convers. Biorefin.* **2025**. [CrossRef]
26. Da Silva, L.F.; de Pádua, A.P.S.L.; de Oliveira Ferro, L.; Agamez-Montalvo, G.S.; Bezerra, J.D.P.; Moreira, K.A.; de Souza-Motta, C.M. Cacti as low-cost substrates to produce L-asparaginase by endophytic fungi. *World J. Microbiol. Biotechnol.* **2022**, *38*, 247. [CrossRef]
27. Tong, W.H.; Rizzari, C. Back to the future: The amazing journey of the therapeutic anti-leukemia enzyme asparaginase *Erwinia chrysanthemi*. *Haematologica* **2023**, *108*, 2606–2615. [CrossRef]
28. Alam, S.; Pranaw, K.; Tiwari, R.; Khare, S.K. *Green Bio-Processes Enzymes in Industrial Food Processing*; Springer: Singapore, 2019; pp. 55–81. [CrossRef]
29. Da Cunha, M.C.; dos Santos Aguilar, J.G.; de Melo, R.R.; Nagamatsu, S.T.; Ali, F.; de Castro, R.J.S.; Sato, H.H. Fungal L-asparaginase: Strategies for Production and Food Applications. *Food Res. Int.* **2019**, *126*, 108658. [CrossRef]
30. Amena, S.; Vishalakshi, N.; Prabhakar, M.; Dayanand, A.; Lingappa, K. Production, purification and characterization of L-asparaginase from *Streptomyces gulbargensis*. *Braz. J. Microbiol.* **2010**, *41*, 173–178. [CrossRef]
31. Kabeer, S.S.; Francis, B.; Vishnupriya, S.; Kattathayil, H.; Joseph, K.J.; Krishnan, K.P.; Mohamed Hatha, A.A. Characterization of L-asparaginase from *Streptomyces koyangensis* SK4 with acrylamide-minimizing potential in potato chips. *Braz. J. Microbiol.* **2023**, *54*, 1645–1654. [CrossRef]
32. Dumina, M.; Zhgun, A.; Pokrovskaya, M.; Aleksandrova, S.; Zhdanov, D.; Sokolov, N.; El'darov, M. A Novel L-asparaginase from Hyperthermophilic Archaeon *Thermococcus sibiricus*: Heterologous Expression and Characterization for Biotechnology Application. *Int. J. Mol. Sci.* **2021**, *22*, 9894. [CrossRef]
33. Saeed, H.; Hemida, A.; El-Nikhely, N.; Abdel-Fattah, M.; Shalaby, M.; Hussein, A.; Eldoksh, A.; Ataya, F.; Aly, N.; Labrou, N.; et al. Highly efficient *Pyrococcus furiosus* recombinant L-asparaginase with no glutaminase activity: Expression, purification, functional characterization, and cytotoxicity on THP-1, A549 and Caco-2 cell lines. *Int. J. Biol. Macromol.* **2020**, *156*, 812–828. [CrossRef]
34. Nadeem, M.S.; Khan, J.A.; Al-Ghamdi, M.A.; Khan, M.I.; Zeyadi, M.A. Studies on the recombinant production and anticancer activity of thermostable L- asparaginase I from *Pyrococcus abyssi*. *Braz. J. Biol.* **2021**, *82*, e244735. [CrossRef]
35. Saleena, S.K.; Johnson, J.I.; Joseph, J.K.; Padinchati, K.K.; Abdulla, M.H.A. Production and optimization of L-asparaginase by *Streptomyces koyangensis* SK4 isolated from Arctic sediment. *J. Basic Microbiol.* **2023**, *63*, 417–426. [CrossRef] [PubMed]
36. Saleh, A.A.; El-Aref, H.M.; Ezzeldin, A.M.; Ewida, R.M.; Al-Bedak, O.A.M. Enhanced production and purification of L-asparaginase from *Bacillus paralicheniformis* AUMC B-516 with potent cytotoxicity against MCF-7 cell lines. *AMB Express* **2025**, *15*, 80. [CrossRef] [PubMed]
37. Shafqat, I.; Shahzad, S.; Yasmin, A.; Almusallam, S.Y.; Al-Megrin, W.A.I. Efficient Production, Purification and Characterization of Therapeutically Significant L-asparaginase from *Bacillus licheniformis* ASN51. *Poli. J. Environ. Stud.* **2023**, *32*, 4267–4280. [CrossRef]
38. Shahnazari, M.; Bigdeli, R.; Dashbolaghi, A.; Ahangari Cohan, R.; Shoari, A.; Hosseini, H.; Nouri Inanlou, D.; Asgary, V. Biochemical and Biological Evaluation of an L-asparaginase from Isolated *Escherichia coli* MF-107 as an Anti-Tumor Enzyme on MCF7 Cell Line. *Iran. Biomed. J.* **2022**, *26*, 279–290. [CrossRef]
39. Jetli, J.; Jetli, A.; Perla, R. Production of L-Aparaginase by Using *Pectobacterium carotovorum*. *J. Prob. Health* **2017**, *5*, 1000168. [CrossRef]
40. El-Bessoumy, A.A.; Sarhan, M.; Mansour, J. Production, isolation, and purification of L-asparaginase from *Pseudomonas aeruginosa* 50071 using solid-state fermentation. *BMB Rep.* **2004**, *37*, 387–393. [CrossRef]
41. Deepak, V.M.M.; Vaishnavi, L.; Palaniswamy, R. Isolation and screening of L-asparaginase producing *Sphingomonas* sp. from soil sample. *Asian J. Pharm. Clin. Res.* **2025**, *18*, 58–62. [CrossRef]
42. Neto, D.C.; Buzato, J.B.; Borsato, D. L-asparaginase production by *Zymomonas mobilis* during molasses fermentation: Optimization of culture conditions using factorial design. *Acta Sci. Technol.* **2006**, *28*, 151–153. [CrossRef]
43. Elshafei, A.; El-Ghonemy, D.H. Extracellular glutaminase-free L-asparaginase from *Trichoderma viride* f2: Purification, biochemical characterization and evaluation of its potential in mitigating acrylamide formation in starchy fried food. *J. Microbiol. Biotechnol. Food Sci.* **2021**, *11*, e4336. Available online: <https://office2.jmbfs.org/index.php/JMBFS/article/view/4336/346> (accessed on 27 July 2025).
44. Ratuchne, A.; Izidoro, S.C.; Beitel, S.M.; Lacerda, L.T.; Knob, A. A new extracellular glutaminase and urease-free L-asparaginase from *Meyerozyma guilliermondii*. *Braz. J. Microbiol.* **2023**, *54*, 715–723. [CrossRef]
45. Momeni, V.; Alemzadeh, I.; Vosoughi, M. Enhancement of L-asparaginase Production by *Candida utilis* in a 13 L Fermenter and its Purification. *Int. J. Eng.* **2015**, *28*, 1134–1139. Available online: https://www.ije.ir/article_72558.html (accessed on 27 July 2025). [CrossRef]

46. Costa, I.M.; Schultz, L.; de Araujo Bianchi Pedra, B.; Leite, M.S.M.; Farsky, S.H.P.; de Oliveira, M.A.; Pessoa, A.; Monteiro, G. Recombinant L-asparaginase 1 from *Saccharomyces cerevisiae*: An allosteric enzyme with antineoplastic activity. *Sci. Rep.* **2016**, *6*, 36239. [\[CrossRef\]](#)
47. Moguel, I.S.; Yamakawa, C.K.; Brumano, L.P.; Pessoa, A., Jr.; Mussatto, S.I. Selection and Optimization of Medium Components for the Efficient Production of L-asparaginase by *Leucosporidium scottii* L115—A Psychrotolerant Yeast. *Fermentation* **2022**, *8*, 398. [\[CrossRef\]](#)
48. Aktar, B.Y.; Aysan, A.; Turunen, O.; Yağci, T.; Solğun, H.A.; Binay, B. L-asparaginase from *Lachancea Thermotolerans*: Effect of Lys99Ala on Enzyme Performance and in vitro Antileukemic Efficacy. *Biotechnol. J.* **2024**, *19*, e202400507. [\[CrossRef\]](#) [\[PubMed\]](#)
49. Marathe, A.; Charya, L. Goan mangrove yeast: A source of therapeutic enzyme L-asparaginase. *Folia Microbiol.* **2025**. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Da Cunha, M.C.; Aguiar, J.G.D.S.; Orrillo Lindo, S.M.D.R.; de Castro, R.J.S.; Sato, H.H. L-asparaginase from *Aspergillus oryzae* spp.: Effects of production process and biochemical parameters. *Prep. Biochem. Biotechnol.* **2022**, *52*, 253–263. [\[CrossRef\]](#)
51. Elshafei, A.M.; Hassan, M.M.; Abouzeid, M.A.-E.; Mahmoud, D.A.; Elghonemy, D.H. Purification, characterization and antitumor activity of L-asparaginase from *Penicillium brevicompactum* NRC 829. *Micro. Res. J. Inter.* **2012**, *2*, 158–174. [\[CrossRef\]](#)
52. Johar, D.; Hefny, H.M.; Mansy, M.S.; Mekawey, A.A.I.; Abdulrahman, M.S.; Zaky, S. Cytotoxicity of L-asparaginase from eucaryotic *Cladosporium* species against breast and colon cancer in vitro. *J. Egypt. Natl. Cancer Inst.* **2025**, *37*, 33. [\[CrossRef\]](#)
53. El-Gendy, M.M.A.A.; Awad, M.F.; El-Shenawy, F.S.; El-Bondkly, A.M.A. Production, purification, characterization, antioxidant and antiproliferative activities of extracellular L-asparaginase produced by *Fusarium equiseti* AHMF4. *Saudi J. Biol. Sci.* **2021**, *28*, 2540–2548. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Freitas, M.; Souza, P.; Cardoso, S.; Cruvinel, K.; Abrunhosa, L.S.; Ferreira Filho, E.X.; Inácio, J.; Pinho, D.B.; Pessoa, A.; OMagalhães, P. Filamentous Fungi Producing L-asparaginase with Low Glutaminase Activity Isolated from Brazilian Savanna Soil. *Pharmaceutics* **2021**, *13*, 1268. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Ali, D.I.; Ouf, S.A.; Eweis, M.; Soliman, D.M. Optimization of L-asparaginase Production from Some Filamentous Fungi with Potential Pharmaceutical Properties. *Egypt. J. Bot.* **2018**, *58*, 355–369. [\[CrossRef\]](#)
56. Da Silva Duarte, S.M.; de Carvalho Silva, A.K.; Assunção Borges, K.R.; Borges Cordeiro, C.; Lindoso Lima, F.J.; da Silva, M.A.C.N.; de Souza Andrade, M.; do Desterro Soares Brandão Nascimento, M. Production, Characterization Purification, and Antitumor Activity of L-asparaginase from *Aspergillus niger*. *Fermentation* **2024**, *10*, 226. [\[CrossRef\]](#)
57. Fazeli, N.; Alimadadi, N.; Nasr, S. Screening and Optimization of Process Parameters for the Production of L-asparaginase by Indigenous Fungal-Type Strains. *Iran. J. Sci. Technol. Trans. A Sci.* **2021**, *45*, 409–416. [\[CrossRef\]](#)
58. Ali, S.; Maqsood, S.; Ahmad, M.U.; Shabbir, I.; Raish, M.; Batool, F.; Rehman, A.U.; Liaqat, I.; Abbasi, B.B.K.; Irfan, A.; et al. Enhanced production of extracellular L-asparaginase in batch culture via nitrous acid-induced mutagenesis of *Aspergillus oryzae*. *Microb. Cell Factories* **2025**, *24*, 172. [\[CrossRef\]](#)
59. Rabelo, N.G.; Simões, L.A.; de Andrade Teixeira Fernandes, N.; Souza, A.C.; Fernandes, M.L.P.; Veríssimo, L.A.A.; Schwan, R.F.; Dias, D.R. Optimization of L-asparaginase Production from *Aspergillus caespitosus*: Solid-State and Submerged Fermentation Using Low-Cost Substrates and Partial Purification. *Appl. Microbiol.* **2025**, *5*, 19. [\[CrossRef\]](#)
60. Guo, J.; Coker, A.R.; Wood, S.P.; Cooper, J.B.; Chohan, S.M.; Rashid, N.; Akhtar, M. Structure and function of the thermostable L-asparaginase from *Thermococcus kodakarensis*. *Acta Crystallogr. Sect. D Struct. Biol.* **2017**, *73*, 889–895. [\[CrossRef\]](#)
61. Sharma, A.; Kaushik, V.; Goel, M. Insights into the Distribution and Functional Properties of L-asparaginase in the Archaeal Domain and Characterization of *Picrophilus torridus* Asparaginase Belonging to the Novel Family Asp2like1. *ACS Omega* **2022**, *7*, 40750–40765. [\[CrossRef\]](#)
62. Zuo, S.; Xue, D.; Zhang, T.; Jiang, B.; Mu, W. Biochemical characterization of an extremely thermostable L-asparaginase from *Thermococcus gammatolerans* EJ3. *J. Mol. Catal. B Enzym.* **2014**, *109*, 122–129. [\[CrossRef\]](#)
63. Narayana, K.J.; Kumar, K.G.; Vijayalakshmi, M. L-asparaginase production by *Streptomyces albidoflavus*. *Indian J. Microbiol.* **2008**, *48*, 331–336. [\[CrossRef\]](#) [\[PubMed\]](#)
64. El-Naggar, N.E.; El-Shweihy, N.M. Bioprocess development for L-asparaginase production by *Streptomyces rochei*, purification and in-vitro efficacy against various human carcinoma cell lines. *Sci. Rep.* **2020**, *10*, 7942. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Rath, G.; Nivedita, S.; Behera, S.S.; Behera, H.T.; Gouda, S.K.; Raina, V.; Achary, K.G.; Behera, S.K.; Ray, L. L-asparaginase producing novel *Streptomyces* sp. HB2AG: Optimization of process parameters and whole genome sequence analysis. *3 Biotech* **2023**, *13*, 201. [\[CrossRef\]](#)
66. Vimal, A.; Kumar, A. Biotechnological production and practical application of L-asparaginase enzyme. *Biotechnol. Genet. Eng. Rev.* **2017**, *33*, 40–61. [\[CrossRef\]](#)
67. Chand, S.; Mahajan, R.V.; Prasad, J.P.; Sahoo, D.K.; Mihooliya, K.N.; Dhar, M.S.; Sharma, G. A Comprehensive Review on Microbial L-asparaginase: Bioprocessing, Characterization, and Industrial Applications. *Biotechnol. Appl. Biochem.* **2020**, *67*, 619–647. [\[CrossRef\]](#)

68. Eyvazi, S.; Hakemi-Vala, M.; Hashemi, A.; Bejestani, F.B.; Elahi, N. Emergence of NDM-1-Producing *Escherichia coli* in Iran. *Arch. Clin. Infect. Dis.* **2017**, *13*, e62029. [\[CrossRef\]](#)
69. Ashok, A.; Doriya, K.; Rao, J.V.; Qureshi, A.; Tiwari, A.K.; Kumar, D.S. Microbes Producing L-asparaginase free of Glutaminase and Urease isolated from Extreme Locations of Antarctic Soil and Moss. *Sci. Rep.* **2019**, *9*, 1423. [\[CrossRef\]](#)
70. Costa-Silva, T.A.; Camacho-Córdova, D.I.; Agamez-Montalvo, G.S.; Parizotto, L.A.; Sánchez-Moguel, I.; Pessoa, A., Jr. Optimization of culture conditions and bench-scale production of anticancer enzyme L-asparaginase by submerged fermentation from *Aspergillus terreus* CCT 7693. *Prep. Biochem. Biotechnol.* **2018**, *49*, 95–104. [\[CrossRef\]](#)
71. Muneer, F.; Siddique, M.H.; Azeem, F.; Rasul, I.; Muzammil, S.; Zubair, M.; Afzal, M.; Nadeem, H. Microbial L-asparaginase: Purification, characterization and applications. *Arch. Microbiol.* **2020**, *202*, 967–981. [\[CrossRef\]](#)
72. Lefin, N.; Miranda, J.; Munhoz Costa, I.; Pedroso Reynaldo, A.; Monteiro, G.; Zamorano, M.; Pessoa, A., Jr.; Farias, J.G. Optimized Amino Acid-Enhanced Medium for Efficient L-asparaginase II Production in *E. coli*: From Shake Flask to Bioreactor. *Fermentation* **2025**, *11*, 239. [\[CrossRef\]](#)
73. Yang, X.; Rao, Y.; Zhang, M.; Wang, J.; Liu, W.; Cai, D.; Chen, S. Efficient production of L-asparaginase in *Bacillus licheniformis* by optimizing expression elements and host. *Chin. J. Biotechnol.* **2023**, *39*, 1096–1106. [\[CrossRef\]](#)
74. Suresh, J.V.; Raju, K.J. Studies on the Production of L-asparaginase by *Aspergillus Terreus* MTCC 1782 Using Agro-Residues under Mixed Substrate Solid State Fermentation. *J. Chem. Biol. Phys. Sci. JCBPS* **2012**, *3*, 314.
75. Soni, S.K.; Soni, R. Policy and Regulatory Frameworks for Green Biorefinery: Driving Sustainable Waste Valorization. In *Green Biorefinery Solutions: Transforming Biodegradable Waste into Resources*; Springer: Berlin/Heidelberg, Germany, 2025; pp. 315–349. [\[CrossRef\]](#)
76. Lefin, N.; Miranda, J.; Beltrán, J.F.; Belén, L.H.; Effer, B.; Pessoa, A., Jr.; Farias, J.G.; Zamorano, M. Current State of Molecular and Metabolic Strategies for the Improvement of L-asparaginase Expression in Heterologous Systems. *Front. Pharmacol.* **2023**, *14*, 1208277. [\[CrossRef\]](#)
77. Brumano, L.P.; da Silva, F.V.S.; Costa-Silva, T.A.; Apolinário, A.C.; Santos, J.H.P.M.; Kleingesinds, E.K.; Monteiro, G.; de Oliveira Rangel-Yagui, C.; Benyahia, B.; Junior, A.P. Development of L-asparaginase Biobetters: Current Research Status and Review of the Desirable Quality Profiles. *Front. Bioeng. Biotechnol.* **2019**, *6*, 212. [\[CrossRef\]](#)
78. Chakraborty, M.; Shivakumar, S. Bioprospecting of the Agaricomycete *Ganoderma Australe* GPC191 as Novel Source for L-asparaginase Production. *Sci. Rep.* **2021**, *11*, 6192. [\[CrossRef\]](#)
79. Correa, H.T.; Vieira, W.F.; Pinheiro, T.M.A.; Cardoso, V.L.; Silveira, E.; Sette, L.D.; Pessoa, A.; Filho, U.C. L-asparaginase and Biosurfactants Produced by Extremophile Yeasts from Antarctic Environments. *Ind. Biotechnol.* **2020**, *16*, 107–116. [\[CrossRef\]](#)
80. Gurunathan, B.; Sahadevan, R. Design of Experiments and Artificial Neural Network Linked Genetic Algorithm for Modeling and Optimization of L-asparaginase Production by *Aspergillus Terreus* MTCC 1782. *Biotechnol. Bioprocess Eng.* **2011**, *16*, 50–58. [\[CrossRef\]](#)
81. El-Naggar, N.E.-A.; Hamouda, R.A.; Elshafey, N. Artificial Intelligence-Based Optimization for Extracellular L-Glutaminase Free L-asparaginase Production by *Streptomyces Violaceoruber* under Solid State Fermentation Conditions. *Sci. Rep.* **2024**, *14*, 29625. [\[CrossRef\]](#)
82. Moguel, I.S.; Yamakawa, C.K.; Pessoa, A., Jr.; Mussatto, S.I. L-asparaginase Production by *Leucosporidium Scottii* in a Bench-Scale Bioreactor with Co-Production of Lipids. *Front. Bioeng. Biotechnol.* **2020**, *8*, 576511. [\[CrossRef\]](#)
83. Šelo, G.; Planinić, M.; Tišma, M.; Tomas, S.; Koceva Komlenić, D.; Bucić-Kojić, A. A Comprehensive Review on Valorization of Agro-Food Industrial Residues by Solid-State Fermentation. *Foods* **2021**, *10*, 927. [\[CrossRef\]](#)
84. Nunes, J.C.; Cristóvão, R.O.; Freire, M.G.; Santos-Ebinuma, V.C.; Faria, J.L.; Silva, C.G.; Tavares, A.P. Recent Strategies and Applications for L-asparaginase Confinement. *Molecules* **2020**, *25*, 5827. [\[CrossRef\]](#)
85. Veríssimo, N.V.; Vicente, F.A.; de Oliveira, R.C.; Likozar, B.; de Souza Oliveira, R.P.; Pereira, J.F.B. Ionic Liquids as Protein Stabilizers for Biological and Biomedical Applications: A Review. *Biotechnol. Adv.* **2022**, *61*, 108055. [\[CrossRef\]](#)
86. Veríssimo, N.V.P.; Mussagy, C.U.; Bento, H.B.S.; Pereira, J.F.B.; de Carvalho Santos-Ebinuma, V. Ionic Liquids and Deep Eutectic Solvents for the Stabilization of Biopharmaceuticals: A Review. *Biotechnol. Adv.* **2024**, *71*, 108316. [\[CrossRef\]](#) [\[PubMed\]](#)
87. Jia, R.; Wan, X.; Geng, X.; Xue, D.; Xie, Z.; Chen, C. Microbial L-asparaginase for Application in Acrylamide Mitigation from Food: Current Research Status and Future Perspectives. *Microorganisms* **2021**, *9*, 1659. [\[CrossRef\]](#) [\[PubMed\]](#)
88. Orabi, H.M.; El-Fakharany, E.M.; Abdelkhalek, E.S.; Sidkey, N.M. L-asparaginase and L-Glutaminase: Sources, Production, and Applications in Medicine and Industry. *J. Microbiol. Biotechnol. Food Sci.* **2019**, *9*, 179. [\[CrossRef\]](#)
89. Sahu, S.; Agrawal, S.; Sahu, A. Regulatory Policies on Use of Food Enzymes. In *Enzymes Beyond Traditional Applications in Dairy Science and Technology*; Elsevier: Amsterdam, The Netherlands, 2023; pp. 519–535. [\[CrossRef\]](#)
90. Veríssimo, N.V.; Mussagy, C.U.; Oshiro, A.A.; Mendonça, C.M.N.; de Carvalho Santos-Ebinuma, V.; Pessoa, A.; de Souza Oliveira, R.P.; Pereira, J.F.B. From Green to Blue Economy: Marine Biorefineries for a Sustainable Ocean-Based Economy. *Green Chem.* **2021**, *23*, 9377–9400. [\[CrossRef\]](#)

91. Rojas, L.F.; Zapata, P.; Ruiz-Tirado, L. Agro-Industrial Waste Enzymes: Perspectives in Circular Economy. *Curr. Opin. Green Sustain. Chem.* **2022**, *34*, 100585. [\[CrossRef\]](#)
92. Sharma, V.; Tsai, M.-L.; Nargotra, P.; Chen, C.-W.; Kuo, C.-H.; Sun, P.-P.; Dong, C.-D. Agro-Industrial Food Waste as a Low-Cost Substrate for Sustainable Production of Industrial Enzymes: A Critical Review. *Catalysts* **2022**, *12*, 1373. [\[CrossRef\]](#)
93. Sarker, T.C.; Azam, S.M.G.G.; Bonanomi, G. Recent Advances in Sugarcane Industry Solid By-Products Valorization. *Waste Biomass Valorization* **2017**, *8*, 241–266. [\[CrossRef\]](#)
94. Ravindran, R.; Hassan, S.S.; Williams, G.A.; Jaiswal, A.K. A Review on Bioconversion of Agro-Industrial Wastes to Industrially Important Enzymes. *Bioengineering* **2018**, *5*, 93. [\[CrossRef\]](#)
95. Bharathiraja, S.; Suriya, J.; Krishnan, M.; Manivasagan, P.; Kim, S.-K. Production of Enzymes from Agricultural Wastes and Their Potential Industrial Applications. In *Advances in Food and Nutrition Research*; Elsevier: Amsterdam, The Netherlands, 2017; Volume 80, pp. 125–148. ISBN 1043-4526. [\[CrossRef\]](#)
96. Rodrigues, R.P.; Gando-Ferreira, L.M.; Quina, M.J. Increasing Value of Winery Residues through Integrated Biorefinery Processes: A Review. *Molecules* **2022**, *27*, 4709. [\[CrossRef\]](#)
97. Kapoor, M.; Panwar, D.; Kaira, G.S. Bioprocesses for Enzyme Production Using Agro-Industrial Wastes: Technical Challenges and Commercialization Potential. In *Agro-Industrial Wastes as Feedstock for Enzyme Production*; Elsevier: Amsterdam, The Netherlands, 2016; pp. 61–93. [\[CrossRef\]](#)
98. Leite, P.; Sousa, D.; Fernandes, H.; Ferreira, M.; Costa, A.R.; Filipe, D.; Gonçalves, M.; Peres, H.; Belo, I.; Salgado, J.M. Recent Advances in Production of Lignocellulolytic Enzymes by Solid-State Fermentation of Agro-Industrial Wastes. *Curr. Opin. Green Sustain. Chem.* **2021**, *27*, 100407. [\[CrossRef\]](#)
99. Lalor, F.; Fitzpatrick, J.; Sage, C.; Byrne, E. Sustainability in the Biopharmaceutical Industry: Seeking a Holistic Perspective. *Biotechnol. Adv.* **2019**, *37*, 698–707. [\[CrossRef\]](#)
100. Sarkis, M.; Fyfe, A.T.; Kontoravdi, C.; Papathanasiou, M.M. Towards a Net Zero, Socially Sustainable and Eco-Efficient Biopharma Industry: How Far Are We? *Curr. Opin. Chem. Eng.* **2024**, *44*, 101027. [\[CrossRef\]](#)
101. Baskar, G.; Aiswarya, R.; Renganathan, S. Applications of Asparaginase in Food Processing. In *Green Bioprocesses: Enzymes in Industrial Food Processing*; Springer: Berlin/Heidelberg, Germany, 2018; pp. 83–98. [\[CrossRef\]](#)

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.