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Aspergillus Oryzae: Multifunctional Powerhouse Revolutionizing Food Industries and Gut Health

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Abstract

Aspergillus oryzae, commonly known as "Koji mold," is a filamentous fungus extensively utilized in various food industries due to its robust enzymatic activity and generally recognized as safe (GRAS) status. This review explores its diverse applications, focusing on its role in fermented food production, enzyme generation, and contributions to gut health. In the production of fermented foods, such as soy sauce, miso, and sake, A. oryzae serves as a crucial microorganism, breaking down complex carbohydrates and proteins into simple sugars and amino acids, and enhancing flavor and nutritional profiles. Its ability to produce industrial enzymes, including amylases, proteases, and lipases, has made it indispensable in brewing, baking, and the dairy industry. Additionally, A. oryzae-derived enzymes are widely used in developing food thickeners and flavor enhancers, underscoring their biotechnological significance. Emerging studies highlight the potential of A. oryzae in promoting gut health. Fermented foods made with Aspergillus oryzae can support better digestion, balance the gut microbiome, and boost the absorption of nutrients. Its enzymes aid in breaking down complex dietary components, reducing gut inflammation, and supporting overall gastrointestinal well-being. This review consolidates recent advancements in the food industry and explores the symbiotic relationship between A. oryzae and gut health, emphasizing its importance in functional food development.

Keywords: *Aspergillus oryzae*, fermented food, gut health, symbiosis, Microbes

INTRODUCTION

Aspergillus oryzae (A. oryzae) is a multicellular fungus renowned worldwide as a vital biotechnological asset. Its exceptional ability to produce amylase and protease enzymes, which break down proteins and starches into amino acids and sugars, makes it indispensable in the food industry. It is extensively used to create a variety of fermented products, such as miso (soybean paste), shoyu (soy sauce), tane-koji (seed rice malt), douche (fermented and salted black soybean), bean curd seasoning, and vinegar [1]. Historically, *A. oryzae* has been central to koji production in the orient for over two millennia and has been employed in Europe for enzyme production in brewing and baking since the early 1900s [2]. In Japanese cuisine, koji refers to soybeans or cooked grains fermented with a specific mold, later termed koji mold to denote its role in koji fermentation [3].

It plays a crucial role in traditional Japanese fermentation industries, contributing to the production of staples like soy sauce, sake, bean curd seasoning, and vinegar. Its exceptional capacity to secrete a diverse range of enzymes makes it particularly notable among filamentous fungi. Recent advancements in genetic engineering have further enhanced its application in modern biotechnology, particularly in industrial enzyme production. Notably, *A. oryzae* facilitated the first commercial production of a heterologous enzyme in 1988, when it was used to manufacture a lipase for laundry detergents [4].

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Within the *Aspergillaceae* family, *A. oryzae* holds significant importance for Japan's economy due to its enzyme production capabilities. While its utilization is widespread in Japan, its adoption in Western countries has been relatively limited. One notable exception is the amylo-process introduced in France in 1891 by Calmette and Bodin, who explored its potential in the spirit industry. In Japan, *A. oryzae* has been employed for centuries in the production of sake, soy sauce, and miso, generating substantial economic value. For example, in 1912, sake fermentation contributed $41,974,630 in revenue, while soy products accounted for $2,048,141 in taxes, with the total value of products derived from this fungus estimated at $200,000,000. Prof. Kozai of Tokyo Imperial University acknowledged early investigations into the industrial applications of *A. oryzae,* crediting Hoffmann and Korshelt as pioneers in the field.

GRAS STATUS AND SAFETY PROFILE

Aspergillus oryzae has been extensively used in food fermentation industries for centuries, leading to its designation as generally recognized as safe (GRAS) by the FDA in the USA [5, 6]. The World Health Organization (WHO) also supports its safety [7]. Despite its genetic similarity to *A. flavus*, a known producer of the potent carcinogen aflatoxin, *A. oryzae* has no history of producing aflatoxins or other carcinogenic compounds [8]. Foods fermented with *A. oryzae*, including its close relative *A. sojae*, have been confirmed aflatoxin-free [9, 10]. Traditional distinctions between *A. oryzae* and *A. flavus* were based on morphology, physiology, and culture characteristics [11], but recent DNA-based techniques have improved their differentiation [12–14]. Studies by Zhang et al. revealed that aflatoxin biosynthesis genes in *A. oryzae* are not expressed even under conditions favorable for aflatoxin production in *A. flavus* and *A. parasiticus* [15].

While *A. oryzae* has provided immense benefits for thousands of years, its unique cultivation methods, species origin, and isolation processes remain subjects of intrigue. Despite its economic importance, traditional research on *A. oryzae* has been limited due to its multinucleate conidia and the absence of a sexual cycle. Genomics holds promise for uncovering its evolutionary history, molecular mechanisms for high secretion, and potential applications in unexplored fields.

APPLICATIONS OF *ASPERGILLUS ORYZAE* IN FOOD INDUSTRIES

It plays a pivotal role in the food industry due to its exceptional enzymatic capabilities and GRAS (generally recognized as safe) status. This versatile fungus is integral to the production of a variety of fermented foods, including soy sauce, miso, and sake, where it enhances flavor, nutritional quality, and overall product appeal. Beyond traditional fermentation, its ability to produce significant enzymes, such as amylases, proteases, and lipases has cemented its importance in processes like brewing, baking, and dairy production. These applications highlight the indispensable role of *A. oryzae* in shaping both traditional and modern food technologies.

ROLE IN FERMENTED FOOD PRODUCTION

Aspergillus oryzae has been a foundational element in fermented food production for centuries, thanks to its ability to convert complex carbohydrates and proteins into simpler compounds. This enzymatic process not only enriches the taste and aroma of products like soy sauce, miso, and sake but also boosts their nutritional value. By facilitating the release of simple sugars, amino acids, and other bioactive compounds, *A. oryzae* plays a critical role in creating high-quality, flavorful, and health-promoting fermented food products, making it indispensable in traditional and contemporary food preparation.

Soy Sauce Fermentation

In the production of soy sauce, cooked soybeans and roasted crushed wheat are mixed in varying ratios (6:4 to 4:6) and inoculated with a pure starter culture of *Aspergillus oryzae*, known as koji starter or seed mold. The mixture is then transferred to a koji cultivation room, where it is spread on large stainless steel trays and incubated at 25–30ºC for two to three days. During this time, temperature, moisture, and aeration are carefully controlled to promote the growth of the mold and enzyme production. Stirring is performed twice during the incubation period to maintain optimal conditions and prevent the temperature from exceeding 35ºC, which would kill the mold. The product, koji, is characterized by fungal growth covering the raw materials, resulting in a clear yellow to yellowish-green color (Figure 1) [16].

The *A. oryzae* mold is crucial for soy sauce production due to its high productivity of α-amylase, which helps break down starches into sugars, and proteases that hydrolyze proteins into amino acids and peptides. *Aspergillus oryzae* is also integral to the production of other traditional Japanese fermented foods, including miso and sake [16–18].

In the mash production phase, koji is mixed with water and salt, creating a mixture called moromi, which is transferred to large fermentation tanks for four to eight months. During fermentation, enzymes from the koji mold continue to break down starches and proteins, with the remaining starch converted into simple sugars. As the fermentation progresses, lactic acid bacteria and yeasts ferment these sugars into lactic acid and alcohol, lowering the pH of the mash [17, 18].

After fermentation, the mash is pressed under high pressure to extract the liquid, which becomes the raw soy sauce. The residue, known as soy sauce cake, is used as animal feed. The liquid is then refined by separating it into three layers – sediment, a clear middle layer, and an oily top layer. The oil is removed, and the middle layer is pasteurized at high temperatures to denature enzymes, coagulate proteins, and develop the characteristic color and aroma of soy sauce. The final product is then filtered, bottled, and ready for market [16, 17].

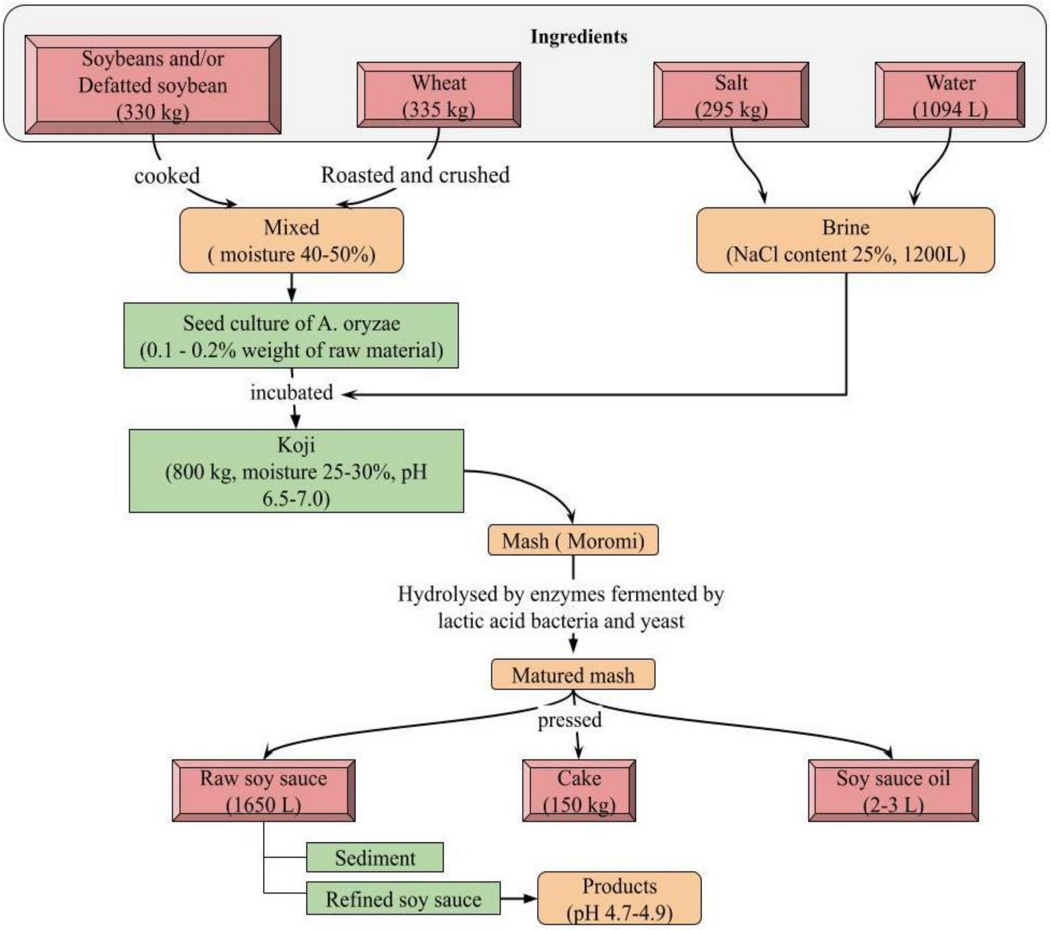

Figure 1. The manufacturing process of soy sauce (koikuch-shoyu), which is a representative of fermented soy sauce in Japan.

Soybean Paste (MISO) Fermentation (Rice-Miso and Barley-Miso)

Whole yellow soybeans are used to make ordinary miso, while dehulled soybeans or soybean grits are used for white or pale-yellow rice-soybean paste (Figure 2). The process involves soaking soybeans in water, followed by cooking or steaming. For koji cultivation, *A. oryzae* is applied to rice, barley, or rye at 35ºC to 38ºC for 40–48 hours, sometimes reaching 40ºC in the final stage. The resulting koji is mixed with salt (30% by weight) to halt mold growth. After mixing with cooked soybeans, salted rice- or barley-koji, and necessary microbial inoculants, fermentation occurs at 30ºC for 1–3 months. The role of *A. oryzae* is critical in the production of koji, where it promotes enzyme activity, particularly for starch breakdown and fermentation. After fermentation, the paste is blended, mashed, and pasteurized to complete the process [16, 18].

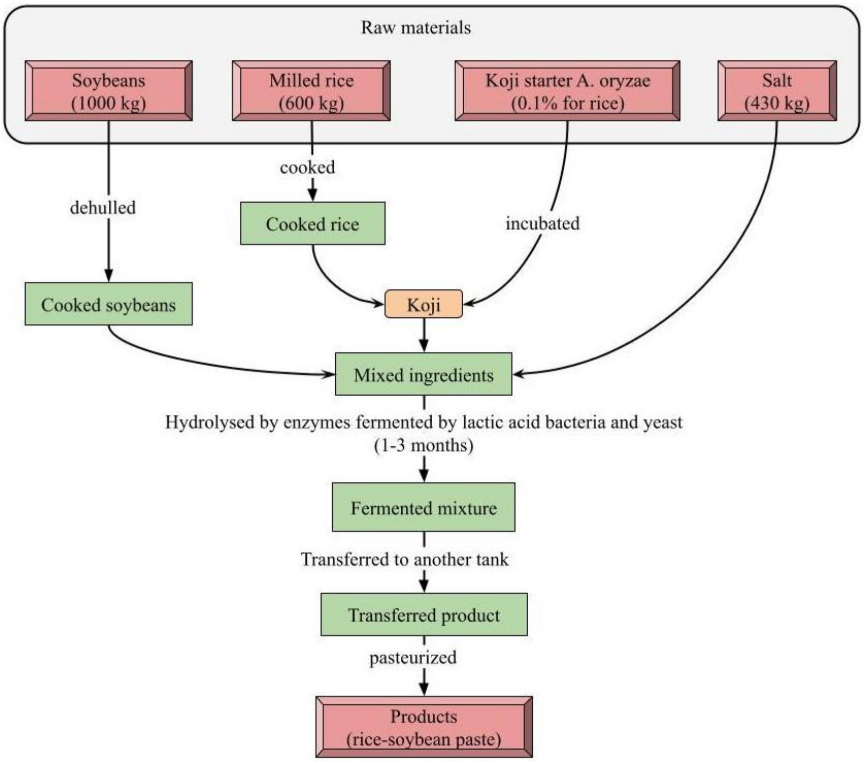

Figure 2. The manufacturing process of soybean paste (miso).

Sake (Japanese Rice Wine) Fermentation

In sake production, *Aspergillus oryzae* (koji mold) is essential for converting the starch in rice into fermentable sugars through saccharification. The process begins with inoculating steamed rice with conidiospores of *A. oryzae* in a koji-making room maintained at around 30ºC with high humidity. The mold grows over the rice grains, producing amylolytic enzymes, such as α-amylase and glucoamylase, which break down starches into fermentable sugars. This step is critical because the production of amylolytic enzymes is optimal at temperatures above 35ºC, which is higher than the temperature required for protein-degrading enzymes in soy sauce production. The final product of this stage, koji, is white and faintly smells of sweet chestnuts (Figure 3) [19, 20].

In sake fermentation, *A. oryzae* continues to contribute by facilitating the simultaneous saccharification and ethanol fermentation process. Koji enzymes liquefies and saccharify starch in rice, producing sugars that are later fermented into ethanol by sake yeast (*Saccharomyces cerevisiae*). The quality of the koji used in sake making differs from those used in soy sauce or miso production, with a focus on high amylolytic activity and minimal proteolytic and peptidase activity. This tailored enzyme profile ensures efficient starch breakdown without excessive protein degradation, contributing to the overall quality of the same [19, 20].

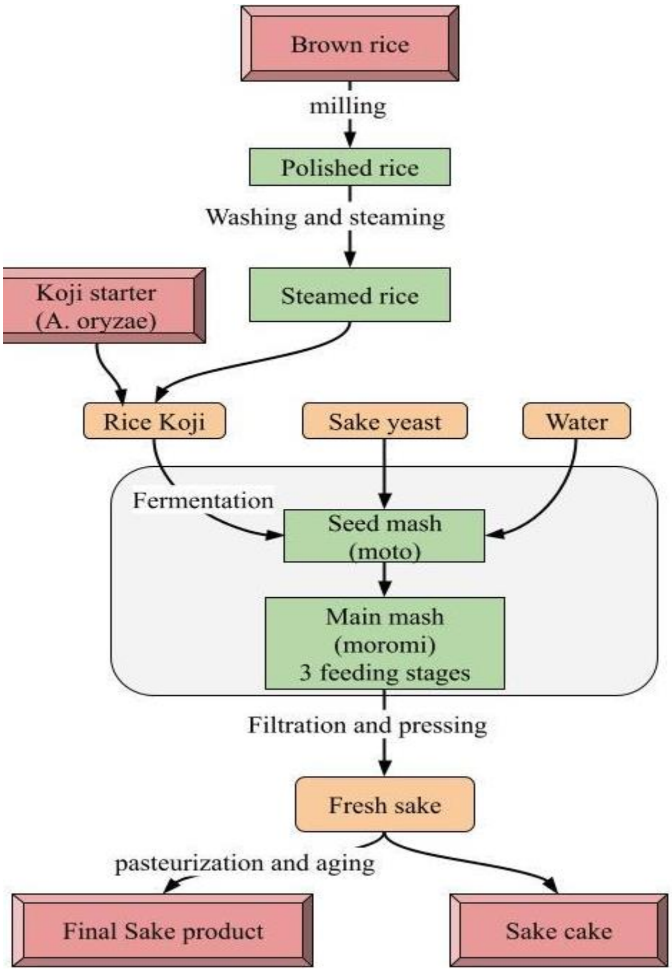

Figure 3. The manufacturing process of sake (Japanese rice wine).

Industrial Enzyme Production

There is increasing potential for utilizing low-cost and safe resources to produce valuable byproducts. Enzymes of microbial origin are preferred for industrial applications over those obtained through conventional methods due to various advantages, including economic viability, low toxicity, eco-friendliness, reduced energy requirements, enhanced efficiency, and superior product quality (Table 1) [21]. Additionally, the ability of microbes to grow on solid substrates enables the conversion of agricultural byproducts into valuable materials, contributing to both sustainable agriculture and environmental conservation. Among microbial enzymes, those derived from fungi are particularly favorable due to their hyphal growth form, tolerance to low water activity (0.5–0.6 aw), and ability to thrive under high osmotic conditions [22, 23]. Enzyme production is typically carried out using either solid-state fermentation (SSF) or submerged fermentation (SmF) methods [24]. *Aspergillus oryzae*, having GRAS (generally recognized as safe) status [25], is widely and safely utilized as a source for numerous industrial enzymes.

Liang et al. (2009) and Chancharoonpong et al. (2012) reported that *Aspergillus oryzae* produces significant amounts of hydrolytic enzymes, including amylase, proteases, and glutaminase, in soybean koji [26, 27]. Proteases, widely used in food, pharmaceutical, and biotechnological industries, account for 60% of the global enzyme market and are favored for their extracellular nature and ease of recovery [28, 29]. *A. oryzae* proteases have been used for protein hydrolysis, enhancing antioxidant activity [30], and as a potential therapy for gluten intolerance [31].

Amylases, including α-amylase, are critical in food and detergent industries, with *A. oryzae* producing them on substrates like wheat bran and oil cakes [32, 33]. Glucoamylases are used in syrup and alcohol production [34], while lipases contribute to food, cosmetics, and medicine applications [35, 36]. Additionally, cellulases and pectinases from *A. oryzae* are employed in various industries, including textiles, fuel, and food processing [37, 38]. Enzymes like β-galactosidase aid in lactose intolerance management [39], and asparaginase helps hydrolyze asparagine [40].

Table 1. Some industrial enzymes originated from *A. oryzae* and their substrates.

Enzyme	**Substrate**	**Production Yield**	**References**
Neutral protease	Soybean	84.38 U/g	[27]
Alkaline protease	Soybean Wheat bran	41.35 U/g 600 U/ml	[27, 41]
α-amylase, glucoamylase	Coconut oil cake Soybean Wheat bran Wheat bran Wheat bran and sugar cane bagasse	3388 U/g 200 U/g 1986 U/g 36.31 U/ml 330 µg/ml/min	[27, 32, 42–44]
Prolyl endopeptidases	Wheat gliadin	22 U/ml	[31]
Cellulase	Corncobs	38.80 U/ml	[45]
Asparaginase	Asparagine	282 U/ml	[46]
Lipase	Sorghum	35.66 U/ml	[36]
Pectinase	Soybean residue Cellulose	120 U/ml 2.03 U/ml	[47, 48]
β-galactosidase	Wheat bran and rice husk	386.6 U/ml	[39]

Improving the Quality of Bakery Products

Exogenous microbial enzymes, such as amylases and proteases, have long been used in industrial baking [49–52]. Recently, hemicellulases, particularly endo-xylanases, have been incorporated to improve dough, bread, biscuits, cakes, and other bakery products [52]. While the exact mechanism of action of endo-xylanases is not fully understood, it is believed that they hydrolyze arabinoxylan in dough, aiding water redistribution, which enhances dough handling, bread volume, texture, and stability [52, 53]. Additionally, adding endo-xylanases increases arabinoxylan oligosaccharides in bread, which may have health benefits. Enzymes like arabinases, α-L-arabinofuranosidases, and esterases also play significant roles in improving the texture, quality, and sensory properties of bakery products [52]. The optimal combination of these enzymes is crucial for maximizing benefits during baking.

Beer Brewing

This technology relies on enzymes activated during malting and fermentation. Malting of barley involves seed germination, triggering the biosynthesis of enzymes like α- and β-amylases, carboxypeptidase, and β-glucanase, which hydrolyze seed reserves (Table 2). However, many breweries use poor-quality or un-malted barley due to seasonal variations, cultivars, or poor harvests, which contain low β-glucanase levels. These grains, with 6–10% nonstarch polysaccharides (NSP), primarily soluble β-glucan, cause filtration issues, slow runoff, and haze formation. To tackle this issue, microbial β-glucanases from species like *Penicillium emersonii*, *Aspergillus niger*, *Bacillus subtilis*, and *Trichoderma reesei* are introduced to lower wort viscosity [54].

Pajunen (1986) found that the β-glucanase from *Trichoderma* had the best cost/performance ratio [55]. Oksanen et al. (1985) showed that *Trichoderma* enzymes, including endoglucanase II and cellobiohydrolase II, significantly reduced polymerization and wort viscosity, improving filtration by 30% and decreasing β-glucan content by 90% [56]. Further studies confirmed that *Trichoderma* β-glucanase outperformed others in pilot and industrial trials, yielding high-quality beer without compromising flavor [55, 57]. Thus, *Trichoderma* β-glucanase is ideal for brewing with poor-quality barley.

Table 2. Cellulases, hemicellulases and pectinases in brewery and wine biotechnology.

Enzyme	Function	Application	References
b-Glucanase/ glucanolytic yeast	Hydrolysis of b-1, 3, and b-1, 4 glucans; reducing the viscosity and releasing reducing sugars during primary fermentation.	Improvement in primary fermentation, filtration, and quality of beer	[54–57]
Pectin esterase	De-esterification and gelling of pectins.	Improvement in the clarification of cider.	[58]
Macerating enzymes (cellulases, hemicellulases and pectinases)	Hydrolysis of plant cell wall polysaccharides.	Improvement in skin maceration and color extraction of grapes; quality, stability, filtration, and clarification of wines.	[54, 58, 59]
β-Glucosidase	Modification of aromatic residues.	Improvement in the aroma of wines.	[60, 61]

Wine Production

This biotechnological process involves both yeast cells and enzymes, which play critical roles in wine production. Over the past four decades, efforts have focused on improving yeast strains for grape juice fermentation and incorporating exogenous microbial enzymes. The primary enzymes used in winemaking are pectinases, β-glucanases, and hemicellulases, offering several benefits: enhanced skin maceration and color extraction, easier clarification and filtration, and improved wine quality and stability (Table 2) [54]. Recently, β-glucosidase has gained attention for enhancing wine aroma by modifying glycosylated precursors [60, 61].

The first microbial enzyme used in winemaking was pectinase from *Aspergillus*, which includes pectin esterase, polygalacturonase, pectin lyase, and small amounts of hemicellulose [54]. Adding pectinase during grape crushing or to the must improves juice extraction, reduces clarification time, and increases terpene content in wine. Pectinase with high pectin lyase and low pectin methyl esterase activities is preferred to minimize methanol production during fermentation [54].

In the early 1980s, *Trichoderma* β-glucanase was identified as useful for winemaking from Botrytis cinerea-infected grapes, which produce problematic β-glucan during filtration [62, 63]. A β-glucanase from *Trichoderma harzianum* was patented to hydrolyze these glucans and improve filtration [54]. Combining macerating enzymes resulted in improved grape pressability, juice yield, and settling rate compared to pectinase alone [64].

Galante et al. (1998b) assessed a commercial enzyme preparation, Cytolase 219, and found significant improvements in wine production, including a 10–35% increase in juice extraction, up to 180% improvement in filtration rate, and energy savings during cooling [54]. This enzyme technology offers substantial benefits to the wine industry, and further advancements in enzyme development are expected to bring additional advantages for both producers and consumers.

SYMBIOSIS WITH GUT HEALTH

Emerging research highlights the symbiotic relationship between *Aspergillus oryzae* and gut health, showcasing its potential as a key player in gastrointestinal well-being. Fermented foods produced using *A. oryzae* have been shown to improve digestion, modulate the gut microbiome, and enhance nutrient absorption. Its enzymes contribute to breaking down complex dietary components, reducing gut inflammation, and supporting a balanced microbial environment. This underscores the significance of *A. oryzae* in promoting digestive health and its growing relevance in the development of functional foods aimed at improving overall gut health.

Primary and Secondary Metabolites Produced by *Aspergillus oryzae* Fermentation

Comprehensive analytical studies, employing both targeted and untargeted methods, have revealed that *Aspergillus oryzae* produces a diverse range of primary and secondary metabolites (Table 2).

Primary metabolites are essential for the organism's growth and physiological processes, while secondary metabolites, although not directly involved in growth or development, contribute to virulence, host defense, and environmental adaptation [65–67].

A. oryzae has a rich history in fermentative metabolite production, with kojic acid being a notable secondary metabolite. First isolated in 1907 from koji culture [68], kojic acid serves multiple applications, including as an antibiotic [69, 70], food preservative [71], antioxidant [72, 73], and tyrosinase inhibitor [74]. Its role in cosmetics for skin lightening [75] and medical treatments for chloasma [76] underscores its versatility. The production of kojic acid by *Aspergillus oryzae* is well-documented and thoroughly understood [77–79].

Aspergillus species are key producers of organic acids, including 1, 4-dicarboxylic acids like succinic, malic, and fumaric acids, which play essential roles in the tricarboxylic acid (TCA) cycle. These acids, recognized as high-value chemicals derived from biomass by the U.S. Department of Energy, have broad industrial applications [80]. While other *Aspergillus* strains are favored for fumaric and malic acid production under stress conditions [81], *A. oryzae* shows significant potential for succinic acid production under optimized fermentation conditions [82–85]. Malic acid, commonly used in food and beverages for its flavor-enhancing qualities, also finds applications as a cleaning agent and an additive in animal feed [86–88].

Secondary metabolite production by *Aspergillus* species is influenced by fermentation conditions and methods [89]. *A. oryzae* generates various secondary metabolites, including terpenoids, coumarins, and oxylipins, which are detailed in (Table 3).

Table 3. Metabolites found in *Aspergillus oryzae* fermentation products and their described bioactivity.

Metabolite	Chemical Class/Family	Occurrence	Bioactivity	References
Asperorydines A-M	Alkaloid	Fermentation of *A. oryzae* strain L1020 in potato dextrose broth at 25°C for 7 days	Neuroprotective effect	[90]
L-ergothioneine	Amino acid derivative	Fermented rice bran with *A. oryzae*	Antioxidant activity, cytoprotective effects	[91–93]
Asperfuran	Furan	Fermentation of *A. oryzae* strain HA 302–84 on YMG-medium at 23°C for 48h	Chitin synthase inhibitor	[94]
Aspirochlorine	Gliotoxin	Fermentation of *A. oryzae* strain IAM 2613 on a medium mainly containing sucrose, peptone, and yeast at 29°C for 20 days	Antibiotic activity, antifungal activity	[95, 96]
Dihydroxymethoxycoumarin	Isocoumarin derivative	Fermentation of *A. oryzae* strain KCCM 12698 on malt extract agar plates or broth at 28°Cover 16days	Antimicrobial activity	[89]
Agmatine	Polyamine	Fermentation of *A. oryzae* strain RW (rice wine) on steamed rice	Antidepressant-like effect, anxiolytic activity, antihyperalgesic effects	[97–100]

Modulation of Gut Microbiota

The molecular composition of a potential *Aspergillus oryzae* postbiotic is heavily influenced by the method of inactivation used. Various drying techniques can modify the profiles of primary and secondary metabolites [101–103], influence enzymatic activities [104, 105], and alter the morphology, structure, and molecular weight of polysaccharides [106, 107].

The *Aspergillus oryzae* postbiotic is a fermentation product derived from a specific strain of *Aspergillus oryzae* (NRRL 458), combined with 4–5% wheat bran as a carrier. The fermentation process occurs in a two-stage liquid fermenter system under closed and sterile conditions, using standard production media. After the second fermentation, the supernatant – comprising the broth and cell solids – is sprayed onto food-grade wheat bran at a ratio of 4–5% w/w. It is then air-dried at 55°C until the moisture content drops to below 10%, typically averaging around 7.2%. The final commercial product contains approximately 7×10^6 intact *Aspergillus oryzae* conidiospores, along with 0.2% citric acid, 2.3 mg riboflavin, 202.0 mg niacin, 23.2 mg pantothenic acid, 8.5 mg pyridoxine hydrochloride, 0.9 mg folic acid, 0.4 mg biotin, and 1.2 µg cobalamin per kg. It also includes enzymatic activities of 3.0 IU cellulase and 40.0 IU amylase per kg [108].

The health-promoting potential of *Aspergillus oryzae* has traditionally focused on its probiotic [109–112] and prebiotic effects [113–115]. Recent studies, however, have begun exploring its role as a progenitor microorganism for postbiotic production, highlighting its ability to modulate gut health. For example, Nomura et al. (2022) showed that heat-killed *Aspergillus oryzae* spores notably boosted the population of the anti-inflammatory gut bacterium *Bifidobacterium pseudolongum* in mice, helping to reduce colitis symptoms and improve gut health [116]. In a similar study, Ríus et al. (2022) discovered that feeding *A. oryzae* postbiotics to dairy calves enhanced intestinal barrier function and water absorption, although it had minimal impact on systemic inflammation markers [117].

Other studies provide indirect evidence of *A. oryzae* postbiotic potential in gut modulation. Hymes-Fecht and Casper (2021) reported improved nutrient absorption and feed efficiency in livestock fed dried *A. oryzae* fermentation products, while Gomez-Alarcon et al. (1990) demonstrated enhanced rumen digestibility and fiber utilization in Holstein cows [118, 119]. These findings underscore *A. oryzae's* capacity to support gastrointestinal health by improving nutrient utilization, promoting beneficial gut microbes, and mitigating inflammatory responses in animals.

Modulation of the Resident Gut Microbiota

Postbiotics can directly and indirectly influence the composition and functionality of the intestinal microbiome [120]. Components, such as short-chain fatty acids (SCFAs) have direct effects on the gut microbiota [121], while exopolysaccharides (EPS) from organisms like *Bifidobacterium* interact with gut flora [122]. Additionally, metabolites like bacteriocins [123] and organic acids [124] can inhibit pathogenic activity in the gut. Postbiotics often include indigestible fibers, which have a prebiotic-like effect by fostering beneficial microbiota growth. This dual functionality highlights how postbiotics can simultaneously offer prebiotic benefits.

A. oryzae is a rich source of various polysaccharides, such as β-glucans [125, 126], galactomannan [127, 128], chitin, and chitosan [129, 130], which act as prebiotics. β-galactosidase from *A. oryzae* has been used to produce galactooligosaccharides from lactose, which enhance the growth of *Bifidobacterium infantis* at higher concentrations [114]. Additionally, koji glycosylceramide from *A. oryzae* serves as a prebiotic, promoting the abundance of *B. coccoides* in the gut, as demonstrated in mouse studies [113]. These findings underscore the potential of *A. oryzae* in supporting gut health through microbiota modulation.

Enhancement of Epithelial Barrier Function

The integrity of the gut epithelium is essential for overall health, acting as a vital barrier against pathogens and harmful substances [131]. Strategies to enhance epithelial barrier function include stimulating the secretion of proteins like HM0539 [132], reducing inflammation [133], supporting tight junction function [134], and protecting against LPS-induced damage [135]. These effects have been well-documented for postbiotics derived from *Lactobacillus plantarum* [136], *Lactobacillus rhamnosus* GG [132], and *Bifidobacterium longum* [137]. Such postbiotics contribute to the strengthening and functionality of the epithelial barrier, which is critical for gut health and pathogen defense. Enzymes produced during *Aspergillus oryzae* fermentation may also play a role in this process, potentially enhancing the digestibility and bioavailability of nutrients that support epithelial health [138, 139]. Xylanase supplementation has been shown to improve intestinal health in broiler chickens, particularly by mitigating barrier dysfunction caused by *Clostridium perfringens* infections, as reported by Liu et al. (2012) [140]. In another study, Petry et al. (2020) demonstrated that xylanase supplementation also improves gut barrier integrity in growing pigs [141].

Enhancing the gut barrier can lead to improved nutrient absorption and overall health in pigs, demonstrating the wide applicability of xylanase across different species. Further studies have emphasized the role of phytase in regulating the expression of intestinal tight junction and nutrient transporter genes in pigs [142]. Additionally, research by [143] demonstrated that phytase supplementation improves intestinal health in broiler chickens, likely by modulating the gut microbiota [143]. It promotes the growth of beneficial bacteria while reducing harmful bacteria, leading to better intestinal morphology. These changes are linked to increased nutrient digestibility and enhanced bone development, highlighting a direct connection between enzyme supplementation and improved physiological growth in poultry. Moreover, potential components of *Aspergillus oryzae* postbiotics, such as specific furans and alkaloids, may directly influence tight junction proteins [144]. Additionally, the prebiotic-like effects of *A. oryzae* preparations could also affect epithelial barrier function by altering the gut microbiome [145, 146].

Modulation of Systemic Metabolic Responses

Postbiotics influence systemic metabolic responses through metabolites and enzymes within inactive microorganisms [147]. For instance, *Lactobacillus johnsonii* supernatant, a postbiotic, can transform benign bile constituents into toxic compounds against *Giardia duodenalis*, highlighting its role in bile acid metabolism and parasitic infection management. Similarly, *A. oryzae* fermentation products, such as koji glycosylceramide, have shown potential in modulating cholesterol and bile acid metabolism. Feeding koji glycosylceramide to obese mice significantly reduces liver cholesterol levels, likely by promoting cholesterol conversion into bile acids.

The secondary metabolites synthesized by *Aspergillus oryzae* exhibit antioxidative, antimicrobial, and antitumor properties (refer to Table 2), suggesting their potential role in modulating systemic metabolic pathways. However, the mechanisms underlying these effects remain unclear. The concentration and bioavailability of these metabolites in postbiotic formulations likely determine their biological efficacy, making them critical considerations in optimizing health benefits.

FUTURE DIRECTIONS

The promising applications of *Aspergillus oryzae* in the food industry and gut health open several avenues for future research and innovation. Advanced studies should focus on optimizing the fermentation processes involving *A. oryzae* to enhance the nutritional and therapeutic properties of food products. Genetic engineering approaches can be employed to develop strains with improved enzyme productivity and specificity, catering to diverse industrial needs. Exploring the interactions between *A. oryzae*-derived metabolites and the gut microbiome may yield novel insights into its role in promoting gastrointestinal health and preventing disorders. Additionally, research on the potential application of *A. oryzae* in personalized nutrition and functional foods tailored to individual gut microbiota profiles could revolutionize health-oriented dietary strategies. Finally, expanding the scope of *A. oryzae's* applications beyond the food industry, such as in pharmaceutical and agricultural domains, warrants further investigation to maximize its biotechnological potential.

CONCLUSIONS

Aspergillus oryzae, celebrated for its enzymatic versatility and GRAS status, remains an indispensable organism in the food industry and functional food development. Its pivotal role in producing fermented foods and industrial enzymes demonstrates its unmatched biotechnological potential, enhancing flavor, nutritional value, and food processing efficiency. Beyond its industrial significance, emerging research underscores its contributions to gut health, particularly through digestion improvement, gut microbiome modulation, and gastrointestinal well-being. By consolidating recent advancements, this review highlights *A. oryzae* as a cornerstone in both traditional practices and innovative food technologies, paving the way for future applications in health-oriented food products.

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