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Implementation of Metabolomic Approaches on Fermented Cereals

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ABSTRACT
Fermentation is a widely utilized food processing technique that enhances sensory characteristics, extends shelf life, and increases product diversity, particularly in cereals. Furthermore, fermentation processes can generate bioactive metabolites that confer various health benefits. Metabolomics is employed to assess changes in the nutritional composition and metabolite profile of fermented cereal products. This research contributes to identify and synthesize findings from various studies that evaluate the effects of fermentation on the quality of the final product using a metabolomics approach, with particular emphasis on metabolic pathways and the metabolites formed. The research highlights that metabolomics, through sophisticated analytical techniques, has successfully identified metabolites produced during fermentation, which are categorized into volatile compounds (such as organic acids, alcohols, and aldehydes) and non-volatile compounds (including polyphenols, amino acids, fatty acids, free sugars, esters, and amides). These compounds play a pivotal role in enhancing the sensory properties of the product and exhibit bioactive potential that can help prevent the development of metabolic diseases, including diabetes, obesity, digestive disorders, and liver cancer. Despite its potential, the metabolomic approach faces challenges due to the large and intricate datasets it produces. Recent technological advancements have led several studies to incorporate Artificial Intelligence (AI) to enhance the accuracy of data derived from metabolite databases. Moreover, integrating other omics disciplines, such as foodomics, is crucial for achieving more detailed research results, encompassing metabolite composition, sensory attributes, and their effects on health. Such integration would contribute to a more holistic understanding and help bridge the gaps in contemporary metabolomic research.

KEYWORDS
Cereal; Fermentation; Food processing; Metabolomic

1. INTRODUCTION

Cereals are a critical source of calories and essential nutrients for the growing human population. With a total production exceeding two billion tons annually, cereals represent one of the most important food sources worldwide [1]. Cereals, commonly referred to as grains, are members of the Poaceae family, cultivated mainly for their seeds or kernels, which serve as a major source of carbohydrates. In general, cereals are high in carbohydrates, contain significant amounts of protein, are low in fat, and are an excellent source of dietary fiber. In addition to their primary macronutrients, cereals are also abundant in vitamins, such as vitamin E, vitamin A, and the B-complex vitamins, as well as essential minerals like iron, magnesium, and zinc [2]. Epidemiological studies have shown that regular consumption of wheat-based cereals can reduce the risk of non-communicable diseases. However, their digestibility and bioavailability are limited due to the presence of antinutritional factors, which can be mitigated through processing methods such as soaking, cooking, or fermentation. According to Tsafrakidou et al., (2019) [3], the fermentation process in cereal-based products not only extends shelf life but also enhances the content of phenolic compounds and vitamins, while improving the digestibility of proteins and carbohydrates in the final product. Moreover, the fermentation of cereals and pseudocereals can lead to several beneficial

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changes in the nutritional composition of food products, including increased protein, amino acid, and fatty acid content, the removal of toxins, and partial hydrolysis of starch and gluten [4].

Currently, metabolomics is a rapidly advancing field with significant applications in food science, food quality, and food safety. The metabolomic approach is particularly valuable as it enables the identification of qualitative characteristics of fermented products, such as taste, nutritional composition, functional properties, and shelf life [5]. The techniques and tools commonly used in metabolomic studies include NMR, FTIR, LC-MS, GC-MS, and CE-MS. Each of these techniques has its own advantages and limitations, such as sensitivity and sample preparation requirements. According to Utpott et al., (2022) [6], the application of metabolomics in fermented food products facilitates the prediction of sensory characteristics and nutritional quality, as well as the monitoring of metabolic changes throughout fermentation. Additionally, this approach allows for the identification of metabolites linked to health benefits, aroma, and taste, thereby providing a deeper understanding of microbial activity and its impact on the final product, while also enabling better control of processing times.

The various benefits and advantages demonstrated by research using metabolomics in cereal fermentation can be discussed in detail. This is highlighted in the review by Gupta & Gaur, (2021) [7], which explores the levels of food safety, nutrition, and food quality, as well as their health effects in cereal fermentation products, using a metabolomic approach focused specifically on LC-MS. Similarly, the review by Adebo et al., (2021) [8] summarizes findings from cereal and legume fermentation, concentrating exclusively on a metabolomic approach utilizing GC-MS. Many reviews focus solely on specific metabolomics techniques, particularly LC-MS and GC-MS. However, several studies have utilized a wider variety of metabolomics approaches. This review seeks to offer a deeper understanding of the alterations in metabolite profiles and uncover the nutritional potential of fermented cereals, analyzed through a broader spectrum of metabolomics tools. Therefore, this review aims to focus on the metabolomic process in cereal fermentation, employing a range of analytical instruments, with an emphasis on final product quality, metabolite identification, and the metabolic pathways involved. This systematic literature review serves as a valuable reference and guide for researchers, industry professionals, and relevant stakeholders in the development and diversification of processed cereal products.

2. MATERIALS AND METHODS

The material collection process involved gathering relevant research papers for analysis [9]. A database search was conducted using a set of keywords related to the research question. These keywords included "fermentation metabolomics," "cereal fermentation," "nutritional content," "metabolomics," "metabolites," "sensory properties," "metabolomic data analysis," "NMR cereal," "LC-MS cereal," "GC-MS cereal," and "FTIR cereal." The search was limited to studies published within the last 10 years and restricted to peer-reviewed journal articles in English. The most recent update was conducted in July 2024. To ensure a comprehensive search, various major databases were selected, including Elsevier, Scopus, Springer, Web of Science, Wiley, Taylor & Francis, and PubMed. The steps used in this study are visualized in Figure 1.

3. RESULTS AND DISCUSSION

3.1. Cereal Fermented

Fermentation plays a crucial role in the production of cereal-derived products, significantly influencing sensory characteristics such as taste, aroma, and texture. Solid-state fermentation is widely used in cereal fermentation and is considered a promising alternative for biotransformation in industrial applications due to its minimal water requirement, which facilitates the concentrated production of metabolites [10]. In addition to solid-state fermentation, several studies also employ submerged fermentation, as well as cold fermentation, using two methods: unchanged stepping liquor and modified stepping liquor, which differ in the alteration of the brewing liquid during fermentation. Common microorganisms used in the fermentation process include *Rhizopus*, *Lactobacillus*, *Streptococcus*, *Aspergillus*, and *Bacillus*. The fermentation process is typically divided into several stages (Figure 2),

including substrate preparation, medium preparation, inoculum preparation, cereal inoculation, incubation, and the monitoring and control of the fermentation process.

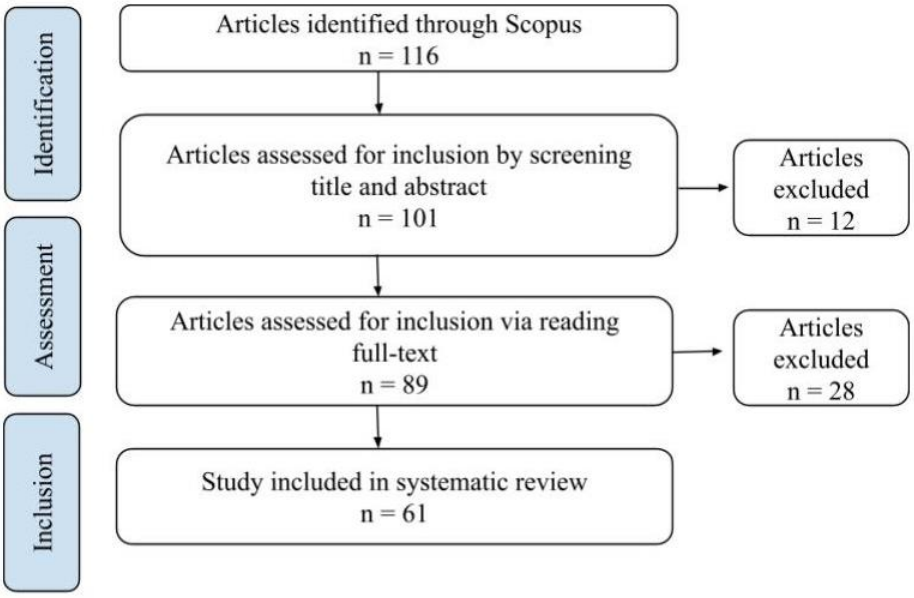

Figure 1. Flowchart of articles selection for review.

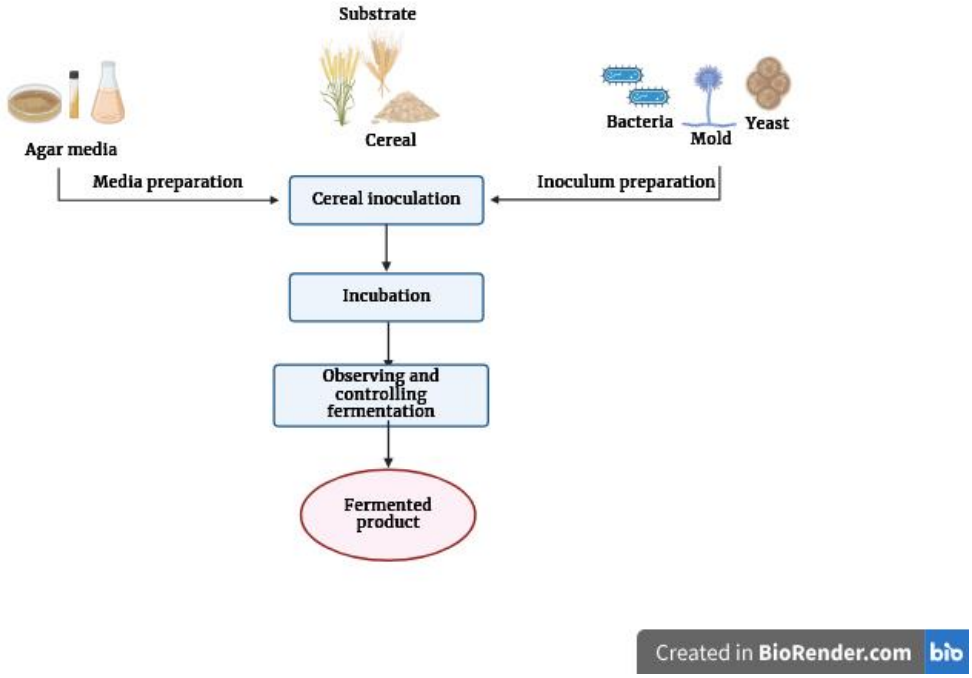

Figure 2. Flowchart of the fermentation process in cereals.

The initial preparation of cereals involves sorting, washing, and cleaning, followed by pre-treatment steps such as processing and sterilization. To convert cereals into a suitable substrate for fermentation, grinding is one of the most commonly used methods. In addition to grinding, cooking is another widely employed step before fermentation. A commonly used medium in fermentation is deMan, Rogosa, and

Sharpe (MRS) agar, which supports the growth of species such as *Lactobacillus*, *Limosibacillus*, *Weissella cibaria*, *Lacticaseibacillus*, *Lactiplantibacillus*, and *Streptococcus*. Cereal substrates that utilize MRS medium during fermentation include Kamut (Khorasan wheat), sorghum, rye, red rice, quinoa, black barley, buckwheat, oats, rice, millet, and barley. Other agar media used in the fermentation process include Yeast Extract Peptone Dextrose (YPD), Plate Count Agar (PCA), Rose Bengal Chloramphenicol, and Potato Dextrose Agar (PDA). The next step involves inoculating the microorganisms onto the cereal substrate. The starter concentration varies depending on the cereal type, but typically, the concentration of microorganism ranges from 10^5 to 10^9 CFU/mL. The fermentation substrate is then incubated in an environment with 90-95% humidity and temperatures ranging from 30-37 °C, with continuous monitoring. Incubation may also be conducted in an incubator. Fermentation time varies depending on the type of cereal used and the desired product.

The incorporation of cereals can enhance the sensory properties of fermented food products, making them more distinctive and appealing. This is supported by studies indicating that barley-based yogurt is more widely accepted than traditional yogurt [11]. The taste and aroma of yogurt are primarily influenced by the presence of organic acids. Sensory evaluation of yogurt involves the assessment of several attributes, including taste, aroma, color, and overall product quality. The addition of superoxide dismutase to millet yogurt improves viscosity, prevents whey precipitation, and contributes to a unique taste, smooth texture, white color, and glossy appearance [12]. Furthermore, fermentation with *Lactiplantibacillus plantarum* plays a critical role in flavor development, enhancing the formation of volatile metabolites that contribute to the yogurt's aroma quality.

Fermentation of cereals and legumes also gives rise to a variety of both alcoholic and non-alcoholic beverages. This review discusses six types of beverages derived from the fermentation of these substrates. Baijiu, a traditional Chinese alcoholic beverage, is produced through the fermentation of sorghum. The presence of functional microbes plays a crucial role in flavor development, with aldehydes and glucose identified as key compounds. During fermentation, the concentrations of alcohol, alditol, and 1-hexadecanol increase. Additionally, to produce a light and pleasant flavor in Baijiu, several organic acids that form esters are elevated [13]. Another alcoholic beverage produced through fermentation is derived from millet. In a study by Majumder et al., (2021) [14], tongba is described as a traditional fermented beverage from the regions of India, Nepal, and Bhutan. This millet-based beverage is uniquely served by mixing the fermented product with boiling water in wooden or bamboo cups. Another millet-based alcoholic drink, raksi, also originates from Nepal, although this beverage includes additional ingredients, such as rice [15].

3.2. Metabolomics in Cereal Fermented Products

Metabolomics has been developed to comprehensively analyze small molecules (metabolites with molecular weights < 1500 Da) in biological systems. As one of the primary "omics" tools, the extensive metabolomics approach has achieved significant success in addressing a wide range of scientific challenges across biology, biomedicine, agriculture, nutrition research, drug discovery, disease diagnosis, and plant physiology [16]. Metabolomics offers an in-depth analysis of chemical components [17], defining quality by both qualitatively and quantitatively identifying thousands of metabolites, particularly those that cannot be detected through conventional methods [18]. Figure 3 illustrates the analytical procedure employed in metabolomics to obtain accurate measurements of specific metabolites in research. This procedure encompasses sample preparation, instrumental analysis, data processing, statistical analysis, and data interpretation, all of which directly influence the final results and biological insights. The methods used in sample treatments such as centrifugation, solid-phase microextraction, extraction, and/or concentration, along with critical analytical techniques, have successfully detected contaminants and identified key biomarker compounds at very low concentrations in food product metabolism [19].

Recent studies have demonstrated that metabolomic analysis can be used to identify bioactive compounds and assess microbial metabolic pathways involved in cereal fermentation. For example, research by Khakpour et al., (2023) [20], highlighted the antibacterial potential of plant extracts derived from *Juglans regia*, *Citrus sinensis*, *Vicia faba*, and *Urtica urens*. These extracts were found to exhibit

antimicrobial properties that could contribute to the safety and stability of fermented cereal products by inhibiting harmful bacteria. Such findings underscore the potential of metabolomics in identifying functional metabolites that may enhance the nutritional and microbiological quality of fermented cereals.

Additionally, Kalavari et al., (2023) [21] explored the enrichment of dough, a traditional fermented dairy beverage, with olive leaf extract and evaluated its physicochemical, microbial, and sensory properties. Their study found that the inclusion of olive leaf extract significantly reduced pH and microbial load while enhancing stability and extending shelf life. These findings further support the application of metabolomics in optimizing fermentation processes and improving the functional quality of fermented products.

The analysis of complex and diverse samples composed of small molecules with distinct physicochemical properties requires the use of various analytical techniques. Among the 61 research articles reviewed, the most commonly employed metabolomic analysis technique was Liquid Chromatography-Mass Spectrometry (LC-MS), followed by Gas Chromatography-Mass Spectrometry (GC-MS), with some studies also utilizing Nuclear Magnetic Resonance (NMR). LC-MS combines liquid chromatography to separate sample components with mass spectrometry as the detector. This technique is widely used in metabolomic studies of cereal and legume fermentation because it is not limited to volatile molecules (typically with molecular weights below 500 Da) and can also analyze highly polar analytes, requiring relatively simple sample preparation [22]. The basic components of liquid chromatography include a pump system, a separation column, and an ionization interface. In contrast, Gas Chromatography-Mass Spectrometry (GC-MS) is a hybrid method that integrates Gas Chromatography (GC) and Mass Spectrometry (MS). Gas Chromatography is a separation technique used to analyze volatile or easily vaporized compounds, while Mass Spectrometry serves as an analytical method to identify and characterize sample components by determining the relative mass of the molecular components and their fragments. The main components of a GC-MS system typically consist of four parts: the gas chromatograph, the interface, the mass spectrometer, and the data processing system.

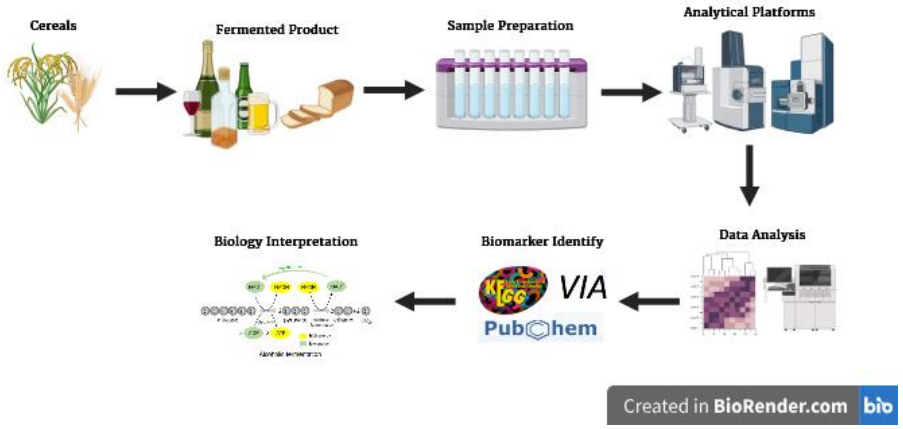

Figure 3. Flowchart of Metabolomic Analysis in Cereal Fermentation.

Raw data from LC-MS and GC-MS instrument analyses typically consist of ion intensity spectra obtained at various time points, plotted against the m/z ratio. These spectra can be annotated to identify the chemical structures of metabolites and quantify compounds in cereal fermentation samples. Annotation can be performed using public databases, which are categorized into two types: compound-centric databases and pathway-centric databases. The most commonly used databases for GC-MS and LC-MS are compound-centric, where unknown spectra are matched with reference spectra from databases such as the Human Metabolome Database (HMDB), PubChem, Phenol Explorer, NIST, Wiley, FooDB, and METLIN, using computational search algorithms like "find-by-formula" [23][24]. Statistical analysis is essential for understanding the effects of fermentation on a system. Multivariate analysis is particularly suited for

datasets with large numbers of variables. The most commonly applied techniques in the studies reviewed are Principal Component Analysis (PCA) and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA).

Although Nuclear Magnetic Resonance (NMR) provides several benefits, including straightforward sample preparation, reduced analysis time, ease of metabolite identification, and enhanced sample recovery, it also has certain drawbacks. These include its limited ability to detect a broader range of metabolite variations, as well as the restricted availability of comprehensive metabolite databases for NMR-based metabolomics. Similarly, techniques like Raman and FTIR also face selectivity limitations. Mass Spectrometry (MS)-based approaches can analyze a broader spectrum of metabolites following chromatographic separation, with access to various extensive metabolite databases. However, the sensitivity of MS platforms is contingent upon the chromatographic separation techniques employed. Advancements in Gas Chromatography-Mass Spectrometry (GC-MS) offer superior chromatographic resolution for metabolites, while Liquid Chromatography-Mass Spectrometry (LC-MS) is the preferred method for achieving greater metabolite coverage and sensitivity. However, GC-MS is not without its limitations, such as longer analysis times and its restriction to thermally stable, volatile compounds that are prone to degradation [25].

Due to the inherent limitations of each analytical technique, it is crucial to acknowledge that no single standard exists in quantitative metabolomics. The selection of an appropriate method should be guided by various factors, including the specific sample type, the analytes of interest, and the available instrumentation. As novel technologies continue to emerge and become more accessible, they are poised to drive significant advancements in metabolomics. Techniques such as ion mobility spectrometry and ionization methods have now been introduced, offering new opportunities for analysis. For instance, ion mobility separation can serve as a valuable complement to the limitations of chromatographic separation in mass spectrometry. Additionally, to align metabolite concentration data across biological samples, there is an ongoing need for the development of comprehensive databases that facilitate accurate metabolite identification [26].

3.3. Identification of Metabolites in Cereal Fermented Products

Metabolites are categorized into primary and secondary metabolites. Primary metabolites are molecules involved in the basic functions of organisms, such as growth, development, and reproduction, and are found in all types of organisms. Examples include amino acids, nucleotides, vitamins, ethanol, organic acids, and sugars. In contrast, secondary metabolites are molecules that contribute to the protective functions of an organism and do not directly participate in essential processes like growth, reproduction, or development. Examples of secondary metabolites include phenolics, terpenoids, alkaloids, bacteriocins, and polyketides. To facilitate the discussion of metabolites found in cereal fermentation products, they will be categorized into volatile and non-volatile compounds. A summary of the metabolites identified through metabolomic analysis of fermentation products is provided in Table 1.

Flavor results from the interaction of various volatile and non-volatile components, each with distinct chemical properties. Non-volatile compounds primarily contribute to taste characteristics. Polyphenols, one of the non-volatile compounds found in cereal fermentation products, are secondary metabolites in plants and are present in a wide range of foods. Based on their chemical structure, polyphenols can be classified into several groups, depending on the number of phenolic rings and the structural elements that form the ring bonds. The most common classification of polyphenols includes five main classes: phenolic acids, stilbenes, flavonoids, lignans, and others. Among these, flavonoids are the most abundant phenolic compounds, synthesized through the phenylpropanoid pathway [27].

Amino acids have been identified in the fermentation of various substrates, including corn, soybeans, red rice, rye, and black glutinous rice, resulting in products such as doenjang, angkak, pixian, meju, wine, shuanjiang, and yogurt. The amino acids identified in these products include glutamic acid, arachidonic acid, methionine, agmatine, proline, alanine, isoleucine, glycine, phenylalanine, tyrosine, aspartic acid, and asparagine. In addition to contributing to sweetness, proline and alanine have physiological effects, such as preventing apoptosis and scavenging free radicals. Isoleucine imparts a bitter taste, glycine contributes to

sweetness, while phenylalanine and tyrosine are associated with savory or umami flavors. Beyond their role in flavor, amino acids offer several health benefits. Agmatine is known to enhance cardiovascular health [28], while arachidonic acid plays a role in the prevention of diabetes and tumors [12]. L-methionine, an essential amino acid, helps protect the liver and is of significant value for human health.

In addition to amino acids, fatty acids are another important non-volatile compound found in fermented products. Fatty acids are hydrocarbon chain molecules containing carboxyl groups [29]. The fermentation process can increase the fatty acid content through lipid hydrolysis. This increase is associated with the dissociation of lipid complexes and the activity of lipolytic enzymes, which enhance fatty acid extraction. Beyond their nutritional value, fatty acids also influence the taste and sensory properties of fermented food products. Fatty acids identified in fermented cereals and legumes include palmitic acid, stearic acid, linoleic acid, oleic acid, hexadecanoic acid, and lauric acid. Palmitic acid and oleic acid, in particular, can affect yeast activity, thereby influencing the flavor of wine.

Table 1. Summary of cereal fermentation with metabolomic analysis.

Cereals	The aims	Metabolomic instrumen	Metabolites result	Metabolit/ biomarker	Statistic methods	Results	Ref
Barley malt	To further evaluate the phenolic content based on antioxidant activity and a metabolomic approach	UPLC-ESI-QTOF-MS	102 metabolites	Flavonoid, phenolic acids (trans-ferulic acid, p-coumaric acid, ferulic acid, caffeic acid, protocatechuic acid, 3-7-dimethyl quercetin, tetramethyl scutellarin isomer 3, gentisic acid, phlorizin, naringenin 7-O-glucoside), polyphenols, lignans, stilbenes, 4-hidroksi hipurat	PCA, OPLS-DA	1. SSF BSG with *Aspergillus oryzae* has been shown to be efficient in the release of antioxidant bioactive compounds; furthermore, variations in malt affect the fermentation process 2. PC is influenced by the enzyme β-glucosidase. Metabolites act as preventatives for age-related chronic diseases, including cardiovascular, neurodegenerative, diabetes, and cancer	[10]
Sorghum	To identify unique active functional microbes involved in flavor formation and provide new insights into the optimization of Niulanshan	LC-MS, GC-MS, HS-SPME-GC-MS	83 metabolites	Organic acids (lactic acid, linoleic acid, aldehyde, heptanoic acid, succinic acid). Etil butirat, ester asetat, benzil alkohol.	PCA, OPLS-DA	Fermentation samples were taken on days 16 and 27. Fourteen microbes contributed to the production of volatile NLS flavors, including *Lactobacillus*, *Saccharomyces*, *Aspergillus*, and *Streptococcus*.	[13]

Cereals	The aims	Metabolomic instrumen	Metabolites result	Metabolit/ biomarker	Statistic methods	Results	Ref
	baijiu fermentation					Ethyl esters, alcohols, and aldehydes were correlated with the unique flavor of NLS	
Mixed grain	To determine the metabolic profile and investigate the nutritional and functional properties of fermented mixed grains	CE-TOF-MS	165 metabolites	Amino acids (Spermidine, agmatine) Phenolic compounds (Homovanillic acid; 2,5-dihydroxybenzoic acid and p-hydroxybenzoic acid)	Heat map	Metabolites involved in the urea cycle and polyamine pathway are altered through fermentation, with arginine being used as a precursor to produce citrulline, ornithine, and agmatine. FMG incubated for 36 hours showed higher total phenolic content and free radical scavenging activity	[28]
Barley	To determine the antioxidant activity, total phenolic content, and flavonoid compounds in the product	UPLC-MS/MS	27 metabolites	Cyclic adenosine monophosphate (cAMP), glycerophosphocholine, organic acids, phenylpropanoids, polyketides, flavonoids, isoflavonoids	OPLS-DA	Barley with soy protein concentrate combined with *L. bulgaricus* VHProbi R03 and S. thermophilus VHProbi R08 produced a good yogurt as a source of probiotics and antioxidants, with a viable bacterial count of 1.9 x 10^8 CFU/mL after 4 hours of fermentation	[11]
Barley	1. To explain the biotransformation role of *L. plantarum* dy-1 2. Exploration of the potential of barley fermentation	UPLC-HRMS	124 metabolites	Saccharides, nucleosides, amino acids, organic acids, glucose-6-phosphate, gluconic acid, indole-3-lactic acid, phenyllactic acid, homovanillic acid, 2-hydroxyvaleric acid, etc	PCA, heatmap, PLS-DA	During fermentation, nutrients are partially bound or conjugated by bioactive molecules, and several important metabolites are produced as a	[30]

Cereals	The aims	Metabolomic instrumen	Metabolites result	Metabolit/ biomarker	Statistic methods	Results	Ref
	as a functional food					result of the growth and metabolism of *L. plantarum* dy-1	
Barley	To determine the effect of *S. thermophilus* fermentation on the metabolic profile and antioxidant activity of barley juice	GC-MS, UPLC-QTOF-MS	71 metabolites	Pentanoic acid, vanillin, benzyl alcohol, ethanone, ethanol, 2-pentanone, 2,3-pentanedione, benzoic acid. S-adenosylmethionine, uracil, hypoxanthine.	PCA, OPLS-DA	Barley juice fermented with *S. thermophilus* 7G10 exhibited increased free radical activity. Sensory evaluation of barley juice fermented with *S. thermophilus* 7G10 showed the most appealing results for consumers	[31]
Quinoa and black barley	To investigate the nutritional characteristics, composition and quantity of bioactive components, as well as the in vitro activity of black barley and quinoa fermentation	UPLC-Q-TOF-MS	41 metabolites	Flavonoids (saponin, tyrosine, phenylalanine) Polyphenols (methyl chlorogenate, phenylpropanoic acid, ρ-coumaric acid)	PCA, OPLS-DA	Fermentation increases protein content and total dietary fiber. Furthermore, the increase in flavonoid levels leads to in vitro bioactivity such as α-amylase and α-glucosidase inhibition. Metabolites are involved in pathway analysis, particularly in the biosynthesis of flavonoids, anthocyanins, and phenylpropanoids	[32]
Red rice	To determine the GABA content, antioxidant activity, and bioactive compounds in red rice	UHPLC-Q-TOF-MS/MS	78 metabolites	Amino acids (GABA, methionine, histidine, valine, tryptophan, leucine, lysine). Phenolics (β-caroteneol, eugenol, apigenin, 6-gingerol, cinnamic acid). Organic acids (lipoic acid, pyrazinoic acid, malonic acid, ascorbic acid, gluconic acid, p-coumaric acid, malic acid).	ANOVA and PCA	Fermentation with *Lactobacillus reuterii* resulted in an increase in amino acids, phenolic compounds, organic acids, fatty acids, and peptides in red rice. Consumption of fermented red rice *L. reuterii* AKT1 can meet	[33]

Cereals	The aims	Metabolomic instrumen	Metabolites result	Metabolit/ biomarker	Statistic methods	Results	Ref
				Fatty acids (stearic acid, linoleic acid, valeric acid, lauric acid).		the requirements for optimal health benefits	
Sorghum	To determine the metabolomic profile extracted from sorghum varieties (low tannin and high tannin) and their fermentation derivatives (ting) to provide metabolic variation in the samples using a metabolomic approach	GC-HRTOF-MS	34 metabolites	Fatty acid ester, metalaxyl, fenol, keton, 4-chlorobenzonitrile, 2-hidroksil-5-metil fenil, 2(5H)-furanon, tannin, 1-(2-hidroksil-5-metil-fenil)	PCA, OPLS-DA	Tannin content affects the composition of sorghum and WG-ting samples. Fermentation leads to an increase in fatty acid and fatty acid ester content. The metabolic composition of raw sorghum causes different metabolic changes during fermentation. The presence of fermentation affects the sustainability of substrates for fermentation. The best fermentation is achieved using *Lactobacillus fermentum* FUA 3321	[34]
Rice and oat	To evaluate the fermentation efficiency of bioactive compounds in rice and oats by *Lactobacillus apis* (L. apis), a new strain isolated from honeybee gut, and two types of Lactobacillus isolated from breast milk (L.BM) and camel milk (L.CM)	SPME-GC-MS	35 metabolites	Amino acids (Lysine, threonine, valine, methionine, isoleucine, phenylalanine, histidine, arginine, aspartate. Volatiles (Ester, terpene, furan, ketone, aldehyde). Organic acids (Dodecanoic acid, hydroxycinnamic acid, acetic acid, lactic acid).	Heat map	L.CM and L.BM produced the highest levels of phenolics, flavonoids, volatiles, alcohols, and organic acids from different substrates and exhibited the highest antioxidant capacity. The best pattern was obtained with L.BM, followed by *L. apis* and L.CM, compared to the *L. plantarum* and non-fermented samples.	[35]

Cereals	The aims	Metabolomic instrumen	Metabolites result	Metabolit/ biomarker	Statistic methods	Results	Ref
						L.BM and L.CM significantly increased the volatile components	
Oat	Combining various analyses to understand the mechanism by which starter culture affects oat beverage, monitored from chemical, physical, metabolic, and sensory aspects.	GC-MS	60 metabolites	Aldehyde (pentanal, hexanal, octanal, nonanal, decanal dan benzaldehyde) Ketones (Diacetyl (2,3-buta-nedione), acetylpropionyl (2,3-pentanedione), Acids (acetic acid, hexanoic acid, 2-methylbutanoic acid). 2-butylfuran, 2-pentylfuran.	PCA	The concentration of preferred volatile compounds such as diacetyl and acetoin increased during fermentation. The growth of various vegan starter cultures and changes in the consortium bacterial composition affect the sensory development and texture of oat beverages.	[36]
Millet	Developing millet-flavored yogurt enriched with SOD	HPLC-MS, HS-SPME-GC-MS	54 metabolites	2-phenyl ethanol, hesperidin, N-acetylornithine and L-methionine, L-threonine, allopurinol, arachidonic acid, adenosine, nonanoic acid, oxalic acid. Flavonoid (hesperetin, 2-phenyletanol)	PCA	SOD-rich millet yogurt is known to neutralize free radicals, efficiently break down superoxide dismutase into oxygen and hydrogen peroxide. *Lactiplantibacillus plantarum* has good probiotic potential. The addition of enzymatic hydrolysis of millet in yogurt fermentation enriches the flavor and nutrition.	[12]
Buckwheat	Investigating the differences and changes in the volatile profile of buckwheat (soba) soksungjang inoculated	SPME-GC-MS	83 metabolites	3-metilbutano, 2,3-metilbutanal, 2-feniletanol, 2-etilheksa-1-ol, dimetil karbonat, asam pentanoat. Fatty acids (methyl heptanoate, methyl octanoate, methyl	PLS-DA	The volatile profile of the soba soksungjang samples depends on the fermentation period and the microbial combination.	[37]

Cereals	The aims	Metabolomic instrumen	Metabolites result	Metabolit/ biomarker	Statistic methods	Results	Ref
	with various microbial starters during fermentation			butanoate), esters, fusel aldehydes		Generate complex metabolic processes such as glycolysis, lipolysis, pyruvate metabolism, and proteolysis	

3.4. Metabolic Pathway

The Kyoto Encyclopedia of Genes and Genomes (KEGG) database is widely used to explore metabolic pathways and elucidate the mechanisms underlying metabolic changes during fermentation [38]. Enrichment and topological analyses are then conducted to identify key pathways most relevant to the metabolites involved. The identification of metabolic pathways suggests that fermentation can enhance the nutritional value and nutrient content of fermented cereal and legume products. Among these pathways, starch metabolism, sucrose metabolism, alanine metabolism, aspartate metabolism, glutamate metabolism, and the pentose phosphate pathway are particularly significant, as they are associated with the pre-fermentation processes that influence wine color. Wine fermentation begins with the degradation of polysaccharides. As the glycolysis/gluconeogenesis pathway progresses, glucose is converted into pyruvate, which enters the TCA cycle via acetyl-CoA, leading to the production of various organic acids. In rice fermentation for the production of angkak, four key metabolic pathways include glycolysis, pyruvate metabolism, α-linolenic acid metabolism, and linoleic acid metabolism. Pyruvic acid, α-linolenic acid, and linoleic acid are essential in the transformation of these compounds into secondary metabolites during angkak production.

3.5. Challenges and Future of Metabolomics

Metabolomics is an analytical approach that offers significant benefits and potential for the development of fermented cereals products. However, this technology also faces certain challenges and limitations. A major obstacle with LC-MS instrumentation is the time-consuming nature of analysis preparation. Standardizing the pre-analytical phase is essential for minimizing variability across biological samples and preserving the integrity of metabolite profiles. This involves aligning protocols for sample collection, storage, and preparation, in compliance with recognized analytical methodology standards [39]. Consequently, to achieve the desired outcomes in metabolomic research, meticulous selection of sample preparation methods is vital for ensuring reliable and reproducible biological data.

Additionally, the large variation in data generated after the chromatographic process often leads to suboptimal data processing. The variability in data can be attributed to several factors, including differences in operator handling, instrument construction, and changes in operational conditions, such as laboratory temperature [7]. Annotation and metabolite identification present additional challenges, particularly in untargeted analysis. Data libraries often struggle when faced with new compounds and data generated from novel procedures and instruments. This is consistent with the findings of Diez-Simon et al., (2019) [40], who highlighted that the limited availability of comprehensive libraries containing food metabolites significantly hinders metabolite identification. A key limitation is the absence of well-established standard procedures for pre-treatment and normalization of metabolomic data, as well as for its integration into a consolidated dataset for subsequent statistical analysis and modeling. Numerous challenges in data standardization typically occur at various stages, such as data acquisition, initial processing, addressing missing values, detecting outliers, data normalization, centering and scaling, data transformation, and statistical analysis [41]. Given the complexity and scale of metabolomic data, it is crucial to map and preprocess the data before employing AI-driven machine learning techniques. This preprocessing is

essential, as machine learning models depend on the precision and reliability of the data for generating accurate outcomes.

From an instrumental perspective, the mass analyzer in a mass spectrometer must operate in a high-vacuum environment, as collisions with air molecules can disrupt the ion flow from the source detector, leading to signal loss. Therefore, careful and precise handling of metabolomics instruments is crucial. According to Harmita et al., (2019) [22], the primary issues in the operation of mass spectrometers include air leaks, solvent contamination, and disturbances in the detection of small mass readings. Regardless of the instrument type, regular cleaning of the interface, ion source, and mass analyzer is essential. The mass spectrometer source in LC-MS systems typically experiences fewer contamination issues compared to GC-MS systems, as contamination in GC-MS is often caused by filament aging and the combustion of hot vapor from the GC oven.

The complex standardization and dynamic processes involved in metabolomics reveal several limitations due to the various factors at play and the diverse information required for research. Therefore, when complemented with chemometric tools, metabolomics can be used to objectively evaluate both the fermentation process and the final products [42]. The potential of metabolomics in metabolite analysis remains promising, as it enables the simultaneous analysis of thousands of metabolites in a short time, identification of potential biomarkers, and quantitative measurement of metabolites involved in metabolic pathways. Efforts to standardize sample preparation methods and establish reporting guidelines are essential for advancing the field.

Advancements in automation can help reduce reliance on manual handling, thereby enhancing the efficiency and reliability of instruments. Innovations aimed at mitigating matrix effects will contribute to more accurate data. Additionally, improvements in instrumental components, such as column technology, will increase the versatility of metabolomics. According to Gupta & Gaur, (2021) [7] artificial intelligence-based metabolite annotation is now available to assist in compound identification. Metabolomics and Artificial Intelligence (AI) work synergistically to enhance research outcomes. Metabolomics produces extensive datasets, encompassing hundreds to thousands of metabolites with intricate interconnections. Numerous studies have demonstrated the effective use of AI across multiple facets of metabolomic analysis, such as analytical detection, data preprocessing, biomarker identification, predictive modeling, and the integration of multi-omics data [43]. Aligning accuracy and improving data quality are essential for fostering collaboration, accelerating scientific progress, and gaining a deeper understanding of complex biological processes like fermentation. Further efforts are needed to enhance spectral databases, particularly through the development of accurate automatic identification algorithms.

The cereal-based fermented products noted the growing importance of emerging technologies, such as metabolomics, in elucidating the relationship between the microbial communities, metabolic pathways, and the functional properties [44]. The unique microbial communities involved in traditional fermentation processes can contribute to the enhanced nutritional value and potential health benefits of the final products [45][46]. The study of fermented foods highlighted the potential of this approach to provide a deeper understanding of the complex metabolic processes involved in fermentation, as well as its ability to identify biomarkers for the potential health benefits of these products. These studies have highlighted the potential of metabolomics to provide a comprehensive evaluation of the microbial diversity, metabolic profiles, and functional attributes of traditional fermented foods, paving the way for a better understanding of their relevance and potential in modern food science and human health [6].

4. CONCLUSION

A metabolomic approach reveals that fermentation influences the quality of the final fermentation products of cereals. Using metabolomics, it is possible to investigate the effects of fermentation on biological systems and develop fermentation protocols that can later be adopted by governments, industries, and stakeholders to create fermentation-based cereal and products. Fermentation of cereals produces a broad spectrum of beneficial metabolites, which improve both shelf life and sensory qualities, as shown by metabolomic analysis. It is expected that further research will examine a greater diversity of cereal varieties, enabling their potential as alternative food sources and supporting their development for large-scale

industrial production. However, metabolomic research needs to be integrated with other omics technologies, such as foodomics, to gain a more comprehensive understanding and address the research gaps left by metabolomics alone. Additionally, studies comparing metabolomic profiles of cereal products processed by various methods, beyond fermentation, are needed. This will enhance our knowledge of effective techniques to improve the quality of cereal products for public consumption.

AUTHOR CONTRIBUTION

All author participated actively to this paper. Final paper was read and approved by all authors. **Yani Magfiroh**: Writing (concepting & editing), writing (original draft), supervision. **Laksmi Hartajanie**: Writing (review & editing), evaluating analysis result. **Lindayani**: Validation, formal analysis. **Dyah Wulandari**: Investigation, designing graphical abstract, validation, funding acquisition.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest to declare.

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REFERENCES

[1] M. Verni, C. G. Rizzello, and R. and Coda, "Fermentation biotechnology applied to cereal industry by-products: nutritional and functional insight," *Front. Nutr.*, vol. 6, 42, 2019, https://doi.org/10.3389/fnut.2019.00042.

[2] H. Widiastuti, *Pengolahan hasil nabati komoditas serealia dan kacang-kacangan*. Penerbit Mitra Cendekia Media, 2021.

[3] P. Tsafrakidou, A.-M. Michaelidou, and C. and Biliaderis, "Fermented cereal-based products: nutritional aspects, possible impact on gut microbiota and health implications," *Foods*, vol. 9, 734, 2019, https://doi.org/10.3390/foods9060734.

[4] P. Petrova and K. Petrov, "Lactic acid fermentation cereals and pseudocereals: ancient nutritional biotechnologies with modern applications," *Nutrients*, vol. 12, 1118, 2020, https://doi.org/10.3390/nu12041118.

[5] G. Yaxin, H. Lizhen, G. Jie, L. Danfeng, T. Zhiliang, F. Bei, W. Fengzhong and L. Shuying, "Metabolomics approaches for the comprehensive evaluation of fermented foods: a review," *Foods*, vol. 10, p. 2294, 2021, https://doi.org/10.3390/foods10102294.

[6] M. Utpott, E. Rodrigues, A. Oliveira Rios, G. M. Mercalli, and S. H. Flores, "Metabolomics: an analytical technique for food processing evaluation," *Food Chem.*, vol. 366. 13068, 2022, https://doi.org/10.1016/j.foodchem.2021.130685.

[7] R. Gupta and S. Gaur, "LC-MS investigated as a tool to study the metabolomic characteristics of cereal fermentation," *Appl. Food Res.*, vol. 4. 100365, 2021, https://doi.org/10.1016/j.afres.2023.100365.

[8] O. A. Adebo, S. A. Oyeyinka, J. A. Adebiyi, X. Feng, J. D. Wilkin, Y. O. Kewuyemi, A. M. Abrahams and F. Tugizimana, "Application of gas chromatography mass spectrometry (GC-MS)-based metabolomics for the study of fermented cereal and legume foods: a review," *Int. J. Food Sci. Technol.*, vol. 56, no. ue 4, pp. 1514–1534, 2021, https://doi.org/10.1111/ijfs.14794.

[9] E. Koberg and A. Longoni, "A systematic review of sustainable supply chain management in global supply chains," *J. Clean. Prod*, vol. 207, pp. 1084–1098, 2019, https://doi.org/10.1016/j.jclepro.2018.10.033.

[10] I. C. Maia, T. D'Almeida, D. Freire, E. Calvancanti, L. Cameron, J. Dias, and M. Ferreira, "Effect of solid-state fermentation over the release of phenolic compounds from brewer's spent grain revealed by UPLC-MS," *LWT-Food Sci. Technol.*, vol. 133 . 1101, 2020, https://doi.org/10.1016/j.lwt.2020.110136.

[11] K. Li, Z. Duan, J. Zhang, and H. Cui, "Growth kinetics, metabolomic changes, antioxidant activity of probiotics in fermented highland barley-based yogurt," *LWT-Food Sci. Technol.*, vol. 173, p. 114239, 2023, https://doi.org/10.1016/j.lwt.2022.114239.

[12] X. Fan, X. Li, T. Zhang, Y. Guo, Z. Shi, Z.Wu, X. Zheng, and D. Pan, "Novel millet-based flavored yogurt enriched with superoxide dismutase," *Front. Nutr.*, vol. 8, Article, 2022, https://doi.org/10.3389/fnut.2021.791886.

[13] Y. Pan Y. Wang, W. Hao, S. Zhou, C. Duan, Q. Li, J. Wei, and G. Liu, "Exploring the role of active functional microbiota in flavor generation by integrated metatranscriptomics and metabolomics during niulanshan bijiu fermentation," *Foods*, vol. 12, p. 4140, 2022, https://doi.org/10.3390/foods12224140.

[14] S. Majumder, S. Chakraborty, A. Ghosh, and M. Bhattacharya, "The himalayan ethnic beverage tongba with therapeutic properties in high-altitude illnesses and metabolomics similarities to japanese sake," *Acta Univ. Sapientiae, Aliment.*, vol. 15, pp. 67–83, 2021, https://doi.org/10.2478/ausal-2022-0006.

[15] S. Majumder, A. Ghosh, S. Chakraborty, S. Saha, and M. Battacharya, "Metabolomics affirms traditional alcoholic beverage raksi as a remedy for high-altitude sickness," *J. Ethn. Foods*, vol. 8, no. 17, 2021, https://doi.org/10.1186/s42779-021-00094-4.

[16] F. R. Pinu, S. A. Goldansaz, and J. Jaine, "Translational metabolomics: current challenges and future opportunities," *Metabolites*, vol. 9, no. 6, 2019, https://doi.org/10.3390/metabo9060108

[17] X. Zhang, C. Zhang, L. Xiao, X. Wang, K. Ma, and F. Ji., "Gas chromatography-mass spectrometry and non-targeted metabolomics analysis reveals the flavor and nutritional metabolic differences of cow's milk fermented by Lactiplantibacillus plantarum with different phenotypic," *Food Biosci.*, vol. 60. Articl, 2024, https://doi.org/10.1016/j.fbio.2024.104433.

[18] J. Wang D. Wang, M. Huang, B. Sun, F. Ren, J. Wu, and J. Zhang, "Identification of nonvolatile chemical constituents in Chinese Huangjiu using widely targeted metabolomics," *Food Res. Int.*, vol. 172, Artic, 2023, https://doi.org/10.1016/j.foodres.2023.113226.

[19] N. Reyes-Garces and E. Gionfriddo, "Recent developments and applications of solid phase microextraction as a sample preparation approach for mass-spectrometry-based metabolomics and lipidomics," *TrAC Tends Anal. Chem.*, vol. 113, pp. 172–81, 2019, https://doi.org/10.1016/j.trac.2019.01.009.

[20] A. Khakpour, N. A. Shadmehri, H. Amrulloh, and H. Kioumarsi, "Antibacterial effect of Juglans regia, Citrus sinensis, Vicia faba , and Urtica urens extracts under in vitro conditions," *Bioactives*, vol. 1, no. 2, pp. 74–80, 2023, https://doi.org/10.47352/bioactivities.2963-654X.195.

[21] L. Kalavari, N. Nasiri, F. Ahmadian, and H. Kioumarsi, "Enrichment of doogh with olive leaf extract and investigation of its physiochemical, microbial and sensory properties during storage," *J. Multidiscip. Appl. Nat. Sci.*, vol. 3, no. 1, pp. 34–42, 2023, https://doi.org/10.47352/jmans.2774-3047.143.

[22] A. A. K. Harmita, Y. Harahap, and Supandi, *Liquid chromatography-tandem mass spectrometry (LC-MS/MS*), ISBN-978-602-17850-7-2, 2019, Jakarta: Penerbit PT. ISFI Penerbitan.

[23] W. Mancino, P. Carnevali, V. Terzi, P. G. Perez, L. Zhang, G. Giuberti, L. Morelli, V. Patrone, and L. Lucini, "Hierarchical effects of lactic fermentation and grain germination on the microbial and metabolomic profile of rye doughs," *Foods*, vol. 12, p. 998, 2023, https://doi.org/10.3390/foods12050998.

[24] S. Bocchi, G. Rocchetti, M. Elli, L. Lucini, C.-Y. Lim, and L. Morelli, "The Combined effect of fermentation of lactic acid bacteria and in vitro digestion on metabolomic and oligosaccharide profile of oat beverage," *Food Res. Int.*, vol. 142, p. 110216, 2021, https://doi.org/10.1016/j.foodres.2021.110216.

[25] D. Magro, M. Venezia, and C. R. Balistreri, "The omics technologies and liquid biopsies: Advantages, limitations, applications," *Med. Omi.*, vol. 11, 100039, 2024, https://doi.org/10.1016/j.meomic.2024.100039.

[26] N. Ghafari and L. Sleno, "Challenges and recent advances in quantitative mass spectrometry-based

metabolomics," *Anal Sci Adv*, vol. 5:e2400007, 2024, https://doi.org/10.1002/ansa.202400007.
[27] O. Lopez-Fernandez, R. Dominguez, M. Pateiro, P. E. S. Munekata, G. Rocchetti, and J. M. Lorenzo, "Determination of polyphenols using liquid chromatography-tandem mass spectrometry technique (LC-MS/MS): a review," *Antioxidants*, vol. 9, p. 479, 2020, https://doi.org/10.3390/antiox9060479.
[28] S. J. Heo, A. H. Kim, M.-J. Park, K. Kang, and D. Y. Soung, "Nutritional and functional properties of fermented mixed grains by solid-state fermentation with Bacillus amyloliquefaciens 245," *Foods*, vol. 9, p. 1693, 2020, https://doi.org/10.3390/foods9111693.
[29] J. O. Odukoya S. Saeger, M. Boevre, G. OAdegoke, F. Devlieghere, S. Croubels, G. Antonissen, O. A. Adebo, S. Gbashi, and P. B Njobeh "Mycotoxin reduction and metabolite profiles of ogi produced using traditional fermentation methods," *Food Hydrocoll. Heal.*, vol. 4, 100160, 2023, https://doi.org/10.1016/j.fhfh.2023.100160.
[30] Y. Zhao, C. Wu, Y. Zhu, C. Zhou, Z. Xiong, A. S. Eweys, H. Zhou, Y. Dong, and X. Xiao, "Metabolomics strategy for revealing the components in fermented barley extracts with Lactobacillus plantarum dy-1," *Food Res. Int.*, 2020, https://doi.org/10.1016/j.foodres.2020.109808.
[31] B. Mao, W. Guo, M. Chen, X. Tang, Q. Zhang, J. Zhao, H. Zhang, and S. Cui, "Effects of Streptococcus thermiphilus fermentation on the flavors and antioxidant properties of barley juice," *Fermentation*, vol. 9, p. 263, 2023, https://doi.org/10.3390/fermentation9070623.
[32] H. B. Jiang, P. Nie, Z. H. Lin, C. Zhu, L. Y. Zhong, F. F. Wei, Y. Wu, and L. H. Song, "The polyphenols profile of co-fermented quinoa and black barley with Lactobacillus kisonensis and its in vitro bioactivities," *Food Biosci.*, vol. 58, 103712, 2024, https://doi.org/10.1016/j.fbio.2024.103712.
[33] A. Tyagi, S.-J. Yeon, E. B.-M. Daliri, X. Chen, R. Chelliah, and D.-H. Oh, "Untargeted metabolomics of korean fermented brown rice using UHPLC-Q-TOF-MS/MS reveal an abundance of potential dietary antioxidative and stress-reducing compounds," *Antioxidants*, vol. 10, p. 626, 2021, https://doi.org/10.3390/antiox10040626.
[34] O. A. Adebo, E. Kayitesi, F. Tugizimana, and P. B. Njobeh, "Differential metabolic signatures in naturally and lactic acid bacteria (LAB) fermented ting (a southern african food) with different tannin content, as revealed by gas chromatography mass spectrometry (GC-MS)-based metabolomics," *Food Res. Int.*, vol. 121, pp. 326–335, 2019, https://doi.org/10.1016/j.foodres.2019.03.050.
[35] M. Ghamry, L. Li, and W. Zhao, "A metabolomics comparison of Lactobacillus communities isolated from breast milk and camel milk and Lactobacillus apis isolated from bee gut during cereals-based fermentation vs. Lactobacillus plantarum as a reference," *LWT-Food Sci. Technol.*, vol. 146, p. 111400, 2021, https://doi.org/10.1016/j.lwt.2021.111400.
[36] M. L. Kutt, K. Orgusaar, I. Stulova, R. Priidik, D. Pismennoi, H. Vaikma, A. Kallastu, A. Zhogoleva, I. Morell, and T. Krisciunaite, "Starter culture growth dynamics and densory properties of fermented oat drink," *Heliyon*, vol. 9, e15627, 2023, https://doi.org/10.1016/j.heliyon.
[37] M. K. Park, H. S. Choi, Y. S. Kim, and I. H. Cho, "Comparison of volatile profiles in Fagopyrum esculentum (buckwheat) soksungjang prepared with different starter cultures during fermentation," *Food Sci Biotechnol*, 2019, https://doi.org/10.1007/s10068-018-00549-6.
[38] C. Wang, S. Wei, M. Jin, B. Liu, M. Yue, and Y. Wang, "Integrated microbiomic and metabolomic dynamics of fermented corn and soybean by-product mixed substrate," *Front. Nutr.*, vol. 9, Article, 2022, https://doi.org/10.3389/fnut.2022.831243.
[39] V. I. Nakhod, T. V. Butkova, K. A. Malsagova, D. V. Petrovskiy, A. A. Izotov, K. S. Nikolsky, and A. L. Kaysheva, "Sample preparation for metabolomic analysis in exercise physiology," *Biomolecules*, vol. 14, p. 1561, 2024, https://doi.org/10.3390/biom14121561.
[40] C. Diez-Simon, R. Mumm, and R. D. Hall, "Mass spectrometry-based metabolomics of volatiles as a new tool for understanding aroma and flavour chemistry in processed food products," *Metabolomics*, vol. 15, no. 41, 2019, https://doi.org/10.1007/s11306-019-1493-6.
[41] J. Sun and Y. Xia, "Pretreating and normalizing metabolomics data for statistical analysis," *Genes Dis.*, vol. 2532–3042, 2023, https://doi.org/10.1016/j.gendis.2023.04.018.
[42] C. R. Balcazar-Zumaeta, E. M. Castro-Alayo, I. S. Cayo-Colca, G. Idrogo-Vasquez, and L. D. Munoz-Astecker, "Metabolomics during the spontaneous fermentation in cocoa (Theobroma cacao

L.): an exploraty review," *Food Res. Int.*, vol. 163, p. 112190, 2023, https://doi.org/10.1016/j.foodres.2022.112190.

[43] J. Chi, J. Shu, M. Li, R. Mudappathi, Y. Jin, F. Lewis, A. Boon, X. Qin, L. Liu, and H. Gu, "Artificial intelligence in metabolomics: a current review," *Trends Anal. Chem.*, vol. 178, p. 117852, 2024, https://doi.org/10.1016/j.trac.2024.117852.

[44] X. Cai, T. Wijesekara, and B. Xu, "New insights into recent development, health benefits, emerging technologies, and future trends of cereal-based fermented products," in *Process Biochemistry*, Elsevier BV, 2024, https://doi.org/10.1016/j.procbio.2024.10.010.

[45] B. W. Lemi, "Microbiology of Ethiopian traditionally fermented beverages and condiments [review of microbiology of Ethiopian rraditionally fermented beverages and condiments," *Int. J. Microbiol.*, vol. 1, 2020, https://doi.org/10.1155/2020/1478536.

[46] R. H. Mallappa, C. Balasubramaniam, B. H. Nataraj, C. Ramesh, S. Kadyan, D. Pradhan, S. K. Muniyappa, and S. Grover, "Microbial diversity and functionality of traditional fermented milk products of India: Current scenario and future perspectives," *Int. Dairy J.*, vol. 114, p. 104941, 2020, https://doi.org/10.1016/j.idairyj.2020.104941.