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Changes of volatile flavored compounds during lactic acid fermentation in cereal-based products

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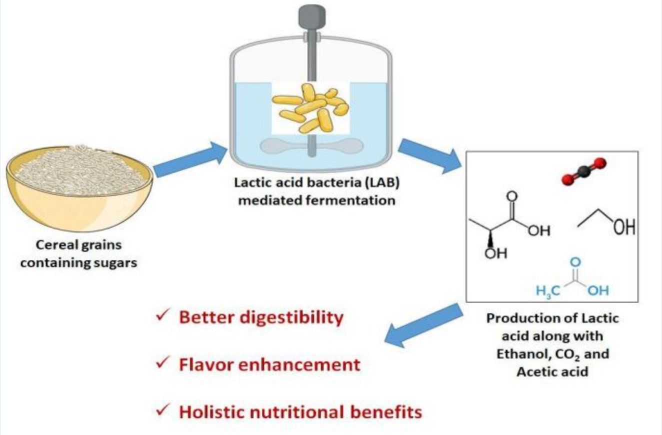
ABSTRACT

Lactic acid bacteria (LAB)-mediated fermentation of cereals is a preservation technique that enhances food flavor, nutrition, and safety. LAB naturally exists in cereals and plays a significant role in fermentation converting sugars into lactic acid and lowering the pH levels to create a distinctive sour taste and aroma. This process increases nutritional value and produces compounds like ethanol, acetic acid, and carbon dioxide, contributing to texture and appearance. LAB fermentation breaks down complex carbohydrates and proteins, improving digestibility and nutrient absorption. Common LAB-fermented cereal-based foods include sourdough bread, fermented cereal porridges, yogurt and kefir. Effective monitoring of volatile flavor compounds during fermentation is crucial for ensuring the quality, safety, and consumer acceptance of these foods.

Keywords: Cereal-based food, Volatile flavor compounds, Lactic acid bacteria, Fermentation, Amino acid metabolism, HPLC, GC-MS



Graphical Abstract: Changes of volatile flavored compounds during lactic acid fermentation in cereal-based products

INTRODUCTION

Consuming cereals has become a primary energy source providing essential protein, carbohydrates, fiber and minerals [1]. However, the application of cereal fermentation is limited due to the poor sensory properties. Three methods are employed to enhance nutritional quality: germination, milling, and fermentation. Among this fermentation, utilizing lactic acid bacteria (LAB) or probiotics, is the oldest and most effective. LAB fermentation increases cereal-based products' bioavailability, digestibility, and organoleptic properties [2]. During lactic acid fermentation (LAF), volatile flavor compounds undergo significant changes, impacting the final product's sensory appeal, appetite stimulations, and profile. The specific changes depend on the LAB strain and cereal used. LAB produces enzymes breaking down complex carbohydrates and proteins into smaller molecules, such as organic acids, (propionic acid, acetic and lactic acid) and metabolites (diacetyl, acetaldehyde, and ethanol). This study may provide insight into the changes in volatile flavor compounds during LAF in cereal-based products.

Process for Identification of Volatile Compounds: For identification of the volatile flavoring compounding compounds, the first step is fermentation. Modifications in the fermentation process can be made to yield diverse results. For instance, fermenting various cereals with the same microorganism [3-5] and or using the same cereal by different bacteriological bacterial or fungal strains on the same cereal [6-8] produces a range of similar volatiles with variations. Analyzing these variations and their concentrations help determine the optimal cereal-strain combination for maximum biotechnological benefits and pharmaceutical applications.

Mass spectrometry is employed to identify volatile compounds in fermented food products. The analytical method involves: Solid Phase Extraction (SPE), Gas chromatography with mass spectrometry (GC-MS). The procedure includes the sorption of volatile compounds using 6-amyl-a-pyrone, at 900 rpm for one hour. The absorbed volatiles are then desorbed using a twister and thermal unit, followed by GC-MS analysis. By comparing the retrieved data with standard values [9-11] and retention indices, the evolved volatile compounds during fermentation can be identified.

Post-extraction derivatization and analysis of fatty acids in fermented cereals: Following extraction, fatty acids present in fermented cereal samples undergo derivatization. The sample is treated with heneicosanoic acid and vortexed with a 10:5:2 (v/v) methanol: chloroform: distilled water mixture, followed by sonication [12]. Subsequent steps include ultralow-temperature centrifugation, supernatant evaporation and concentration, and high-performance liquid chromatology (HLPC), followed by Gas chromatography-mass spectrometry (GC-MS) for structural confirmation.

Sensory evaluation of volatile compounds: Sensory evaluation is crucial for researchers studying volatile flavor compounds. Figure 1 represents the process for the identification of volatile organic compounds. Researchers use two tests, conducted by trained, unbiased judges, first to assign attributes based on the aftertaste, aroma, and original taste. The second test assigns a characteristic to each component, aiding in the organoleptic assessment of the flavor profile and identifying volatile compounds that impart flavor. This process identifies volatile compounds released during cereal fermentation, providing descriptive and quantitative data. The panel is informed of the range maxima and minima [13]—table 1 lists common characteristics assigned to attributes and is suitable for evaluating volatiles released upon cereal fermentation.

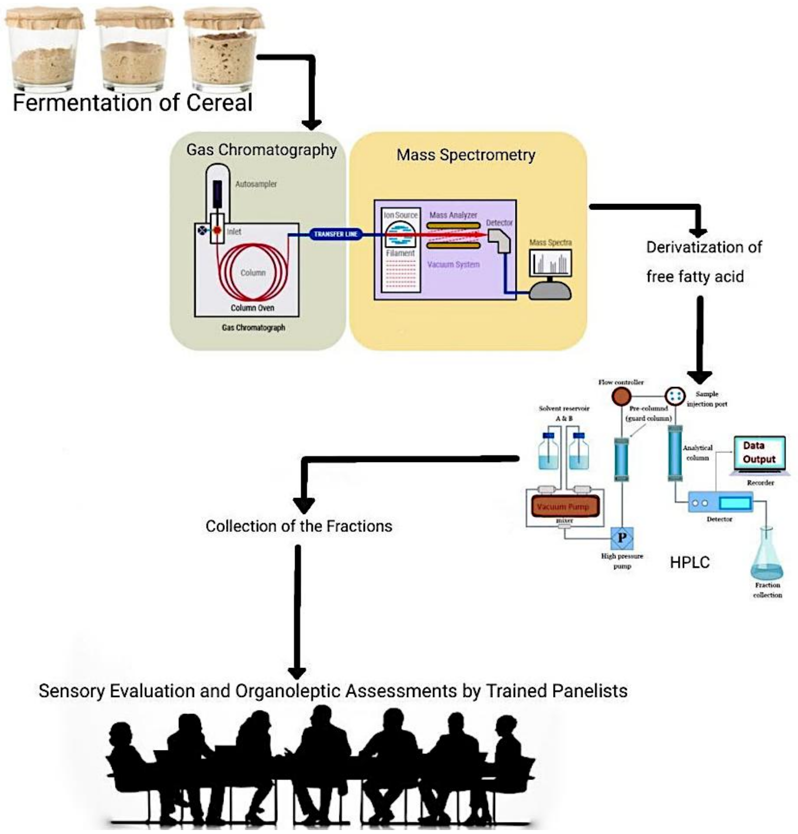


Figure 1: Process for identification of Volatile Organic Compounds

Table 1. Attribute based Characteristics assigned [14]

Characteristic(s) Assigned	Attribute	References
Sticky toffee like	Caramel	[13-14]
Grassy herb like, root like	Worty	
Foul-Drain	Sulphur	
Peach like, subtle	Fruity	
Floral	Apple like	
Taste sensation related to iron nails	Metallic	
Taste sensation related with sugary items	Sweet	
Caffeine/ tonic like	Bitter	
Warming, spirit like	Alcohol	
Malty/ raw Horlicks	Malt	
Citrusy, Lemony	Sour	
Stinging, Burning, Heat	Spicy	
Butterscotch like	Diacetyl	

Types of VCs (Volatile Compounds): Fermentation of cereal-based food items produces volatile flavoring compounds, with aldehyde and ketone-based compounds making up 70% of total volatile compounds. Other prominent compounds include benzene, hydrocarbons, furans, nitrogen, sulfur-containing compounds, and lactones. Aldehyde and ketones, derived from cereal lipids, exhibit tautomeric properties. Their concentrations vary among cereals, with brown rice exhibiting higher levels due to its bran layer, while other cereals have a higher ratio of aldehydes and ketones attributed to their increased fat, lipid content, and presence of bran layers [15]. Studies show that lactones have a coconut-like, floral-fruity smell that intensifies during fermentation. [16]. Extensive literature review reveals that volatile compounds can be classified into 10 distinct segments/types. Table 2 summarizes the most reported volatile compounds within each type.

Table 2. Commonly reported volatile compounds

Type of Volatile	Compounds	References
Aldehyde	Methyl-1-butanal, hexanal, heptanal, (E)-2-hexanal, furfural, benzaldehyde, octanal, (E,E)-2,4-heptandial	[3,4,6,7,8,14,17,18]
Ketones	Diacetyl, acetoin, Acetol, acetophenone, 6-methyl-3,5-heptadiene-2-one 2-heptone	
Alcohols	Ethanol, 2-methyl butanol, 2-methyl-1-propanol, hexanol, 2-butoxyethanol, octan-3-ol Benzomethanol	
Esters	Ethyl hexdecanoate, methyl hexdecanoate, Ethyl hexanoate, 1-methyl-2-hydroxypropanoate	
Hydrocarbons	Styrene, 1,3-xylene Pentadecane, heptadecane 1,4-Xylene, toluene, Lycopene	
Furans	2-Pentylfuran, Butylfuran, 2-propylfuran	
Phenols	(4-vinyl)-2meth-oxyphenols	
Sulphur rich	Benzothiazole	
Nitrogen rich	N,N-dibutylformldehyde	
Lactones	δ-dodecalactone, γ-decalactone, γ-nonalactone	

Pathway of formation of volatile compounds and effects of multiple strains on them: The fermentation process involves multiple metabolic pathways, including carbohydrate metabolism, which yields volatile compounds. The Embden-Meyerhof-Parnas pathway produces fermentation compounds like butanediol and ethanol, with upregulation of enzymes like dehydrogenase and decarboxylase observed via Heat Maps [19]. When compared to the microorganisms used for fermentation, reports state that concentrations of aldehyde were greater when Aspergillus oryzae was used, while the use of L. plantarum increased the final quantities of acetic acid; however, none of these compounds were detected in the unfermented samples. An overall conclusion can be drawn that fermentation is carried out using fungi such as S. cerev*isiae*, *Ryzopus* sp. *Aspergillus* sp. increased the total by-products that can be formed in the fermentation process of carbohydrates, particularly ethanol and ethyl acetate, rocketing at about 200 and 25 folds compared to the control.

When delved into the fermentation of butanediol, the volatiles acetoin i.e., 3-hydrixybutan-2-one and diacetyl - butane2,3-dione needed the enzyme alpha-acetolactate decarboxylase and at the end the dehydration by the enzyme 2,3-butanediol dehydrogenase [20]. Literature portrays that higher yield of acetoin can be obtained by using various species of LAB [21]. Molecules such as sulphur and nitrogen can only be a part of the volatile by-products of fermentation if the metabolic pathway of utilizing amino acids is involved [22]. Nitrogen is sourced from amino acids, while sulfur comes from cysteine and methionine. Amino acids can produce branched chain alkanes upon metabolism, while aromatic rings can be found in tryptophan, phenylalanine, and tyrosine. Methylbutanal derivatives are detected more frequently when fungi are used than LAB, possibly due to a lack of weak vital enzymes [23].

Lipid metabolism is also a pathway that produces several VCs, such as lactones, furans, and many more, via the degradation of fatty acids, ketone glycerol, and esters. The core conversion of these long carbon chain compounds into two carbon segments of acetyl-Co-A happens via beta-oxidation. At the same time, the following stages are carried out by specific enzymes [24]. In the sample that is fermented, the presence of unsaturated fat such as linolenic acid and oleic acids leads to increased Lactone formation using the β-oxidations and hydroxylation of the fatty acids [16], which imparts the products arising from these fermentations a fruity-floral odor. The fermented cereal-based products often have a strong smell that mimics the sweet scent of rose flowers. This is a demarcation feature of 2-phynylethanol [25]. Other volatile benzoic compounds, such as styrene, phenyl-ethan-1-one, are derived from phenylalanine-containing cinnamic acids [26]. Notably, 4-etgyl-2-methoxyphenol is the primary phenolic compound cited in the fermentation of rice and cereals, imparting a characteristic smoky odor. Different possible metabolic fates of biomacromolecules like sugars and amino acids are represented in Figure 2.

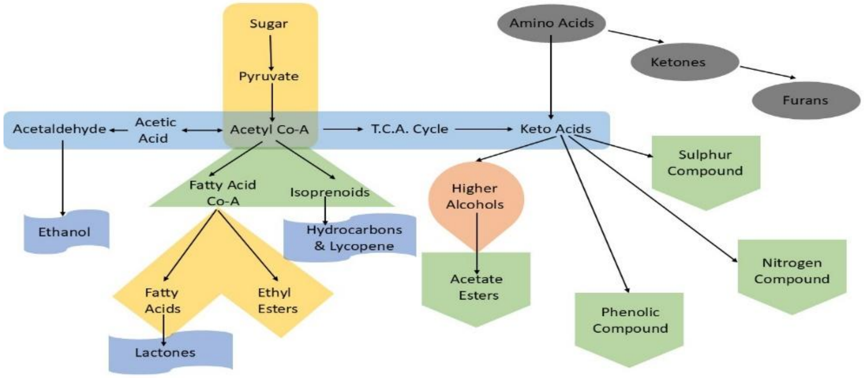


Figure 2. Metabolism of sugar and amino acids to pre-volatile compounds.

Various strains of LAB, along with its specific volatile compounds and its properties: Cereals, grown on 73% of global land, contribute over 60% of worldwide production providing essential nutrients, fiber, protein, energy, minerals, and vitamins. Fermented cereals, particularly in African and Asian countries, are integral to traditional foods like sourdough bread, injera, and ogi, offering highly nutritional value, cultural significance, and importance in global diets [18]. The cereal fermentation process yields end and intermediate products with volatile aromatic compounds, contributing to a harmonious aroma and flavor. Key aroma compounds exhibit a dilution factor of flavor ≥16, indicating their potency and significance in the overall appeal, taste, and smell of food. These compounds confer functional benefits to consumers. Functional foods are natural or processed items containing known or unknown biologically active compounds, providing clinically proven health benefits for chronic disease prevention, management, or treatment. Key volatile compounds identified include vanillin, sotolon, 2-phenyl ethanol, β-damascenone, acetic acid, and furaneol [27,28]. Analytical methods involve microextraction with solid-phase in headspace, gas chromatography, and GC-MS confirmation [3]. Alternatively, automatic headspace gas chromatography (HS-GC) is used, varying by cereal type [7]. Table 3 presents various lactic acid bacteria strains, and their corresponding volatile compounds produced during cereal fermentation.

Table 3: Strain of Bacteria and their Volatiles.

Strains of Bacteria	Cereals/Products	Volatile Compounds	Properties	Reference
Lactobacillus bulgaris	Rice, maize	Ester, ethanols	Aromatic compounds	[8]
Streptococcus thermophillus	Rice, Maize	Ester, ethanols	Aromatic compounds	[8]
Lactobacillus plantarum	Maize, millet sorghum Cocoa, Rice	(Aldehydes, ketones, esters and alcohols	Aroma and flavors	[29]
L. paracasei	Rice, Millet	γ-decalactone, γ-nonalactone, β-lactones, δ-lactones	Flavor and Aroma	[16]
Enterococcus genera.	Sorghum, finger millet	Ethanol, Lactic acid	Alcoholic flavored contribution to bushera	[6,7]
Lactobacillus paracasei	maize	2,3-butanedione and 2-butanone, 3-hydroxy	Flavor compounds	[4]
Lactobacillus fermentum	Maize millet sorghum Cocoa	(Aldehydes, ketones, esters and alcohols	Flavor and aroma	[4,29]
Lactobacillus brevis	Togwa production	3-methyl-1-butan-1-ol, 2-methyl-1-propan-1-al, 3-methyl-1- butanal, 2-methyl-(1)-butanal, 2-methyl butan-1-ol and 2-methyl-1-propan-1-ol		[3]
Lactobacillus delbrueckii subsp. bulgaricus	Maize, millet, kefir grains	hydrocarbons, ketone, ester, aldehydes, organic acid, alcohols, compounds containing sulfur and benzoic compounds	Flavor compounds,	[5]
Lactobacillus spp.	White rice, brown rice	1-octanol 1-hexanol, 1-heptanol, nonanol	Aromas and Flavor	[17]
Lactococcus lactis	Quinoa, oats, barley, rice	Ketone, aldehyde	Aromas and flavor	[4]
Lactobacillus cellobiosus	Togwa	3-methyl-1-butan-1-ol, 2-methyl-1-propan-1-al, 3-methyl-1- butanal, 2-methyl-(1)-butanal, 2-methyl butan-1-ol and 2-methyl-1-propan-1-ol	Helps to yield butanoic acid	[3]
Pediococcus pentosaceus	Emmer	3-methyl-1-butan-1-ol, 2-methyl-1-propan-1-al, 3-methyl-1- butanal, 2-methyl-(1)-butanal, 2-methyl butan-1-ol and 2-methyl-1-propan-1-ol	Helps to yield butanoic acid	[3]
Lactobacillus casei	Sorghum, rice, glutinous rice, wheat bran	Acetoin	Has a key role in formation of the dominant flavor in cereal vinegars	[18]
Acetobacter pasteurianus	Sorghum, rice, glutinous rice, wheat bran	Acetoin	It has a crucial aspect in forming the dominant flavor in cereal vinegars	[18]

Strain-specific contribution to the fermentation of cereals: Fermentation with LAB is a centuries-old preservation process that converts sugars into lactic acid, lowering the food pH and creating a hostile environment for spoilage and pathogenic microorganisms. This process extends shelf life and enhances the nutritional value and sensory properties of fermented foods. LAB produces antimicrobial metabolites like acetic acid, propionic acid, and bacteriocins, *Lactiplantibacillus plantarum* and *Lacticaseibacillus rhamnosus* are conditionally heterofermentative, while *Lacticaseibacillus casei* and Pediococcus *pentosaceus* are predominantly homofermentative, and *Levilactobacillus brevis* is heterofermentative [30].

In cocoa fermentation, a complex microbiota of acetic acid bacteria, yeasts, and LAB produces key components like acetic acid, lactic acid, and ethanol, contributing to flavor and aroma. Secondary metabolites, such as esters, alcohols, and acids, shape the complex flavor profile, dependent on strain, temperature, pH, and pathway. These metabolites are crucial for developing chocolate's potent aroma and characteristic flavor. The diversity of microbial populations and fermentation conditions influence the sensory profile. Furthermore, enzymes responsible for malt and fermenting microorganisms exploit their proteolytic activity to create precursors of aromatic compounds, such as amino acids. Optimal fermentation microorganisms have been identified. For instance, *Streptococcus thermophilous* Conversely, *Pediococcus spp. and Lactobacillus plantarum* dominate corn dough fermentation, causing rapid acidification [18].

Flour from corn, oats, and barley has been extensively reported in literature for producing fermentable cereal-based products. LAB play a crucial role in developing desirable properties in cereal- and pseudocereal-based beverages, including lipid conversion, water-soluble vitamin synthesis (B vitamins), enzymatic breakdown of phytic acid, and enhanced protein digestibility. Pseudocereal-based drinks can also be fortified through mechanisms beyond phytic acid polyphenol degradation, with LAB fermentation altering protein and amino acid content [4]. The food industry is developing functional beverages to meet growing demand for health-benefiting and disease-reducing products. Fermented foods, alcoholic and non-alcoholic beverages based on sorghum or millet, are gaining popularity. For instance, Obutoko type obushera (sorghum koji) made from Secedo red sorghum (Sorghum bicolor (*L. Moench*)) flour exhibits signature ethanol production and bold alcoholic taste [7].

Impacts of using Volatile compounds: Naturally present in fermented products, volatile compounds offer preservative properties [31], potentially reducing chemical preservative use. These compounds have been linked to cancer prevention [32], type 2 diabetes management [33-34], and neurodegenerative disorder prevention [35], potentially reducing the use of chemical-based preservatives. Antimicrobial activity, seen in (E)-2-hexen-1-al, makes them valuable biocontrol agents against pathogens [36]. However, some VOCs may pose health risks, including ENT irritation, headaches, nausea, kidney damage, liver damage, cancer, cardiac arrhythmia, vagal inhibition, respiratory system collapse, and sudden death [37]. Fortunately, their production in fermentation processes is typically negligible and statistically insignificant

Table 4: VOC containing Functional foods in health

Food	Cereal	Fermentation Process	Health Benefits
Idli and Dosa	Rice, Urad Dal	Lactic acid fermentation	Rich in probiotics, aids digestion reduce obesity [35]
Tempeh	Soybeans	Fungal fermentation	High in protein, fiber, and vitamins, especially B vitamins [35]
Miso	Soybeans, rice, barley	Fungal fermentation	High in protein, fiber, and antioxidants, supports gut health
Natto	Soybeans	Bacterial fermentation	High in protein, fiber, and vitamin K2, aids bone health [34]
Kefir	wheat, barley	Bacterial and yeast fermentation	Probiotic-rich, supports gut health, improves digestion [36]
Sourdough Bread	Wheat, rye,	Lactic acid and yeast fermentation	Easier to digest, may improve gut health, contains beneficial prebiotics [35]
Beer	Barley	Yeast fermentation	Contains antioxidants and polyphenols, reduces risk of heart disease and certain cancers [38]
Wine	Grapes	Yeast fermentation	Contains antioxidants and polyphenols [4]

Fermentation of cereals and legumes alters antioxidant levels and properties, transforming them into functional food ingredients. Research has consistently shown that this process imparts anti-obesity properties [4,35,38-39]. Moreover, the probiotics and prebiotics resulting from lactic acid fermentation (LAF) have been found to alleviate hangover symptoms and support live health, preventing diseases and disorders [32-34,40]. Black Mahalab, a cereal seed native to subtropical and tropical regions, has been a staple in Sudanese traditional meals for centuries. Fermentation is a key cooking method, rendering it a functional food component. Studies have revealed its antimicrobial and laxative properties, relieving irritable bowel syndrome (IBS) and other stomach conditions [17,41]. These examples demonstrate the potential of fermented cereal-based foods as functional ingredients, highlighting their role in maintaining health and combating disease. These foods have contributed to human well-being for centuries, underscoring the importance of preserving traditional fermentation practices and exploring their modern applications.

Functional Effects of Fermented Cereal VOCs- Prospects of Use: Research volatile flavorings have revealed promising potential in disease prevention, yet the industry has been slow to develop these compounds for therapeutic applications [42-43].

However, growing interest in antimicrobials found in fermented cereal-based foods has sparked innovation in functional food development, positioning this area as a hub for health-focused research [35,44]. Fermented plant-based foods are an abundant source of volatile aromatic flavoring compounds, which have shown impressive results in improving shelf life [45], enhancing health benefits [46,47], exhibiting fungicidal and insecticidal properties domain [43,48,49], and defending against microorganism-based decay in wounds (animals and plants) [45].

The antimicrobial effects of these volatiles are attributed to the simultaneous interactions of multiple volatile molecules, rather than single compounds [46]. Three major categories of volatiles with preservative potential have been identified: phenolic and phenol compounds, alkaloids, and terpenoids [50]. These findings underscore the vast potential of volatile compounds in fermented foods, warranting further research and development in the food-pharma interface.

Research has unveiled the antimicrobial potential of various volatile compounds found in foods. For instance, E-2-hexenal, an aldehyde from raw green cocoa beans, exhibits potent antimicrobial activity against pathogens [51]. Linalol and a-Terpineol, volatiles from barley and hop, inhibit the growth of cariogenic microorganisms and periodontopathic bacterial species [38]. Linalol's anti-histamic and anti-inflammatory properties make it a valuable ingredient in pharmaceuticals [52] while its precursor, non-volatile geraniol, has been proposed as a component for treating neurological disorders [47,53]. Fermented beans and cereals contain significant bioactive volatiles, including vanillin, vanillic acid, and alcohol derivatives. These compounds are renowned for their anticarcinogenic properties, anticlastogenic properties [54], respiratory inhibition of pathogenic species, such as *L. plantarum, L. innocua, and E. coli* [55], and promising inhibitory effect on sickle cell disease. The pharmaceutical industry utilizes these compounds as natural flavoring agents and bioactive ingredients, harnessing their potential to develop innovative treatments.

CONCLUSION

Whole wheat and wheat-based cereals offer significant nutritional value, booasting dietary antioxidants and high esterified phenolic acid content. Fermented barley, rich in β-glucan, has been shown to control hypoglycemia and hypercholesterolemia, while also reducing colon cancer risk associated with harmful food chemicals. Other cereals, such as sorghum, finger millet, and oats, serve as excellent prebiotics, providing long-term bacterial support. India is home to various fermented functional foods, including idli, dosa, dhokla, selroti, khaman, ambeli, sez, vada, anarshe, balam, kurdi, naan, bhatura, kulcha, and jalebi. These fermented pulses and cereals enhance total protein content, oil-holding capacity, sensory attributes, volatile saponins, and phenolics, all essential for overall health support. LAF, a process where bacteria convert sugars into lactic acid, is integral to fermented foods like yogurt, sauerkraut, and kimchi. This process yields volatile flavoring compounds, including aldehydes, alcohols, ketones, benzoids, acids, and sulphur. The type and amount of volatile organic compounds (VOCs) produced depend on the bacteria involved and cereal-derived food type.

Abbreviations: LAB, Lactic acid bacteria; LAF, lactic Acid fermentation; GC-MS, Gas chromatography/Mass spectrometry; Rpm, Revolutions per minute; HPLC, High Performance Liquid Chromatography; VOCs, Volatile organic compounds.

Authors Contribution: Sagnik Saha: Research analysis, interpretation of results designing of figure, and manuscript preparation, Shyarshree Putatunda: manuscript preparation, Rajib Majumder: manuscript preparation Arpita Das: Study conception and design.

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