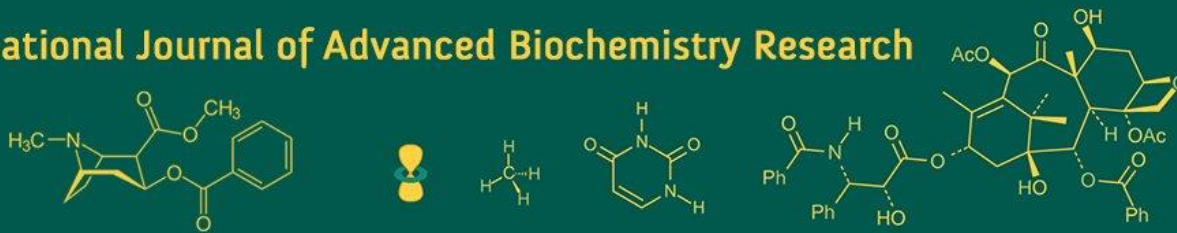


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Lacto-fermented agricultural by-products as potential bio-preservatives: A comprehensive review

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Abstract

The potential of lacto-fermented agricultural byproducts as natural bio-preservatives in the food industry is gaining momentum due to indiscriminate use of chemical preservatives. Agricultural byproducts, often considered waste, can be valorised through lactic acid fermentation to produce compounds with antimicrobial and antioxidant properties. The review examines various agricultural byproducts suitable for this process, including fruit and vegetable residues. It discusses the mechanisms of lactic acid fermentation and the bioactive compounds produced, such as organic acids, bacteriocins, and exopolysaccharides. The antimicrobial efficacy of these fermented byproducts against common food spoilage and pathogenic microorganisms is evaluated, along with their potential applications in different food systems. Additionally, the review addresses the challenges and opportunities in scaling up production and consumer acceptance of these novel bio-preservatives. This comprehensive analysis highlights the promising role of lacto-fermented agricultural byproducts in developing sustainable, clean-label food preservation solutions, potentially reducing food waste and improving environment and food safety.

Keywords: Lactic acid bacteria, bio preservation, byproducts, food, spoilage

Introduction

Lactic acid bacteria (LAB) are generally regarded as safe (GRAS) and have long been utilized in the production of fermented foods, including fermented milk, sourdough, cereals, vegetables, and beverages. LAB encompasses several genera, such as *Lactococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Streptococcus*, which are typically found in nutrient-rich environments and are distinguished by their production of lactic acid, ethanol, and bacteriocins. With over 30 genera and 300 species, LAB represent a diverse bacterial group isolated from various habitats, such as plant surfaces, fruits, vegetables, phyllosphere, and fermented products. Additionally, they have been employed as probiotics to enhance gastrointestinal health by improving the bioavailability of vitamins and fatty acids. LAB also exhibit antibacterial and antifungal properties, making them effective green preservatives. They are Gram-positive, non-spore-forming, catalase-negative microorganisms essential in producing a wide range of food and beverages (Konings, 2000) [34]. Historically, LAB have played a pivotal role in food preservation and fermentation by producing lactic acid, which inhibits the growth of spoilage and pathogenic microorganisms. LAB naturally reside in the guts of humans and animals and are also found in raw vegetables, meat, and cereals. The food industry widely uses them as starter cultures or non-starter LAB, contributing to the development of flavors and textures in products like fermented milk, cheese, meat, fish, vegetables, bread, wine, and beer (Aguilar, 1991) [2]. In Asia, LAB are particularly crucial in traditional fermented foods such as kimchi, rice wine/beer, and rice cakes, where they contribute both to preservation and unique flavor profiles (Rhee *et al.*, 2011) [53].

Various methods, including physical, chemical, and biological approaches, have been employed to control post-harvest fungal diseases in fruits and vegetables. Growing concerns over fungicide residues on raw produce have driven research toward using natural products to reduce postharvest disease incidence. Bio-preservation, which involves using natural methods to control fungal growth, is a promising and environmentally friendly alternative. LAB and their metabolites, used as natural disinfectants, have shown promising results in

extending shelf life and enhancing the quality and sensory properties of fruits and vegetables (Marín *et al.*, 2019) ^[42]. LAB inhibit fungal growth through the production of organic acids, fatty acids, and antifungal peptides. For example, *L. plantarum* has been shown to significantly reduce the incidence of *Botrytis cinerea* in grapes, while *L. paracasei* and *L. plantarum* isolated from fruits and vegetables effectively control *Colletotrichum capsici* in chili peppers. LAB isolates from fresh vegetables have also been used to preserve cucumbers (Sathe *et al.*, 2007) ^[57]. Cell-free supernatants derived from LAB fermentation offer a promising alternative to conventional antimicrobials. As a result, LAB fermentation (LAF) is recognized for its significant potential in enhancing the functional, nutritional, and sensory properties of both plant-based and animal-based foods (Luti *et al.*, 2020) ^[41]. Beyond their role in food production, LAB are also valued for their probiotic properties, particularly in protecting against opportunistic pathogens within the gut microbiome of infants (Aguilar, 1991) ^[2]. The production of lactic acid and other antimicrobial compounds by LAB creates an environment that selectively favours beneficial bacteria while inhibiting harmful microorganisms (Zacharof & Lovitt, 2010) ^[75].

To meet the growing demand for LAB and their metabolites, researchers have focused on optimizing growth conditions, developing efficient cultivation techniques, and exploring innovative applications for LAB in the food and beverage sectors

Fruit and Vegetable Production in India

India's diverse climate and fertile soil have established the country as a significant producer of fruits and vegetables, integral to its agricultural economy. In 2022, India's fruit production was estimated at 102.48 million tonnes, with a total horticultural output reaching 204.61 million tonnes (Ministry of Agriculture & Farmers Welfare, GoI, 2022). Among vegetables, tomato ranks second in production after potato, with an estimated output of 18 million tonnes from 0.8 million hectares of cultivated area. India is the second-largest producer of tomatoes globally, both in terms of area and yield, reflecting the vegetable's widespread cultivation and increasing production (Verma *et al.*, 2020) ^[69]. Chhattisgarh, a state in central India, is a significant contributor, producing 868.60 MT of tomatoes across 52.89 hectares (Verma *et al.*, 2020) ^[69]. Globally, tomato production has grown by 33% in the past decade (Dhivya *et al.*, 2023) ^[17]. In 2022, India exported approximately 89 thousand metric tons of fresh and chilled tomatoes, driven by favorable climatic conditions that allow tomato cultivation across various regions (Singh *et al.*, 2019) ^[59]. Horticulture, particularly fruits and vegetables, occupies a vital position in India's agricultural sector, representing nearly 90% of the country's total horticulture output. As the world's second-largest producer of these commodities, India's prominence in the horticultural landscape is undeniable (Choudhary *et al.*, 2017) ^[17]. The country's horticulture production reached 310.74 million tonnes in 2018-19, marking a slight increase from the previous year (Verma *et al.*, 2020) ^[69]. Hilly regions, like Uttarakhand,

benefit from unique agro-climatic conditions, offering distinct advantages for fruit and vegetable cultivation (Choudhary *et al.*, 2017) ^[17]. India also leads in the production of key horticultural crops, including mango, banana, papaya, cashew nut, areca nut, potato, and okra (Choudhary *et al.*, 2017) ^[11]. Despite this growth, India's tomato productivity still lags global benchmarks (Dhivya *et al.*, 2023) ^[17].

While India's horticultural sector has shown consistent progress over the past decade, there is significant potential for further development, especially in regions with comparative advantages due to favourable agro-climatic conditions (Choudhary *et al.*, 2017) ^[11]. The sector's upward trend underscores the importance of continued investment and research to enhance productivity and capitalize on the country's strengths (Dhivya *et al.*, 2023) ^[17].

Post-harvest Losses of Fresh Fruits and Vegetables

Post-harvest losses of fresh fruits and vegetables pose a substantial challenge in both developed and developing nations. Research indicates that farmers lose between 30% to 40% of their produce's value before it even reaches consumers (Fatty *et al.*, 2021) ^[19]. These losses occur across multiple points in the supply chain, including during harvesting, packing, transportation, and at wholesale and retail stages due to inefficient handling (Fatty *et al.*, 2021) ^[19]. In India, post-harvest losses are particularly concerning, with estimates indicating that up to 40% of horticultural output is wasted before reaching consumers (Fatty *et al.*, 2021) ^[19]. The underlying causes of these losses in India include inadequate infrastructure, a lack of suitable storage and transport facilities, and limited awareness among farmers and supply chain participants regarding best practices for post-harvest management. The repercussions of these losses are profound, leading to economic setbacks and food insecurity. In developing nations, it is estimated that 32% of agricultural produce is lost, yet only 5% of research funding is directed toward post-harvest handling strategies (Ibrahim *et al.*, 2022) ^[30]. This disparity underscores the urgency of addressing post-harvest losses to enhance food availability and security. While in developing countries, quantitative losses (e.g., physical damage and spoilage) take precedence, in developed countries, qualitative losses (e.g., diminished flavor or nutritional content) are more pressing as consumer dissatisfaction with produce quality contributes significantly to overall post-harvest losses. Enhancing the flavor, nutritional value, and safety of fresh produce could boost consumption and lead to improved health outcomes (). Addressing post-harvest losses necessitates a multifaceted strategy. This includes applying current knowledge to refine handling practices and preserve the quality and safety of horticultural perishables, alongside the development of new cultivars with enhanced flavor and nutritional profiles. Additionally, addressing socioeconomic constraints such as poor infrastructure, inadequate marketing systems, and limited research and development capacity is vital to reducing post-harvest losses, particularly in developing countries.

Table 1: Losses in the harvest and post-harvest operations (percent) in India

Crop	Storage losses	Farm level operation losses	Total loss
Maize	0.85%	3.8%	4.65%
Sorghum	0.99%	5%	5.99%
Paddy	0.86%	4.67%	4.93%
Wheat	0.86%	4.07%	5.53%
Mango	2.24%	6.92%	9.16%
Apple	1.31%	9.08%	10.39%
Potato	0.78%	6.54%	7.32%
Tomato	3.03%	9.41%	12.44%

(Source: Report on the assessment of quantitative harvest and post-harvest losses: ICAR-CIPET, March 2015)

Post-harvest losses of fruits and vegetables due to fungal and bacterial diseases

Post-harvest losses of agricultural produce, particularly fresh fruits and vegetables, are a significant global issue. Losses can reach up to 40% worldwide, with developing countries experiencing even higher rates (Desai *et al.*, 2014)^[16]. Postharvest deterioration and rots in fresh produce are primarily caused by various fungal and bacterial species, such as *Aspergillus*, *Fusarium*, *Colletotrichum*, *Macrophomina*, *Penicillium*, and *Rhizopus* (Desai *et al.*, 2014)^[16]. These pathogens can thrive during storage and transportation, leading to substantial economic losses for farmers and disruptions in food supply chains. Post-harvest losses of fresh agricultural produce are a multifaceted problem, with several contributing factors. In developed countries, consumer preferences for high-quality, blemish-free produce can lead to significant losses as produce that does not meet aesthetic standards is often discarded (Onwude *et al.*, 2020)^[48]. Additionally, inadequate storage and transportation infrastructure in many developing nations exacerbates the issue, allowing fungi and bacteria to proliferate and cause rapid deterioration (Desai *et al.*, 2014)^[16]. Advanced technologies and improved handling practices are urgently needed to mitigate these losses and increase the availability of fresh fruits and vegetables globally (Onwude *et al.*, 2020)^[48]. These losses have a substantial impact on the farm economy and food security, as they reduce the availability of fresh produce for consumption (Onwude *et al.*, 2020)^[48]. Factors such as consumer preferences, particularly in developed countries, can also drive post-harvest losses through a reduction in quality and safety standards (Onwude *et al.*, 2020)^[48]. Food loss has implications that go far beyond monetary value. Around 7% of the world's total greenhouse gas

(GHG) footprint is accounted for by the carbon emissions from food that is thrown out after being eaten. From production and transportation to processing, distribution, and consumption, it encompasses the entire supply chain. Addressing post-harvest losses is crucial to increasing the supply of fresh produce and improving the sustainability of agricultural systems. Advanced technologies are required to reduce the losses of fruits and vegetables in the postharvest supply chain (Onwude *et al.*, 2020)^[48].

Environmental effects of chemical preservatives

Over the past two decades, chemical methods have been extensively employed due to their straightforward application and low cost. However, recent studies have highlighted the negative impacts of pesticide use, such as the emergence of pesticide-resistant fungal strains, genetic mutations in consumers, environmental pollution, and the persistence of toxic residues (Palou *et al.*, 2018)^[49]. Post-harvest losses are influenced by factors including the type of produce, handling practices, and the nature of the decay. Soft or watery breakdowns are typically more detrimental than dry rots or minor fruit blemishes, as the latter often have minimal impact on the internal quality of the produce. Consequently, efforts to reduce post-harvest losses primarily target pathogens responsible for soft or watery breakdowns. Research indicates that *Penicillium* species account for approximately 30% of all fruit spoilage. Additionally, significant fruit losses are attributed to species from the genera *Botrytis*, *Rhizopus*, *Monilinia*, *Diplodia*, *Phomopsis*, and *Alternaria*. These fungi also contribute substantially to the degradation of vegetables. According to the same study, bacterial soft rot is responsible for 36% of all vegetable spoilage, with the most damaging pathogens belonging to the genera *Erwinia* and *Pseudomonas*.

Table 2: Effect of chemical preservatives on humans

Preservative	Class	Negative effect on humans
Sodium and potassium benzoate, benzoic acid	Antimicrobial	Aggravates asthma and suspected to be a neurotoxin and carcinogen may cause foetal abnormalities
Methyl and propyl paraben	Antimicrobial	May cause neurological damage.
Nitrites and nitrates	Antimicrobial	May lower oxygen carrying capacity of blood, may combine with other substances to form nitrosamines that are carcinogens
Sulfites and sulfur dioxide	Antimicrobial	May induce gastric irritation nausea, diarrhoea, asthma attacks, skin rashes

Source: (Yadav *et al.*, 2021)

Fermentation process employing LAB

Since they are engaged in the creation of numerous fermented meals from raw materials of animal (mostly milk) and vegetable origin, as well as in feed silage fermentations, lactic acid bacteria (LAB) comprise a group of microorganisms of enormous industrial significance (Avila and Carvalho, 2020)^[4]. The sensory, technical, nutritional, and functional qualities of the resulting fermented meals and

feeds are significantly influenced by the metabolic activity of LAB on other substrates. LAB are highly specialised in the bioconversion of the carbohydrates in lactic acid, rendering as well as minor amounts of other organic acids which reduce the pH and are a natural way of conservation. The "domestication" or choice of bacterial lineages well-suited to the fermented products has been favoured by conventional methods of raw material preservation (Li and

Gänzle, 2020)^[36]. Currently, starter and adjunct cultures from controlled fermentations are used in the production of a wide range of fermented foods as a result of this empirical, or unintentional, usage of LAB. *Streptococcus thermophilus*, *Lactococcus lactis*, *Leuconostoc spp.*, and *Lactobacillus spp.* are the LAB cultures frequently utilised in controlled food manufacturing for dairy products. However, the genera *Pediococcus*, *Oenococcus*, and *Weissella* also play a crucial role in plant-based fermented goods (Wuyts *et al.*, 2020)^[73].

Alternate Media for growing LAB

The cultivation of lactic acid bacteria has been a subject of significant interest in various fields, including food production, probiotics, and biotechnology. Traditional methods have relied on the use of specialized media, but there is a growing need to explore alternative media that can offer cost-effective, sustainable, and diverse options for the growth of these microorganisms. Lactic acid bacteria are known for their versatility, with the ability to metabolize a wide range of sugars and their resistance to high concentrations of lactic acid (Juturu & Wu, 2015)^[33]. However, these bacteria are also characterized as being fastidious, requiring specific nutrient sources, particularly in terms of nitrogen sources (Toe *et al.*, 2019)^[66]. Recent studies have highlighted the potential of utilizing alternative nitrogen sources, such as waste products from agricultural and industrial processes, to support the growth of lactic acid bacteria (Toe *et al.*, 2019; Liu *et al.*, 2019)^[66, 39]. One such example is the use of yeast extract, which has been extensively reported to enhance biomass production of various *Lactobacillus spp.* (Galante *et al.*, 2023)^[21]. For instance, studies have shown the successful cultivation of lactic acid bacteria using cheese whey, a byproduct of the cheese-making process, as well as municipal solid waste and wood molasses (Litchfield, 1996)^[38]. These substrates have been demonstrated to support the growth of lactic acid bacteria and, in some cases, can even result in higher productivity and yields compared to traditional media. One potential alternative media for the growth of lactic acid bacteria is the use of waste or byproduct streams from various industries, such as agricultural residues or dairy processing effluents. These alternative media can provide a cost-effective and environmentally friendly solution, as they can repurpose waste materials while supporting the growth of valuable lactic acid bacteria.

Agricultural By-products and their Valorization

Agricultural activities and food processing industries generate a substantial number of by-products and waste materials, which, if not properly managed, can pose significant environmental challenges. However, these by-products and waste streams can be viewed as a valuable resource, as they often contain a range of valuable compounds and materials that can be extracted and utilized in various industries. The manufacturing of plant-based foods is currently growing and producing a lot of by-products. The shift to a bioeconomy must include improved waste management to reduce the damaging environmental effects of the processing of fruits and vegetables. Because of this, there is a shared interest in developing novel methods of valorizing these substrates, and green valorization strategies that result in an integrated biorefinery platform have been established. The occurrence of these by-products

and waste materials is vast, with estimates suggesting that more than 100 million metric tons are generated annually during the industrial processing of agricultural products into beverages alone. These by-products and waste streams can originate from a variety of sources, including cultivation, livestock, and aquaculture (Obi *et al.*, 2016)^[47]. While the economic value of these materials may be lower than the cost of collection, transportation, and processing, their potential for beneficial use is significant (Obi *et al.*, 2016)^[47]. Agricultural and food processing by-products have garnered significant interest as researchers and industries explore ways to extract and utilize the valuable components within them. Numerous research efforts have been directed towards the extraction, characterization, and application of the valuable components found in agricultural and food processing by-products. These efforts have explored the use of animal-based and plant-based biomaterials in the food and non-food industries, as well as the potential for energetic valorization through approaches such as anaerobic digestion, bio-char production, and the recovery and use of bioactive molecules (Rawdkuen & Ketnawa, 2020; Kusch-Brandt *et al.*, 2019)^[52, 65].

The valorization of these agricultural by-products and waste materials presents an opportunity to reduce environmental impact, while also generating economic benefits. Numerous efforts are currently underway to explore the potential applications of these materials, including the extraction and characterization of valuable compounds, such as bioactive molecules, and the development of innovative utilization strategies (Rawdkuen & Ketnawa, 2020; Kusch-Brandt *et al.*, 2019)^[52, 65].

Banana Peel

Bananas are among the most widely consumed fruits globally, with a production exceeding 114 million tons in 2020 (Campos *et al.*, 2020)^[9]. Although the fruit itself serves as a significant food source, the by-products generated during banana processing, such as banana peels, remain largely underexploited (Acevedo *et al.*, 2021)^[1]. Banana peels account for approximately 40% of the total fruit mass, making them a substantial agricultural waste (Acevedo *et al.*, 2021)^[1]. Typically discarded or incinerated, these peels contribute to environmental pollution. However, banana peels hold considerable potential for value-added applications due to their rich composition, which includes carbohydrates, vitamins, lignocellulosic materials, and beneficial phytochemicals (Acevedo *et al.*, 2021; Campos *et al.*, 2020)^[1, 9]. The composition of banana peels is marked by a high concentration of carbohydrates, predominantly in the form of starch and cellulose, along with significant levels of vitamins, minerals, and antioxidant-rich phytochemicals (Campos *et al.*, 2020)^[2]. This chemical makeup can vary depending on factors like the banana variety, its stage of maturity, and its geographical origin (Cordeiro *et al.*, 2004)^[12]. In general, banana peels contain cellulose, hemicellulose, lignin, and a variety of other compounds, including starch, protein, and organic acids (Cordeiro *et al.*, 2004)^[12]. Recent research has highlighted the potential of using banana peels as a medium for cultivating lactic acid bacteria. The fermentation process, initiated by inoculating the peels with lactic acid bacterial strains, can yield valuable lactic acid and other metabolites. Additionally, composting inoculated banana peels has been

shown to improve the nutrient content and agronomic quality of the compost, making it a useful soil amendment. One promising approach to utilizing banana peels is their use as a growth medium for lactic acid bacteria. Lactic acid bacteria, widely used in the production of fermented foods like cheese, yogurt, and sauerkraut, offer numerous health benefits. The high carbohydrate content in banana peels, particularly starch and sugars, provides an ideal substrate for the cultivation of these bacteria.

Watermelon rind

Watermelon is a widely consumed fruit across the globe, with a global production of 118 million tons recorded in 2017, accounting for 7% of the total cultivated area for fruits and vegetables. Known for its high-water content and nutrient richness, watermelon serves as an important source of hydration as well as essential vitamins and minerals. Beyond the edible flesh, the rind of watermelon also presents significant opportunities for value addition. Research indicates that watermelon rind contains various beneficial compounds, such as antioxidants and pectin (Pérez *et al.*, 2021) ^[51]. Both the seeds and rind have been found to possess antioxidant properties (Pérez *et al.*, 2021) ^[51]. Pectin, a linear polysaccharide primarily made up of anhydro galacturonic acid units with partially esterified carboxyl groups, is one of the key components found in the rind (Pérez *et al.*, 2021) ^[51]. Despite the primary use of watermelon for local juice production, which generates substantial waste without appropriate disposal, the underutilized rind holds considerable potential for conversion into pectin and other valuable products, thus mitigating the environmental impact of melon processing (Pérez *et al.*, 2021) ^[51]. The antioxidant properties of watermelon rind are attributed to its rich phytochemical profile (Pérez *et al.*, 2021) ^[51].

Further research is needed to optimize the extraction of bioactive compounds like citrulline from watermelon rind and explore their use in developing new functional food products. Another promising application of the rind is its use as a growth medium for lactic acid bacteria. These insights expand our understanding of sugar and organic acid metabolism in watermelon, which is vital for assessing the rind's viability as a substrate for microbial fermentation (Table 3). The significant quantities of watermelon residues generated as a byproduct of juice production hold great promise for pectin production and other value-added applications (Pérez *et al.*, 2021) ^[51].

Orange Peel

Orange peel, a byproduct generated by the citrus processing industry, has gained considerable interest as a viable feedstock for producing high-value products. One key application is its use as a cultivation medium for lactic acid bacteria, organisms extensively utilized in the food, pharmaceutical, and beverage sectors (Dedenaro *et al.*, 2016) ^[15]. The composition of orange peel includes structural carbohydrates such as cellulose, hemicellulose, and lignin, along with other bioactive components (Campos *et al.*, 2020; Hussain *et al.*, 2022) ^[9, 29]. It is also abundant in bioactive substances like essential oils, flavonoids, and dietary fibers (Hussain *et al.*, 2022) ^[29]. These elements have demonstrated antimicrobial, antioxidant, and prebiotic capabilities, establishing orange peel as a promising substrate for lactic acid bacteria cultivation (Godara *et al.*,

2020) ^[25]. Furthermore, its high content of structural carbohydrates, such as cellulose and hemicellulose, can be harnessed for producing various organic acids, including lactic acid.

Utilizing orange peel as a medium for lactic acid bacteria cultivation offers multiple advantages. Firstly, it provides a sustainable approach to managing fruit waste by converting a byproduct into a valuable asset. Secondly, as an affordable and readily available resource, using orange peel can lower the costs associated with lactic acid production. Numerous studies have assessed the viability of orange peel as a substrate for lactic acid fermentation, focusing on optimizing fermentation conditions—such as pH, temperature, and nutrient supplementation—to maximize lactic acid yield. Additionally, enzymatic-assisted extraction techniques have been explored to enhance the extraction of key compounds from the orange peel matrix.

For instance, a study on lactic acid production from cheese whey using *Bifidobacterium longum* showcased the potential of alternative substrates for lactic acid fermentation. Similarly, research into the valorization of pineapple by-products for organic acid production, including lactic acid, underscored the adaptability of fruit-based waste streams as feedstocks for microbial fermentation (Campos *et al.*, 2020; Martillanes *et al.*, 2018) ^[9, 43]. By analyzing the composition and characteristics of such byproducts, researchers can identify opportunities for valorizing agricultural waste, contributing to the development of more sustainable and circular food systems.

Table 3: Mineral composition of water melon peels

Mineral	Rind composition
Iron	1.29 mg/100g
Manganese	1.42 mg/100g
Calcium	29.15 mg/100g
Copper	0.45 mg/100g
Zinc	1.29 mg/100g
Magnesium	1.48 mg/100g
Ash %	0.46
Carbohydrate %	4.2
Protein %	0.63

Source: (Gladvin *et al.*, 2017) ^[25]

Biological Methods for Food Preservation

Food preservation has been a crucial challenge for human societies throughout history, with the advancement of effective techniques being essential for ensuring food security and enabling the extended storage and transportation of perishable goods. While traditional methods such as drying, salting, and smoking have been employed for centuries, recent decades have seen a growing interest in advanced biological approaches to food preservation. A promising area of research involves the use of natural antimicrobial compounds, derived from both plant and microbial sources, to inhibit the growth of spoilage and pathogenic microorganisms in food products (Juneja *et al.*, 2012; Lucera *et al.*, 2012; Tiwari *et al.*, 2009; Teneva & Denev, 2023) ^[32, 40, 65, 64]. These naturally occurring compounds, including essential oils, phenolic compounds, and bacteriocins, exhibit potent antimicrobial activity against a broad spectrum of microorganisms, offering appealing alternatives to synthetic preservatives (Juneja *et al.*, 2012; Lucera *et al.*, 2012) ^[32, 40]. The application of beneficial microorganisms, particularly lactic acid bacteria,

has also emerged as a significant strategy in the field of biopreservation (Tiwari *et al.*, 2009; Teneva & Denev, 2023) ^[65, 64]. These microorganisms and their metabolites can prolong the shelf-life of food products and enhance safety by suppressing the growth of undesirable microbes. Bacteriocins, for instance, are antimicrobial peptides produced by lactic acid bacteria that have been extensively investigated for their potential as natural preservatives (Tiwari *et al.*, 2009) ^[65].

Moreover, researchers have explored the use of plant-derived extracts, which contain compounds such as saponins, flavonoids, and terpenoids, as effective natural preservatives (Teneva & Denev, 2023) ^[64]. These phytochemicals exhibit a range of antimicrobial, antioxidant, and other beneficial properties that contribute to the preservation of food products. With the increasing consumer demand for natural and minimally processed foods, the development of effective biological preservation methods has become an important research focus (Juneja *et al.*, 2012; Tiwari *et al.*, 2009; Teneva & Denev, 2023) ^[32, 65 64]. By leveraging naturally occurring antimicrobial compounds and beneficial microorganisms, researchers and food producers aim to create safer, more sustainable, and longer-lasting food products that align with evolving consumer preferences.

Lactic Acid fermentation

Lactic acid fermentation is a pervasive metabolic process that significantly impacts various industries, including food production and biofuel generation. Lactic acid bacteria (LAB) are the principal agents in this fermentation, demonstrating notable versatility and adaptability (Hatti Kaul *et al.*, 2018) ^[27]. These microorganisms are integral to the preservation and fermentation of diverse food products such as dairy, vegetables, meat, and animal feed (Hatti Kaul *et al.*, 2018) ^[27]. The prominence of lactic acid, a key product of this fermentation, is growing due to its extensive applications across multiple sectors, including food, chemicals, cosmetics, and pharmaceuticals. Moreover, lactic

acid is crucial for producing biodegradable and biocompatible polylactic acid (PLA) polymers, which are used in a range of products from packaging to fibers and foams, contributing to market expansion (Hatti Kaul *et al.*, 2018) ^[27].

The production of lactic acid through fermentation has garnered significant interest due to its wide array of applications. Lactic acid is a natural organic acid with diverse uses in the pharmaceutical, food, and chemical industries, serving as an acidulant, preservative, and substrate to produce biodegradable plastics and other organic acids (Li *et al.*, 2006) ^[37]. Historically, the use of lactic acid bacteria in food fermentation dates back over a million years, with ancient civilizations employing these techniques in the production of fermented dairy products, vegetables, and alcoholic beverages (Vos, 2011; Hatti Kaul *et al.*, 2018) ^[70, 27]. In contemporary settings, the role of lactic acid bacteria has expanded beyond traditional food applications to include their use as biological catalysts for generating various organic compounds, including biofuels, biochemicals, and pharmaceuticals. The adaptability of LAB, which allows them to thrive in diverse environments such as the human and animal gut, underscores their value as probiotic cultures and their widespread application in the food and feed industries (Table 4). Traditionally, lactic acid has been produced by fermenting sugar-containing substrates like cheese whey with strains such as *Lactobacillus helveticus* and *Lactobacillus casei* (Li *et al.*, 2020) ^[37]. Recent advancements in genetic and metabolic engineering have significantly enhanced the capabilities of lactic acid bacteria, leading to the development of strains with improved traits, including resistance to bacteriophages, enhanced production of bacteriocins, and better carbohydrate metabolism (Daly *et al.*, 1998) ^[14]. These technological and health benefits have been extensively researched, with recent progress in genomics and genetic engineering further expanding the industrial potential of these microorganisms (Hatti-Kaul *et al.*, 2018) ^[27].

Table 4: Antimicrobials secreted by LAB for bio preservation

Antimicrobials	Source	Food bio-preservation mechanism
CO ₂	Heterofermentative LAB produces CO ₂ as a by-product of sugar fermentation.	Creation of an anaerobic environment and antagonistic effects specifically against aerobic bacteria and produces carbonic acid
Diacetyl (2,3-butanedione)	LAB as a by-product of metabolic activity	Antibacterial activity against <i>Listeria</i> , <i>Salmonella</i> , <i>Escherichia coli</i> , <i>Yersinia</i> , and <i>Aeromonas</i> .
Hydrogen peroxide	Produces by LAB in presence of oxygen and action of flavoprotein oxidases or NADH peroxidase	Antibacterial effect through oxidative damage of proteins and increase of membrane permeability.
Reuterin	Low molecular- weight antimicrobial compound produced by <i>Lactobacillus reuteri</i> and some other LAB.	Antimicrobial activity against bacteria as well as yeasts and molds by inhibiting DNA synthesis.
Reutericyclin	Reutericyclin-producing strains of LAB.	Reutericyclin acts as a proton ionophore and dissipation of the proton motive force against gram-positive bacteria including <i>Lactobacillus</i> spp., <i>Bacillus subtilis</i> , <i>Bacillus cereus</i> , <i>Enterococcus faecalis</i> , <i>Staphylococcus aureus</i> and <i>Listeria innocua</i> .

Diverse Sources of Lactic Acid Bacterial Isolation

Lactic acid bacteria (LAB) are a highly versatile group of microorganisms that have garnered significant attention for their extensive applications across several industries, including food processing, agriculture, and biotechnology. Known primarily for their ability to produce lactic acid, LAB also have the potential to generate antimicrobial

substances known as bacteriocins. Traditionally, LAB have been isolated from natural sources such as dairy products, fermented vegetables, and the gastrointestinal tracts of humans and animals. In the food industry, LAB are widely utilized as starter cultures or as non-starter cultures to enhance the preservation and flavor of various fermented products. Additionally, the probiotic properties of certain

LAB strains have led to their incorporation into foods and animal feeds as adjunct cultures. In recent years, there has been a growing interest in exploring alternative sources for LAB isolation to expand the diversity of strains available for industrial applications (Table 5). One notable source is the human vaginal tract, where LAB play a critical role in maintaining an acidic environment that helps protect against pathogenic microorganisms (Aguilar, 1991) [2]. The human gut microbiome is another rich source of LAB, with diversity and abundance varying across different species, ages, and regions within the gastrointestinal tract. Recent research has also investigated alternative sources for LAB isolation, such as plant biomass and industrial waste

streams. For example, studies have demonstrated that Lactobacilli can be effectively propagated using various carbohydrate sources derived from plant biomass, optimizing growth strategies for lactic acid and bacteriocin production (Zacharof & Lovitt, 2010) [75]. Additionally, microbial production of lactic acid from plant biomass fractions, such as cellulose, has been explored as a sustainable alternative to chemical production methods, which often result in racemic mixtures of lactic acid isomers (Juturu & Wu, 2015) [33]. These innovative approaches highlight the potential for harnessing diverse sources to enhance the industrial application and sustainability of LAB.

Table 5: Antimicrobial activity of Lactic Acid Bacteria (LAB) isolated from plant material and food product

Source	LAB	Pathogen inhibited	References
Himachal Pradesh traditional pickles	<i>E. faecalis</i> , <i>L. plantarum</i> , <i>P. pentosaceus</i> , <i>L. mesenteroides</i> , <i>L. lactis</i> , and <i>Enterococcus sp.</i>	<i>B. cereus</i> , <i>E. coli</i> , <i>S. aureus</i> and <i>S. dysenteriae</i>	Monika <i>et al.</i> , 2017 [45]
Fermented milk	<i>L. plantarum</i>	<i>A. flavus</i>	Ahlberg <i>et al.</i> , 2017 [3]
Corn products	<i>B. bifidum</i> , <i>L. fermentum</i>	<i>A. parasiticus</i> , Aflatoxins	Ghazvini <i>et al.</i> , 2016 [22]
Apple	<i>L. plantarum</i> , <i>Ln. mesenteroides</i> , <i>W. soli</i>	<i>S. enteritidis</i> , <i>L. monocytogenes</i> and <i>E. coli</i>	Siroli <i>et al.</i> , 2015 [60]
Corn stubble	<i>L. plantarum</i> , <i>P. pentosaceus</i> , <i>E. mundtii</i> , <i>W. cibaria</i> , and <i>Ln. pseudomesenteroides</i>	<i>S. entérica</i> , <i>M. luteus</i> and <i>E. coli</i> .	Li <i>et al.</i> , 2020 [36]
Fresh vegetables and fermented products	<i>Lactobacillus fermentum</i> , <i>Lactobacillus plantarum</i> , and <i>Weisella cibaria</i>	<i>S. aureus</i>	Wong <i>et al.</i> , 2015 [72]
Rhizosphere of olive trees and desert truffles	<i>L. mesenteroides</i> , <i>W. halotolerans</i> , and <i>E. faecium</i>	<i>S. maltophilia</i> , <i>P. agglomerans</i> , <i>P. savastanoi</i> , <i>S. aureus</i> , <i>L. monocytogenes</i> , <i>B. cinérea</i> , <i>P. expansum</i> , <i>V. dahliae</i> , and <i>A. niger</i>	Fhoula <i>et al.</i> , 2013 [20]
Fruits and vegetables	<i>L. mesenteroides</i> , <i>Ln. citreum</i> , <i>L. plantarum</i> , <i>L. lactis</i> , <i>W. cibaria</i> , and <i>E. mudti</i>	<i>X. campestris</i> , <i>E. carotovora</i> , <i>P. expansum</i> , <i>M. laxa</i> , and <i>B. cinérea</i>	Trias <i>et al.</i> , 2008 [67]

Growing conditions of LAB

The *Lactobacillus* genus is diverse and its members may thrive in temperatures ranging from 2 to 53 °C and pH range between 4.5 and 6.5, with some strains able to survive in even lower pH. The ideal temperature and pH parameters for lactobacilli growth are 30-40 °C and 5.5-6.2, respectively. Growth kinetics characteristics such as specific growth rate and lag phase duration, which is the period during which bacteria adapt to new media but do not proliferate, can be affected by culture conditions and the composition of the fermentation medium in which *Lactobacillus spp.* bacteria are cultured. Rogosa agar, Lactobacilli Selective Agar (LBS), and De Man Rogosa Sharpe (MRS) are the medium most frequently employed for the culture of *lactobacilli*. It is commonly acknowledged that these media, particularly MRS agar, are expensive and unsuitable for large-scale production despite being appreciated and widely used

Effects of LAB fermentation

One of the best methods for unlocking the biogenic/functional potential of plant matrices and enhancing them with bioactive substances is thought to be the use of lactic acid bacteria and, more specifically, the lactic acid fermentation (Pellati *et al.*, 2004) [50]. In fact, the antibacterial, antioxidant, and immune-modulating properties of various cereal, pseudo-cereal, and leguminous flours as well as of medicinal plants like *Echinacea spp.*

were greatly enhanced by the fermentation by chosen lactic acid bacteria (Rizzello *et al.*, 2013) [54]. Compared to fresh leaves, the pickles and relish made from *S. scabrum* and *S. villosum* had a much higher mineral content after fermentation, Ca (442 mg/100 g DW) and Fe (12 mg/100 g DW) concentrations in fresh *S. villosum* were found to be lower than those found in this investigation. Pickles and relishes now contain more minerals because of LAF (lactic acid fermentation). The content of iron, Zn, Ca, S, P, Cu, and Ni all increased by 0.58 to 2.01 times, respectively. Amaranth, pumpkin, and capwood leaf fermentation produced higher levels of Ca, Mg, Zn, Fe, Se, and Cu (Ifesan *et al.*, 2014) [31]. The breakdown of antinutrients by microbial enzymes occurs because of LAB, which enhances the nutritional quality and bioavailability of micronutrients in food. LAF has a low pH, it can be thought of as a method to improve nutritional and functional quality by activating endogenous enzymes (Rollán *et al.*, 2019) [55]. Additionally, LAB can manufacture several enzymes that hydrolyse the food matrix into palatable nutritional and sensory qualities. It has been demonstrated that environmental conditions are critical in the development of fungi that cause fruit diseases (Safari *et al.*, 2021) [56]. Among these fungi, *Alternaria spp.* causes tomato fruit rot, which manifests as smooth, slightly sunken lesions that range in colour from dark brown to black and have a hard feel. The lesions can grow to reach several centimetres in diameter (Yang *et al.*, 2020) [74].

The infection caused by *Fusarium oxysporum* fruit rot, which shortens post-harvest life and hence lowers market value, is particularly likely to affect tomatoes (Safari *et al.*, 2021)^[56]. A prevalent disease in tomato fruit, *Fusarium* fruit rot is brought on by *Fusarium oxysporum*, and it can lead to fruit deterioration both in the field and during storage. To prevent this disease, synthetic fungicides like carbendazim, chlorothalonil, and mancozeb are frequently applied. However, due to chemical residues and an increase in infection resistance, this management method has a severe influence on human health and the environment. A soil-borne pathogen that can survive for many years in the soil without a host is the cause of fusarium wilt. The population surrounding infected tomato debris is where most infections start. If the soil in which they are grown contains the pathogen, healthy plants may become infected by it (Farr *et al.*, 1989; Mijatovi *et al.*, 2007)^[18, 44].

Bio preservation employing LAB

Lactic acid bacteria have long been used in the food industry for their ability to preserve and enhance the safety of various food products (Brashears *et al.*, 2005)^[6]. This includes their application in the preservation of fruits and vegetables, where their antimicrobial properties can help extend the shelf life and inhibit the growth of harmful pathogens (Brashears *et al.*, 2005; Castellano *et al.*, 2017)^[6, 10].

Lactic acid bacteria are a group of Gram-positive, anaerobic bacteria that are known for their production of lactic acid as a byproduct of carbohydrate fermentation (Daba & Elkhateeb, 2020)^[13]. This metabolic characteristic is one of the key reasons for their widespread use in food preservation, as the acidic environment created by lactic acid can inhibit the growth of many spoilage and pathogenic microorganisms (Tenea & Yépez, 2016; Daba & Elkhateeb, 2020)^[13]. *Lactobacilli*, a group of lactic acid bacteria, have been extensively utilized in food biotechnology due to their versatility and effectiveness (Giraffa *et al.*, 2010)^[23]. These microorganisms possess several desirable characteristics, such as the production of lactic acid, enzymes, and natural antimicrobial compounds known as bacteriocins (Zacharof & Lovitt, 2010)^[75]. The use of *Lactobacilli* as starter cultures or non-starter lactic acid bacteria has been a crucial aspect of food preservation, including the fermentation of dairy products, vegetables, fish, and meat products (Giraffa *et al.*, 2010)^[23]. The biopreservation approach, which refers to the extended storage life and enhanced safety of food using natural or controlled microflora, has gained significant attention in recent years (Brashears *et al.*, 2005)^[6]. Lactic acid bacteria, with their ability to inhibit the growth of foodborne pathogens, have been explored as a natural and sustainable alternative to traditional chemical preservatives (Brashears *et al.*, 2005)^[6].

One of the key advantages of using lactic acid bacteria for the biopreservation of fruits and vegetables is their production of antimicrobial compounds, such as lactic acid and bacteriocins (Castellano *et al.*, 2017)^[10]. These compounds can effectively suppress the growth of harmful bacteria, including *Listeria monocytogenes*, a common foodborne pathogen (Castellano *et al.*, 2017)^[10]. The application of lactic acid bacteria in the biopreservation of fruits and vegetables can be a promising approach to enhance food safety and extend shelf life, while minimizing the use of synthetic preservatives.

Conclusion

Lacto-fermented agricultural byproducts have emerged as promising candidates for natural bio-preservatives. These fermented products, derived from various agricultural waste streams, offer a sustainable solution to food preservation challenges while addressing the growing consumer demand for clean-label ingredients. Moreover, the use of lacto-fermented agricultural byproducts as preservatives aligns with the principles of a circular economy. By valorising waste, this approach not only reduces environmental impact but also creates value-added products from what would otherwise be discarded. This sustainability aspect, coupled with the natural origin of these preservatives, resonates strongly with environmentally conscious consumers and food manufacturers seeking to reduce their reliance on synthetic additives. However, several challenges that need to be addressed for widespread adoption of these bio-preservatives. These include the need for standardized production processes to ensure consistent quality and efficacy, optimization of extraction methods for bioactive compounds, and the development of scalable, cost-effective production techniques suitable for industrial applications. Additionally, more extensive research is required to fully elucidate the mechanisms of action of different bioactive compounds and to explore potential synergistic effects when combining various fermented byproducts or integrating them with other natural preservatives. As research in this field progresses, these innovative preservatives could revolutionize food preservation strategies, contributing to improved food safety, reduced food waste, and a more sustainable food supply chain. The successful implementation of these bio-preservatives could mark a pivotal shift towards more natural, efficient, and environmentally friendly food preservation methods, aligning with global efforts to create a more sustainable and health-conscious food industry.

Conflict of interest

The authors have no conflict of interest.

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