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Review Article

A Review of fermentation and the nutritional requirements for effective growth media for lactic acid bacteria

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Abstract
Lactic acid bacteria are useful microorganisms that are well-known to have probiotic effects and provide foods with unique sensory qualities such as aroma and taste (flavor). Probiotic bacteria such as *Lactobacillus delbrueckii* subsp. *bulgaricus* are found in many fermented food products and confer several human health benefits. Probiotic strains help to strengthen and boost the human immune system, increasing the body's resistance to a wide range of disease conditions. The food industry's effort to meet customers' sensory and health demands in dairy and fermented food items has boosted the need for probiotic starter cultures with superior performance and health-beneficial qualities. One of the crucial dairy starter cultures for producing fermented dairy products such as yogurt and cheese is lactic acid bacteria, particularly *L. bulgaricus.* An enhanced fermentation media improves the generation of essential metabolites, such as lactic acid and the sensory attribute of fermented food. Therefore, this review aims to present an overview and the importance of lactic acid bacteria in fermentation. The review also presents information on specific nutritional requirements of growth media for fermentation purposes as well as new classifications and views on the present commercial applications of these healthy bacteria.

Keywords
lactic acid bacteria (lab), *lactobacillus delbrueckii* subsp. *bulgaricus*, growth media, fermentation, nitrogen sources

Abbreviations
CFU – coliform forming unit; CSL – corn steep liquor; DPM – date palm media; FAO – Food and Agricultural Organization; GRAS – generally recognized as safe; HPLC – high-performance liquid chromatography; LAB – lactic acid bacteria; MRS – de man rogosa and Sharpe; SPM – sweet potato media; WHO –World Health Organization

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Introduction

Lactic acid bacteria (LAB) are essential microorganisms that primarily create lactic acid as a by-product of metabolic activity (Ayivi et al. 2020). Lactic acid bacteria have several applications in agriculture, food, medicine, etc., and are used in the production of many food products, particularly fermented foods. In fermentation, employing this bacterium is one of the most traditional and well-known methods of food preservation. LAB has also been described as microorganisms with the potential for improving human health (Ayivi et al. 2020; Hayek and Ibrahim 2013; Hayek et al. 2019). LAB also have unique nutritional characteristics, along with improved adhesion adaptive traits, which allow the bacteria to survive in a variety of habitats, including dairy-based products, fermented dairy products, vegetables and the human gut Bintsis (2018). Lactic acid bacteria create organic acids and other metabolites during fermentation that increase taste enhancement in food and prevent spoiling, making them highly helpful in a variety of applications, particularly in the food and dairy industries The food industry benefits greatly from lactic acid bacteria; thus, validating the possibility of lactic acid bacteria as starter cultures is critical, since the role of dairy starter cultures has a substantial effect on product quality and sensory appeal (Hati et al. 2013). *L. bulgaricus* is among the most significant LAB since it combines with *Streptococcus thermophilus* as a starter culture to produce yogurt. *L. bulgaricus* was isolated from Bulgarian yogurt, and today, the yogurt manufacturing industries use it in addition to *S. thermophilus* for fermentation. *L. bulgaricus* plays an important role during yogurt production regarding organoleptic, hygienic and sensory characteristics. As a result, it is a safe probiotic with several advantageous qualities (Ayivi et al. 2020). According to the FAO/WHO (2002), probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host." Mani-López et al. (2014) reported that the food sector, especially the dairy industry, is looking to enhance its knowledge of different probiotic bacteria to produce products that offer health benefits at a reasonable cost. However, the development of starter cultures for fermented dairy products depends on the microbial symbiosis of several lactic acid bacteria with excellent fermentation abilities (Urshev et al. 2006). Several studies have confirmed that the most significant fermentation properties of industrial or commercial yogurt starter cultures include rapid acidification, texturing capacities, specific flavor compounds, weak post-acidification, and health benefits with minimal dietary needs (UmaMaheswari et al. 2021). Although the dietary needs of starter cultures such as LAB vary depending on the strain, excellent bacterial growth may be adopted in favorable and rich fermentation media under the right environmental circumstances. In general, nitrogen sources and other growth-promoting substances are added to the fermentation medium to improve its nutritional quality, which in turn promotes bacterial growth. However, the high cost of nitrogen sources makes them prohibitively expensive for fermentation processes, which is thus a limiting factor regarding supplementing a fermentation medium with significant amounts of nitrogen Hayek and Ibrahim (2013). Despite the high cost of nitrogen sources, several studies have shown that LAB species may produce large amounts of lactic acid in the presence of amino acids, vitamins and yeast extract. The production of both primary and secondary metabolites by LAB species can thus be considerably influenced by the optimization of a growth medium that satisfies all nutritional needs (Hayek et al. 2019). Therefore, this review discusses lactic acid bacteria and the dietary requirements for lactobacilli alternate growth media for fermentation.

Characteristics of Lactic Acid Bacteria

Basic function of *L. bulgaricus* as LAB. *L. bulgaricus* has been known to be one of the most useful probiotics in human health as well as the food (dairy) industries and health sectors due to its resourcefulness. *L. bulgaricus* is a gram-positive, rod-shaped bacterium that is naturally found in the gastrointestinal tract of humans and other animals Bintsis (2018). The discovery of this useful probiotic (*L. bulgaricus*) is credited to a famous scientist in the field of microbiology by the name of Stamen Grigorov, a Bulgarian-born microbiologist who isolated the strain from yogurt in 1905 in Geneva and subsequently gave the microorganism the name *"L. bulgaricus"* in honour of his native land Bulgaria. After careful consideration by (Weiss, Schillinger and Kandler 1983), the bacteria's previous name (*L. bulgaricus)* was then

reformed to *L. bulgaricus* following several different research which then stayed to date. *L. bulgaricus* is a thermophilic genus probiotic with an ideal temperature range between 43-46°C Bintsis (2018). This organism can metabolize energy without oxygen due to its anaerobic characteristic; however, it can also live in both aerobic and anaerobic environments. The most common method for enumeration of *L. bulgaricus* is to use the MRS (de Mann, Rogosa and Sharpe) agar with a modified pH of 4.6 and anaerobically incubate it at 42°C (Tharmaraj and Shah 2003; Oyeniran et al. 2020).

One of the main functions of *L. bulgaricus* is to convert lactose, the sugar found in milk, into lactic acid through a process known as lactic acid fermentation. This helps to create the characteristic tangy taste and thick texture of yogurt. In addition, *L. bulgaricus* also produces other organic acids such as acetic acid, which contribute to the flavor and preservation of dairy products Bintsis (2018). Another important role of *L. bulgaricus* is in the digestive system. This bacterium is part of the natural microbiota of the human gut and helps to maintain a healthy balance of microorganisms in the digestive tract (Hati et al. 2013). It has been shown to benefit the immune system, reducing the risk of infections and inflammation. *L. bulgaricus* is a bacteria species that also plays an important role in the production of dairy products such as yogurt and cheese. The use of *L. bulgaricus* in the production of dairy products is widespread and the bacterium is often used in combination with *S. thermophilus* to create a starter culture Ayivi and Ibrahim (2022). The starter culture is added to milk, which is then incubated at a specific temperature for several hours to allow the bacteria to ferment the lactose and create yogurt. In addition to its use in the food industry, *L. bulgaricus* has also been the subject of numerous studies investigating its potential health benefits. These studies have shown that consuming *L. bulgaricus* may positively affect digestive health, reduce the risk of certain infections, and improve immune function Ayivi and Ibrahim (2022).

L. bulgaricus can also be problematic in certain situations despite its many benefits. For example, lactose-intolerant individuals may experience discomfort or digestive issues when consuming dairy products containing *L. bulgaricus*. In addition, excessive consumption of yogurt or other dairy products containing *L. bulgaricus* can lead to overgrowth of the bacteria in the gut, which may cause diarrhea or other digestive issues Kok and Hutkins (2018).

Lactobacillus in fermentation. LAB such as lactobacillus have been well known for their wide range of utilization during fermentation in different food (Hayek and Ibrahim 2013; Hayek et al. 2019; Yeboah et al. 2023). The production of fermented foods has been an old practice over the past years without any/proper comprehension of any microbiology. However, although the nature of fermentation used in ancient times was misunderstood, the process was a feasible user experience. Over the past centuries, we have learned crucial information about food fermentation through the discovery of microorganisms and the growth of microbiology as a scientific discipline. This knowledge has allowed better insight into sing inoculants known as starter cultures to initiate fermentation. Lactic acid fermentations are often low cost, and their preparation often requires little or no heat, making them fuel efficient. Fermentation has multiplied the possible combinations of LAB and their growth variables (such as temperature, salinity, and moisture) through transformation and preservation facilitated by this unique biological process (fermentation), thus resulting in a plethora of fermented product types such as acidified milk, cheese, leavened bread, fermented sausages, wine, beer, vinegar, sauerkraut, kimchi, and soy sauce, yogurt, dahi, kefir and laban, among others Binda and Ouwehand (2019). LAB produces acid during fermentation, which helps to lower the pH and therefore contributes to the preservation of the food as well as the creation of a gel-like texture and an acid flavor Fraqueza and Patarata (2019). Although most LABs are quite specialized and sensitive to artificial media, they grow swiftly in most food substrates and quickly lower pH levels to a point where competing species cannot live. *Leuconostocs* and lactic *Streptococci* lower the pH to around 4.0 to 4.5, whereas *Lactobacilli* and *Pediococci* also do so to about 3.5 before inhibiting their growth. By oxidizing reduced nicotinamide adenine dinucleotide (NADH) with flavin nucleotide, which combines effectively with ambient oxygen, lactobacilli can contribute to the generation of hydrogen peroxide in addition to lactic acid. Hydrogen peroxide is produced by flavoproteins such as glucose oxidase, which has an antimicrobial

effect on bacteria that cause food degradation. Lactobacilli are often resistant to hydrogen peroxide Steinkraus (1992).

Table 1. Taxonomic reclassification of some common LAB species (Zheng et al. 2020)

Former name	Novel name
Lactobacillus rhamnosus	*Lacticaseibacillus rhamnosus*
Lactobacillus paracasei	*Lacticaseibacillus paracasei*
Lactobacillus casei	*Lacticaseibacillus casei*
Lactobacillus helveticus	*Lactobacillus helveticus*
Lactobacillus iners	*Lactobacillus iners*
Lactobacillus delbrueckii subsp. *delbrueckii*	*Lactobacillus delbrueckii* subsp. *delbrueckii*
Lactobacillus johnsonii	*Lactobacillus johnsonii*
Lactobacillus acidophilus	*Lactobacillus acidophilus*
Lactobacillus jensenii	*Lactobacillus jensenii*
Lactobacillus crispatus	*Lactobacillus crispatus*
Lactobacillus gasseri	*Lactobacillus gasseri*
Lactobacillus pentosus	*Lactiplantibacillus pentosus*
Lactobacillus salivarius	*Ligilactobacillus salivarius*
Lactobacillus plantarum	*Lactiplantibacillus plantarum*
Lactobacillus brevis	*Levilactobacillus brevis*
Lactobacillus kefiri	*Lentilactobacillus kefiri*
Lactobacillus fermentum	*Limosilactobacillus fermentum*
Lactobacillus reuteri	*Limosilactobacillus reuteri*

Types of Fermented Foods

There have been a lot of fermented foods including local dairy fermented foods. Many of the starchy foods frequently found in Africa are usually fermented before consumption, with yeasts and bacteria, mainly LAB being the most prevalent and common microorganisms involved in this kind of fermentation (Olasupo et al. 1997). Lactobacillus has been the most important LAB group, making it one of the most beneficial and useful microorganisms in food manufacturing and processing due to its useful applications in a wide range of foods, making it possible to produce many fermented foods (Olasupo et al. 1997) such as yogurt, cheese, kefir, "kenkey," "fufu," "ogi", "Kunu-Zaki," etc. Some fermented foods from LAB fermentation (*Lactobacillus*) have been discussed below. Table 1 lists some common LAB used in food fermentation.

Yogurt. The history of yogurt dates to the year 5000 BC and the discovery of this fermented food is attributed to the ancient nomads who resided in the Middle East and massively absorbed it into the human diet (Fisberg and Machado 2015; Rul 2017). Yogurt is derived from the Turkish term "*yoghurtak*", which means to thicken or coagulate. Yogurt, an ancient, fermented yogurt, is known by many different names across the world, including dahi (India), laban (Iraq and Lebanon), zabadi (Egypt), and matsoni (Georgia, Russia, and Japan) Fisberg and Machado (2015). Yogurt is also a low-calorie (about 90 kcal per serving) fermented milk food item containing living bacteria that has several uses, and these fermented milk products have been proven to be safe Rul (2017).

The production of yogurt some years back was aimed at extending the shelf life of milk Tamime and Robinson (2007). Although this product (yogurt) is currently mostly consumed as a dairy product, it was first developed as a pharmaceutical product. Yogurt is a fermented milk product that is often produced by exposing milk to sour at a temperature of 40-45°C Lourens-Hattingh and Viljoen (2001). The fermented milk product, which subsequently becomes a fluid is the result of a well-controlled fermentation method that includes ingredients such as milk, milk powder, sugar, fruit, flavors, colouring, emulsifiers, stabilizers, and specific pure cultures of LAB (*S. thermophilus* and *L. bulgaricus*) which convert lactose (milk sugar) into lactic acid, giving yogurt its characteristics tangy taste and the texture Lourens-Hattingh and Viljoen (2001).

According to the Codex Alimentarius, yogurt requires the presence of two LAB, *L. bulgaricus* and *S. thermophilus*, in a minimal viable count (sum of

microorganisms, min 10^7 CFU.g^{-1}) Binda and Ouwehand (2019). Interestingly, the latter is the only *Streptococcus* species employed in the food industries, and it is likely to be one of the most often consumed bacteria by humans Binda and Ouwehand (2019). The bacterial inhabitants of yogurt are responsible for its pleasant flavor, unique texture, and some of its health benefits and this makes yogurt a very popular dairy product (Marette et al. 2017). Consumption of yogurt has health benefits such as helping to prevent lactose maldigestion due to the production of lactase-like enzyme β-galactosidase and *L. bulgaricus* (Guarner et al. 2005). It was however seen that the regular intake of yogurt showed a 14% lower risk of diabetes in those individuals who consumed between 80 and 125 g of yogurt per day compared to non-consumers (Gijsbers et al. 2016). Epidemiological studies have also proved that frequent intake and regular consumption of fermented and dairy products lower the risk of high blood pressure (Chen et al. 2014; Rai and Sanjukta 2015).

Yogurt can be consumed plain or flavored with various fruits, honey, or other sweeteners. It is also a versatile ingredient that can be used in many recipes, such as smoothies, dips, dressings, and baked goods. Many types of yogurts are available, including regular, low-fat, and non-fat options. Greek yogurt, which is strained to remove excess liquid and has a thicker consistency and higher protein content, has become particularly popular in recent years. While yogurt is generally considered a healthy food, choosing varieties that are low in added sugars and artificial ingredients is important. Some people may also be sensitive to dairy or lactose intolerance and should opt for lactose-free or non-dairy alternatives such as coconut or almond milk yogurt (Sekar et al. 2020). Figure 1 shows the process of yogurt production.

Cheese. Cheese is the collective name for a group of fermented milk-based dietary products that are produced globally and come in a variety of flavors, textures, and shapes (Fox et al. 2017). These milk-based foods are made by converting milk in the form of liquid into curd, which is a solid substance that retains casein and fat from the milk but has lost most of the water and in most cases, the whey proteins. The process is achieved by adding rennet to coagulate the casein gel, as a result, the cheese curd acts as the foundation for the final product (the cheese) which is subsequently modified by operations such as pressing, salting, and ripening Lomholt and Qvist (1999).

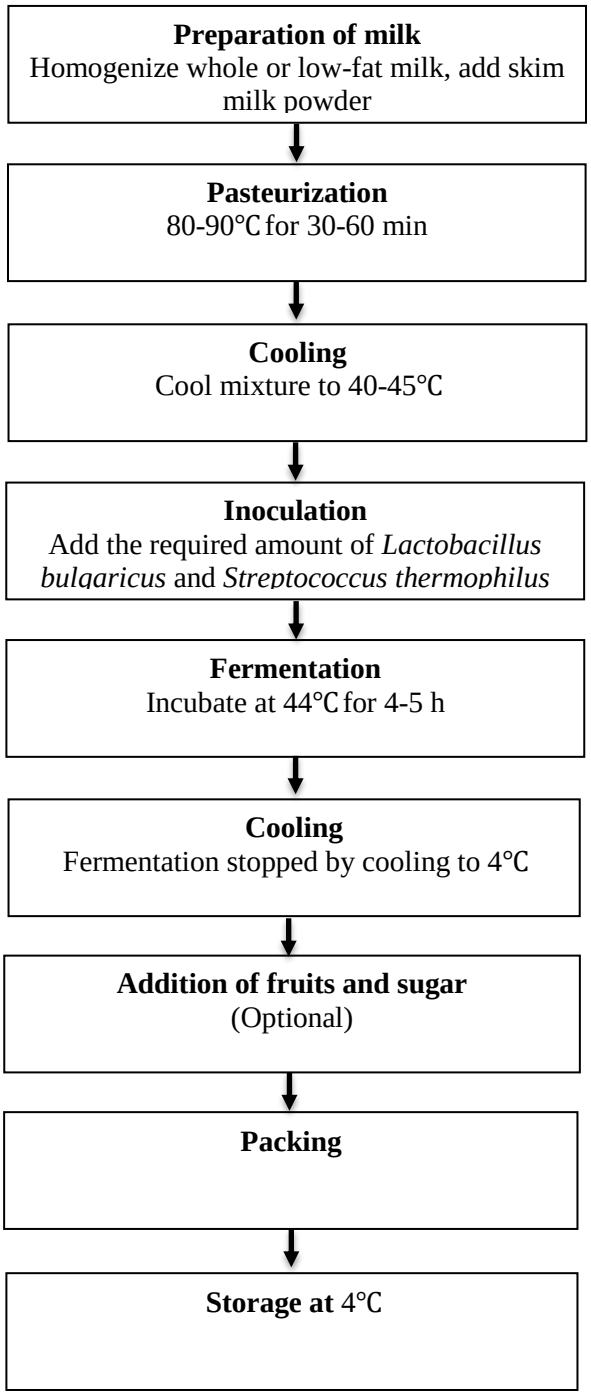


Figure 1. Flowchart representation of yogurt production

Cheese mostly comes in different varieties in various parts of the world, however, despite all the different variations, the basic production method remains the same. What usually differs between the various cheese varieties sometimes are the differences in the starter cultures, the presence or absence of molds, and the whey separation procedures (straining, pressing, and heating) Binda and Ouwehand (2019). Differences in ripening processes, such as time and environmental conditions, the use of ripening cultures, smearing the cheese surface with various preparations, etc., as well as the use of different types of milk (pasteurized or unpasteurized) also have a significant impact on the final product. Some common examples of cheese include fresh cheese (e.g., cottage cheese, mozzarella, and feta (-like) cheese), and soft cheese (e.g., Camembert and Brie), usually in addition to LAB, these cheeses also contain a surface mold of *Pencillium camemberti* and *Geotrichium candidum.* Hard cheese, these cheeses vary from semi-hard (such as Gouda and Edam) to hard (such as Parmesan) Binda and Ouwehand (2019). In most nations, cheese consumption has increased over the last decade, regardless of the country's socioeconomic status. (Gomes da Cruz et al. 2009) reported that cheese production reached 17,778 million tons (t) in 2004, an increase of over 3272 t over the previous ten years. Cheese consumption has been proven to be associated with many health benefits to the individual. According to research, the regular consumption of cheese has been proven to reduce the risk of cardiovascular disease as well as cardiovascular mortality and a reduced risk of stroke (Chen et al. 2017; Farvid et al. 2017; Gille et al. 2018). Some cheeses may differ in their composition during their processing method.

A study conducted by (Stankov et al. 2023) examined the composition of artisan sheep's milk cheese as it ripened. The researchers reported that the cheese's main physicochemical parameters changed during the ripening period. The study also observed a smooth acidification process, which matched the high number of lactic acid bacteria. The fatty acid analysis revealed that the cheese had 66.0% saturated fatty acid composition and 34.0% unsaturated fatty acids. However, on the 60th day of the test period, during the biochemical ripening, the study found 0.12% of linoleic polyunsaturated fatty acid, and this shows that artisan sheep cheese is safe to eat after being aged 60 d (Stankov et al. 2023). The composition of the cheese's fatty acids changes during the aging process, and unsaturated fatty acids are present at the end of the period. Overall, the study confirms that using domestic technologies to make sheep's cheese does not pose any health risks. The key to ensuring a high-quality final product is closely monitoring and controlling the technological processes involved in production (Stankov et al. 2023).

Ivanova et al. (2023) also conducted a study on the effects of chilled curd on the microbiological, functional, textural, and sensory characteristics of Kashkaval cheese. Their findings revealed that salting the curd in a hot solution led to a greater reduction in microflora from the starter culture in Kashkaval cheese made from fresh curd. *Streptococcus* ssp. exhibited a higher survival rate than *Lactobacillus* ssp. during the early stages of maturation, but this trend shifted as the number of *Lactobacillus* ssp. increased while *Streptococcus* ssp. remained constant and even slightly decreased in both samples studied as seen in Table 2 (Ivanova et al. 2023). The melting and textural properties of the two samples at the end of the maturation process did not differ significantly. Therefore, the study's results demonstrate that "Cagliata" can be a suitable alternative to fresh curd in the production of Kashkaval cheese without compromising its microbiological, functional, textural, and sensory quality (Ivanova et al. 2023).

The level of contamination in certain cheeses sampled from the market has been established. (Studenica et al. 2022) investigated the presence of bacterial contaminants (*Escherichia coli, Staphylococcus aureus, Listeria monocytogenes, and Salmonella* spp.) in 116 artisanal cheese samples sold in informal markets of Kosovo, a city in Bulgaria. The researchers reported a significant number of bacterial contaminants in artisanal cheese sold in Kosovo's informal markets. *Escherichia coli* was present in 64.7% of samples, *Staphylococcus aureus* in 39.7% and *Listeria monocytogenes* in during winter. This information, therefore, denotes that public health institutions should increase attention and control measures to improve the safety of artisanal cheese in informal markets to ensure the safety of consumers. Fig. 2 shows the schematic representation of cheese production.

Table 2. Microbiological analysis of kashkaval from chilled and fresh curd (Ivanova et al. 2023)

Component	Kashkaval from chilled curd		Kashkaval from fresh curd	
	Beginning of ripening	End of ripening (60^{th} d)	Beginning of ripening	End of ripening (60^{th} d)
Lactobacillus spp.,	3.29 ± 0.30^{a}	4.12 ± 0.30^{b}	2.96 ± 0.22^{a}	4.22 ± 0.25^{b}
Streptococcus spp.,	3.97 ± 0.20^{a}	3.70 ± 0.20^{a}	4.94 ± 0.12^{b}	3.77 ± 0.24^{a}
Coliforms, log cfu.g^{-1}	-	-	-	-
Moulds and yeasts, cfu.g^{-1}	< 10	< 10	-	-

^{a,b} *Means with different letters within a row are significantly different ($p < 0.05$)*

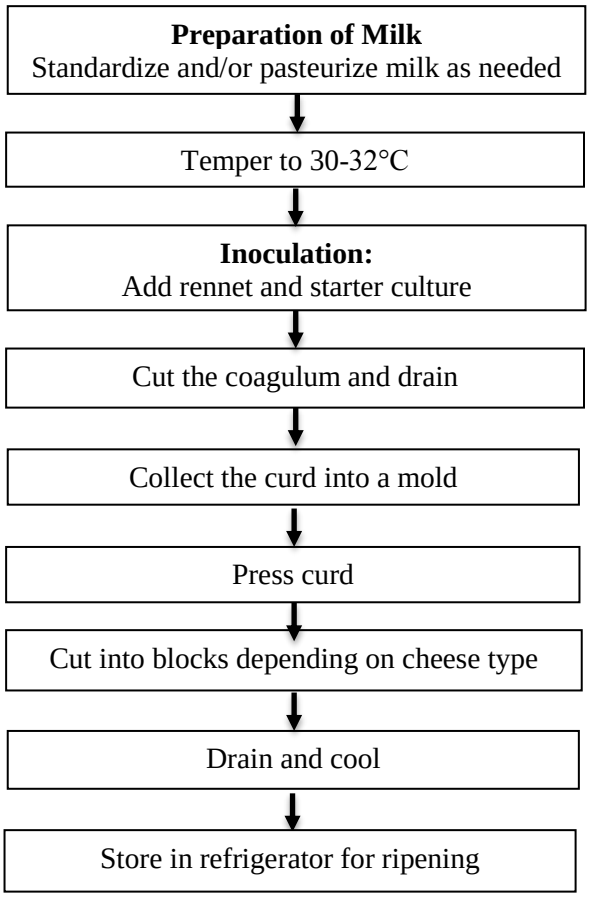


Figure 2. A schematic representation of the general cheese production process

Kenkey. Kenkey is one of the indigenous fermented food products which is commonly produced in the coastal areas of Ghana, West Africa. It is believed that the modern-day patronage of this fermented food by the coastal natives in Ghana descends from the ancestral history some years back. Two ethnic groups in Ghana - the Gas in the Greater Accra Region and the Fantis in the Central and Western Regions - produce this well-liked and popular traditional fermented food. Ga-kenkey (also known as Komi) and Fanti-kenkey (dokono) are the two separate variations of this native meal that are produced by the two ethnic groups (Gas and Fantis). Interestingly, both types of kenkey are produced by fermenting maize dough into a sourdough that is then cooked and wrapped in either maize husks or plantain leaves, depending on whether they are called Ga-kenkey or Fanti-kenkey respectively, even though the organoleptic qualities and processing techniques for these two types of kenkey differ slightly from one another (Hui et al. 2004). The Fanti-kenkey ferments for a little longer than the Ga-kenkey and salt is added to the latter throughout the process while salts are added to the former only in the initial stage. These indigenous fermented foods are rich in nutritional content; however, the nutritional quality is mostly determined by the type of maize used and the processing technology employed. According to (Hui et al. 2014), maize is more protein-rich than other staple foods including cassava, cocoyam, yams and plantains and significantly improve the overall colorific and protein content of the diet of individual consumers.

Traditional varieties of maize, however, are deficient in lysine, tryptophan and B vitamins (Hui et al. 2014). Maize is anticipated to provide over 70% of the dietary proteins and 90-95% of the total calories for some coastal populations (Capurso 2021). Therefore, on dry matter, Ga-kenkey is reported to have roughly 8.9-9.8% protein, 1.3-3.2% fat, and 74.3-87.1% total carbohydrates (Hui et al. 2014). Cleaning, steeping, milling, fermentation of the dough, preparation of the aflata, the blending of the aflata with the raw dough, shaping and packaging, and cooking into kenkey are all the processes involved in the traditional preparation and the production of Ga and Fanti kenkey (Hui et al. 2004). Fig. 3 (A and B) displays local Ga and Fanti kenkey respectively consumed in Ghana. Fig. 4 shows the schematic representation of the general production of the two varieties of kenkey in Ghana.



Ghanaian Ga Kenkey (**a**)



Ghanaian Fanti Kenkey (**b**)

Figure 3. Ghanaian traditional fermented foods, Ga Kenkey (**a**) and Fanti Kenkey (**b**)

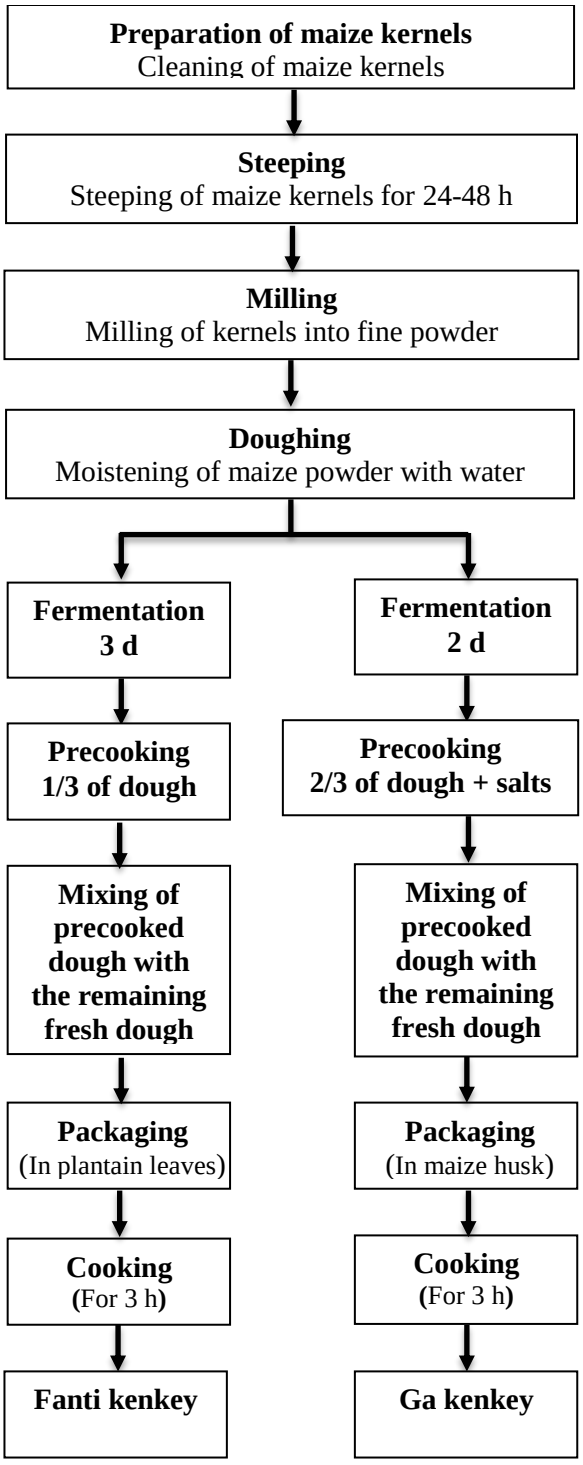


Figure 4. A schematic representation of the production of two varieties of kenkey in Ghana

Types of Microorganisms in Fermented Foods

There have been a lot of microorganisms involved in fermentation both in the commercial and industrial manufactured foods to our local foods. *Lactobacillus* and other related species have been utilized as starter cultures for fermentation operations in a wide range of industries (Yeboah et al. 2023) these microorganisms are usually classified as the most important element in the fermentation process due to their vital role in converting the sugars in the food into useful end products. These organisms are useful in diverse ways during the fermentation process because their wide range of properties imparts a beneficial effect. Such effects are preservation, flavor enhancement, organoleptic properties, etc. to foods like yogurt, beer, pickles, cheese, wine, bread, etc. and as such making their life useful for humans (Yeboah et al. 2023). There are over thousands of microorganisms found in the ecosystem however, the groups of these organisms mainly involved in the fermentation process include bacteria, yeast and mold.

The bacteria group of microorganisms involved in fermentation is the LAB, these groups of microorganisms have widely been applied in food fermentation worldwide and this is a result of the LAB being well-known and GRAS microorganisms (Widyastuti et al. 2014). Another reason this useful probiotic is generally accepted and used worldwide is its fermentative ability and thus enhancing food safety, improving organoleptic attributes, enriching nutrients and increasing health benefits (Panesar 2011; Liu et al. 2011). This group of LAB is usually used during milk fermentation and the main reason behind fermenting of milk is due to their high perishability thus they (milk) are being fermented to increase the shelf life to prolong the nutritious components of the products. Additionally, it is recognized that fermenting milk with LAB would surely result in high-quality goods with coveted organoleptic qualities (Widyastuti et al. 2014; Yeboah et al. 2023). The ability of these LAB to acidify the milk and produce flavor and other properties such as texture by converting milk protein due to their proteolytic activities are the most crucial characteristics of these LAB during these fermentations, which have been discussed to cause milk fermentation (Mäyrä-Mäkinen and Bigret 2004; Griffiths and Tellez 2013; Kongo 2013).

Table 3. Functional benefits of lactic acid bacteria (LAB) in different fermented products

LAB culture	Product name	Origin	Functional benefit	Reference
L. rhamnosus CAN-1	Probiotic yogurt	Ontario, Canada	Nutrition and immune function for people living with HIV	(Hemsworth et al. 2011)
L. brevis	Ewe milk, traditional yogurt	Iran	Cholesterol reduction	(Iranmesh 2013)
L. plantarum, L. brevis *L. paracasei* subsp. *paracasei*, *L. casei* subsp. *pseudoplantarum*	Ayran (yogurt from goat milk)	Turkey	High exopolysaccharides	(Widyastuti 2014)
E. faecalis, E. faecium	Kumis	West Columbia	ACE Inhibitor	(Chaves-López 2011)
L. casei strain Shirota	Fermented milk	Japan	Maintenance treatment for myelopathy/tropical spastic paraparesis (HAM/TSP) patients	(Widyastuti 2005)
L. plantarum, L. fermentum, *L. acidophilus, L. paracasei*	Kule Naoto (Maasai)	Kenya	Diarrhea and constipation	(Mathara 2004)

Aside from acidifying the milk, these LAB also have the potential to release antimicrobial metabolites known as bacteriocins and both these bacteriocins and acids are good sources of safe natural preservatives for foods. Some common examples of LAB which are mainly involved in fermentation include *Lactobacillus bulgaricus, Streptococcus thermophilus* (these two are especially for yogurt production), *Lactococcus lactis, L. acidophilus, L. mesenteroide, L. plantarum, L. fermentum, L. paracasei.* Table 3 shows some LAB, the food product they ferment and their functional benefits to the individual. Another group of microorganisms greatly involved in fermentation is yeast. Despite some yeast being pathogenic for humans, some species such as *Saccharomyces cerevisiae* are very helpful for fermentative activities, and they are usually characterized by alcoholic fermentation to produce end products such as wine and beer. One of the earliest human technologies is yeast fermentation, which has roots in the Neolithic era and uses a variety of plant carbohydrate sources. The intricacy of the gene expression regulatory networks underlying alcoholic fermentation is still far from being fully understood, even though yeasts are now necessary for numerous biotechnological processes, including the fermentation of beer, wine, and biofuels (Compagno et al. 2014). Yeasts such as *Saccharomyces cerevisiae* typically take over as the dominant species in certain habitats. These yeasts' capacity to quickly convert carbohydrates to ethanol under both anaerobic and aerobic circumstances is one of their most notable and distinguishing characteristics and is probably a winning property, making them more suitable for most alcoholic fermentations.

The other group of microorganisms involved in fermentation is mold. Mold, specifically the Rhizopus species, has been one of the microorganisms involved in a variety of fermentations resulting in many different food products. These filamentous fungi have been used greatly to produce different kinds of foods in different parts of the world such as China, Japan and Southern Asia while Africa, the Middle East and Mid Asia use bacteria and yeasts in their fermentations Hachmeister and Fung (1993). Molds not only produce foods in Asia but also provide some Western civilizations with diverse products ranging from tempeh and Orgi. The production of enzymes by mold, which hydrolyses soybean components and aids in the formation of a product's ideal texture, taste, and aroma, is a crucial mechanism that enables various fermentation processes Hachmeister and Fung (1993). Molds are usually mixed up with other organisms such as bacteria and yeast to produce products with good sensory attributes such as flavor, and texture. The nutritional value of the fermented product may be enhanced by enzyme hydrolysis, which also has the potential to reduce or remove antinutritional components.

Functional Properties of Microorganisms in Fermented Foods

The biological capabilities of fermented foods, which are enhanced by several health-promoting advantages for consumers, are their most distinctive feature and this is due to the associated functional microorganisms. Microorganisms have been linked to various benefits ranging from health, preservation, improvement of food quality, etc. (Ibrahim et al. 2023). All these useful benefits come about because of specific attributes/characteristics ascribed to these individual-specific microorganisms. Some offer health benefits such as improving the gut microbiome, others are used in food production through fermentation and by so doing exert several functional properties to the foods such as lowering pH to extend shelf life, enhancing flavor, texture, etc. (Yeboah et al. 2023). These benefits are from the various microorganisms used in food production and some are being discussed.

Acidification. LAB is extremely significant and is usually used for milk acidification and flavor development in these days' dairy industries. When we consider the production of yogurt and other dairy products, the main probiotics which are used as starter cultures in this process are *S. thermophilus* and *L. bulgaricus*. The capacity of these two microbes to quickly develop and acidify milk is a desirable property that is critical for their usage in various applications Courtin and Rul (2003). As a result, LAB has been attributed with a variety of superior advantages and features that might be used in a wide range of applications while also improving the quality of dairy products. LAB strains are significant starter cultures that are associated with

high fermentation ability and therefore making them a suitable microbe for several different uses in several processes. Many stakeholders in the food and dairy industries have long strived to obtain desired product attributes through LAB. Quick acidification is one of the most significant and important characteristics of LAB, and it is necessary throughout many fermentation processes to contribute to the formation of texture, flavor and the safety of the product (Grattepanche et al. 2008).

Controlling and having starter cultures of desired and preferred qualities to supply and satisfy the essential criteria for both producers and customers at large is vital and fundamental in maintaining the quality of the products and their consistency as well as avoiding low fermentation rates in general. It is however important to identify the impacts of process parameters on the kinetics of the bacteria's growth pattern to fully exploit the potential of LAB acidification activities. Harvesting time and pH are the two important process factors that have a direct influence on the physiological condition of the LAB following the fermentation process (Liu and Shen 2008). Yogurt production is an important fermentation process that relies on starter cultures as well as other parameters such as milk composition (Liu and Shen 2008). The high demand of the consumer for yogurt with desirable product features is increasing, putting the obligation for scientists to fully explore several capabilities of the LAB strains as the right option to perform this purpose. The symbiotic relationship between *S. thermophilus* and *L. bulgaricus* is critical for the acidity of fermented dairy products, particularly yogurts. In the milk medium, *S. thermophilus* produces pyruvic acid, formic acid, and CO_2, which support the development of *L. bulgaricus.* Because *S. thermophilus* is a poor proteolytic bacterium as compared to *L. bulgaricus*, the latter creates peptides and amino acids which stimulate the growth of S. thermophilus Courtin and Rul (2004). Even though the capacity of starter cultures to acidify fast is essential in many dairy sectors, it is important to ensure that the fermentation time required to attain the required pH does not vary significantly between batches. As a result, there are three essential factors associated with selecting a starter culture with exceptional fermentation characteristics: their acidification rate, acid concentration and fermentation time (Ayivi et al. 2020).

Biological preservation. One major characteristic which is of great importance to LAB is their biological preservation functions; this important and well-known feature of the organism is due to its capacity to create and produce acid, which has antibacterial properties. Acid production by these LAB protects milk against bacteria causing spoilage and pathogens proliferation. As a result of the metabolism of carbohydrates, the homofermentative species of LAB generally convert milk's carbohydrates into lactic acid, whereas the heterofermentative species primarily convert lactose into lactic acid, acetic acid, ethanol, and CO_2 (Widyastuti et al. 2014). However, lactic acid production by LAB is usually dependent on a particular strain. (Widyastuti et al. 2014) reported that some newly isolated *Lactobacillus paracasei* subsp. *paracasei CHB2121* produced a large concentration of L (+)-lactic acid effectively and confirmed that these *lactobacillus* species may be excellent for the industrial production of lactic acid. Also, a study by (Widyastuti et al. 2014) on two LAB (*Lactococcus lactis*) strains for their capacity to inhibit the growth of bacterial pathogens demonstrates that these organisms strongly inhibit the pathogenic strains of *Salmonella enteritis* and E. *coli* tested, with the main inhibition effect associated with quick acid production, which resulted in rapid pH reduction. The addition of 2% (v/v) *Lactobacillus casei AST18* also fully prevented the growth of *Penicillium sp.*, which was utilized as an indicator fungus during a test on yogurt preservation. This proved that the antifungal compounds lactic acid and cyclo-(Leu-Pro) are produced by *L. casei AST18* (Widyastuti et al. 2014). LAB also produces antimicrobial metabolites known as bacteriocins Hayek and Ibrahim (2013), these bacteriocins have a lot of potential for usage as natural preservatives in food. Bacteriocins are formed during the first phase of bacterial development and have a lot of antibacterial properties.

Biological enhancement of nutritional value. One major potential and functional property of LAB in various fermented foods is their ability to enhance the nutritional content of these foods. LAB fermentation enhances the aroma and flavor of food products, making them more appealing. In terms of consumer acceptability (Blandino et al. 2003) confirmed that these organoleptic qualities made

fermented foods more desirable than unfermented foods. LAB is one of the main elements responsible for these beneficial characteristics in meals but the exact mechanism by which flavor is formed is still subject to investigation (Chelule et al. 2010). Various ingredients, such as citric acid in lemon juice, were added to foods to mimic and reproduce the low pH of these foods and examine their sensory features, however, these foods failed to produce the same desired outcome compared to naturally fermented foods. This means that regular acidification of food does not result in a direct enhancement in the organoleptic and sensory aspects of the meal, consequently, fermentation is unique in that it alters unfermented food in a variety of ways, resulting in novel sensory attributes in the fermented product Leroy and De Vuyst (2004). However, not all bacteria and molds help improve food sensory qualities like taste. They may cause food deterioration in some cases because their enzymes stimulate the formation of fermented digests with disagreeable odors or flavors, rendering the food undesirable. Several foods, such as cereals, are nutritionally deficient, even though they make up a considerable part of staple meals in some low-income nations. The nutritional content and digestibility of these foods have thus been reported to be improved by LAB fermentation. Temperatures between 22 and 25°C are ideal for microbial enzyme activity since the acidic nature of fermented products encourages it Mokoena (2005). These enzymes, which comprise amylases, proteases, phytases, and lipases, change the main dietary constituents by hydrolyzing appropriate amounts of polysaccharides, proteins, phytates, and lipids. Food's antinutrients like phytic acid and tannins are reduced by LAB fermentation in addition to boosting enzyme activity. This increases the bioavailability of nutrients like protein, vitamins, and simple carbohydrates as well as minerals like iron Chelule (2010). Several techniques, such as nutrient supplementation have been used in the past to improve the nutritional value of foods; however, these are insufficient and often unaffordable in meeting the demands of low-income populations, leading to the adoption of low-cost methods, such as microorganism fermentation (Chelule et al. 2010).

Nutritional Requirements of *Lactobacillus*

Fastidious nutritional requirements, which are well known for playing an essential role in the survival and the life of LAB may differ within species and even strains. LAB has a unique and distinct nutritional requirement because these organisms are of different species and are sensitive. Polak-Berecka et al. (2011) also reported that the type of growth media these organisms (LAB) found themselves in affects their ability to proliferate. The growth media of these probiotics is usually made up of carbohydrates (carbon source), protein, which is mostly composed of amino acids and peptides (nitrogen source), and buffer, in addition to tweens, minerals, vitamins, and fatty acids. These components play the role of supporting the growth and development, and the metabolic activities of microorganisms for the microbe to continue being useful to the host Ummadi and Curic-Bawden (2008).

Typically, the metabolic activities of these LAB result in the development and production of numerous valuable and useful substances, including organic acids, antibiotic substances, and specialized enzymes that may break down complex organic molecules into simpler usable ones. Instead of this, it can therefore be deduced that the environments that are both biochemical and biophysical may have a great effect on the growth and metabolic processes of LAB and both these biochemical and physical environments, which are sometimes known as nutrients or nutritional needs are made available through growth media that the bacteria utilize for their growth (Hayek et al. 2019). A wide range of bacteria growth media has been developed with various functions in mind, depending on the unique nutritional needs of various LAB species. The most common method for determining the nutritional needs of LAB is to remove one key element of the whole medium at a time. One or two of the key elements in the medium for LAB may be left out, which might cause either poor growth, no growth, or a reduction in the viability of the strains after fermentation. When conducting single omission experiments components that can synthesize can be left out without fully inhibiting growth. Wegkamp (2010). To obtain the desired growth, it can also be essential to sub-cultivate the culture a few times in newly created medium or novel formulae to investigate the nutritional requirements of LAB.

The major nutritional requirements of LAB are discussed below.

Carbohydrate (Carbon source). In the modern food era, lactic acids are being produced by LAB mostly by fermenting carbohydrates, this, therefore, makes carbohydrates a very key component in producing a growth culture media for LAB Carbohydrates are the primary source of carbon and sugar (energy) for the bacteria and therefore their inclusion in the growth media for the organism is essential for the development and operation of the LAB bacteria. The cell walls of these LAB are made up of dextrose (carbohydrate), and therefore the addition of these simple sugars (dextrose/ glucose) in the media supports the growth of the bacteria by contributing to the strength and stability of their cells walls to promote their growth. The predominant source of carbon and energy for the bacteria comes from this simple sugar (carbohydrate), even though carbon can also come from other components including nitrogen sources and glycerol (Hayek et al. 2019). Although several sugars, including maltose, fructose, raffinose, and others, may be employed in the preparation of media for LAB and exploited by various bacterial strains for growth and development, dextrose (glucose) has often been the most frequently used and preferred carbohydrate for bacterial growth (Hayek et al. 2019). However, certain LAB strains have also demonstrated preferences for sugars like maltose, raffinose, lactose, etc. As a result, LAB strains differ in their capacity to ferment various sugars, which may have an impact on their growth and development. According to Hayek and Ibrahim (2013), *L. acidophilus* grew more effectively when the carbon source in a media was changed from glucose to maltose, salicin, raffinose, or melibiose.

Amino acids and peptides (Nitrogen sources). LAB, known for their complex nutritional requirement, also needs a rich protein hydrolysate (nitrogen sources) for their growth. These protein hydrolysates are typically utilized in the production of LAB media as sources of amino acids, peptides, nucleic acid derivatives, minerals, and vitamins. Peptones, beef extract, and yeast extract are a few examples of the nitrogen sources they contain. These components can enrich LAB media with the prerequisite amount of protein needed for their growth. Milk, skim milk powder, whey protein, and reconstituted whey are increasingly frequently employed as sources of peptides and amino acids in the dairy industry. Peptides are converted into free amino acids and other substances for later use in the action of protease or proteolysis, which produces amino acids and peptides Hayek and Ibrahim (2013). Peptides can either be necessary growth factors or stimulatory factors, depending on the variations in peptide needs across LAB strains; certain strains can even thrive without them Letort and Juillard (2001). According to research on the different lactobacilli species' requirements for amino acids, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus lactis* have more flexible requirements than *Lactobacillus plantarum*, *Lactobacillus pentoses* and *Lactobacillus curvatus* (Hébert et al. 2004). Different organic nitrogen sources can be used to make amino acids and peptides, including papain, digested skim milk, yeast extract, tryptone (casein that has been treated with trypsin), soy peptones, peptones derived from animals, beef extract, corn steep liquor, liver extracts, whey protein hydrolysates, etc. (Hayek et al. 2019). For the typical development of LAB, however, peptone, beef extract, and yeast extract are more frequently used and seem to be practicable (Vazquez et al. 2004). Most LAB strains may be satisfied with a variety of amino acids and peptides found in these nitrogen sources, which can meet their nutritional needs. Additionally, carbon, minerals, and vitamins can also be found in peptone, beef extract, and yeast extract (Calderon et al. 2001). According to Hayek and Ibrahim (2013) a high concentration of these amino acids and peptides in a media result in the depression of bacterial growth. It is, therefore, crucial to use a balance of yeast extracts, beef extracts, and peptone in a LAB media culture to ensure good growth of the strains and their appropriate functionality.

Buffering agents (Buffers). During the preparation of media for the cultivation of bacteria, buffering agents are added as one of the major ingredients. These buffers are usually added because bacterial growth produces acid, and therefore buffering agents must be included to keep the pH at the perfect range to enhance growth. When LAB is growing, they create acid (mostly lactic acid) which lowers the pH of the medium, this can slow down or inhibit the growth of the bacteria strains especially when the pH drops below 4.6 Hayek and Ibrahim (2013).

Therefore, to keep the pH at the ideal level and range for the growth and development of the strains, it is crucial to incorporate buffering agents in the bacteria growth media to keep the pH of the media in the optimum range to ensure continuous growth of the strains. For most LAB genera, pH 4.4 may inhibit growth or noticeably reduce growth rate. Although they may also grow at a pH as low as 4.4, most *Lactobacillus* strains thrive in an optimum pH range of 5.0 to 6.0 Hayek and Ibrahim (2013). As a result, it's critical to include buffering agents in the culture media to maintain the proper pH for LAB growth. The most often used buffers in LAB media, such as MRS and M17, are sodium acetate, trisodium citrate, and di-sodium-glycerophosphate. According to research on Lb. strains, sodium acetate was discovered to be necessary for their growth and development (DeMan et al. 1960). As a result, removing sodium acetate reduced *Lb. plantarum* growth because of the quick pH drop (Sawatari et al. 2006). Other ingredients used in LAB medium with buffering action include disodium phosphate (K_2HPO_4), ammonium citrate ($NH_4C_6H_5O_7$), trisodium phosphate (Na_3PO_4), potassium biphosphate (KH_2PO_4), magnesium phosphate tribasic $Mg_3(PO_4)_2$, calcium carbonate ($CaCO_3$), and dipotassium phosphate (Na_2HPO_4) (Hayek et al. 2013).

Tweens. Tweens, which are a type of polysorbate emulsifiers are another group of ingredients that are very useful and being utilized by the food and pharmaceutical sectors as well as being used in media for LAB. Tweens usually function as surfactants and have vital roles such as shielding cells from harmful elements, boosting nutrition intake, and promoting the growth of LAB (Hayek et al. 2019). Tween 20 (Polyoxyethylene (20) sorbitan monolaurate), Tween 40 (Polyoxyethylene (20) sorbitanmonopalmitate), Tween 60 (Polyoxyethylene (20) sorbitanmonostearate), and Tween 80 (Polyoxyethylene (20) sorbitanmonooleate) are a few classes of Tweens that are readily accessible. Tweens, such as Tween 80 and 85, which contain oleic acid and hence promote the development of LAB and many other bacterial groups are vital for bacterial growth but not all Tweens perform this purpose. Lauric acid is another ingredient in Tween 20 that supports LAB development. The capacity of LAB to recover its tolerance to bile and its metabolic activity have all been shown to be significantly impacted by Tween 80 Hayek and Ibrahim (2013).

Vitamins. Another essential resource that LAB needs for their growth and development is vitamins. Vitamin needs for LAB are divided into three categories: necessary vitamins, which when absent from growth media, reduce growth by 67%; stimulatory vitamins, which reduce growth by 34-66% when omitted; and non-essential vitamins, which reduce growth by less than 67% when omitted from growth media. For several LAB strains, particular vitamins are necessary, including nicotinic acid and riboflavin pantothenic acid. Wegkamp et al. (2010) reported that the absence of riboflavin from a growth medium severely inhibited the development of *L. plantarum*. While ascorbic acid has little influence on LAB, vitamins like thiamine and biotin are necessary for its development.

Minerals. Minerals are crucial for microbial development and have a great impact on the activity of bacterial enzymes (Foucaud et al. 1997). The poor growth of some bacterial (LAB) strains in the absence of minerals (metal ions) demonstrates the significance of minerals for their growth (Foucaud et al. 1997). Important metal ions are beneficial to bacteria in several ways, including as cofactors or activators of different enzymes, components of membrane transport, and constituents of molecules or structural complexes (Hébert et al. 2004). According to reports most organisms including LAB, require just small amounts of the mineral element manganese and magnesium (Mn^{2+} and Mg^{2+}), respectively, for their growth and metabolic activity (Hayek et al. 2019). These Mg^{2+} and Mn^{2+} were added to the minimal medium to ensure that Lactobacillus plantarum would grow (Wegkamp et al. 2010). Fe^{2+}, Mg^{2+}, Ca^{2+}, Fe^{2+}, K^+, and Na^+ were frequently required by LAB as necessary or stimulatory factors for nutrition transportation and enzymatic activity since *S. thermophilus'* growth was unaffected by the removal of Fe^{2+}, $Zn2^+$, Co^{2+}, and Cu^{2+} from a chemically defined media Letort and Juillard (2001).

Standard Laboratory Growth Media

The growth rate and development of micro-organisms in the laboratory is an important and major concerns since doing so will enable

researchers to study them. However, one needs to be aware of the type of culture medium being used to cultivate these microorganisms. The necessary nutrients such as carbon, nitrogen, buffer, tweens, and minerals are essential for strains' development and therefore should be present in the growth medium. The unique organism and its metabolism determine how these nutrients are metabolized in a particular culture medium (Charalampopoulos et al. 2002). The growth of an organism may also be influenced by additional parameters including temperature, pH, and incubation time Gibson (1988). At some point, a medium can promote the growth and development of one microbe while inhibiting the growth of another, in which case that medium is selective to that organism. De Man Rogosa and Sharpe, M17, Whey Medium, and other media were developed since no one medium can sustain all microorganisms' growth and metabolism in an equal manner.

DeMan Rogosa and Sharpe (MRS). The MRS growth medium was created by De Man, Rogosa and Sharpe in 1960, primarily for the cultivation and production of lactobacilli species. In the first half of the 20th century, numerous investigations on the dietary requirements and growth environment of LAB were conducted (Hayek et al. 2019). After the necessary nutrients that can support the growth of LAB were identified, it was possible to develop and produce a medium with predefined components. Thus, in the 1960s, the lactobacillus MRS was developed to aid in the selective growth of *Lactobacillus* species (De Man et al. 1960). Although several media for lactobacilli were being developed at that time such as the popular tomato juice medium of Briggs, it was however discovered that several lactobacilli strains belonging to many species did not grow very well in this medium McLaughlin (1946). Consequently, there was a need to develop a non-selective medium that was required to promote healthy lactobacilli development in general. This medium produces practically all LAB with excellent productivity, but because it is not selective in its original form, it must be adjusted to have a pH between 6.2-6.5 (Hayek et al. 2019).

Table 4. Chemical compositions of original and commercial lactobacilli MRS broth (Hayek et al. 2019)

	Composition of MRS, $g.L^{-1}$				
Component	**Original**	**Merck**	**Neogen**	**Sigma-Aldrich**	**BD Difco**
Peptone					
Oxoid peptone	10	-	-	10	-
Enzymatic digest of casein		1.0	-	-	-
Enzymatic digest of animal tissue	-	-	10	-	-
Proteose peptone No. 3	-	-	-	-	10
Beef extract	10	8	10	8	10
Yeast extract	5	4	5	4	4
Dextrose (Glucose)	20	20	20	20	20
Sodium acetate (CH_3COONa)	5	5	5	5	5
Tween 80	1.0	1.0	1.0	1.0	1.0
Dipotassium phosphate (K_2HPO_4)	2.0	2.0	2.0	2.0	2.0
Ammonium citrate ($NH_4C_6H5O_7$)	2.0	2.0	2.0	2.0	2.0
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	0.2	0.2	0.1	0.1	0.1
Manganese sulphate ($MnSO_4 \cdot 5H_2O$)	0.05	0.04	0.05	0.05	0.05
Final pH at 25℃	6.2±6.6	5.7±0.2	6.5±0.2	6.5±0.2	6.5±0.2

MRS is the base media that is mostly used for many of these processes, including fermentation. For instance, Saeed et al. (2013) produced a medium for lactobacillus using MRS and sweet potato as a fundamental component (carbon source), which resulted in better growth and cell viability than the original MRS made with all conventional components. The compositions of original and commercially available MRS are shown in Table 4.

Ascorbic acid, di-sodium-glycerophosphate (M17). The most widely used standard media, M17 and MRS, have been very helpful and have continued to be essential in a wide range

For instance, they consistently show development for LAB (Hayek et al. 2019). Because M16 had a weak buffering capability, M17 is a medium that was produced from it.

Table 5. Chemical compositions of original and commercial M17 broth (Hayek et al. 2019)

	Composition of MRS, g.L^{-1}				
Component	**Original**	**Merck**	**Neogen**	**Sigma-Aldrich**	**BD Difco**
Tryptone Peptone	5	5	2.5	2.5	5
Polypeptone	5	-	-	-	-
Soya peptone	0	5	5	5	5
Beef extract	5	5	5	5	5
Yeast extract	2.5	2.5	2.5	2.5	2.5
Beef extract	0.5	0.5	0.5	0.5	0.5
Ascorbic acid	1.0	0.25	0.25	0.25	0.25
Di-sodium-glycerophosphate	19.0	19.0	19.0	19.0	19.0
Lactose	5.0	0	5.0	5.0	0
Meat peptone	0	0	2.5	2.5	0
Final pH at 25 °C	7.2±0.1	6.9±0.2	7.0±0.2	7.2±0.2	6.9±0.2

The M16 medium was created because of Lowrie and Pearce's observation that not all LAB strains particularly streptococci, could grow well on MRS media in the 1970s Lowrie and Pearce (1971). However, a novel medium called M17 with a stronger buffering capacity was developed because the pH quickly decreased when streptococci were growing in M16. Since that time, MRS and M17 have continued to be the two standard media that are most frequently used for all bacteria cultures. The compositions of the original and commercially available M17 are shown in Table 5.

Whey medium. Proteins found in cheese whey possess valuable chemical, physical, and functional properties, as pointed out by (Pires et al. 2021). These proteins play a crucial role in nutrition and perform specific physiological actions such as binding metals, aiding the immune and digestive systems, and providing essential amino acids (Mollea et al. 2013). They are also abundant in branched-chain amino acids, which are essential for maintaining muscle health (Pires et al. 2021). Compared to egg, meat, and soy proteins, the composition of whey proteins provides a superior source of essential amino acids (Dullius et al. 2018; Smithers 2015). However, with the increase in food consumption and the implementation of strict environmental regulations, the agri-food industries are struggling to manage food waste and by-products, which leads to substantial economic costs for their treatment or disposal. It's estimated that 9-10 L of whey is generated from producing 1 kg of cheese, and if not properly treated, it poses a significant environmental challenge (Castelli and Du Vale 2013; Jelen 2003). This has led to the development of cheese whey medium, which aims to reduce pollution caused by cheese whey and utilize low-cost by-products from industries for media production to cut costs Hayek (2019).

Cheese whey is generated from cheese with high carbohydrate, protein and fat contents. It typically consists of about 5-6% lactose, 0.8-1.0% protein and 0.06% fat content (De Souza et al. 2010). It is a low-cost and nutrient-dense raw material with a high production rate and yield for lactic acid fermentation. In industrialized nations, LAB has been utilized to ferment whey, a byproduct of cheese manufacturing that is high in lactose and nitrogen, to lower the expense and environmental risks associated with its disposal. When whey was added with other nitrogen sources such as yeast extract and ammonium sulphate [$(NH_4)_2SO_4$], utilizing glucose as the carbon source, higher output was seen (De Souza et al. 2010).

Ghasemi et al. (2009) conducted a study using two *Lactobacillus bulgaricus* (ATCC 8001 and PTCC 1332), the impact of 5 different media with change in volume percent of whey and nutrient was investigated at 32±0.5°C. For this investigation, several time intervals were used to measure substrate consumption and lactic acid generation (0, 12, 24, 36, 48, 60 and 72 h). The medium with 80% whey and 20% nutrient had the greatest results for lactic acid generation (20.8 $g.L^{-1}$), as well as the highest lactose consumption (96.1%), highest yield (54.7%) and the highest rate of productivity (0.304 $g.L^{-1}$). In this situation, a high lactose content was observed which led to an inhibiting effect. This inhibitory effect demonstrates that batch fermentation cannot make use of the high feed (sugar) concentration, and whey without supplementation was not an effective medium for bacterial growth and lactic acid generation. Because whey is so readily available and inexpensive, agricultural enterprises that produce dairy products should concentrate on figuring out how to increase the percentage of whey and reduce the number of nutrients added to promote the creation of lactic acid.

Alternative Low-Cost Media Ingredients

The quantity of probiotic products available on the market has significantly increased over the past few years along with a similarly significant increase in fermented dairy products containing probiotics Karimi (2012). Standard media such as these cannot be used for the culture of LAB due to low cell densities, the need for quality control, the use of MRS (as a laboratory medium) and whey- and skim-milk-based bulk media, as well as other factors (Hayek et al. 2019). To enhance cell densities at a reduced and subsidized price, there has been much study on alternate media, notably those based on agricultural byproducts. Due to the wide range of environmental adaptability, LAB is primarily used in probiotics. This makes them extremely suitable for widespread use in probiotic supplements and various food products. Their wide range of environmental adaptability also gives them the priority to be used in different media, including plant and animal-based alternative low-cost media (Zimmerman et al. 2021).

The past several decades have seen the development of many media for a variety of uses. For instance, talking of microbiologist Walker L. Kulp created a peptone sugar agar medium in 1932, however, the *L. bulgaricus* and *L. acidophilus* grew ineffectively in this kind of media. Not only did this media fail to give consistently good results, but the novel tomato juice agar developed also did not produce reliable findings. The poor growth of the bacteria in these media could be attributed to the results of these novel media lacking one or more specific nutrients needed by the bacteria. The BRIGGS modification was also used to develop the Lactic Acid-Elliker medium, but some LAB did not grow in this type of media, which led to the development of the modern standard MRS. The MRS, which is currently a standard media, seemed to be one of the best media for these bacteria, however, not all the bacteria could grow and survive in it thus an alternative one known as M16 was developed to supplement the presence day MRS making these two media standards.

Because these M16 growth media had a poor buffering capacity, M17 was created to substitute for it, leaving MRS and M17 the standards for LAB growth medium. However, the inclusion of meat, yeast, and beef-based nitrogen sources makes MRS an expensive medium. The handling and production of starter cultures also need further processes and the use of qualified personnel. Additionally, extra quality control practices must be implemented to guarantee that LAB achieves the highest growth and viability levels.

With regards to the growth and development of LAB, a variety of nitrogen sources are needed due to these bacteria being a sensitive culture. As a

result, tryptone, peptone, and yeast extracts are somewhat added to the usual medium preparation together with beef extracts. This is because the various sources of nitrogen give these LAB the necessities for efficient development. However, these nitrogen sources are costly leading to the high prices of the developed media. Due to this high price, various studies have sought to create an alternative low-cost media using low-cost components to both lower the price of these media and achieve larger cell densities.

These have led to researchers looking into novel plant and animal-based alternative media with low cost but can however provide effective growth to LAB (Hayek et al. 2019).

Plant-based media. Research on inexpensive substances that can contribute to the development and mass production of LAB cells has been ongoing for the past few years. These substances may include waste from agriculture such as crop leftovers, woody materials, and by-products from the food industry. The synthesis of lactic acid which is one of the most important industrial components of LAB may also be produced using these waste products from agriculture Zimmerman (2021). Additionally, these waste products can be used for the bulk production of cells, the synthesis of advantageous chemicals, or as inexpensive media sources (Hayek et al. 2019)

These agricultural waste products including the residue of crops and food by-products contain specified nutritional benefits such as carbon (carbohydrate) and nitrogen (protein), vitamins and mineral sources that can be used as an alternative for the high-cost carbon (dextrose/glucose) and nitrogen sources (beef extract, yeast extract and peptones). Some of the agricultural waste and by-products usually used for these purposes may include corn steep liquor, date, sweet potato, cassava and sugar cane bagasse, pineapple peel juice, malt, barley and wheat, etc.

Date fruit. It has been identified that the date (*Phoenix dactylifera* L.*)* fruit is a very nutrient-dense food that provides a variety of useful therapeutic benefits to the health of individuals. Dates fruits are a nutrient-rich dense plant that includes proteins, fiber, vitamins, minerals, and carbohydrates (mainly glucose, sucrose and fructose) that are necessary to support or stimulate the growth and development of *Lactobacillus spp*. Dates are rich energy sources and it is estimated that for each 100 g of date, the amount of energy being supplied is estimated to be 314 kcal (Ibrahim et al. 2021). A substantial amount of dates fruits is lost during several processes such as harvesting as well as during post-harvest activities including processing, marketing, and handling each year. Out of this, more than 55,000 t of these products are classified as "by-products". The date fruit industries also produce a lot of by-products, which are either always wasted or only sometimes utilized. *Lactobacillus reuteri* was cultivated in date by-products as a base medium, and Ayad et al. (2016) investigated the impact of various nitrogen sources on the cell mass production of this organism. For this work, three separate *L. reuteri* strains were employed, and each was individually inoculated into batches of MRS and Date Palm Medium (DPM) before being incubated for 18 hours. The bacterial population's results indicated that date by-products, when combined with nitrogen sources such as phytone peptone, might be utilized as a cheap alternative for the LAB growth medium. Therefore, DPM might be an appropriate medium for the development of LAB.

Sweet potato. *Ipomoea batatas*, which is locally known as the sweet potato, is a rich and common agricultural crop that is grown in many nations and contains a variety of nutrients. Sweet potato is one of the agricultural products that have high nutrient sources. They contain dietary fiber, vitamins, minerals, some amino acids, carbohydrates (starch and sugars), vitamins A, C, thiamine, riboflavin, and vitamin E, as well as calcium, iron, magnesium, phosphorus, potassium, sodium, and zinc (Hayek et al. 2013). But unless you utilize excellent cooking methods, it's usually impossible to obtain the full nutritional advantages of sweet potatoes. One of the greatest ways to prepare sweet potatoes that release these many nutrients is often baking in a convection oven (Buratti et al. 2020).

To reduce the cost of the Lactobacillus media, sweet potatoes may contribute to a substantial amount of it since the growth of Lactobacillus strains involves the utilization of different nutrients such as carbohydrates, amino acids, vitamins, and minerals. Additionally, triglycerides, oleic acid, linoleic acid, and palmitic acid are among the minor nutrients found in sweet potatoes which can contribute

immensely to the effective growth and proliferation of Lactobacillus. Oleic acid is a recognized critical growth factor for Lactobacillus strains, whereas antioxidants can promote Lactobacillus development (Hayek et al. 2013). Additionally, North Carolina produces the sweetest potatoes in the US, accounting for almost 61% of the entire production Stanford (2020). Based on their nutritional makeup, sweet potatoes have a strong chance of replacing all or a portion of the exorbitant components in *Lactobacillus* medium, which would reduce prices.

According to (Hayek et al. 2013), the utilization of sweet potatoes as a foundational element in producing a medium for Lactobacillus culture was examined. A sweet potato medium (SPM), which also contained 0, 4, or 8 $g.L^{-1}$ of each nitrogen source, was created by mixing beef extract, yeast extract, and protease peptone #3 with baked sweet potato extract. Batches of the De Man, Rogosa, and Sharpe (MRS) and sweet potato medium were each individually inoculated with ten different Lactobacillus strains. MRS was utilized as the control medium. The studied Lactobacillus strains were discovered to have growth patterns that matched those in MRS when grown on a sweet potato medium. The findings from this research indicated that the sweet potato medium can be a suitable medium to grow bacterial strains, especially *L. bulgaricus* and can provide an alternative medium at a low cost (Hayek et al. 2013).

Corn steep liquor. Wet milling leaves behind a substance known as corn steep liquor (CSL), which is predominantly composed of organic acids, amino acids, sugars, and vitamins (Hofer and Herwig 2017). Its ubiquitous usage as a low-cost source of nitrogen, carbon, or vitamins for the biotechnological synthesis of antibiotics, glutamic acid, lactic acid, and other chemicals is often enabled by these qualities. The ability to enhance LAB media with corn steep liquor's amino acid and vitamin content makes it a potentially beneficial resource for replacing yeast extract Hofer and Herwig (2017). A partial or full substitute for yeast extract has been described in the literature as open source from inexpensive agricultural wastes (Yu et al. 2008). The modified medium produced 30.4% more lactic acid than that of the yeast extract medium when corn steep liquor was employed in place of yeast extract entirely (Yu et al. 2008). Corn Steep Liquor is a cheap byproduct that contains nutrients and minerals that are useful for fermentation Hofer and Herwig (2017).

Cucumber and bell pepper. Cucumbers generally contain about 2% fermentable sugar, and almost all of them are reduced sugars. The two most common reducing sugars are glucose and fructose, both of which are easily fermentable by LAB (Lu et al. 2002). Typically, high-performance liquid chromatography (HPLC) is used to measure the reduction of the naturally occurring sugars in the fruits, primarily glucose and fructose, as well as the generation of lactic acid, acetic acid, and ethanol to determine when a cucumber fermentation by LAB has completed (Ucar et al. 2020). However, at the industrial level, measuring pH over time is typically done and this is to track the development of a fermentation process for cucumbers.

Citrulline, trehalose, cellobiose, xylose, lyxose, gentiobiose, and lactic acid were among the 92 compounds that changed when cucumbers were anaerobically fermented and spoilt by Lactobacillus buchneri, according to Ucar et al. (2020)'s research on the metabolic profile of the damaged cucumbers. To create effective starter cultures that could complete the bioconversion and get rid of secondary energy sources, it was postulated that a greater understanding of the significance of the energy sources present in cucumber fermentations for LAB would be necessary. In general, many bacteria prefer glucose as an energy source to fructose and other carbon sources. In an experiment, glucose and fructose were used by the starter culture simultaneously, according to Lu et al. (2002), but glucose depletion was slightly faster than fructose depletion during the exponential growth and stationary phases, but then it slowed down and eventually stopped before fructose depletion was fully accomplished. The initial sugar concentration, the additional buffer used, and the fermentation period are only a few of the many variables that affect LAB's capacity to thoroughly ferment cucumber sugars. LAB fermentation is improved by adding a buffer to cucumber juice, according to experiments. The composition of cucumber and bell pepper juice supplemented with buffer for LAB fermentation is found in Table 6.

Animal-based media. To produce complex media for the culture and propagation of microorganisms, several animal-based products that are beneficial sources of nutrients have also been employed as sources of nitrogen. LAB requires protein sources to support their growth, thus animal waste or byproducts that contain a significant number of amino acids may be employed as a potential supply of the protein the bacteria require to thrive. Ram horn, cow tail ray (*Trygon sephen*) viscera, de-lipidated egg yolk and yeast autolysate, auto-hydrolyzed fish viscera hydrolysates and other animal-based products are among the ingredients being utilized to make alternative, low-cost media. Over 50% of fish material, or roughly 32 million tons of trash yearly, is not utilized as food, as reported by Kristinsson and Rasco (2000).

Table 6. Composition of cucumber and bell pepper media for LAB fermentation ($g.L^{-1}$)

Cucumber media	**Bell pepper media**
Potassium phosphate - 2 g	Potassium phosphate - 2 g
Sodium acetate - 5 g	Sodium acetate - 5 g
Magnesium sulphate - 0.2 g	Magnesium sulphate - 0.2 g
Manganese sulphate - 0.05 g	Manganese sulphate - 0.05 g
L-cysteine - 1 g	L-cysteine - 1 g
Calcium chloride - 0.35 g	Calcium chloride - 0.35 g
Ammonium citrate - 2 g	Ammonium citrate - 2 g
Tween 80 - 1 ml	Tween 80 - 1 ml
*Cucumber juice	*Bell Pepper juice

***Vegetables used*

These byproducts, which include the brain, viscera, skin, bone, and some muscular tissue, are high in proteins and, if thrown without treatment, degrade chemically and microbiologically. There is a need for novel techniques for processing fish waste, animal waste, and byproducts as environmental restrictions become more stringent. Hydrolyzing the waste to produce fish protein hydroxylates (FPH), which contain proteins with good functional characteristics, is one of the intriguing methods. The need for microbial mediums is rising in the biotechnological fermentation sector. Usually, nitrogen is often the costliest part of a microbial growth medium (Aspmo et al. 2005). Some bacteria like LAB are fastidious and therefore require a wide variety of amino acids, vitamins, and other growth elements in their media to grow and become viable. Almost all the media being developed are compared to MRS because it is the current standard media for LAB. However, when supplemented with modest quantities of nitrogen sources, many of the developed media showed significant improvement over MRS in terms of LAB growth and functioning (Hayek et al. 2013).

Fish. By-products from the processing of fish, meat, plants, agricultural waste and the dairy sector are all cheap sources of nitrogen that can be substituted to produce LAB media. For instance, fish processing by-products including heads, viscera, chitinous material and wastewater are great sources of nutrients for microbial development (Vázquez et al. 2004). Additionally, these by-products can be employed in the production of enzymes such as protease, lipase, chitinolytic, and ligninolytic enzymes Rebah and Miled (2013). For instance, the head of yellowfin tuna (*Thunnus albacares*) is a good source of nitrogen and can effectively promote the growth of LAB through enzymatic hydrolysis (Safari et al. 2012). According to (Vazquez et al. 2004), the largest cell biomass production of LAB was obtained from auto-hydrolyzed fish viscera, which typically support the production of biomass and bacteriocins that is comparable to or greater than that produced by MRS. Low hydrolysis periods

and little impact of hydrolysis pH on final levels also contributed to this. Cow tail ray, a byproduct of the fish industry, was ensiled using a 3% (v/w) mixture of propionic and formic acids 1:1 (v/v). This procedure allowed for the creation of inexpensive microbiological peptones (Hayek et al. 2019). Comparing crude peptones generated from cow tail ray viscera to commercial peptones, microbial growth was found to be more significant (Hayek et al. 2019). Additionally, it was observed that the performance of the resulting hydrolysates was significantly influenced by the proteolytic enzyme that was employed to hydrolyze the fish waste (Safari et al. 2012).

Conclusion

To summarize, one group of bacteria which require rich complex conditions to thrive normally is the LAB. As a result, LAB-cultivating media have been intensively researched to support developing LAB applications, notably in the dairy sector. In addition to fermented carbohydrates and other required supplements, the LAB culture media contains nitrogen sources, minerals, and buffering agents, as well as Tween 80. Although many culture media for LAB have been developed in the time past, MRS and M17 remain the most often used standard media. Unfortunately, such conventional growth mediums have various drawbacks that limit their usage for various purposes, including high costs, complicated preparation methods, and lengthy incubation times. As a result, new low-cost media or upgrading existing media is needed. Food byproducts, agricultural products, agricultural wastes, and low-cost, high-nutrient sources, for instance, are becoming increasingly prevalent as part of an attempt to produce low-cost cultivation media. It has been demonstrated that the carbon and nitrogen sources obtained from agro-industrial and plant-based byproducts are suitable to produce lactic acid, reducing production costs and promoting more environmentally friendly procedures that adhere to the principles of green chemistry. It is possible to add the carbon and nitrogen from these agricultural by-products without affecting the production of lactic acid, and this has proven to be a successful replacement for yeast and beef extracts while also enhancing process performance and productivity. These low-cost substances have the potential to replace, at least in part, the complicated and expensive nitrogen additions that are often utilized for the development of LAB strains. Even though several low-cost items have been presented as nitrogen replacement sources in LAB media in several works of literature, the creation of an adequate LAB cultivation medium remains a pressing topic. As a result, greater scientific attention is required to produce a novel cultivating medium that could effectively enhance the development and functionality of LAB while meeting industry standards.

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