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Use of Probiotic/ Postbiotic along with Starter Bacteria for Enhancing Fermentation Processes –A Review

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Abstract

Postbiotics or/ and inactivated probiotics recently have driven attention and it seems they can overcome the problems of probiotics. This paper explores a comparison between inactivated probiotics (postbiotics) and probiotics containing starter bacteria in fermented products. The study focused on the potential synergies and benefits of incorporating inactivated probiotic cells into the fermentation process especially to enhance the viability of starter cultures, physicochemical and nutritional properties, and elevate overall product quality. In this context, the substitution of probiotics with postbiotics is being considered.

Keywords: Postbiotics; Probiotics; Starter Culture; Fermentation Process

Introduction

Fermentation, an ancient method of food preservation, is now widely used around the world to create fermented foods on both small and large scales. The fermentation process in dairy involves the conversion of milk into various dairy products like yogurt, cheese, and kefir through the action of beneficial bacteria or yeast. First of all, the fermentation process begins with the addition of a starter culture containing specific strains of lactic acid bacteria (LAB) or yeast. These cultures are responsible for converting lactose, the sugar present in milk, into lactic acid through fermentation. The inoculated milk is then incubated at a controlled temperature to allow the fermentation process to take place. During fermentation, the LAB in the starter culture produces lactic acid as a byproduct of metabolizing lactose. This acidification process lowers the pH of the milk, causing it to thicken and develop a tangy flavor characteristic of fermented dairy products. In some dairy products like yogurt and cheese, the acidification of milk by LAB also leads to coagulation or thickening of the milk proteins. This coagulation helps in forming the desired texture and structure of the final product. As the fermentation progresses, the beneficial bacteria or yeast release enzymes that break down proteins and fats in the milk, leading to the development of complex flavors and aromas in the dairy product. After the initial fermentation, some dairy products undergo additional ripening or aging processes to further develop their flavor, texture, and appearance. During ripening, specific enzymes continue to act on the proteins and fats, resulting in unique characteristics of the final product. Once the fermentation and ripening processes are complete, the dairy product is cooled to stop further fermentation and then packaged for distribution and consumption [1,2], the fermentation steps are represented in Figure 1.

Microbial starter cultures play key roles in extending shelf life, enhancing safety, and improving the sensory characteristics of food products through fermentation. Lactic acid bacteria, including species from Lactococcus, Leuconostoc, Pediococcus, Streptococcus, and Lactobacillus genera, have been successfully employed for many years in fermenting milk products. These bacteria can be categorized based on their morphology and growth requirements into cocci and rods, as well as mesophilic and thermophilic cultures that thrive at different temperatures [3].

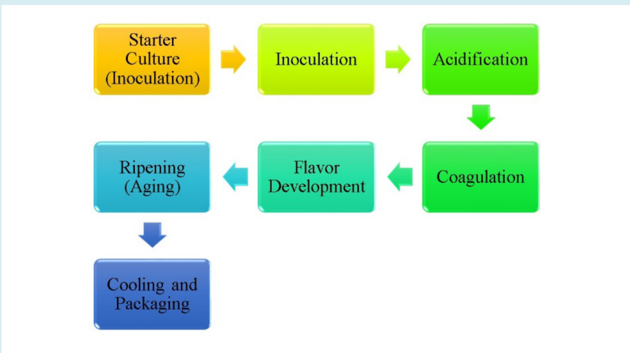

Figure 1: The fermentation process in dairy.

Starter cultures like *lactococci, lactobacilli*, and *streptococci* are commonly used in dairy production and have complex nutritional needs. When combined in a medium with limited nutrients, these cultures can exhibit various growth patterns that can influence the environment by inhibiting the growth of harmful or spoilage microorganisms [4]. This inhibitory activity is attributed to factors such as lactic acid production, bacteriocins, nutrient competition, and altered oxidation-reduction potential [5]. The intricate interplay between postbiotics and probiotics with starter bacteria in fermented products is discussed in this regard.

Probiotics and Starter Bacteria

The selection of starter cultures for probiotic dairy products should aim to minimize antagonistic interactions between the starters and probiotics. Some rod/coccus culture combinations have been found to have different effects on lactic acid production rates compared to single-strain cultures. The concept of probiotics was introduced in 1908 by Metchnikoff, who observed that consuming fermented milk products could lead to increased longevity. Probiotics are defined as viable microorganisms that, when consumed in sufficient quantities, confer health benefits.

Probiotics are defined as live microorganisms by the WHO and FAO that, when consumed in adequate quantities, can improve human health by addressing conditions such as ulcerative colitis, Crohn's disease, weight management, urinary tract infections, lactose and gluten intolerance, and irritable bowel syndrome. While the consumption of probiotics is highly recommended, there may be potential side effects for certain individuals, such as immunological stimulation in sensitive populations like pregnant women, the elderly and critically ill patients, as well as concerns about gene transfer and gastrointestinal issues [6-8].

To ensure probiotics remain viable from product storage to colonizing the gut, various studies have suggested encapsulating probiotics to shield them from harsh conditions and enhance their tolerance by incorporating prebiotics or culturing them under challenging environments. However, introducing probiotics alongside live microorganisms in fermented foods could negatively impact starter cultures through competition for nutrients or the production of antimicrobial substances, posing limitations on probiotic food production. Nevertheless, selecting the appropriate combination of probiotics and starter cultures can influence factors such as acidification rate, pH decrease rate, and product texture [9-11].

In the context of probiotics interacting with starter cultures, various scenarios can unfold. In inhibitory interactions, due to having the capacity for the production of antimicrobial components against pathogenic and spoiler bacteria [12], probiotics also may hinder the growth or activity of the starter culture, potentially leading to delays in the fermentation process or affecting the final product's quality., According to the study by Vinderola, et al. [13], who investigated the interaction between probiotic bacteria and starter bacteria, the cell-free supernatants (CFS) of certain strains of *Lb. delbrueckii* subsp. bulgaricus weakly inhibited the growth of some *Lb. acidophilus* strains. Additionally, *Bifidobacterium* and *L. casei* strains weakly inhibited the growth of S. thermophilus and Lc. lactis strains. However, all *Lb. delbrueckii* subsp. bulgaricus strains were completely inhibited by all *Lb. acidophilus* strains and by certain strains (A5, A10, DC1, 175, and 176) of S. thermophilus. For example, *Lb. delbrueckii subsp. bulgaricus* Ab1 caused a delay in the growth of *L. casei* and *S. thermophilus* A10. Conversely, inert interactions may occur where probiotics and starter cultures coexist without a significant impact on each other. For instance, the cell-free supernatants of *Lc. lactis* did not affect the growth of *S. thermophilus* and *Lb. delbrueckii* subsp. bulgaricus strains. Stimulatory interactions can also occur, where probiotics enhance the growth or activity of the starter culture, leading to improved fermentation outcomes and potentially enhancing the functional properties of the final product. For example, *Lb. acidophilus* CNRZ 1881 was found to have a stimulating effect on *B. bifidum* A12 and *B.b. BBI*. Additionally, the interaction between *Lb. acidophilus* A3 and *B.b. BBI* could stimulate the growth of each other [13].

Texture and Sensory Effect of Probiotics on Dairy

The incorporation of probiotic bacteria into dairy products can impact the texture, rheology, and sensory attributes of the products. Texture plays a crucial role in food perception and enjoyment, directly influencing consumer preferences. It can either enhance product liking or lead to rejection, potentially causing aversion. The flavor of dairy products, resulting from proteolysis, lipolysis, and glycolysis processes, is a complex interplay of numerous compounds, categorized as volatile (such as aldehydes, ketones, alcohols, organic acids) and nonvolatile (including peptides, free amino acids, fatty acids, and organic acids).

Probiotic bacteria can produce enzymes during storage that alter the flavor profile of products like cheese compared to non-probiotic controls. The texture of food items is influenced by the specific bacterial strains used in fermentation, as well as processing parameters. Some lactic acid bacteria (LAB) can enhance texture by generating metabolites in the medium or breaking down added fibers during food processing [14,15].

Similarly, research has shown that the inclusion of bifidobacteria in cheddar cheese did not exhibit robust metabolic activity and did not impact the flavor, texture, or appearance over a 24-week storage period [16]. Additionally, Marchetti, et al. [17] found that while the addition of *L. acidophilus* to Mina's fresh cheese increased proteolysis during storage; it did not affect the texture and flavor throughout its 15-day shelf life at 5°C. On the contrary, probiotic bacteria can have a positive influence on the sensory attributes of dairy products. In a study by Tomar, Tulum cheese made with *L. acidophilus* received the highest ratings for texture and overall acceptability, whereas Tulum cheese produced with *B. animalis* subsp. lactic had the highest flavor score [15]. However, some studies have suggested that incorporating probiotic bacteria into cheese may reduce the sensory acceptability (particularly in terms of flavor) compared to non-probiotic versions. For instance, cheeses containing *L. casei* and a combination of ABC culture (*L. acidophilus* 4962, *B. longum* 1941, and *L. casei* 279) received lower acceptance scores [18]. Furthermore, as lactic acid bacteria (LAB) cheese cultures and their proteolytic enzymes play a role in breaking down casein and whey proteins into peptides and free amino acids (FAAs) [19], cheeses with added probiotic adjuncts tended to have higher bitterness, sour-acid taste, and vinegary taste scores compared to those without probiotic bacteria. According to Ong, et al. cheeses containing *L. casei* 279 and *L. paracasei* LAFTI® L26 had significantly higher bitterness scores than the control cheese [18].

Some studies have indicated that incorporating probiotic bacteria into cheese and yogurt with the right culture composition and formulation may not significantly alter the final product's flavor or sensory characteristics compared to non-probiotic versions.

Kailasapathy, who examined the impact of probiotic strains (*Lactobacillus acidophilus* and *Bifidobacterium lactis*) on the sensory characteristics of yogurt, found that the addition of these cultures did not significantly alter the appearance, color, acidity, flavor, or aftertaste of the yogurts during storage. However, there were notable differences in the smoothness of the yogurt texture [20]. Similar findings were reported regarding the sensory evaluation of low-fat and full-fat yogurts with and without the addition of *Bifidobacterium lactis* and *Lactobacillus acidophilus* [21,22]. These results were consistent with studies conducted by Allgeyer, et al. [23] and Atunes, et al. [24]. Additionally, Akalin, et al. [25] investigated the effects of combining a probiotic culture (*Bifidobacterium animalis ssp. Lactis*) with fortification using whey protein concentrate (WPC), which resulted in a firmer coagulum with increased firmness and adhesiveness values in the yogurt. These changes were primarily attributed to the fortification with WPC rather than the presence of the probiotic culture.

In frozen dairy desserts, maintaining the viability and survivability of probiotic bacteria faces limitations due to various factors such as high sugar, additives, fat content, high redox value, intrinsic environmental parameters, and process conditions like damage during freezing and oxygen toxicity. However, studies suggest that incorporating probiotic strains into frozen dairy desserts does not significantly impact taste, texture, or overall acceptance of the product. The addition of different flavoring agents may enhance the flavor and acceptance of the probiotic ice cream Different sugars, particularly lactose and sucrose commonly used in dairy desserts and ice cream as flavor enhancers, can have varied effects on probiotic viability in frozen products during storage. The osmotic stress induced by sugar can limit the viability of probiotic bacteria in sweetened products, with the impact depending on the type and concentration of sugars, probiotic organism type, freezing conditions, and rate. Enhancing fermentation conditions through feeding or fortification can help maintain a higher number of viable starter cultures and probiotic cells during manufacturing and shelf life [26,27]. Research by Shah explored the effects of L-cysteine, whey protein concentrate, acid casein hydrolysate, and tryptone on the viability of specific probiotic strains in frozen dairy desserts. They found that *Lactobacillus delbrueckii* ssp. bulgaricus experienced a 2-cycle reduction in viability during storage, while Streptococcus thermophilus remained more stable. Additionally, *Lactobacillus acidophilus* and Bifidobacteria showed a similar 2-cycle decrease in viability [28].

Prebiotic

Prebiotics are food ingredients that humans cannot digest but can have beneficial effects on the host by promoting the growth or activity of specific bacteria in the colon, thereby improving health through the modulation of intestinal flora [29]. Prebiotics play a crucial role in supporting the growth and function of starter cultures and maintaining bacterial viability in fermented milk. However, the effectiveness of prebiotics can be influenced by various factors such as the type, purity, and chain length of the prebiotic, the type of microorganisms involved, and the properties of the product (including pH, acidity, milk solids, fat content, storage conditions, and shelf-life) [30,31]. Studies have shown that adding prebiotics to fermented milk can significantly impact the acidification rate and growth of starter cultures, which in turn affects flavor and texture [32]. Probiotics also play a key role in flavor development by producing metabolites like acetic acid, which can lead to a 'vinegary' taste at high concentrations during fermentation [33,34].

Research has shown that prebiotics can alter the post-acidification rate of starter cultures and pH stability in fermented products, affecting flavor changes during shelf life. For example, fortifying fermented skimmed milk with oat beta-glucan has been found to influence acidification rate and gelation kinetics [35]. Additionally, studies evaluating the viability and growth of probiotic strains (such as *Lactobacillus paracasei* B117) in yogurt starter cultures have shown that certain prebiotics, like beta-glucan, can enhance the viability of probiotics during cold storage. However, findings from studies by Guven, et al. [36] suggest that adding inulin to fat-free yogurt does not significantly impact titratable acidity, pH, or acetaldehyde content [36].

While prebiotics indirectly influence sensory attributes, they can also directly affect characteristics like flavor and texture. Research by Khodear et al. found that the sensory properties of yogurt containing inulin were comparable to yogurt made with commercial stabilizers [37]. However, Guven et al. noted that increasing inulin concentration could lead to a decline in yogurt's sensory quality [36]. Aryana and McGrew reported that low molecular weight prebiotics did not hurt the sensory properties of yogurt [38].

Postbiotics

Postbiotics are bioactive compounds that are produced by probiotic bacteria during fermentation. They consist of metabolites, cell wall components, enzymes, peptides, and organic acids that benefit the host's health as illustrated in Figure 2. Postbiotics can influence the gut microbiota, enhance the immune system, reduce inflammation, and promote overall well-being. Various forms of postbiotics, such as soluble factors, metabolites, bacteriocins, and cell-free supernatants, are as effective as live probiotics, particularly those related to *Lactobacilli* [39,40]. It highlighted the importance of postbiotics in maintaining colonic health and suggested their potential as a safer alternative to live bacteria [41,42]. It was also proposed the use of postbiotics to improve the quality of life in patients with colorectal cancer [43]. Tsilingiri, et al. [44] recommended utilizing postbiotics for the treatment and prevention of gut-related diseases like inflammatory bowel disease [44].

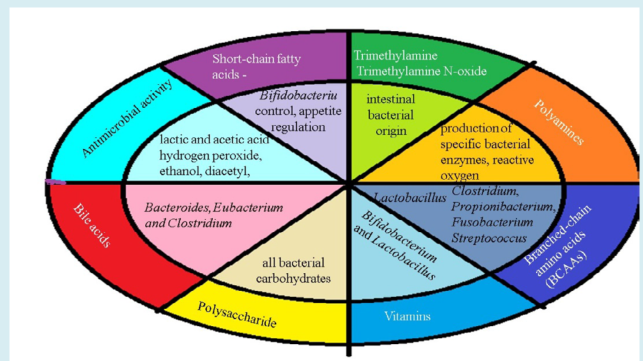

Figure 2: Different postbiotics from different probiotic.

Postbiotics have been extensively studied in rats [45,46], poultry [47,48], and pigs [49,50]. These postbiotics have shown growth- and health-promoting effects when used as replacements for antibiotics in animal feeds. The *L. plantarum* strains produce various beneficial compounds like lactic acid, acetic acid, and bacteriocin [51,52]. Bacterial cell inactivation methods include physical (mechanical disruption, heat treatment, irradiation, high pressure, freeze-drying, and sonication) and chemical (acid deactivation) methods [53]. While these methods may alter microbial structures or functions, they allow the bacteria to retain their health benefits without the ability to grow.

Application of Postbiotics in Food

It has been noted that the inactivation of bacteria can result in the release of various components such as peptidoglycans, exopolysaccharides, and lipoteichoic acids. These components offer several health benefits, including immunomodulatory and inhibitory effects against pathogenic microorganisms and diseases. Additionally, these components do not interact with other elements in the food matrix, are stable across a wide range of pH levels and temperatures, and are easy to handle and commercialize industrially. This has paved the way for the development of paraprobiotic functional food products, as highlighted in studies by de Almada, et al. [54] and Sugawara, et al. [55].

Organic acids are byproducts of LAB growth that play a variety of roles in fermented foods. One way they preserve food is by lowering pH, which also limits or prevents undesirable microbial growth [56] the other way is by adding to the aroma and flavor of food, which increases consumer acceptance and appeal. Additionally, they can exert bacteriostatic or bactericidal effects on potentially pathogenic bacteria in the intestine after consumption [57].

In a recent study by Parvarei, et al. [58], probiotic yogurt containing *Lactobacillus acidophilus* ATCC SD 5221 and *Bifidobacterium lactis* BB-12 was compared to paraprobiotics, which are inactivated probiotics achieved through heating at 121°C. The results revealed that the yogurt with inactivated *L. acidophilus* added before fermentation exhibited the highest mean pH drop rate, mean acidity increase rate, mean redox potential increase rate, final acidity, and final redox potential. The highest lactic acid content was found in samples prepared by adding the paraprobiotic form of *L. acidophilus* after fermentation and storing it for 28 days. Moreover, incorporating inactivated probiotic cells into yogurt led to reduced syneresis and increased water-holding capacity compared to probiotic yogurt samples. The key finding of this study is that the addition of paraprobiotics enhanced the viability of starter cultures.

In another study by Parvarei, et al. [59], the results indicate that the addition of inactivated probiotics through pre-fermentation treatments did not lead to a significant reduction in pH drop compared to the control group. Interestingly, the control treatment and the inclusion of inactivated *Bifidobacterium lactis* resulted in the least increase in acidity compared to the other treatments. Additionally, based on the FTIR data, the consumption of carbohydrates and alanine by starter cultures was found to be linked to the paraprobiotic samples. The study showed that adding inactive probiotic cells to yogurt led to reduced whey separation, improved water retention, and increased viscosity in comparison to regular probiotic yogurt, likely due to the release of EPSs from paraprobiotics. This suggests that inactivated probiotics may have a stabilizing effect on acidity levels during fermentation processes.

In another study, fifty-two *Leuconostoc* strains were assessed for their postbiotic potential, α-glucosidase inhibitory activity, response to technological stress conditions, and safety characteristics in dairy products. The findings revealed the presence of biogenic amines at concentrations exceeding 200 mg/L and the production of streptomycin, clindamycin, erythromycin, and chloramphenicol by Leuconostoc strains. The levels of tryptophan, ornithine, and γ-aminobutyric acid varied among strains and growth media. The predominant organic acids produced were lactic, acetic, and propionic. Furthermore, it was suggested to utilize *Leuconostoc* strains for the production of postbiotic compounds in sheep's milk [60].

Moreover, Mei Zhi Alcine and Shao-Quan declared that *S. boulardii* postbiotic as an appropriate prebiotic or substrate in food products could be modified to create synbiotic formulations, potentially enhancing probiotic survival, sensory characteristics, and overall effectiveness of starter bacteria and probiotics [61].

Overall, the results suggest that the starter cultures utilized in fermented production exhibited increased fermentative activity; potentially attributed to the probiotic cell wall components (postbiotics) creating a nutrient-rich environment with amino acids, sugars, vitamins, and minerals that supported the activity of the starter cultures.

Further research is warranted to explore the mechanisms behind this observation and to determine the potential benefits of using inactivated bifidobacteria in food fermentation, however.

Effect of Postbiotics in Health

Different natural reactions observed with heat-killed probiotic bacterial cells (paraprobiotic) may not have antimicrobial effects but could potentially have immunomodulating effects. According to Homayouni-Rad, et al. [62], postbiotics are more easily absorbed through metabolism and have a strong signaling capacity to various organs and tissues, triggering beneficial biological responses in consumers as shown in Table 1. For example, fermented milk containing heat-inactivated *L. gasseri* CP2305 has been shown to regulate bowel function in individuals prone to constipation. It is worth noting that inactive probiotics exhibit greater suppressive potential than live probiotics [63]. Studies have demonstrated that using high doses of dead probiotics can significantly reduce tumors compared to live probiotics alone. In one study, inactivated *L. plantarum* was found to result in fewer colonic tumors, longer colons, and less weight loss than pure live probiotics, attributed to the suppression of inflammation, apoptosis, and increased IgA secretion [64,65]. Additionally, research by K. Nishida et al. on the impact of the parapsychobiotic *Lactobacillus gasseri* CP2305 on stress-related symptoms and sleep quality suggested that the anti-stress properties of this paraprobiotic strain may be linked to specific cell components that regulate stress responses. Moreover, CP2305 was found to influence the growth of Faecal Bacteroides vulgatus and Dorea longicatena, which are involved in intestinal inflammation [66].

Postbiotic	Diseases	References
Lactobacillus paracasei	inflammatory bowel disease	[67]
Clostridium perfringens	immunomodulatory diseases	[68]
Lactobacillus	Salmonella Infectious	[69]
Micrococcus luteus	dermatological health	[70]
Lactobacillus acidophilus	colorectal cancer	[71]
Lacticaseibacillus rhamnosus GG	Diarrhea and Oxidative Stress	[72]

Table 1: Effect of Postbiotics on Health.

In conclusion, postbiotics as a functional ingredient could enhance health and be consumed as a drug, especially in purified from.

Conclusion

The study discussed the interaction of postbiotics and probiotics with starter bacteria in fermented dairy production and provided valuable insights into the potential benefits of incorporating inactivated probiotic cells, or postbiotics, into the fermentation process. The findings demonstrate that the addition of postbiotics can enhance the viability of starter cultures, leading to improved fermentation kinetics and overall product quality. The results also suggest that postbiotics may have a positive impact on the physicochemical properties of products, such as reduced technological limitation and an increase in nutrition value. Further research is warranted to explore the mechanisms underlying these interactions and to investigate the potential applications of inactivated probiotics in the food industry.

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