IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

ISSN PRINT 2319 1775 Online 2320 787

Research Paper , Journal Volume 11, Iss 02, 2022

Probiotic Fortification in Functional Foods: Trends and Challenges

Ranjit Kumar, Assistant Professor, Ajeenkya D Y Patil University, Pune

Email – Ranjit.kumar@adypu.edu.in

Sakshi Sanjay Kolpe, Student Department of B.Tech Food Biotechnology, Ajeenkya D Y Patil University, Pune.

Abstract:

This research delves into the burgeoning field of integrating probiotics into functional foods, a concept that's gaining substantial traction in the health and nutrition industry. This trend is driven by increasing consumer awareness of the health benefits associated with probiotics, which include improved gut health, enhanced immune response, and potential benefits in managing various chronic diseases. The paper begins by outlining the current trends in the functional food market, emphasizing the growing demand for food products that not only satisfy hunger but also offer specific health benefits. The focus then shifts to the concept of probiotic fortification, which involves adding beneficial live microorganisms to foods. The paper highlights various food categories that are common targets for probiotic fortification, such as dairy products, beverages, and cereals. However, the integration of probiotics into functional foods is not without challenges. The paper discusses the technical difficulties in ensuring the viability and stability of probiotics during food processing, storage, and consumption. Factors such as temperature, pH, and exposure to oxygen can significantly impact the survival of probiotic cultures. The paper also explores the regulatory landscape, which varies by region and presents its own set of challenges regarding the labelling and marketing of probiotic-fortified foods.

Keyword: Probiotic Fortification, Functional Foods, Consumer Health Awareness, Food Processing Challenges, Microbial Viability, Regulatory Landscape, Health-Oriented Food Products

1. Introduction

The concept of Probiotic Fortification revolves around enriching various food products with probiotics, which are live microorganisms known to confer health benefits when consumed in adequate amounts. This emerging field in nutrition science aims to address the growing consumer demand for health-enhancing food options, beyond traditional nutrient provision. Probiotic fortification is driven by the need to improve gut health and overall well-being through diet. Probiotics, primarily certain strains of bacteria and yeast, have been recognized for their role in enhancing gut flora, thereby contributing to a healthier digestive system. They are instrumental in promoting the balance of gut microbiota, which is crucial for nutrient absorption, immune function, and even mental health.

The advantages of probiotic fortification are manifold. First and foremost, it offers a convenient way to incorporate beneficial bacteria into the daily diet without the need to consume separate supplements. This is particularly advantageous for individuals who are not in the habit of taking supplements or who prefer to obtain their nutrients from food sources. Additionally, fortified foods can provide a more enjoyable and varied way of ingesting probiotics, as they can be incorporated into a wide range of products like yogurts, cheeses, beverages, and cereals.

Moreover, probiotic-fortified foods have the potential to reach a wider segment of the population, including those who might not otherwise consider probiotic supplements. This widespread accessibility can contribute to improved public health outcomes on a larger scale. Lastly, the fortification of foods with probiotics aligns with the growing trend of functional foods, which are designed to provide health benefits beyond basic nutrition, thereby catering to the increasingly health-conscious consumer market.

Typically, to enhance the nutritional value of food, bioactive compounds extracted from natural sources are integrated into the food matrix. The methodologies for formulating functional foods and extracting bioactive compounds are broadly categorized into several groups as follows.

1. **Pre-treatment Methods**
    - *Physical Methods*:
        - Traditional: Techniques like milling, thermal drying, mechanical pressing, maceration, moderate-speed centrifugation (between 5,000 and 30,000 rpm), freeze-drying, and microfiltration.
        - Advanced: Methods such as foam mat drying, electro-osmotic dewatering, and treatment with low-temperature plasma.
    - *Physico-Chemical Methods*:
        - Traditional: Processes like steam explosion, ammonia fiber explosion, CO_2 explosion, and high-pressure hot water treatment.
    - *Chemical Methods*: Including treatments with acid, alkali, oxidative delignification, ozonolysis, organosolv processes, and wet oxidation.
    - *Biological Methods*: Enzymatic treatments and microbial degradation.
2. **Macro and Micro-molecule Separation**
    - *Traditional Methods*: Techniques like alcohol and isoelectric precipitation, roasting, and ultrafiltration.
    - *Advanced Methods*: High-speed methods such as ultracentrifugation (exceeding 70,000 rpm), density gradient centrifugation, and differential centrifugation.
3. **Extraction Techniques**
    - *Traditional Methods*: Including hydro-distillation, steam distillation, Soxhlet extraction, Kumagawa extraction, and vacuum distillation.
    - *Advanced Methods*: Techniques like microwave-assisted, ultrasonic-assisted, high hydrostatic pressure-assisted, high-pressure homogenizer-assisted, and high-voltage electrical discharge-based extractions.
4. **Isolation Techniques**

ISSN PRINT 2319 1775 Online 2320 787

Research Paper **© 2012 IJFANS. All Rights Reserved**, Journal Volume 11, Iss 02, 2022

- *Traditional Methods*: Nanofiltration, electrodialysis, and adsorption chromatography.
- *Advanced Methods*: Approaches like magnetic particle-assisted extraction, aqueous two-phase separation, and ion-exchange membrane chromatography.

Traditional technologies are increasingly seen as less efficient due to growing consumer demands and lower yields. Consequently, a range of advanced or emerging technologies has been developed to address these limitations. The term "emerging" refers to the enhancement of traditional methods rather than a specific characteristic. These advanced technologies often feature a smaller equipment footprint, higher throughput, and increased energy efficiency. Additionally, the synthesis of biomolecules from precursors using enzymatic and microbial routes is an emerging area. These advanced methods not only intensify the process but are also more eco-friendly compared to traditional techniques. While the discussion here provides some examples of the development of functional foods and bioactive compounds, the field is not limited to these alone.

2. Literature survey

Probiotics have long been recognized as an integral component of functional foods. However, their application has expanded beyond food products to include the biopharmaceutical and cosmetic industries. Innovative cosmetic ingredients that offer skin moisturizing, skin brightening, and antioxidant properties are being developed [1]. This innovation involves blending Limosilactobacillus reuteri ferment filtrate, a probiotic lactic acid bacterium, with alginite, a naturally occurring organo-mineral rock. This combination, enriched with lactic acid derived from lactose through probiotic fermentation and mixed with alginite's humic and fulvic acids, enhances the biochemical efficacy of these cosmetic products.

The concept of microencapsulation of bioactive compounds is gaining attention. This technique aims to meet the growing demand for food ingredients with enhanced functionality and value. In microencapsulation, tiny particles or droplets are encased in a thin film or matrix. The encapsulation of substances [2] like polyunsaturated fatty acids, pigments, and other bioactive compounds is increasingly popular in the food and biopharmaceutical sectors. This method protects these compounds from degradation, allowing for controlled release in various environments, including food matrices and the digestive tract.

The management of food waste has become a crucial aspect of sustainability in the food system. Annually, around 1.3 billion tons of food, approximately one-third of global food production for human consumption, is wasted. According to a 2019 report by the Food and Agriculture Organization (FAO) of the United States, different stages of the food supply chain contribute variously to this waste: 11–23% during harvest, 17–19% in industrial processing, 8–17% in retail, and over 50% at the consumption stage [3][. The United Nations Environment Programme (UNEP) highlighted that 17% of total global food production is wasted, with disparities among regions. For instance, developing countries (population 6.2 billion) waste less than 630 million tons of food, while developed countries (population 1.4 billion) waste

ISSN PRINT 2319 1775 Online 2320 787
Research Paper , Journal Volume 11, Iss 02, 2022

around 670 million tons. Food processing waste, rich in carbohydrates, proteins, and lipids, is typically used as animal feed [4]. However, with nearly 3 billion people unable to afford a healthy diet and 690 to 829 million people facing hunger, repurposing food waste into edible forms has become a focus. Over the past two decades, the food industry has shifted towards recovering and transforming bioactive compounds from food waste. This approach not only addresses hunger issues but also generates significant revenue from the sale of these bioactive compounds [5-7].

2.1 Research Gaps in Probiotic Fortification and Bioactive Compounds

There's limited research on the specific health benefits of different probiotic strains in fortified foods. Identifying and characterizing the unique properties of various strains can lead to more targeted and effective probiotic applications. Maintaining the stability and viability of probiotics in different food matrices throughout processing, storage, and digestion is a significant challenge [8-10]. More research is needed to enhance these aspects, ensuring that consumers receive the full health benefits. The bioavailability and efficacy of bioactive compounds when ingested through fortified foods or cosmetic applications are not fully understood. Research is required to optimize the extraction, preservation, and delivery of these compounds [11].

2.2 Microencapsulation Challenges

Selecting appropriate materials for microencapsulation that are food-grade, biocompatible, and capable of protecting and releasing bioactive compounds efficiently is a complex challenge. Many microencapsulation techniques are still not scalable or cost-effective for widespread commercial use [12]. Research into more economically viable production methods is necessary. Despite the potential, the efficient conversion of food waste into valuable products, including the extraction of bioactive compounds, is still underdeveloped. Innovative and more efficient methodologies are required. There are significant socio-economic and regulatory hurdles in implementing food waste management strategies on a large scale. Research into policy development, consumer behavior, and economic incentives is needed to promote food waste reduction and reuse [13]. The disparity in food waste management between developed and developing countries presents unique challenges. Tailored strategies that consider local contexts and resources are necessary.

2.3 Integration of Technologies and Systems

There is a need for integrated research that combines probiotic fortification, bioactive compound extraction, microencapsulation, and food waste management to create comprehensive, sustainable food systems [14]. The development of new technologies that can bridge the gaps in these areas is crucial. This includes advancements in biotechnology, materials science, and process engineering. By addressing these research gaps and problems,

ISSN PRINT 2319 1775 Online 2320 787
Research Paper **© 2012 IJFANS. All Rights Reserved**, Journal Volume 11, Iss 02, 2022

the potential benefits of probiotic fortification, bioactive compound utilization, microencapsulation, and efficient food waste management can be fully realized. This will not only enhance food quality and health benefits but also contribute to sustainability and resource efficiency in the food industry.

3. Microbiota in Intestine

Elie Metchnikoff, Nobel Prize winner in 1908, suggested that aging might be linked to toxins produced by certain intestinal bacteria or the release of proteolytic enzymes from Clostridium spp. His hypothesis paved the way for the development of the first dairy industry, with fermented milk from Bacillus bulgaricus (now known as Lactobacillus delbrueckii subsp. Bulgaricus). Probiotics, initially used in dairy and food, later found applications in improving health. In 1989, Fuller defined probiotics as dietary supplements containing live microbes beneficial to the host's gut microbial balance. Subsequent definitions emphasized their positive effects on humans and animals. A widely accepted definition characterizes probiotics as microorganisms that, when administered in sufficient quantities, confer health benefits.

In the proximal small intestine, a diverse microbial community exists, including both strictly anaerobic and facultative anaerobic bacteria. The dominant genera belong to the Bacteroidota, Bacillota, and Actinomycetota phyla. Among these, Bacteroidota is the most versatile, capable of thriving in various pH conditions and digesting both proteins and carbohydrates. These bacteria aid in food digestion and produce beneficial metabolites. However, certain circumstances can lead to pathogenic behavior, exemplified by Bacteroides fragilis. Importantly, the composition of gut microbiota is associated with various health outcomes, including the risk of colon cancer. Bacteroides thetaiotaomicron, a key species, plays a role in carbohydrate metabolism, including dietary carbohydrates and those derived from the host organism, contributing to the overall gut ecosystem.

Bacteroides thetaiotaomicron's digestive adaptability plays a crucial role in maintaining intestinal balance, allowing the gut microbiota to adjust to dietary changes without disrupting its microbial composition. In infants, B. thetaiotaomicron produces enzymes for digesting breast milk carbohydrates. These enzymes suppress the host's defense mechanisms, permitting glucan digestion. However, non-degraded β-glucans can induce immune responses by binding to Toll-like receptors, activating macrophages and dendritic cells. Indigestion can occur if fructans and glucans are not metabolized, leading to gastrointestinal issues often mistaken for gluten intolerance, highlighting the importance of this bacterium in carbohydrate metabolism

ISSN PRINT 2319 1775 Online 2320 787

Research Paper **© 2012 IJFANS. All Rights Reserved, Journal Volume 11, Iss 02, 2022**

Patients with perceived gluten sensitivity often experience symptoms due to fructan consumption rather than gluten. Bacterial enzyme-mediated immune suppression, independent of glycan binding to receptors like TLR and Dectin-1, is a protective mechanism. It aids in energy absorption from oligosaccharides, dampens inflammation, fosters immune tolerance to microorganisms, and guards against allergies. The Gram-positive phylum Bacillota, specifically Clostridia spp., interacts with the immune system, protecting against gastrointestinal inflammatory responses like colitis and colon cancer by inducing tumor cell apoptosis. Faecalibacterium prausnitzii, a prominent Clostridia species, increases anti-inflammatory molecule production. While Clostridium butyricum is beneficial, some Clostridia spp. can become harmful, causing infections like botulism and necrotizing enterocolitis. Clostridioides difficile, an opportunistic bacterium, poses life-threatening risks, often affecting hospitalized or antibiotic-exposed individuals. Additionally, Bifidobacterium, abundant in the gastrointestinal tract, plays a vital role in health, protecting colon cells from cancer-related mutations and being transmitted from mother to fetus and through breastfeeding

4. Results discussion on effects in intestine

Probiotics, when consumed in adequate amounts, offer health benefits, especially in the gastrointestinal tract. Prebiotics, on the other hand, are non-digestible food components like inulin and oligosaccharides that promote the growth of beneficial bacteria in the colon.

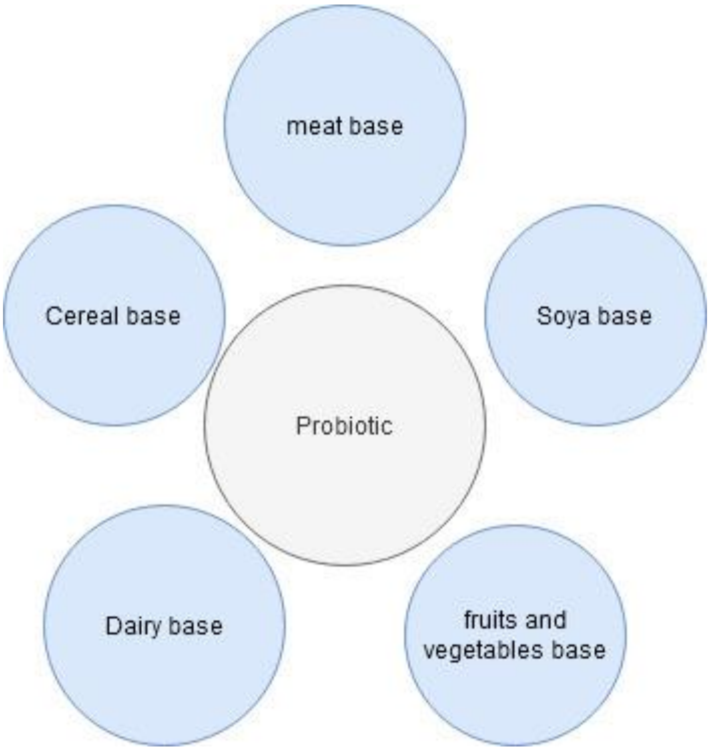

Figure 1: Various probiotic process

Non-dairy Sources of Probiotic Food Products

Foods such as fruits, vegetables, cereals, soy, and meat represent key non-dairy categories that are abundant in nutrients like proteins, minerals, vitamins, dietary fibers, antioxidants, and

ISSN PRINT 2319 1775 Online 2320 787
Research Paper , Journal Volume 11, Iss 02, 2022

other health-promoting compounds. These have been explored for their capacity to support the survival and stability of probiotics. Furthermore, the distinct physiological properties of plants and the fat content in meats contribute to safeguarding probiotic bacteria against various forms of stress. A variety of traditional non-dairy fermented foods, which serve as carriers for probiotics, are showcased in Figure 1. Notably, most of these traditional fermented foods fall under the category of cereals, which, despite being the least examined in the context of developing novel probiotic foods in the contemporary food industry, play a significant role. In contrast, the area of fruits and vegetables garners the most research interest in this domain.

Probiotic Food Products Derived from Fruits and Vegetables

Fruits and vegetables are notable for their nutrient density and distinctive physical structures, like surface pores and irregularities, which may naturally protect probiotic bacterial cells from external stressors. An example of this is the effective pairing of L. paracasei LMGP 22043 with artichoke, which has been observed to enhance the bacterial equilibrium in the gastrointestinal tract (GIT). This benefit is attributed in part to the unique micro-architecture of the artichoke's surface. Such findings highlight the promising potential of fruits and vegetables in probiotic food applications.

The extensive research on using fruits and vegetables as effective carriers for delivering probiotic bacteria has led to the categorization of probiotic foods into three main types: fermented or unfermented fruit juices, fermented vegetables, and minimally processed fruits. Considerable efforts have been devoted to creating probiotic-rich fruit and vegetable juices, incorporating high counts of viable probiotic microbes in various juices and food forms, as detailed in Table 1.

Numerous studies have validated the successful integration of probiotic bacteria into fruits and vegetables. Yet, the persistence and stability of these bacteria in such environments are significantly influenced by the specific strains used, as discussed by Rivera-Espinoza and Gallardo-Navarro in 2010. For instance, the survival of three probiotic bacteria in fermented cabbage juice, stored at 4°C without additional nutrients for four weeks, was examined by Yoon, Woodams, and Hang in 2006. Post-fermentation, these strains initially exhibited high viable cell counts of 10^9 CFU/ml. However, a decline in viability was noted after the cold storage period. L. plantarum C3 and L. delbruekii D7's viability decreased to 4.1 × 10^7 CFU/ml and 4.5 × 10^5 CFU/ml, respectively, while L. casei A4 did not survive in the fermented cabbage juice. In another work, the growth of B. lactis Bb-12, B. bifidum B7, and B. bifidum B 3.2 in fermented carrot juice was assessed, showing high initial viable cell counts of 10^10 CFU/ml. However, the survival of these strains during storage was not monitored, limiting the insights for future product development.

Challenges in Probiotic Technology

The field of probiotic technology has seen significant advancements, particularly in the areas of fermentation, encapsulation, drying, rehydration, and storage. These technologies have been

ISSN PRINT 2319 1775 Online 2320 787
Research Paper **© 2012 IJFANS. All Rights Reserved**, **Journal Volume 11, Iss 02, 2022**

effectively employed to shield probiotics from the environmental stresses found in various non-dairy food matrices. However, there remain considerable technological hurdles (as illustrated in Table 2) in the production and preservation of probiotic foods that need addressing.

Maintaining a high level of viable bacteria in a food product is critical, given that probiotics are live microorganisms. It is not sufficient to merely use bacterial species like Lactobacillus and Bifidobacterium, as their viability in fermented products post-fermentation and during storage is not guaranteed (Holko et al., 2013). Therefore, selecting the right probiotic bacteria and cultures is crucial. Understanding the interaction between bacteria and food matrices under various conditions becomes essential. Additionally, the safety of probiotic products is a paramount concern. For example, while a significant drop in pH during the fermentation of sausages can inhibit the growth of Enterobacteriaceae, it is also important to consider any potential negative effects.

Survival and sensory characteristics of probiotic bacteria in fermented meat products present unique challenges. Challenges, including the formation of biogenic amines through decarboxylation of amino acids or amination and transamination of aldehydes and ketones during ageing of fermented meats. These amines can negatively impact health. Additionally, oxidation of lipids and proteins can lead to a loss in nutritional value, color, and other sensory characteristics. For instance, raw-fermented sausages with L. casei LOCK 0900 were found to develop bitter taste and other undesirable flavors, acrid odor, fatty flavor, and visible fat in the final product.

Processing, storage temperature and time, oxygen content, pH, and external stresses are common technological challenges impacting probiotics in food products. Water activity is another critical parameter influencing bacterial viability. Ingredients like salts and sugar, which bind water, create low water activity environments, enhancing microbial survival. Conversely, high water activity in certain environments, such as fruit juice, can reduce bacterial viability during storage .

Advancements in fermentation processes have greatly improved the viability and yield of probiotic bacterial cells. For instance, many probiotic bacteria have shown good survival, with viable cell counts of 10^8 – 10^9 CFU/ml in fermented juices .However, the application of these techniques in producing non-dairy probiotic foods still faces challenges that need to be addressed individually.

Microencapsulation has been a focus for enhancing the viability of probiotic cells in fruit and vegetable bases Various lyoprotectants have been used to increase the survival of probiotics after freeze-drying. For example, cellobiose, lactose, sucrose, and trehalose have been found effective in enhancing the viability of B. infantis UV16PR by stabilizing cell membranes and preventing intracellular ice formation. The combination of drying methods and formulations plays a significant role in the survival of probiotics in fruit powders during storage. Microencapsulation technologies, while effective in maintaining probiotic viability during storage and gastrointestinal passage, face challenges in production and size of the encapsulated

ISSN PRINT 2319 1775 Online 2320 787
Research Paper © 2012 IJFANS. All Rights Reserved, Journal Volume 11, Iss 02, 2022

probiotics. Moreover, the survival of encapsulated probiotics in different non-dairy foods is also strain-dependent, indicating that a successful formulation for one probiotic might not be suitable for another in developing new probiotic foods.

5. Conclusion

In conclusion, the research on the survival and application of probiotic bacteria in non-dairy food matrices highlights significant advancements and ongoing challenges in the field of food biotechnology. The studies demonstrate that while fermentation, encapsulation, and microencapsulation technologies have been successfully employed to enhance the survival of probiotics in various food products, the effectiveness of these techniques is heavily dependent on the specific strains of bacteria used and the unique environmental conditions of each food matrix. The research underscores the complexity of maintaining the viability and sensory characteristics of probiotics in non-dairy foods, particularly in the context of fermented meat products and fruit and vegetable-based probiotic foods. Issues such as the formation of biogenic amines, lipid and protein oxidation, and water activity are critical factors influencing the survival and quality of probiotics in these products.

Furthermore, the findings point to the need for continued innovation in processing methods and formulation techniques to address the diverse challenges associated with probiotic foods. The strain-dependency of probiotic survival, along with the impact of external factors like storage temperature, pH, and oxygen content, calls for more tailored approaches in the development of probiotic foods. The research also emphasizes the importance of safety and efficacy in the development of probiotic products, underscoring the need for rigorous testing and optimization of production processes. As the demand for probiotic foods continues to grow, ongoing research and development in this area will be crucial for ensuring the delivery of high-quality, health-promoting products to consumers.

References

1. Hasler, C.M. Functional Foods: Benefits, Concerns and Challenges—A Position Paper from the American Council on Science and Health. J. Nutr. **2002**, 132, 3772–3781.
2. Karelakis, C.; Zevgitis, P.; Galanopoulos, K.; Mattas, K. Consumer Trends and Attitudes to Functional Foods. J. Int. Food Agribus. Mark. **2020**, 32, 266–294.
3. Hodges, R.J.; Buzby, J.C.; Bennett, B. Postharvest Losses and Waste in Developed and Less Developed Countries: Opportunities to Improve Resource Use. J. Agric. Sci. **2011**, 149, 37–45.

ISSN PRINT 2319 1775 Online 2320 787

4. Conrad, Z.; Niles, M.T.; Neher, D.A.; Roy, E.D.; Tichenor, N.E.; Jahns, L. Relationship between Food Waste, Diet Quality, and Environmental Sustainability. PLoS ONE 2018, 13, e0195405.
5. Ali, A. A., M. T. Velasquez, C. T. Hansen, A. I. Mohamed, and S. J. Bhathena. 2004. Effects of soybean isoflavones, probiotics, and their inter-actions on lipid metabolism and endocrine system in an animal model of obesity and diabetes. Journal of Nutritional Biochemistry 15 (10):583–90.
6. Alves, M. C. M., A. Peres, M. R. Hernandez-Mendonza, C. P. Bronze, and F. X. Malcata. 2015. Olive paste as vehicle for delivery of potential probiotic Lactobacillus plantarum 33. Food Research International 75 (2015):61–70.
7. Panghal, A.; Janghu, S.; Virkar, K.; Gat, Y.; Kumar, V.; Chhikara, N. Potential Non-Dairy Probiotic Products—A Healthy Approach. Food Biosci. **2018**, 21, 80–89.
8. Rosi, A.; Mena, P.; Pellegrini, N.; Turroni, S.; Neviani, E.; Ferrocino, I.; Di Cagno, R.; Ruini, L.; Ciati, R.; Angelino, D.; et al. Environmental Impact of Omnivorous, Ovo-Lacto-Vegetarian, and Vegan Diet. Sci. Rep. **2017**, 7, 6105.
9. Hill, C.; Guarner, F.; Reid, G.; Gibson, G.R.; Merenstein, D.J.; Pot, B.; Morelli, L.; Canani, R.B.; Flint, H.J.; Salminen, S.; et al. The International Scientific Association for Probiotics and Prebiotics Consensus Statement on the Scope and Appropriate Use of the Term Probiotic. Nat. Rev. Gastroenterol. Hepatol. **2014**, 11, 506–514
10. Saarela, M.; Alakomi, H.L.; Mättö, J.; Ahonen, A.M.; Puhakka, A.; Tynkkynen, S. Improving the storage stability of Bifidobacterium breve in low pH fruit juice. Int. J. Food Microbiol. **2011**, 149, 106–110.
11. Perricone, M.; Bevilacqua, A.; Altieri, C.; Sinigaglia, M.; Corbo, M.R. Challenges for the Production of Probiotic Fruit Juices. Beverages **2015**, 1, 95–103.
12. Markowiak, P.; Śliżewska, K. Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. Nutrients **2017**, 9, 1021.
13. Liu, Y.; Tran, D.Q.; Rhoads, J.M. Probiotics in Disease Prevention and Treatment. J. Clin. Pharmacol. **2018**, 58, S164–S179.
14. Ryma, T.; Samer, A.; Soufli, I.; Rafa, H.; Touil-Boukoffa, C. Role of Probiotics and Their Metabolites in Inflammatory Bowel Diseases (IBDs). Gastroenterol. Insights **2021**, 12, 6.