International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Enhancing Shelf Life and Probiotic Viability in Vegetable Juices Through Lactic Acid Fermentation and Micro-Encapsulation

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ABSTRACT:
This study uses lactic acid bacteria, such as *Lactobacillus plantarum, Lactobacillus fermentum, and Lactobacillus casei,* to produce fermentative functional drinks based on vegetable juice. The probiotic cultures were micro-encapsulated using chitosan beads coated with alginate to improve their stability. This study investigated the microbial population, pH, lactic acid, and glucose changes over a 24-hour fermentation period at 37°C. Additionally, the viability of lactic acid bacteria, pH variations, and lactic acid were assessed during a 28-day storage period at 4°C. The process of lactic acid fermentation not only extends the shelf life of plants but also improves their nutritional value, flavors, and toxicities. Collectively, probiotic-rich fermented fruit and vegetable juices offer health benefits in addition to acting as dietary supplements. Vegetable juice such as beetroot carrot and tomato samples of juice were pasteurized for 20 minutes at 63°C. After inoculation, *Lb. fermentum, Lb. plantarum, and Lb. casei* were cultured for 72 hours at 37 °C. Vegetable juices kept at 4 °C for 5–6 weeks showed higher viability of encapsulated cells than free cells, suggesting that the former had superior cell protection. However, the probiotic beads' addition affected the product's sensory quality by making it harder to swallow and making vegetable juices more turbid due to the remaining encapsulated particles.

Keywords: *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus casei*, Chitosan beads, Alginate, Micro-encapsulated.

INTRODUCTION:

The usage of probiotic products has been increased in the last two decades due to the health awareness of consumers (Menrad,2002). Probiotics are living microbial supplements, which beneficially affect the Host by controlling intestinal infection, serum cholesterol levels, beneficially influencing the immune system, improving lactose utilization in lactose maldigests, and having anticarcinogenic activity (McNaught & MacFie, 2000; Rafter, 2003). Internationally, probiotics are defined as live microorganisms which upon ingestion in certain numbers exert health benefits beyond inherent general nutrition (FAO/WHO, 2001). Many health effects have been reported for probiotics such as anti-carcinogenic and antimutagenic effects (Mortazavian and Sohrabvandi, 2006; Pereira et al., 2011). Cholesterol reduction, reduction of blood ammonia levels (Prado et al., 2008, Saarela et al., 2000; Shah, 2001),stimulation of the immune system (Mazaheri Tehrani et al, 2010),diabetes prevention (Roble et al., 2010), treatment, and prevention of

rotavirus diarrhea (Peres Et al., 2012), restoration of the normal intestinal microflora after antibiotic therapy and increasing lactose tolerance (Prado et al., 2008).Fruit and vegetable extracts are suitable for probiotics transfer due to having minerals, vitamins, dietary fiber and antioxidants (Moraru et al., 2007; Yoon et Al., 2004).The most common probiotic microorganisms used and marketed in food worldwide belong to the genera Lactobacillus and Bifidobacterium (Champagne, Ross, Saarela, Hansen, Charalampopoulos, 2011;Saulnier, Spinler, Gibson, & Versalovic, 2009)The increasing number of individuals with lactose intolerance, dyslipidemia, and vegetarianism reinforces the importance of development of non-dairy probiotic products such as fruit and vegetables (Peres, Peres, Hernández-Mendoza, & Malcata, 2012; Ranadheera, Baines, & Adams, 2010). Fermentation using probiotic strains could improve the aroma and taste profile and increase the shelf-life as the cell culture breaks down fermentable sugars to release by-products such as lactic acid, which has antimicrobial properties, and modifies the final product to have a tangy and sour taste (Huang, H.; Qureshi, N.; Chen, M.H.; Liu, W.; Singh,2015) .Loss of probiotic viability in fermented foods and acidic-bile conditions of the gastrointestinal tract has led researchers to seek better preservation methods. Microencapsulation, a new and effective technique, is now gaining special attention and study. Microencapsulation can Be defined as the process of entrapment/enclosure of microorganisms' cells by means of coating them with proper hydrocolloid(s) in order to segregate the cells from the surrounding environment; in a way that results in appropriate cell release in the intestinal medium (Sultana et al., 2000; Krasaekoopt et al., 2003; Picot and Lacroix, 2003a). Viability of probiotic bacteria in a product at the point of consumption is an important consideration for their efficacy, as they have to survive during the processing and shelf life of food and supplements, transit through high acidic conditions of the stomach and enzymes and bile salts in the small intestine.

PROBIOTICS:

'Probiotics' is defined by the Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO 2001). Two of the most widely used probiotics are from the genera Lactobacillus and Bifidobacterium, as both constitute most of the normal intestinal microbiota in various mammalian species (Vlasova, A.N et al.,2016)

LACTIC ACID BACTERIA

Lactic acid bacteria are group of gram-positive, non-spore forming, non -motile, catalase-negative, and anaerobic or aerotolerant bacteria. This group of bacteria is divided into two sub-groups based on fermentation pathways:

(I) HOMO-FERMENTATIVE

This sub-group of bacteria produces a single fermentation product, i.e., LA *via* the glycolytic (Embden-Meyerhof) pathway (Steinkraus 2002). Members of the genera are *Pediococcus*, *Streptococcus* and *Lactococcus*. The fermentation of one mole of glucose yields two moles of LA.

$$C_6H_{12}O_6 \longrightarrow 2CH_3CHOHCOOH$$

Glucose Lactic acid

(II) HETERO-FERMENTATIVE

This sub-group of bacteria produces LA plus appreciable amount of ethanol, acetate and CO_2 *via* the 6-phosphogluconate/phosphohexose pathway (Steinkraus 2002). Bacteria involved in this group belong to

genera *Leuconostoc* and *Lactobacillus*. The biochemical pathway is as follows.

$C_6H_{12}O_6 \longrightarrow 2CH_3CHOHCOOH + C_2H_5OH + CO_2$

Glucose Lactic acid Ethanol Carbon dioxide

PRINCIPLES OFLACTIC ACID VEGTABLE FERMENTATION:

Thera are three primary conditions under which lactic acid fermentations can occur dry-salted, brined, and non-salted. Salting creates an ideal habitat for LAB growth, which gives food an acidic flavour.

1.DRY-SALTED FERMENTED VEGETABLES

During this process, about 3 kg of dry salt is added to every 100 kg of vegetables to draw out their juice and form brine. After slicing, the vegetables are washed with potable water, drained, and layered in a fermenting container (such as a barrel or keg) to a depth of about 2.5 cm. Each layer is sprinkled with salt, and this is repeated until the container is three-quarters full. Weights, usually stones, are placed on top to compress the vegetables and help brine formation, which takes about 24 hours. Once the brine forms, fermentation begins, indicated by the release of CO_2 bubbles. Fermentation occurs over a period of 1 to 4 weeks, depending on the surrounding temperature. Fermentation is complete When no more bubbles appear, then the pickle can be packaged in a variety of mixtures, i.e., vinegar and spices or oil and spices (Liu et al. 2011).

2.BRINE-SALTED FERMENTED VEGETABLES:

This method involves dissolving salt in water to create a solution of brine (15 to 20% salt solution). Vegetables with a reduced water content by nature are utilized in brine. Best fermentation takes place in brine of about 12.5 to 20° Salometer (Liu et al. 2011). It is crucial that the salt concentration does not fall below 10%; otherwise, conditions will not allow fermentation (Panda et al. 2009). A quick growth of microorganisms is seen in the brine after the vegetables have been brined and the container has been sealed. The temperature and salt content of the brine, the availability of fermentable materials, and the quantity and kinds of microorganisms present at the beginning of fermentation are some of the natural factors that influence the microbial populations of the fermenting vegetables. At highest salt concentrations as 60º Salometer, lactic fermentation

stops and if any acid is detected during brine storage, it is acetic acid, presumably produced by acid-forming yeasts which are still active at this salt concentration (Montet et al. 2006).

3.NON-SALTED LACTIC ACID FERMENTED VEGETABLES:

LAB can ferment certain veggies without the need for brine or salt beforehand. The fermentation process depends on the food being quickly colonized by bacteria that produce LA, which lowers pH and creates an environment that is inappropriate for the growth of spoilage organisms. Additionally, oxygen is not included. because an anaerobic environment is preferred by the lactobacilli. By limiting oxygen, yeasts are prevented from growing.

SELECTION OF PROBIOTICS:

The selection of probiotics strain from the ecosystems (raw or fermented FV) implies several functional properties that are primarily in vitro investigated and proved in animal studies: they must be of human original and a normal inhabitant of the intestine; able to survive in the GIT and

resistant to salivary enzymes, gastric acid, pancreatic juice and bile salts; able to colonize and adhere to the GIT epithelium; able to modulate human immune response, decrease incidences of diarrhoea and improve the GIT health; maintain the mucosa integrity; are non-pathogenic and non-toxic, produce toxins

binding substances and have detoxification activity (Markowiak, Śliżewska, Markowiak, & Śliżewska, 2017). Probiotics must be capable of hydrolysing constituents from FV that cannot be utilized by the host such as the fructans and galactans; fructooligosaccharides (FOS), mannooligosaccharides (MOS), xylooligosaccharides (XOS), inulin and some antinutritional factors such as tannins (Gibson et al., 2017; Mohanty, Misra, Mohapatra, & Sahu, 2018). They must produce beneficial compounds such as vitamins, antioxidants and antimicrobial substances such as organic acids (lactic and acetic acids), hydrogen peroxide, bacteriocins, aldehydes, acetoin, carbon dioxide, reuterin, reutericyclin, phenolic acids, peptides, short chain fatty acids and competitively exclude pathogens. (Derrien & van Hylckama Vlieg, 2015).

ISOLATION AND IDENTIFICATION OF PROBIOTICS:

In order to improve the quality and functional qualities of food systems, researchers have looked at the use of LAB of Lactobacillus, Bifidobacterium, and yeast strains isolated from FV. To reach the target areas in the human GIT, probiotics must be able to withstand the extremely high pH of gastric juice and bile salts in the intestine for probiotics to be effective, they must be transported to the intended locations in a viable and active state. According to reports, probiotics' viability and activity are necessary for attaining health benefits. The recommended probiotics concentration in food to confer the desired health benefit is at least 108 to 109 colony forming unit per milliliter (CFU)/ml to ensure a minimum of 106 to 107 CFU/ml in the colon (Fernando et al., 2018; Shori, 2016, 2017). However, even non-viable culture or heat-treated probiotics are proved to have functional properties, bacteriocin accumulation and immune system stimulation effects (Champagne et al., 2018; Lee et al., 2017). For instance, strains of *Oenococcus oeni*, *Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus salivarius subsp. Thermophilus and yeast (Saccharomyces boulardii)* are qualified potential probiotics (Amorim et al., 2018; Foligné et al., 2010)

ISOLATION:

The first step in characterizing probiotics, submitting a health claim, and marketing them is isolation. The identification of the strain, species, and genus offers valuable insights on the physiological and metabolic characteristics of probiotics. Several protocols have been developed for isolation of probiotics from FV so that a probiotics microorganism can resist gastric conditions of pH 2.5–3 and pepsin enzymes, bile salts, pancreatin enzymes and stimulated GIT peristalsis and adapt colon environment (Amorim et al., 2018; Lee et al., 2016). Additionally, the isolates meet the requirements for auto-aggregation, antibiotic resistance, antibacterial activity, suppression of enteric pathogens, and cell surface hydrophobicity in both in vitro and animal models. Additionally, probiotic isolates are examined for haemolytic activity and the generation of hazardous chemicals for safety reasons. Several LAB strains, including Lactobacillus, Bifidobacterium, and yeast, have been identified, isolated, and stored for use as cultures in manufactured food products.

IDENTIFICATION AND CHARACTERIZATION:

The species level identification is achieved by combination of techniques that includes biochemical information (growth in different media, carbon source, nitrate as well as Gram, catalase, urease and reductase tests), genotype (sequencing of the 16s rRNA gene) and DNA fingerprinting (Repetitive element palindromic PCR, Random Amplified Polymorphic DNA, Restriction Fragment Length Polymorphism

(RFLP), PCR-RFLP and Pulse-Field Gel Electrophoresis (PFGE)) (de Melo Pereira et al., 2018; Lefevre et al., 2017; Mianzhi & Shah, 2017).

DIFFERENT PROCESSES OF LACTIC ACID FERMENTATION:

Latic acid fermentation are classified into following types they are

- Spontaneous fermentation
- Controlled fermentation

SPONTANEOUS FERMENTATION:

Spontaneous fermentation involves allowing naturally occurring lactic acid bacteria to ferment a substrate like vegetables or fruits without the addition of starter culture. The bacteria are normally present on the surface of raw ingredients. These bacteria ferment sugars into lactic acid, which preserves the food, creates a sour taste and contributes to texture. Spontaneous fermentation leads to variations in the sensory properties of the products which differ according to the quality of raw material, temperature and harvesting conditions (Paramithiotis et al. 2010, Wouters et al. 2013).

Some of the LAB isolated from naturally fermented vegetables *are Lactobacillus plantarum, Lb. brevis, Lb. lactis, Lb. paraplantarum, Lb. hilgardii, Pediococcus cerevisiae, Leuconostoc mesenteroides, and Lactococcus lactis,* etc. (Dahal et al. 2005, Tamang et al. 2005, Montet et al. 2006, Ponce et al. 2008, Paramithiotis et al. 2010, Di Cagno et al. 2013). Lactobacilli do not only produce LA but also H2O2 and bacteriocins, which inhibit the growth of pathogens. Recently, metagenomic and metabolomic approaches were used to characterize the microbial community during spontaneous fermentation (Jung et al. 2011).

CONTROLLED FERMENTATION:

Controlled lactic acid fermentation involves managing specific parameters to achieve desired outcomes such as using selected starter cultures (either commercial or adapted local strains) instead of relying on spontaneous fermentation. Quality control is essential for the industrialization of fermentation process (Ray and Sivakumar 2009). For controlled LA fermentation, conditions must

be created which favour the growth of commensal and/or inoculated LAB while excluding other microorganisms (Gardner et al. 2001, Di Cagno et al. 2008a, b, 2011a). Authorized lists of microorganisms with certified use in food fermentations, which cover a wide range of food matrices, including vegetables and fruits, were recently published (Bourdichon et al. 2012). Two main options may be pursued for the controlled LA fermentation of vegetables and fruits: the use of autochthonous or allochthonous starters (Di Cagno et al. 2008a, b, 2009, 2010, 2011b).

COMMERCIAL/ALLOCHTHONOUS STARTERS

Allochthonous starters are microbial cultures such as bacteria that are used in food fermentation processes but are isolated from a different raw material than the one, they are being used to ferment. Allochthonous starters are "foreign or imputed into fermentation environment to control the process and standardize the final product. Majority of the reports show the use of commercial/allochthonous starters in LA fermentation of vegetables and fruits (Gardner et al. 2001, Plengvidhya et al. 2004, Demir et al. 2006, Johanningsmeier et al. 2007). Allochthonous starters (e.g., *Lb. plantarum* RSKK 1062) were also used for making vegetable juices, aiming at favouring the activity of pectolytic enzymes, which increases the juice

yield (Wong 1995), and at rapidly decreasing the value of pH, when the matrix was poorly acid (carrots) (Demir et al. 2006).

AUTOCHTHONOUS STARTERS:

Autochthonous starters are selected microorganisms, like bacteria or yeast, isolated from a specific geographical source of a traditional food product, such as fermented vegetables or fruits. Selection of starter cultures within the autochthonous microbiota of vegetables and fruits should be recommended since autochthonous cultures may ensure prolonged shelf life and targeted nutritional, rheology and sensory properties (Di Cagno et al. 2013). Compared to
selected autochthonous strains, these allochthonous strains showed longer
latency phases of growth and acidification.

MICROENCAPSULATION OF PROBIOTIC BACTERIA:

Microencapsulation is described as a process of enclosing micron-sized particles of solids or droplets of liquids or gasses in an inert shell, which in turn isolates and protects them from the external environment (Ghosh 2006).

Microencapsulation can be used to:

1. shield sensitive substances from the environment;
2. conceal the substance's organoleptic qualities, such as colour, taste, and odour;
3. achieve controlled release of the drug substance;
4. ensure safe handling of toxic materials;
5. achieve targeted release of the drug; and
6. prevent side effects, such as gastric irritation.

ENCAPSULATING MATERIAL FOR PROBIOTICS:

Barrett K. Green, a chemist, was awarded a patent for the microencapsulation process in 1955. This technique, initially applied to typing paper, has since expanded to the pharmaceutical and food industries (M. Sbehat, G. Mauriello, and M. Altamim 2022; M. Peanparkdee, S. Iwamoto, R. Yamauchi 2016). A key factor for Probiotics to exert their biological effects on the host is the Viability and activity of probiotic bacteria, though non-viable Probiotics can also have beneficial biological activity (Q-Y.Dong et al.,2013). Nonviable probiotics can provide health benefits by regulating the Immune system, enhancing adherence to intestinal cells to Inhibit infections, and secreting various metabolites (S.Akter et al.,2020).Probiotics are highly sensitive to environmental conditions Such as oxygen stress, freezing, temperature variations, drying And harsh GIT conditions like low pH and bile salt. Several techniques have been explored to improve Probiotic viability, including selecting bile and acid tolerant Strains, incorporating protective compounds, optimizing Starter cultures, choosing suitable packaging materials, using Two-stage fermentation, enhancing stress adaptability, Including oxygen scavengers, and encapsulation. Among these, Encapsulation is considered one of the most effective methods for preserving probiotic viability (S.Sarkar 2010).

Various methods have been used in probiotic encapsulation they are

1. GELATIN:

Probiotics have been encapsulated using gelatine, either by themselves or in conjunction with other substances. Gelatine, due to its thermo-reversible and Amphoteric properties, is an excellent encapsulating

material When combined with polysaccharides such as gellan gum. Polysaccharides have a net negative charge and repel each, but They are miscible at pH levels above 6. In contrast, gelatine has A positive charge below its isoelectric point, which results in a Strong attraction to negatively charged hydrocolloids (J.A.Rather et al.,2022)

2. ALGINATE

The two structural components of alginate, a ligand heteropolysaccharide that is isolated from various algae species, are D-mannuronic and L-guluronic acids. Calcium alginate
has been widely used for the encapsulation of lactic acid- and probiotic bacteria, mainly in the concentration range of 0.5-4% (Sheu and Marshall, 1991; Sheu and Marshall, 1993; Truelstrup-Hansen *et al*.,2002; Kim *et al*., 1996). Generally, a higher ratio of D-mannuronic to L-guluronic acid results in smaller average pore sizes (R.A.Shirwaiker et al.,2014). Gel beads are created by the quick crosslinking of alginate guluronic units and calcium ions that occurs when an aqueous alginate solution is added to a bath containing calcium.

3. XANTHAN-GELAN GUM

A mixture of xanthangelan gum has been used for the microencapsulation of probiotics (Paquin *et al*., 1990; Sanderson, 1990; Sultana *et al*., 2000; Sun and Griffiths, 2000. The optimum
mixing proportion was 1:0.75 for xanthan: gelan (Sun and Griffiths, 2000). In contrary with alginate, this mixture is resistant to acidic conditions. Also, as opposed to from carrageenan which needs potassium ions for structural stabilization (it is harmful for the body in high concentrations), this gum can be stabilized with calcium ions (Klein and Vorlop, 1985; Sanderson, 1990). It should be noted that although gelan gum is able to generate gel-bead structure for microencapsulation, it is not used on its own for this purpose because of having a high gel-setting temperature (80-90°C for about 1 hr) which results in heat injuries to the probiotic cells (Sun and Griffiths ,2000).

4. CARRAGEENAN

K-carrageenan is a neutral polysaccharide which requires high temperatures (60-90°C) for dissolution especially when applied at high concentrations such as 2-5% (Klein and Vorlop, 1985). Adding monovalent ions such as potassium in the form of KCl leads to the establishment of gel-beads (Krasaekoopt *et al*., 2003). However, KCl has been reported to have an inhibitory effect on some lactic acid bacteria such as *Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (traditional yogurt bacteria) (Audet *et al*., 1988). Due the inhibitory effect of KCI it is replaced with Rb+, Cs+ and NH4+ ions. It has been reported that the proportion of 1:2 for carrageenan-locust been gives a strong gel for microencapsulation (Miles *et al*., 1984; Takata*et al*., 1977).

5. CHITOSAN

The linear polymer chitosan, which is produced by deacetylating chitin, has amine groups that give it a negative charge. Like alginate, it forms a gel structure through ionotropic gelation and is soluble at pH values less than 6. Chitosan polymers can further polymerize by means of cross-link formation in the presence of anions and polyanions (Klien *et al*., 1983). It has reported that mixture of chitosan and hexamethylene diisocyanate or chitosan and glutaraldehyde make stronger coats compared with chitosan alone (Groboillot *et al*.,1993).

TYPES OF MICROENCAPSULATION METHODS:

1. SPRAY DRYING METHOD

Spray drying is the most commonly used microencapsulation method in the food industry, is economical

and flexible, and produces a good quality product (Dziezak, 1988). The process involves the dispersion of the core Material into a polymer solution, forming an emulsion or Dispersion, followed by homogenisation of the liquid, then Atomisation of the mixture into the drying chamber (Jackson and Lee, 1991). This leads to evaporation of the solvent (water) and hence the formation of matrix type microcapsules.

2. THE EXTRUSION METHOD:

Concentrated probiotics are dissolved in an aqueous hydrocolloid solution, and the hydrocolloid cell mixture is then extruded through a droplet-forming nozzle. Extrusion is a low-cost, straightforward technique that produces high probiotic viability without harming probiotic cells. This technique uses sodium alginate, a common ingredient in food, to create gel beads when it comes into contact with Ca2+ solution.

The size of the beads produced is determined by the nozzle diameter, the distance between the outlet, the hardening solution, and the viscosity of the hydrocolloid-cell mixture (L.Gasperini et al.,2014). This process Produces larger encapsulates than any other technique, and is Also effective when working with limited wall materials (N.Choudhury et al.,2021).

3. THE EMULSION METHOD:

Probiotic encapsulation has been effectively accomplished using the emulsion approach. This method is commonly used to Encapsulate enzymes and microorganisms (N.Choudhury et al.,2021). The technique Has the advantage of being simple to scale up, allowing for Flexible adjustment of the resulting capsule size, and providing A high probiotic survival rate (L.Gasperini et al.,2014;M.-J.Chen and K.-N.Chen 2007). The viscosity ratio, emulsifier addition, and energy input during emulsion are the primary parameters that regulate the microbeads' size fall between the dispersed and continuous phases. To further Improve probiotic viability, the microbeads can be coated with A second polymer (K.Kailasapathy 2009). At elevated temperatures (72, 85, and 90 °C) and elevated salt levels, the organism endured longer in the form that is protected. Free cells at 90 °C were totally killed, but cells that were enclosed were decreased by 4.14 log. After 3 hours incubation at 2% bile salt, The free and protected cells showed 5.47 and 2.16 log Reductions, respectively (L.Sabikhi et al., 2010). The percentage viability of the bacteria in the emulsion was 49% at two hours in the model gastric juice, while the viability of the bacteria directly dispersed in the juice was 1.3% even at 0.67 hours. The food sectors may use emulsion systems in a variety of ways. These Studies demonstrated that emulsion technology for probiotic

Encapsulation is an effective method of protecting probiotics From harsh processing conditions and a simulated Gastrointestinal environment (L.Sabikhi et al.,2010).

CONCLUSION:

The present study highlights the potential of *lactic acid bacteria—Lactobacillus plantarum, L. fermentum, and L. casei*—for the development of functional fermented vegetable beverages enriched with probiotics. The use of microencapsulation with alginate–chitosan coatings significantly improved bacterial survival during fermentation and cold storage, demonstrating its effectiveness in protecting cells from environmental stresses and gastrointestinal conditions. Encapsulated cultures consistently showed higher viability compared to free cells, supporting their suitability for functional food applications.

Fermentation not only enhanced the nutritional and sensory qualities of vegetable juices but also contributed to extended shelf life through natural acidification and antimicrobial metabolite production. However, sensory limitations arising from bead turbidity and altered mouthfeel indicate the need for further optimization of encapsulation methods to balance functionality and consumer acceptance.

Overall, the findings reinforce the feasibility of producing non-dairy probiotic beverages using plant substrates and microencapsulated LAB strains. Continued research focusing on improving encapsulation materials, bead size, and sensory characteristics will further advance the development of stable, appealing, and health-promoting fermented vegetable beverages suitable for commercial applications.

RFERENCES:

1. Amorim, J. C., Piccoli, R. H., & Duarte, W. F. (2018). Probiotic potential of yeasts isolated from pineapple and their use in the elaboration of potentially functional fermented beverages. Food Research International, 107, 518–527. doi: 10.1016/J.FOODRES.2018.02.054
2. Audet P, Paquin C, Lacroix C (1988). Immobilized growing lactic acid bacteria with κ-carrageenan-locust bean gum gel. Appl Microbiol Biotechnol. 29: 11-18
3. .Bourdichon, F., S. Casaregola, C. Farrokh, J.C. Frisvad, M.L. Gerds, W.P. Hammes, J. Harnett, G. Huys, S. Laulund, A. Ouwehand, I.B. Powell, J.B. Prajapati, Y. Seto, E. Ter Schure, A. Van Boven, V. Vankerckhoven, A. Zgoda, S. Tuijtelaars and E.B. Hansen. 2012. Food fermentations: microorganisms with technological benefi cial use. International Journal of Food Microbiology 154: 87–97.
4. Champagne, C. P., Ross, R. P., Saarela, M., Hansen, K. F., & Charalampopoulos, D. (2011). Recommendations for the viability assessment of probiotics as concentrated culturesand in food matrices. International Journal of Food Microbiology, 149, 185–193.
5. Champagne, C. P., Gomes Da Cruz, A., & Daga, M. (2018). Strategies to improve the functionality of probiotics in supplements and foods. Current Opinion in Food Science, 22, 160–166. doi: 10.1016/J.COFS.2018.04.008
6. Demir, N., K.S. Bahceci and J. Acar. 2006. The effects of differential initial Lactobacillus plantarum concentrations on some properties of fermented carrot juice. Journal of Food Processing and Preservation 30(3): 352-363.
7. Derrien, M., & van Hylckama Vlieg, J. E. T. (2015). Fate, activity, and impact of ingested bacteria within the human gut microbiota. Trends in Microbiology, 23(6), 354–366. doi: 10.1016/J.TIM.2015.03.002
8. de Melo Pereira, G. V., de Oliveira Coelho, B., Júnior, A. I. M., ThomazSoccol, V., & Soccol, C. R. (2018). How to select a probiotic? A reviewand update of methods and criteria. Biotechnology Advances, 36, 2060–2076. Retrieved from https://www.sciencedirect.com/science/article/pii/S0734975018301605
9. . Di Cagno, R., R. Coda, M. De Angelis and M. Gobbetti. 2013. Exploitation of vegetables and fruits through lactic acid fermentation. Food Microbiology 33: 1–10.
10. Di Cagno, R., R.F. Surico, S. Siragusa, M. De Angelis, A. Paradiso, F. Minervini, L. De Gara and M. Gobbetti. 2008a. Selection and use of autochthonous mixed starter for lactic acid fermentation of carrots, French beans or marrows. International Journal of Food Microbiology 127: 220–228.
11. Di Cagno, R., R.F. Surico, A. Paradiso, M. De Angelis, J.-C. Salmon, S. Buchin, L. De Gara and M. Gobbetti. 2008b. Effect of autochthonous lactic acid bacteria starters on healthpromoting and sensory properties of tomato juices. International Journal of Food Microbiology 128: 473–483.
12 Di Cagno, R., R.F. Surico, G. Minervini, M. De Angelis, C.G. Rizzello and M. Gobbetti. 2009. Use of autochthonous starters to ferment red and yellow peppers (Capsicum annum L.) to be stored at room temperature. International Journal of Food Microbiology 130: 108–116

13 Di Cagno, R., G. Cardinali, G. Minervini, L. Antonielli, C.G. Rizzello, P. Ricciuti and M. Gobbetti. 2010. Taxonomic structure of the yeasts and lactic acid bacteria microbiota of pineapple (Ananas comosus L. Merr.) and use of autochthonous starters for minimally processing. Food Microbiology 27: 381–389

14 Di Cagno, R., G. Minervini, C.G. Rizzello, M. De Angelis and M. Gobbetti. 2011a. Effect of lactic acid fermentation on antioxidant, texture, color and sensory properties of red and green smoothies. Food Microbiology 28: 1062–1071

15 Di Cagno, R., G. Minervini, C.G. Rizzello, R. Lovino, M. Servili, A. Taticchi, S. Urbani and M. Gobbetti. 2011b. Exploitation of sweet cherry (Prunus avium L.) puree added of stem infusion through fermentation by selected autochthonous lactic acid bacteria. Food Microbiology 28: 900–909

16 Di Cagno, R., R. Coda, M. De Angelis and M. Gobbetti. 2013. Exploitation of vegetables and fruits through lactic acid fermentation. Food Microbiology 33: 1–10.

17 Dziezak, J.D. 1988. Microencapsulation and encapsulatedingredients. Food Technol. 42: 36-151.

18 FAO/WHO. 2001. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Cordoba, Argentina: Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report

19 Fernando, W. M. A. D. B., Flint, S. H., Ranaweera, K. K. D. S., Bamunuarachchi, A., Johnson, S. K., & Brennan, C. S. (2018). The potential synergistic behaviour of inter- and intra-genus probiotic combinations in the pattern and rate of short chain fatty acidsformation during fibre fermentation. International Journal of FoodSciences and Nutrition, 69(2), 144–154. doi:10.1080/09637486.2017.1340932

20 Foligné, B., Dewulf, J., Breton, J., Claisse, O., Lonvaud-Funel, A., & Pot, B. (2010). Probiotic properties of non-conventional lactic acid bacteria: Immunomodulation by Oenococcus oeni. International Journal of Food Microbiology, 140(2–3), 136–145. doi:10.1016/J.IJFOODMICRO.2010.04.007

21 Gardner, N.J., T. Savard, P. Obermeier, G. Caldwell and C.P. Champagne. 2001. Selection and characterization of mixed starter cultures for lactic acid fermentation of carrot, cabbage, beet and onion vegetable mixtures. International Journal of Food Microbiology 64: 261–275.

22 Ghosh SK. Functional coatings and microencapsulation: A general perspective, Ch 1. Wiley-VCH, Verlag GmbH & Co. KGoaA, Weinheim. Green BK. 1960. US Patent Re 24:899.

23 Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., … Reid, G. (2017). Expert consensus document: The international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Reviews Gastroenterology & Hepatology, 14(8), 491. doi:10.1038/nrgastro.2017.75

24 Groboillot AF, Champagne CP, Darling GD, Poncelet D (1993). Membrane formation by interfacial cross-linking of chitosan for encapsulation of Lactobacillus lactis. Biotechnol Bioeng. 42:1157-1163.

25 . Huang, H.; Qureshi, N.; Chen, M.H.; Liu, W.; Singh, V. Ethanol production from food waste at high solids content with vacuum recovery technology. J. Agric. Food Chem. **2015**, 63, 2760–2766

26 Jackson, L.S. and Lee, K. 1991. Microencapsultion and the food industry. Food Sci. Technol. 24: 289-297

27 J.A. Rather, N. Akhter, Q. S. Ashraf, S. A. Mir, H. A. Makroo, D. Majid, F. J. Barba, A. M. Khaneghah, B. N. Dar, A comprehensive review on gelatin: Understanding impact of the sources, extraction methods, and modifications on potential packaging applications, Food Packaging and Shelf Life, 2022, 34, 100945, 2022, doi: 10.1016/j.fpsl.2022.100945
28 Johanningsmeier, S., R.F. McFeeters, H.P. Fleming and R.L. Thompson. 2007. Effects of Leuconostoc mesenteroides starter culture on fermentation of cabbage with reduced salt concentrations. Journal of Food Science 72: M166–M172
29 Jung, J.Y., S.H. Lee, J.M. Kim, M.S. Park, J.-W. Bae, Y. Hahn, E.L. Madsen and C.O. Jeon. 2011. Metagenomic analysis of kimchi, a traditional Korean fermented food. Applied and Environmental Microbiology 77: 2264–2274
30 Kim IK, Baek YJ, Yoon YH (1996). Effects of dehydration media and immobilization in calcium-alginate on the survival of lactobacillus casei and Bifidobacterium bifidum. Korean J Dairy Sci.18: 193-198.
31 Klien J, Stock J, Vorlop KD. (1983) Pore size and properties of spherical calcium alginate biocatalysts. Eur J Appl MicrobiolBiotechnol. 18: 86-91
32 Klein J, Vorlop DK (1985). Immobilization techniques cells. In Comprehensive Biotechnology, pp. 542-550, Moo-Yong, C.L. Cooney, AE Humphrey (Eds.). Oxford: Pergamon Press
33 K. Kailasapathy, Encapsulation technologies for functional foods and nutraceutical product development, CABI Reviews, 2009, 1-19, doi: 10.1079/pavsnnr20094033
34 Krasaekoopt W, Bhandari B, Deeth H (2003). Evaluation of encapsulation techniques of probiotics for yoghurt. *Int Dairy J.* 13: 3-13.
35 Krasaekoopt W, Bhandari B, Deeth H (2003). Evaluation of encapsulation techniques of probiotics for yoghurt. Int Dairy J. 13: 3-13
36 Lee, A., Lee, Y. J., Yoo, H. J., Kim, M., Chang, Y., Lee, D. S., … Lee, J. H. (2017). Consumption of Dairy Yogurt Containing Lactobacillus paracasei ssp. paracasei, Bifidobacterium animalis ssp. lactis and Heat-Treated Lactobacillus plantarum Improves Immune Function Including Natural Killer Cell Activity. Nutrients, 9(6), 558. doi:10.3390/nu9060558
37 Lee, K. W., Shim, J. M., Park, S.-K., Heo, H.-J., Kim, H.-J., Ham, K.-S., & Kim, J. H. (2016). Isolation of lactic acid bacteria with probiotic potentials from kimchi, traditional Korean fermented vegetable. LWT - Food Science and Technology, 71, 130–137. doi:10.1016/J. LWT.2016.03.029
38 Lefevre, M., Racedo, S. M., Denayrolles, M., Ripert, G., Desfougères, T., Lobach, A. R., … Urdaci, M. C. (2017). Safety assessment of Bacillus subtilis CU1 for use as a probiotic in humans. Regulatory Toxicology and Pharmacology, 83, 54–65. doi: 10.1016/J.YRTPH.2016.11.010
39 Liu, S.-N., Y. Han and Z.-J. Zhou. 2011. Lactic acid bacteria in traditional fermented Chinese food. Food Research International 44: 643–651.
40 L.Gasperini, J. F. Mano, R. L. Reis, Natural polymers for the microencapsulation of cells, Journal of The Royal Society Interface, 2014, 11, doi: 10.1098/rsif.2014.0817.
41 L. Sabikhi, R. Babu, D. K. Thompkinson, S. Kapila, Resistance of Microencapsulated Lactobacillus acidophilus LA1 to Processing Treatments and Simulated Gut Conditions, Food and Bioprocess Technology, 2010, 3, 586–593, doi: 10.1007/s11947-008-0135-1.
42 Mazaheri Tehrani V, Yegane Azad SM, Moeinfard S, Vahedi S. 2010. Performance and use of various healthy additives in food industry. First published compilation, Mashhad: Ferdowsi University of Mashhad

43 Markowiak, P., Śliżewska, K., Markowiak, P., & Śliżewska, K. (2017). Effectsof probiotics, prebiotics, and synbiotics on human health. Nutrients, 9(9), 1021. doi:10.3390/nu9091021
44 McNaught, C. E. & Mac Fie, J. (2001). Probiotics in clinical practice: a critical review of the evidence. Nutri Res. 21, 343-353.
45 Menrad, K. (2002). Market and marketing of functional food in Europe. J Food Eng. 53, 181-18.
46 Mianzhi, Y., & Shah, N. P. (2017). Contemporary nucleic acid-based molecular techniques for detection, identification, and characterization of Bifidobacterium. Critical Reviews in Food Science and Nutrition, 57(5), 987–1016. doi:10.1080/10408398.2015.1023761
47 Miles MJ, Morris VJ, Carroll V (1984). Carob gum kappa-carrageenan mixed gels-mechanical-poperties and X-ray fiber diffraction studies. Macromolecules 17 2443-244
48 M.-J. Chen and K.-N. Chen, Applications of probiotic encapsulation in dairy product Encapsulation and Controlled Release Technologies in Food Systems, 2007, 83–112. doi: 10.1002/9780470277881.ch4.
49 Mohanty, D., Misra, S., Mohapatra, S., & Sahu, P. S. (2018). Prebiotics and synbiotics: Recent concepts in nutrition. Food Bioscience, 26, 152–160. doi: 10.1016/J.FBIO.2018.10.008
50 Montet, D., G. Loiseau and N. Zakhia-Rozis. 2006. Microbial Technology of fermented vegetables. Volume 1. pp. 309–343. In: R.C. Ray and O.P. Ward (eds.). Microbial Biotechnology in Horticulture. Science Publishers Inc. Enfi eld, New Hampshire.
51 Moraru D, BlancaI, Segal R. 2007. Probiotic vegetable juices, Food Technology **4,** 87- 91.
52 Mortazavian S, Sohrabvandi S. 2006. Probiotics and probiotic food products. First published compilation, Eta Press
53 M. Peanparkdee, S. Iwamoto, R. Yamauchi, Microencapsulation: a review of applications in the food and pharmaceutical industries. Reviews in Agricultural Science, 2016, 4, 56-65, doi: 10.7831/ras.4.56
54 M. Sbehat, G. Mauriello, and M. Altamimi, Microencapsulation of probiotics for food functionalization: an update on literature reviews, 2022.
55 N.Choudhury, M. Meghwal, K. Das, Microencapsulation: An overview on concepts, methods, properties and applications in foods, Food Frontiers, 2021, 2, 426–442, doi: 10.1002/fft2.94
56 Panda, S.H., S. Panda, P.S. Shiva Kumar and R.C. Ray. 2009. Anthocyanin-rich sweet potato lacto-pickle: Production, nutritional and proximate composition. International Journal of Food Science and Technology 44:445-45
57 Paquin C, Lerog M, Lacroix C (1990). Bifidobacterium Longum ATCC 15707 production using free and immobilized cell fermentation in whey permeate based medium. Proceeding of the 23rd International Dairy Federation, Brussels, Belgium; pp. 321
58 Paramithiotis, S., O.L. Hondrodimou and E.H. Drosinos. 2010. Development of the microbial community during spontaneous caulifl ower fermentation. Food Research International 43: 1098–1103
59 Pereira AL, Maciel T, Rodrigues S. 2011. Probiotic beverage from cashew apple juice fermented with Lactobacillus casei. Food Research International **44,** 1276-1283
60 Peres C, Peres C, Hernandez-Mendoza A, Malcata F. 2012. Rrview on fermented plant materials as carriers and sources of potentially probiotic lactic acid bacteria- with an emphasis on table olives, Trends in food science and Tecnology **20,** 1-12.
61 Peres, C. M., Peres, C., Hernández-Mendoza, A., & Malcata, F. X. (2012). Review on fermented plant materials as carriers and sources of potentially probiotic lactic acid bacteria— With an emphasis on table olives. Trends in Food Science & Technology,26, 31–42.

62 Picot A, Lacroix C (2003a). Effect of micronization on viability and thermotolerance of probiotic freeze-dried cultures. *Int Dairy J.* 13: 455-462.
63 Plengvidhya, V., F. Breidt and H.P. Fleming. 2004. Use of RAPD-PCR as a method to follow
64 the progress of starter cultures in sauerkraut fermentation. International Journal of Food Microbiology 93: 287–296
65 Ponce, A.G., M.R. Moreira, C.E. del Valle and S.I. Roura**.** 2008. Preliminary characterization of bacteriocin-like substances from lactic acid bacteria isolated from organic leafy vegetables. LWT—Food Science and Technology 41: 432–441
66 Prado F, Parada J, Pandey A, Socco C. 2008. Trends in non-dairy probiotic beverages, Food Research International **41,** 111–123.
67 Q-Y. Dong, M-Y. Chen, Y. Xin, X-Y. Qin, Z. Cheng, L-E. Shi, Z-X. Tang, Review Alginate-based and protein-based materials for probiotics encapsulation: a review, Nature Reviews Gastroenterology & Hepatology, 2013, 48, 1339–1351, doi: 10.1111/ijfs.12078
68 Rafter, J. (2003). Probiotics and colon cancer. Best Prac Res Cli Gastroenterol. 17, 849-859.
69 Ranadheera, R. D. C. S., Baines, S. K., & Adams, M. C. (2010). Importance of food in probiotic efficacy. Food Research International, 43, 1–7.
70 R. A. Shirwaiker, M. F. Purser, R. A. Wysk, 6 - Scaffolding hydrogels for rapid prototyping-based tissue engineering, R. B. T.-R. P. of B. Narayan, Ed. Woodhead Publishing, 2014.
71 Ray, R.C. and P.S. Shivkumar. 2009. Traditional and novel fermented foods and beverages from tropical root and tuber crops: Review. International Journal of Food Science and Technology 44: 1073–1087.
72 Roble C, Auty M, Brunton N, Gormley R, Butler F. 2010. Evaluation of fresh- cut apple slices enriched with probiotic bacteria, Innovative Food and Emerging Technologies **11,** 203-209
73 Saarela M, Mogensen G, Fonden R, Matto J, Sandholm T. 2000. Probiotic bacteria: Functional and technological properties, Journal of Biotechnology **84,** 197-215.
74 Sanderson GR (1990). Gellan gum. In: Food Gels. pp. 201-233, P. Harris (Ed.)
75 Saulnier, D. M. A., Spinler, J. K., Gibson, G. R., & Versalovic, J. (2009). Mechanisms of probiosis and prebiosis: considerations for enhanced functional foods. Current Opinion in Biotechnology, 20, 135–141.
76 S. Akter, J. H. Park, H. K. Jung, Potential Health-promoting benefits of Para probiotics, inactivated probiotic cells, Journal of Microbiology and Biotechnology, 2020, 30, 477–481, doi: 10.4014/JMB.1911.11019
77 Shah N. 2001. Functional foods from probiotics and prebiotics, Food Technology **55,** 46–53.
78 Sheu TY, Marshall RT (1991). Improving culture viability in frozen dairy desserts by microencapsulation. J Dairy Sci.74: 107-111.
79 Sheu TY, Marshall RT (1993). Micro entrapment of lactobacilli in calcium alginate gel. J Food Sci. 54: 557-561.
80 Shori, A. B. (2016). Influence of food matrix on the viability of probiotic bacteria: A review based on dairy and non-dairy beverages. Food Bioscience, 13, 1–8. doi: 10.1016/J.FBIO.2015.11.001
81 Shori, A. B. (2017). Microencapsulation improved probiotics survival during gastric transit. HAYATI Journal of Biosciences, 24(1), 1–5. doi: 10.1016/J.HJB.2016.12.008
82 S. Sarkar, Approaches for enhancing the viability of probiotics: a review, British Food Journal, 2010, 112, 329–349, doi: 10.1108/00070701011034376

83 Steinkraus, K.H. 2002. Fermentations in world food processing. Comprehensive Reviews in Food Science and Food Safety 1: 23–32.
84 Sultana K, Godward G, Reynolds N, Arumugaswamy R, Peiris P, Kailasapathy K (2000). Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. *Int J Food Microbiol.* 62: 47-55.
85 Sun W, Griffiths MW (2000). Survival of bifidobacteria in yogurt and simulate gastric juice following immobilization in gellanxanthan beads. Int J Foo
86 Takata I, Tosa T, Chibata I (1977). Screening of matrix suitable for immobilization microbal cells. J Solid-phase Biochem. 2: 225-236
87 Tamang, J.P., B. Tamang, U. Schillinger, C.M. Franz, M. Gores and W.H. Holzapfel. 2005. Identifi cation of predominant lactic acid bacteria isolated from traditionally fermented vegetable products of the Eastern Himalayas. International Journal of Food Microbiology 105(3): 347–356.
88 Truelstrup-Hansen L, Allan-wojtas PM, Jin YL, Paulson AT (2002). Survival of free and calcium-alginate microencapsulated Bifidobacterium spp. in simulated gastro-intestinal conditions. Food Microbiol. 19: 35-45
89 Vlasova, A.N.; Kandasamy, S.; Chattha, K.S.; Rajashekara, G.; Saif, L.J**.** Comparison of probiotic lactobacilli and bifidobacterial effects, immune responses and rotavirus vaccines and infection in different host species. Vet. Immunol. Immunopathol. 2016, 172, 72–84
90 Wong, W.S.D. 1995. Food Enzymes. Chapman and Hall, New York, USA, pp. 212–236
91 Wouters, D., N. Bernaertb, W. Conjaertsa, B. Van Droogenbroeckb, M. De Looseb and L. De Vuysta. 2013. Species diversity, community dynamics, and metabolite kinetics of spontaneousleek fermentations. Food Microbiology 33: 185–196
92 Yoon K, Woodams E, Hang Y. 2004. Probiotication of tomato juice by lactic acid bacteria