Asia-Pacific Journal of Science and Technology :Volume :30 Issue :05 Article ID :APST-30-05-03 Research Article

APST

Asia-Pacific Journal of Science and Technology

https//:www.tci-thaijo.org/index.php/APST/index

Published by Research and Innovation Department,
Khon Kaen University, Thailand

Effect of calcium lactate concentrations on morphology and efficiency of encapsulated probiotics in orange juice

Swastika Dewi[1,2], Rafli Zulfa Kamil[1], Hasna Yumnaningsih[1], Setya Budi Muhammad Abduh[1,2*]

[1]Food Technology Study Program, Department of Agriculture, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Jl. Prof Soedarto, S.H., Tembalang, Semarang 50275, Indonesia
[2]Food Sustainability Research Centre (FORCE), Universitas Diponegoro, Semarang, Central Java, 50275, Indonesia
[*]Corresponding author: setya.abduh@live.undip.ac.id

Received 14 March 2024
Revised 27 September 2024
Accepted 19 March 2025

Abstract

Probiotics are good bacteria required by the body but unable to survive in stomach acidic conditions, leading to the need for a coating agent. The technology that can maintain the viability of probiotics is microencapsulation, which includes coating core substances with polymer wall layers into micro-sized particles. Therefore, this research aimed to determine the optimal level of calcium lactate in preparing probiotic orange juice microbeads against pH, yield, encapsulation efficiency, and morphology. Microencapsulation was used with extrusion methods, while probiotic orange juice microbeads were manufactured using 2%, 2.5%, 3%, and 3.5% calcium lactate (% w/w). The parameters tested were pH value, total yield, encapsulation efficiency, and morphology. The results showed that the addition of a higher calcium lactate level led to a significant increase in morphology, encapsulation efficiency, yield value, and pH value but not in the solidity attribute. Calcium lactate of 3% initiated an insignificant difference in morphology, encapsulation efficiency, yield, and pH value compared to a 3.5% level. Based on production costs, 3% calcium lactate was considered more optimal than the 3.5% level for the manufacture of probiotic orange juice microbeads.

Keywords: Probiotics, Orange, Microencapsulation, Microbeads, Calcium lactate

1. Introduction

Functional food is capable of satisfying hunger, providing health advantages, and offering nutrition. These advantages include possessing anti-carcinogenic characteristics, improving immunity, reducing the risk of lactose intolerance symptoms, lowering blood cholesterol, and treating irritable bowel syndrome and inflammatory bowel disease (1). Probiotic food and beverages are among the areas of functional food production experiencing rapid development. The addition of probiotics into a food matrix presents challenges that can affect bacteria viability due to the different physicochemical properties of each matrix. The challenges include temperature, acids, and bile, increased concentrations of certain ions or depletion of nutrients, as well as exposure to osmotic and oxidative stress in the product matrix during gastrointestinal transit. The minimum requirement for probiotics in a product to ensure being beneficial for health is colony forming units (CFU) 10^6-10^8 CFU/g (2).

Several types of bacteria used in the probiotic food and beverage industry include *Streptococcus*, *Bifidobacterium*, and *Lactobacillus* from the genus of lactic acid bacteria (LAB). The characteristics of LAB include lack of spores formation, acid intolerance, gram-positive type, and low guanine (3), while advantages associated with the usage are enhanced synthesis of riboflavin, niacin, thiamine, vitamin B6, vitamin B12, and folic acid, as well as improved digestion of proteins and fats (4). Due to the excess of LAB, many food industries use probiotics in fermented dairy products, which are even found intolerant by some consumers, leading to challenges in developing non-dairy products without fermentation. vegetables, fruits, and cereals are some identified examples of non-dairy products without fermentation. The history of research on functional foods in recent years shows a trend in non-dairy products, namely using fruit juice as the basic ingredient (5). Fruit juice with LAB addition can be developed into functional food, but this product has weaknesses, including easy spoilage and a need for a small amount of LAB to maintain viability in high stomach acid conditions (6). Therefore, technology is needed to preserve fruit juice and bacteria considering that LAB reaches the intestines in quantities correlating with the minimum standards for probiotics in the digestive tract.

Microencapsulation is a technology that can be an alternative to preserving fruit juice without fermentation and maintaining LAB viability in the intestine. This method includes coating active substances with a polymer compound to form spheres in the micrometre to millimeter sizes known as microbeads for viability enhancement and protection of the active substances from damage (7). The active substances used in this research are probiotics, such as *Lactobacillus acidophilus*, *Bifidobacterium longum*, and *Streptococcus thermophiles,* from LAB strains found in Lacto-B probiotic supplements (8). The coating material used is sodium alginate (9), which dissolves in both cold and hot water (10) to form a viscous solution, and has the ability to precipitate in solution with a $pH < 3.5$ (10). Therefore, a liquid base material with a $pH > 3.5$ is needed for the application of alginate as a product mixture. An example of this material is sweet orange juice which has a pH of 4.6 - 4.8 (11), leading to the suitability for combination with alginate. Orange is among the fruits highly demanded in Indonesia because of the sweetness, fresh taste, and affordability (12). To maintain stability, mmicroencapsulation requires the core and coating material, as well as a crosslinking agent including calcium chloride and calcium lactate.

This research used calcium lactate because of the advantage of not changing the taste of the final product, compared to calcium chloride which caused a bitter taste (13). Alginate and calcium lactate form calcium alginate bonds to produce a strong gel matrix that can trap LAB in the calcium lactate solution and maintain the stability of the microencapsulation (14). The combination of LAB, alginate, orange juice, and calcium lactate using the microencapsulation method produces probiotic orange juice microbeads which have the potential to be a functional food innovation. Microencapsulation produces long-lasting fruit juice, maintains LAB viability, supports easy digestion, and forms microbeads that are uniquely shaped in similarity to orange fruit pulp. The combination can affect the morphology, encapsulation efficiency, yield, and pH of probiotic orange juice microbeads due to the addition of different calcium lactate. Therefore, this research aimed to evaluate the effect of adding 2%, 2.5%, 3%, and 3.5% calcium lactate (w/w) on the morphology, encapsulation efficiency, yield, and pH of probiotic orange juice microbeads to determine optimal calcium lactate levels for manufacturing the product.

2. Materials and methods

2.1 Material

The main material used in this research was the *Citrus reticulata* orange variety purchased from the local market, calcium lactate (Galactic, Belgium), and sodium alginate (JRS, Germany). Others were powdered probiotics (Lacto-B, Indonesia) containing the bacteria *Lactobacillus acidophilus, Bifidobacterium longum*, and *Streptococcus thermophilus*, distilled water, de-Man Rogosa Sharpe Agar (MRSA) (Merck, Germany), as well as pH buffer 4 and 7 (Merck KGaA, Germany),

2.2 The production procedure of orange microbeads

The orange was peeled and pressed to obtain the juice, which was pasteurized at 80°C for 3.5 min and cooled down at room temperature. The pasteurized orange juice was combined with 10^9 CFU/g of probiotic (Lacto-B, Indonesia) and 1.25 g of alginate, then homogenized using an overhead stirrer for 15 min. The mixture was dripped into 100 mL of 2, 2.5, 3, and 3.5% calcium lactate solution (%w/w) using a 20 mL syringe (OneMed, Indonesia). Hardening was conducted for 30 min with a stirring speed of 200 rpm to obtain microbeads filtered and washed 3 times using distilled water (15). The research design was carried out with 4 treatments and 5 replicates, generating 20 experimental units.

2.3 Morphology analysis of orange microbeads by Fiji Image-J app version 2.9.0 (USA)

The analysis of microbeads included the evaluation of morphology, area, circularity, solidity, and roundness performed using the Fiji Image-J app version 2.9.0 (USA) (16). An image contained 10 microbeads and color image files were saved as JPEGs, while a black background was used to promote easier separation of objects, and a ruler facilitated distance calibration in pixels.

Images were analyzed using the Fiji Image-J application and the data were extracted through several stages. The first stage was the process of reducing interference in the image using type 8 bite and filtering large structures down to 20 pixels. The second was scale determination by drawing a line of 0 to 1 cm, then setting the scale to ensure correspondence of the Distance column in pixels to the length of the line selection. The third was colored image conversion into a binary image with a threshold value of 75 in the range of 0-255, where 0 represented black. The threshold method was used to separate the background from the object. Pixels with a value of ≤ 75 were changed to 0 which implied black as the background, while >75 was changed to 1 representing white as the object.

Pixels between the 0 and 1 values are called the border pixel to the perimeter value (P), representing the number of pixels around an object, and the total number of white pixels or 1 in the perimeter is the area (A). The maximum ferret is the distance of the farthest point from the object as well as the length of the object (L), while the minimum ferret is the diameter of the object (W). These values are used to obtain a description of the shape, as a circularity of 1 suggests a perfect circle and the shape becomes more elongated near 0.

Roundness is the inverse of circularity which represents the elliptical shape of an object and solidity denotes object density. Solidity is the pixel of an object divided by the number of pixels in the convex imaginer line. Meanwhile, morphological analysis of microbeads through direct observation is carried out by observing the shape, texture, size, and water content.

2.4 Encapsulation efficiency of orange microbeads

The efficiency test was conducted by calculating the total LAB ratio after and before the encapsulation process. Total analysis of LAB before encapsulation was started with 10^9 CFU/g of Lacto-B added to 9 mL of physiological solution (NaCl 0.85%) and homogenized using vortex. The suspension was diluted 10^{-1} and the cascading was increased to reach 10 123^{-7} with duplo dilution. Subsequently, plating was performed on MRS agar using the standard pour plate method, with 5 repetitions (17). The sample was incubated for 48 h at 37°C (18), and the total LAB represented as CFU/g sample was calculated using the following formula:

$$Total\ LAB\ (\frac{CFU}{g}) = number\ of\ colonies \times \frac{1}{dilution\ factor}$$

The total analysis of LAB on microbeads started with microbeads being taken as much as 1 g and then crushed using mortar. The crushed samples were mixed into a centrifuge tube containing 9 mL of physiological solution (NaCl 0.85%) and homogenized using a vortex, then the suspension was diluted 10^{-1} and the cascading was increased to 10^{-7} with duplo dilution. The standard plate count was conducted on MRS agar medium with 5 repetitions (17), while the calculation of microbeads encapsulation efficiency referred to some previous research [14] and(19).

$$Encapsulation\ efficiency\ (\%) = \frac{LAB\ after\ encapsulation\ (log\ CFU/g)}{LAB\ before\ encapsulation\ (log\ CFU/g)} \times 100$$

2.5 Yield of encapsulation

The yield analysis of probiotic orange juice microbeads referred to previous research (20) and the value was obtained by measuring the final weight of the microbeads. This value was compared to the initial weight of ingredients used during the production, and the weighing was performed in 5 repetitions (17).

$$Yield\ (\%) = \frac{Microbeads\ weight\ (g)}{Materials\ weight\ (g)} \times 100$$

2.6 Analysis of orange microbeads pH

The analysis referred to previous research (21), while the pH value of citrus juice, sodium alginate, calcium alginate, and microbeads was tested with a pH meter (ATC, China). The meter was first calibrated using pH 4 and 7 buffers, then dipped into the mashed samples of microbeads.

2.7 Data Analysis

Data in the form of morphology, encapsulation efficiency, yield value, and pH value of probiotic orange juice microbeads were analysed using one-way analysis of variance (ANOVA) [17]. The existence of a real effect was further evaluated by duncan's multiple range test (DMRT) with a significance (α) level of 5% (22).

3. Result and Discussion

3.1 Morphological analysis

Morphological analysis is commonly used to identify the characteristics of the product. Characteristics of probiotic orange juice microbeads including area, circularity, roundness, and solidity were observed with Fiji image-J.

Table 1 Morphological analysis of probiotic orange juice microbeads.

Ca-Lactate (%w/w)	Area (mm^2)	*Circularity*	*Roundness*	*Solidity*
2	14.29 ± 1.67^{a}	0.60 ± 0.09^{a}	0.78 ± 0.86^{a}	0.84 ± 0.02^{a}
2.5	15.37 ± 2.40^{ab}	0.67 ± 0.13^{ab}	0.87 ± 0.18^{ab}	0.85 ± 0.10^{a}
3	17.58 ± 1.04^{b}	0.78 ± 0.09^{bc}	0.89 ± 0.06^{b}	0.86 ± 0.10^{a}
3.5	17.64 ± 2.05^{b}	0.81 ± 0.03^{c}	0.89 ± 0.47^{b}	0.87 ± 0.07^{a}

Note: The data are shown as the average value of 5 repetitions ± standard deviation with different superscript letters in the same column representing a significant difference ($p<0.05$).

Calcium lactate addition had a significant effect ($p<0.05$) on improving the morphological characteristics of microbeads such as area, circularity, and roundness. The highest area values were obtained with the addition of a calcium lactate level of 3.5%, amounting to 17.64 ± 2.05, 0.81 ± 0.03, 0.89 ± 0.47, and 0.87 ± 0.07. Circularity ranged from 0.0-1.0, with the value near 1.0 suggesting that the microbeads were identical to a perfect circle, while the value near 0.0 signified an increasingly elongated circle(23). The results showed that with increasing levels of calcium lactate, the circularity value was nearer to 1.0.

The morphological data show that increasing the calcium lactate level by 3.5% changes the shape of the microbeads to resemble a perfect circle. A roundness value of less than 1.0 suggests that there are sharp corners at the edges, causing the object to deviate from a perfect circle (16). According to Table 1, 2%-3.5% calcium lactate addition generates a roundness value of < 1.0 identified as the presence of sharp corners on the edges of the microbeads. The addition of 3% and 3.5% calcium lactate has a roundness value near 1.0, suggesting a slight deviation from a perfect circle. Calcium lactate has calcium atoms that bind hydroxyl groups to form hydrogen bonds with water molecules (24).

Hydrogen bonds enhance alginate viscosity, leading to a more effective withdrawal of water content by calcium lactate and an increase in the particle diameter (25). Calcium lactate increasing the bond between Ca^{2+} and alginate anions will lead to more dissolution of solids and an increase in the size of solid particles. Larger size elongates the particle diameter, which is the main factor influencing area size, circularity, and roundness. An extension in particle diameter will increase the area, circularity, and roundness values.

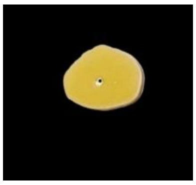
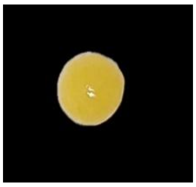
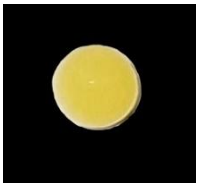
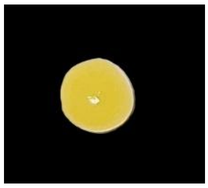
The round shape of microbeads is often caused by the viscosity and surface tension of the liquid, correlating with the opinion that viscosity and surface tension influence the high and low values of roundness (26). Higher levels of calcium lactate increase the viscosity and surface tension to help maintain a more rounded droplet shape. This is consistent with research stating that an increase in Ca^{2+} concentration causes rapid gelation (27), leading to an increase in gel density and viscosity due to rapid ion diffusion. The formation of mono complexes and egg case dimers is promoted by diffusion, thereby generating a dense and elastic gel.

An insignificantly different increase was observed in the average solidity value of microbeads ($p>0.05$). The solidity values resulting from the addition of 2%, 2.5%, 3%, and 3.5% calcium lactate levels were 0.84 ± 0.02, 0.85 ± 0.10, 0.86 ± 0.10, and 0.87 ± 0.07, respectively. The highest solidity value (0.87 ± 0.07) was found in the treatment with the addition of 3.5% calcium lactate and the lowest (0.84 ± 0.02) was detected in the addition of 2% calcium lactate.

Solidity is a representation of density characterized by surface smoothness or porosity which causes the surface of the microbeads to become rough. A solidity value of 1.0 suggests that the edges of the object are smooth or similar to a perfect circle. Meanwhile, a solidity value < 1.0 shows that the edges of the object are rough and irregular. The solidity value for the addition of a calcium lactate level of 2-3.5% is lower than 1.0, signifying the edges of the microbeads to be rough and irregular. The rough edges of the object are caused by the presence of pores on the surface of the microbeads. More Ca^{2+} causes calcium alginate bonds to become more complicated, decreasing porosity and increasing solidity.

Increased Ca^{2+} can affect the density or solidity of microbeads because of ion diffusion during rapid gelation which promotes the formation of more mono complexes and egg cases. When the gelation process has been maximized, all parts of the microbeads completely become harder, denser, and more elastic. This influences the morphological characteristics, including texture, shape, and size of microbeads. Therefore, variations in the addition of calcium lactate levels affect differences in morphological characteristics. In this research, the morphological characteristics of microbeads observed directly included size, texture, shape, and water content.

Table 2 Images of microbeads.

	Calcium Lactate Levels (% w/w)			
	2	2.5	3	3.5
Microbeads				
Dimension scale	24.0 pixels/cm	17.4 pixels/cm	26.3 pixels/cm	18.7 pixels/cm

Note: The images shown are the best 1 of 10 microbeads captured randomly for each treatment based on Fiji Image-J (Version 2.9.0, USA).

Table 2 shows that the addition of a 2% calcium lactate level produces microbeads with characteristics such as oval shape resembling corn kernels, non-uniform size, not chewy material, numerous surface pores, easily crushable skin, and minimal water content. The microbeads are dissolved until all the water comes out to calculate the content with a measuring cup.

Microbeads with a calcium lactate level of 2.5% have characteristics including a more perfect round/uniform shape, more elasticity, fewer surface pores, easily crushable skin, and higher water content. A calcium lactate level of 3% generated a perfectly round and uniform shape, few surface pores, difficult-to-break skin, more supple texture, and high-water content. The addition of a3.5% calcium lactate produced similar characteristics to the 3% level, namely a perfectly round and uniform shape, few surface pores, not easily breakable skin, and nearly the same elasticity, but the water content is higher.

Sodium alginate contains guluronic acid monomers which can bind to calcium cations. The more calcium that binds to guluronic acid, the stronger the gel formed and less easily destroyed due to the larger structure (28). This compound has the function of producing good gel elasticity because alginate and calcium lactate easily bind water. Gel strength and elasticity are influenced by the length and distribution of the Ca^{2+} binding blocks, namely the guluronic acid block (G block) and the non-methoxylated galacturonic acid block (GalA block) (29). More Ca^{2+} binding to the molecular chain increases gel viscosity and elasticity, while a non-uniform appearance was found in microbeads with different calcium lactate levels.

Microbeads produced with a calcium lactate level of 2% show a slightly irregular surface, tend not to be perfectly round, and have a soft texture. However, those using 2.5 - 3.5% calcium lactate has a strong texture, tend to be perfectly round, and do not break easily, with a better general appearance. This difference is influenced by the intrinsic physical properties of the droplets, including surface tension and viscosity. The more calcium lactate causes greater water absorption as a result of more cavities forming, leading to an increase in viscosity. This corresponds with research stating that the enhancement of calcium lactate concentration increases the number of egg boxes to retain water in the granules and improve viscosity (30).

3.2 Encapsulation Efficiency

The encapsulation efficiency of microbeads is a parameter representing the percentage of LAB successfully coated by the coating material. Table 3 shows that calcium lactate levels have a significant effect (p<0.05) on increasing the encapsulation efficiency of microbeads.

Table 3 Encapsulation efficiency test results for probiotic orange juice microbeads.

Calcium Lactate Levels (%w/w)	Total LAB before encapsulation (Log CFU/g)	Total LAB after encapsulation (Log CFU/g)	Encapsulation Efficiency (%)
2		6.8 ± 0.25^{a}	75.07 ± 2.71^{a}
2.5	9.18	7.00 ± 0.60^{ab}	76.30 ± 0.63^{ab}
3		7.15 ± 0.10^{b}	77.93 ± 1.14^{b}
3.5		7.05 ± 0.3^{ab}	76.73 ± 1.11^{ab}

Note: The data shown as the average value of 5 repetitions ± standard deviation with different superscript letters in the same column represents a significant difference (*p*<0.05).

The addition of a 3% calcium lactate produced the highest microbeads encapsulation efficiency of 77.93 ± 1.14 and a 2% calcium lactate had the lowest microbeads encapsulation efficiency of 75.07 ± 2.71. Encapsulation efficiency increases with calcium lactate levels, corresponding with research stating that calcium ions function to replace sodium bonds in alginate and form strong crosslinks (30). Higher calcium lactate levels generate stronger cross-linking, increasing the presentation of the active substances protected by the coating. Microbeads with higher encapsulation efficiency signify a better ability of the coating to protect the active substances (31). However, the encapsulation efficiency decreased with the addition of a 3.5% calcium lactate level due to excessive saturation when calcium binds in the guluronic acid chain, leading to LAB metabolism and respiration (32). Excess calcium ions can cause failure in combining bioactive compounds, leading to a decrease in encapsulation efficiency [25].

The total population of LAB with the addition of 2 - 3 .5% calcium lactate meets the Indonesian National Standard 7552: 2009 which considers a minimum total probiotic of 6 CFU/g in a product to be good. The population of LAB trapped in capsules is influenced by the number of pores, where a high number can increase leakage in bacterial cells. More Ca^{2+} complicates the calcium alginate bonds, decreasing porosity and preventing easy release of the trapped active substances (33).

3.3 Yield

Yield is the ratio of the final weight of the product to the weight of the raw material. Table 5 shows that differences in calcium lactate levels have a significant effect ($p<0.05$) on the yield value of microbeads.

Table 4 Yield of probiotic orange juice microbeads at different calcium lactate levels.

Calcium Lactate Levels (%w/w)	Yield (%)
2	74.91 ± 1.07^{a}
2.5	75.52 ± 0.62^{ab}
3	76.13 ± 0.87^{ab}
3.5	76.57 ± 1.09^{b}

Note: The data shown as the average value of 5 repetitions ± standard deviation with different superscript letters in the same column represents a significant difference ($p<0.05$).

The treatment with the addition of a 3.5% calcium lactate produced the highest yield value of 76.57 ± 1.09 and a 2% calcium lactate had the lowest yield of 74.91 ± 1.07. The yield value was increased by Ca^{2+} ions that originate from calcium lactate forming bonds with the sodium alginate chains. This enhances the network in the microbeads matrix, promoting easier entry of water molecules. Calcium lactate has a calcium atom that binds a hydroxyl group to form hydrogen bonds with water molecules in the alginate, increasing the molecular weight of the alginate. The formed hydrogen bonds improve alginate viscosity, leading to more effective water content withdrawal by calcium lactate (34). Additionally, a higher yield weight of microbeads is initiated by encapsulation efficiency because more trapping of active substances increases the final weight (33).

3.4 pH value

pH is an important factor in preparing microbeads because the value can affect the metabolism of probiotics and the stability of the added polymer. The strength of gel microbeads is influenced by the levels of calcium and alginate salts used at a specific time and pH. The gel volume increases at a pH of 4 - 10 and the low pH of the solution increases the interface voltage, leading to the microbeads becoming spherical with a larger aspect ratio.

Table 5 Test results for the pH value of probiotic orange juice microbeads

Calcium Lactate Levels (%w/w)	pH value
2	5.90 ± 0.39^{ab}
2.5	5.81 ± 0.15^{a}
3	6.21 ± 0.19^{b}
3.5	6.06 ± 0.10^{ab}

Note: The data shown as the average value of 5 repetitions ± standard deviation with different superscript letters in the same column represents a significant difference ($p<0.05$).

Differences in the addition of calcium lactate levels have a significant effect ($p<0.05$) on the pH value of the microbeads. The treatment with the addition of a 3% calcium lactate level produced microbeads with the highest pH value of 6.21 ± 0.19 and 2% calcium lactate generated the lowest pH of 5.90 ± 0.39. The value increases because the neutral pH of calcium lactate reacts with the acidic pH of orange, corresponding with research stating that the pH of a neutral calcium lactate solution is between 6 to 7 (21). The initial pH of orange ranges from 4-5, while the value increases to 5-6 post calcium lactate addition.

The salt bound to calcium alginate reduces acidity, while the added 3.5% calcium lactate has a slightly lower pH than the 3% level because the 3% calcium lactate treatment has a higher initial pH of orange juice. The OH^- group contained in calcium lactate binds with the H^+ group in oranges which is an acid, increasing the pH of microbeads. The pH value of probiotic orange juice microbeads ranging from 5 - 6 affects gel strength and viscosity, as well as gelation speed under optimal conditions. This is consistent with research stating that gel strength and viscosity increase at pH 3.8 - 8.5 due to the number of dissociated carboxylate groups (27). However, high pH > 8.5 reduces the pectin molecular weight because the gelation speed and gel strength of the microbeads decrease. Calcium alginate gel at pH 3.8 produces less homogeneous microbeads with many wrinkles and cavities on the surface, while gel in the range of 5 - 6.8 generates more homogeneous microbeads containing fewer cavities (27).

Orange juice used in this research as well as vegetables are an example of the unconventional matrix for probiotic microorganisms, which can induce stress due to the low pH. Nutrient depletion, low pH, and lactic acid accumulation during storage can inhibit the survival of probiotic bacteria. Therefore, the initial pH of the matrix, pH after inoculation, and any potential changes during storage should be determined. The production of extremely high acid affects the sensory characteristics of the product.

Maintaining the viability of probiotic cultures in fruit juice presents additional challenges due to the high dissolved oxygen concentration in fruit juice. The probiotic microorganisms used in this research are anaerobic, suggesting that the presence of oxygen in the product at significant levels initiates toxicity and loss of viability. Vitamin C in orange juice can reduce oxygen levels, potentially providing a favorable environment for anaerobic bacteria during storage. However, vitamin C is a highly unstable compound, and prolonged storage leads to degradation, damaging the survival of anaerobic cells. Osmotic stress can be a factor affecting probiotics in the food matrix, corresponding with flow cytometry research showing that lactobacilli exposed to various levels of sugar concentration experience a loss of probiotic viability due to osmotic stress.

4. Conclusions

In conclusion, this research showed that an increase in the quantity of calcium lactate correlated with enhanced shape, encapsulation efficiency, yield value, and pH value, without affecting solidity. A calcium lactate concentration of 3% had no significant effect on the shape, encapsulation efficiency, yield value, and pH value of microbeads compared to 3.5%. Based on production costs, the 3% calcium lactate concentration was considered more advantageous than using 3.5% for the manufacture of probiotic citrus juice microbeads.

5. Acknowledgments

This research was funded by LPPM Universitas Diponegoro, with the program scheme of Riset Pengembangan dan Penerapan (RPP) 2023, grant number: 609-11/UN7.D2/PP/VIII/2023.

6. References

[1] Abbas A. The potential of functional foods and their role in improving the health of increasingly vulnerable humans. Teknosains Media Inf Sains Dan Teknol. 2020;14(2):176–186.

[2] Wulandari R, Indriana D, Amalia AN. Hydrocolloid application as an emulsifier in chocolate processing. J Ind Has Perkeb. 2019;14(1):2-8.

[3] Nurhamidah A, Warsidah W, Idiawati N. Isolation and characterization of lactic acid bacteria (LAB) from ale-ale and cincalok. J Laut Khatulistiwa. 2020;2(3):8-15.

[4] Purwandhani SN. Folate Biosynthesis by lactic acid bacteria. Agrotech. 2016;1(1):11–18.

[5] R. Haryo Bimo Setiarto, 2 HDK, Betty Sri Laksmi Jenie TK. Development of microencapsulation technology for probiotic bacteria and its health benefits. J Vet. 2019;19(4):574–589.

[6] Rajagukguk WJ, Dwi W, Sayekti SS. Consumers' attitudes and purchasing decisions on local and imported oranges in bandar lampung. JIIA.2013;1(4):351–357.

[7] Wati RR, Sriwidodo S, Chaerunisa AY. Enhancement of phytoconstituent stability through a microencapsulation approach. Maj Farmasetika. 2022;7(1):3-9.

[8] Abbas P, Mayangsari CP, Gunawan R. The effect of *Lactobacillus acidophilus*, *Streptococcus thermophilus*, and *Bifidobacterium longum* probiotics on bilirubin levels of neonates with hyperbilirubinemia. Sains Med J Kedokt Kesehat. 2023;14(2):5-8.

[9] R. Haryo Bimo Setiarto NW, Octavia ND, Himawan HC. Production of fermented papaya juice (*Carica papaya*) as an antihypercholesterolemia probiotic drink. J Litbang Ind. 2022;2014(2):73–81.

[10] Murdinah, M. The use of alginate in lemon extract effervescent powder production. J Pengolah Has Perikan Indones. 2015;18(2):177–189.

[11] Dwiloka B, Rahman FT, Mulyani S. Nilai pH value, Viscosity and hedonic of sweet orange juice with added milkfish bone gelatin. Agri Health J Agri food Nutr Public Heal. 2022;2(2):10-17.

[12] Hasyim H, Rahim A, Rostiati. Physical, chemical and organoleptic characteristics of jelly candy from soursop fruit juice at varying agar-agar concentrations. J Agrotekbis. 2015;3(4):463–474.

[13] Alahakoon AU, Jayasena DD, Jung S, Kim HJ, Kim SH, Jo C. Antimicrobial effect of calcium chloride alone and combined with lactic acid injected into chicken breast meat. Korean J Food Sci Anim Resour. 2014;34(2):221–229.

[14] Cahyono K, Sumardi S, Irawan B, Wahyuningsih S, Nurcahyani E. Immobilization of lactic acid bacteria using zeolite and sodium alginate. J Ilm Farm Farmasyifa. 2021;4(1):33–40.

[15] Ratnasari N, Kusumawati N, Kuswardhani I. The Effect of sodium alginate concentration as a trap for *Lactobacillus acidophilus* Fncc 0051 cells and storage time on the number of released cells and carrier characteristics. J Teknol Pangan dan Gizi Food Technol Nutr. 2017;13(2):81–86.

[16] Hasan A, Taufik M, Bande LOS, Khaeruni A, Mallarangeng R, Gusnawaty HS, et al. Morphometric analysis of chili leaves with yellow curly symptom using digital image processing approach and data mining algorithm. J Fitopatol Indones. 2023;19(6):231–237.

[17] Prastiti R, Mulyani S, Masykuri. Microbiological characteristics and texture of probiotic ice cream with fresh milk raw material combined with purple sweet potato (*Ipomoea batatas*). Anim Agric J. 2013;18(2(4)):127–136.

[18] Mansur DS, Hidayat MN. Resistance of lactic acid bacteria from broiler digestive tract to pH and bile salts. J Ilmu dan Ind Peternak Anim Husb Sci Ind. 2019;5(1):2-7.

[19] Diza YH, Asben A, Anggraini T. Isolation, identification and preparation of dry preparations of Lactic Acid Bacteria that have the potential as probiotics from dadih from Sijunjung, West Sumatra. J Litbang Ind. 2020;10(2):155-158.

[20] Arifa AN, Suharti PH. The effect of solvent amount on yield in making moringa hand sanitizer (*Moringe Oleifera*). DISTILAT J Teknol Separasi. 2023;7(2):341–346.

[21] Wardani EK, Zulaekah S, Purwani E. The effect of adding pineapple juice (*Ananas comosus*) on the number of lactic acid bacteria (LAB) and the pH value of soyghurt. J Kesehat. 2017;10(1):6-8.

[22] Sumanti DM. The effect of lactobacillus acidophilus bacteria concentration on the characteristics of synbiotic porridge made from composite flour. J. Universitas Padjajaran. 2017;2:2-5.

[23] Zdilla MJ, Hatfield SA, McLean KA, Cyrus LM, Laslo JM, Lambert HW. Circularity, solidity, axes of a best fit ellipse, aspect ratio, and roundness of the foramen ovale: A morphometric analysis with neurosurgical considerations. J Craniofac Surg. 2016;27(1):222–228.

[24] Saria TR, Surjoseputroa S, Setijawatia E. The effect of calcium lactate concentration on the physicochemical properties of rice paper made from ir 64 rice. Teknol Pangan dan Giz. 2020;19(1):22–28.

[25] Maharani AA, Husni A, Ekantari N. The effect of extraction methods on characteristic of sodiumAlginate from brown seaweed sargassum fluitans. Masy Pengolah Has Perikan Indones. 2017;20(3):478–487.

[26] Caoa L, Lua W, Mataa A, Nishinarib K, Fanga Y. Polimer Karbohidrat Gelasi alginat dan pektin berbasis model kotak telur. Sebuah Tinjauan. 2020;24:2-5.

[27] Cao L, Lu W, Mata A, Nishinari K, Fang Y. Egg-box model-based gelation of alginate and pectin: A review. Carbohydr Polym. 2020;242:116389.

[28] Raharjo S, Utama Z. The Effect of particle size and proportion of guluronate-manurnate in alginate on the physical and sensory properties of restructured products from soursop fruit. J Teknol dan Industri Pangan. 2003;14:206-213.

[29] Cao L, Lu W, Mata A, Nishinari K, Fang Y. Egg-box model-based gelation of alginate and pectin: A review. Carbohydr Polym. 2020;2:42-43.

[30] Sari TR, Surjoseputro S, Setijawaty E. The effect of calcium lactate concentration on the physicochemical properties of rice paper made from ir 64 rice. J Teknol Pangan dan Gizi Food Technol Nutr. 2020;19(1):23–28.

[31] Mardikasari SA, Suryani, Akib NI, Indahyani R. Microencapsulation of mefenamic acid using chitosan polymer and sodium alginate by ionic gelation method. J Farm Galen Pharmacy. 2020;6(2):192–203.

[32] Wibowo AA, Suryandari AS, Naryono E, Pratiwi VM, Suharto M, Adiba N. Encapsulation of clove oil within Ca-alginate-gelatine complex: Effect of process variables on encapsulation efficiency. J Tek Kim dan Lingkung. 2021;5(1):71–77.

[33] Kurniawan R, Rahmat D. Controlled-release microencapsulation of lansoprazole using a combination of hydroxypropyl methylcellulose phthalate and sodium alginate via ionotropic gelation. J Ilmu Kefarmasian Indones. 2015;14(1):86–92.

[34] Milla L El, Indrani DJ, Irawan B. Hydroxyapatite alginate scaffold porosity testing and analysis. ODONTO Dent J. 2018;5(1):4-9.