Agrointek Volume 20 No 2 2026: 345-352

p-ISSN: 1907-8056
e-ISSN: 2527-5410

AGROINTEK
Jurnal Teknologi Industri Pertanian

journal homepage: journal.trunojoyo.ac.id/agrointek

Effect of sodium alginate concentration and soaking time on release behaviour of kombucha beads

Dewi Cakrawati[1*], Dwi L. Rahayu[1], Yatti Sugiarti[2], Mustika N. Handayani[2]

[1]*Food Technology, Universitas Pendidikan Indonesia, Bandung, Indonesia*
[2]*Agroindustry Education Technology, Universitas Pendidikan Indonesia, Bandung, Indonesia*

Article history
Received:
28 October 2024
Revised:
19 February 2025
Accepted:
27 August 2025

Keyword
Kombucha, spherification, sodium alginate

ABSTRACT

Encapsulated kombucha was an alternative to be added to food products conveniently. This study investigated the effect of sodium alginate concentration and exposure time on the release of bioactive compounds, namely total phenolic content, antioxidant capacity, and reducing sugar. The stability of kombucha beads in simulated gastric juice was also investigated based on viable bacteria. Kombucha beads was made by mixing kombucha with different concentration of sodium alginate then soaked in calcium cloride at different soaking time. The result shows that a concentration of 1-4% sodium alginate produces tear-shaped beads. The size of kombucha beads increases with the increase in sodium alginate concentration. Low pH value of initial kombucha leads to a strong gel formed during gelation, which results in low release of polyphenolic compounds as well as antioxidant capacity from kombucha beads. The total phenolic content released from kombucha beads was 0.098 ± 0.01 mg GAE/100 g beads. Microbial load after simulated gastric juice was in the range of 178-1398 cfu/g bead. This study highlighted the potential of alginate beads for encapsulating polyphenolic compounds as an innovative approach for food applications. Further study is needed to understand the physical properties of kombucha beads at different concentrations of sodium alginate.

This work is licensed under a Creative Commons Attribution 4.0 International License.

* Corresponding author
Email: dewicakrawati@upi.edu
DOI 10.21107/agrointek.v20i2.27895

INTRODUCTION

Kombucha is a fermented product produced by a mixed culture of yeast and bacteria commonly known as a Synbiotic culture of yeast and bacteria (SCOBY). Kombucha contains polyphenols, primarily catechins; therefore, it offers health benefits and is classified as a functional drink (Cardoso et al. 2020). Recently, the number of companies producing kombucha has increased, along with a variety of kombucha products (Kim and Adhikari 2020). Kombucha is made from a tea solution containing 10% sucrose (Bortolomedi et al. 2022). Fermentation consists of two stages: the conversion of sugar (glucose and fructose) into ethanol by yeast, followed by the oxidation of ethanol into acetic acid by *Acetobacter aceti*. Furthermore, the kombucha starter also contains *Gluconacetobacter*, which converts glucose into glucoronic acid and nanocellulose and converts fructose into acetic acid (Barakat et al. 2023).

Research on the development of kombucha products has been conducted. Raw materials for kombucha can be obtained from snakefruit (Zubaidah et al. 2019), papaya (Sharifudin et al. 2021), and berries, including blackberry, raspberry, and goji berry (Akarca 2021). The development of powdered kombucha products using spray drying techniques, with gum arabic as the encapsulant, has been reported by Mohsin et al. (2022). However, the use of spray drying requires an investment in the purchase of tools that are not inexpensive.

Kombucha has a distinctive flavour and, for some people, is less preferred due to its sourness. Spherification of kombucha helps to reduce the sour taste by encapsulating it. Spherification is a technique that utilises carbohydrate or protein polymers containing ions, added to a solution of divalent ions such as calcium chloride or calcium lactate. Spherification is widely used, especially to encapsulate active ingredients or probiotics, making them more resistant to the digestive system. The application of spherification in encapsulating bioactive compounds is easier than spray drying techniques due to the availability of cheaper equipment. The product produced from the sperification technique is beads or bead juice. Research on sperification has been conducted to develop boba fruit juice products (Nicholas et al. 2022, Samard et al. 2023). Little information is available on kombucha specifications; however, according to Mohsin et al. (2022), several standard qualities should be implied. These include the encapsulation of kombucha, which should preserve the microorganisms in kombucha during digestion and help with the slow release of bioactive compounds in kombucha beads.

Kombucha beads were expected to be conveniently applied to food or drink. Research by Tsai et al. (2017) shows that the use of sodium alginate produces capsules with a smooth surface. However, little information is available regarding the spherification of kombucha. Spherification of kombucha aims to impart a chewy characteristic to kombucha beads while maintaining an acceptable flavour. The spherification technique was employed using sodium alginate at varying concentrations and exposure times to achieve the desired characteristics of kombucha beads. In this study, the effect of hydrocolloid concentration on the stability of boba kombucha at low pH will also be investigated, allowing for a better understanding of the behaviour of kombucha beads in gastric simulation conditions. Kombucha beads were expected to be applied in food or drink conveniently while still containing bioactive compounds, namely phenolic compounds.

METHODS

Materials

Black Tea kombucha was obtained from the Laboratory of Agro-Industry Education Technology, Universitas Pendidikan Indonesia (Bandung, West Java, Indonesia). Sodium alginate was purchased from the local market in Bandung, West Java. Standard compounds, namely cacthecin, gallic acid, trolox, and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, were obtained from Sigma Aldrich (St. Louis, Missouri, USA). While chemical reagents were purchased from Merck (Merck, Darmstadt, Hesse, Germany).

Methods

The research was conducted in three steps: the first is kombucha production, followed by kombucha bead production using the sperification method, and lastly, evaluation of the characteristics of the kombucha beads.

Production of kombucha beads

Kombucha bead production was made according to Gaikwad et al. (2019). Different concentrations of sodium alginate (1%, 2%, 3%,

4%, and 5%) were added to 100 mL of kombucha, and then stirred until the sodium alginate was dissolved. A calcium chloride (CaCl2) solution was prepared by dissolving 3% (w/v) calcium chloride in 100 mL of distilled water. A mixture of Kombucha and sodium alginate was dropped using a syringe into 3% of $CaCl_2$. The mixture was then soaked in a $CaCl_2$ solution at different times (3, 6, 9, and 12 minutes) and then separated from the $CaCl_2$ solution. Kombucha beads were then washed using distilled water and stored at 4 °C for further analysis.

Evaluation

Release of bioactive compounds of kombucha beads

One gram of kombucha beads was dissolved in 10 mL of sodium citrate and stirred for 3 hours at 37 °C (Silveira et al. 2023).

Total phenolic content (TPC) in kombucha beads

The free phenolic content was measured from the phenolic dissolved in the sodium citrate solution. The total phenolic compound was estimated using the modified Folin-Ciocalteau method reported by. Briefly, 0.1 mL of a 0.1 mL sample was added to 0.1 mL of Folin-Ciocalteau reagent. The mixture was set aside for 6 minutes to allow the reaction to occur. The mixture was then supplemented with 1 mL of 7% NaCO3 and 1 mL of distilled water. After 90 minutes, the absorbance was measured at 760 nm using a Mapada UV-100 spectrophotometer. The phenolic content in flour extract was calculated using a standard curve for gallic acid. Results were expressed as mg gallic acid equivalents per 100-gram beads (mg GAE/ 100 g).

Trolox Equivalent Antioxidant capacity (TEAC) in kombucha beads

The antioxidant capacity was analysed following Re et al. (1999). A mixture of 5 mL of 7 mM ABTS and 88 µL of 140 mM $K_2S_2O_8$ was prepared in the dark and kept in 24 h at room temperature to generate $ABTS^{\bullet+}$. The absorbance of $ABTS^{\bullet+}$ was adjusted to 0.700 ± 0.020 at 734 nm by dilluting $ABTS^{\bullet+}$ stock solution in phosphate-buffered saline (PBS). Diluted sample of 5 µL was mixed with 1 mL of diluted $ABTS^{\bullet+}$ and incubated at 4 min at 30 °C to observed the sample discolorization. The absorbance was measure at 734 nm using a UV-100 Mapada spectrophotometer. The absorbance of the sample was then plotted to the standard curve made using trolox wat 0.5 to 4 mM as standard.

Stability of kombucha beads in simulated gastric juice

The stability of kombucha beads in the simulated gastric juice is defined as the number of viable bacteria present in the simulated gastric juice. The determination of kombucha bead stability was conducted following the method described by Khajehie et al. (2023). Kombucha beads of 3 grams were dissolved in 10 mL of 0.2% sodium chloride solution, pH 2, and stirred at 37 °C for 2 hours. One millilitre of supernatant was taken for measuring the viability of bacteria using de Man-Rogosa-Sharpe (MRS) agar by serial dilution.

Statistical Analysis

Research was conducted in two separate replications and analysed using analysis of variance.

RESULTS AND DISCUSSION

Kombucha was made, then transformed into beads using the spherification method, which involves applying sodium alginate as a hydrocolloid. Kombucha beads were analysed to investigate the release of phenolic compounds and microorganisms during the simulated gastric phase using low pH.

Kombucha beads were observed to have different sizes and shapes at different concentrations of sodium alginate and exposure time (Figure 1). Kombucha beads produced with 1% sodium alginate showed uniform shape and had a diameter of 3.22 ± 0.57 mm (Figure 1A). This result was in agreement with Bevan et al. (2023). Increasing the concentration of sodium alginate led to an increase in the diameter of the beads. The results showed that kombucha beads produced with 2% and 3% sodium alginate have diameters of 5.74 ± 0.67 and 7.15 ± 0.57, respectively (Figure 1B and 1C). However, there was no difference in the diameter of kombucha beads produced by 3% and 4% sodium alginate.

Beads produced at a concentration of 1-4% sodium alginate will form a tear-like sphere (Bennacef et al. 2021). However, at concentrations higher than 4%, the mixture of kombucha and sodium alginate exhibited high viscosity and displayed a strong gel-like behaviour (Farrés et al. 2013).

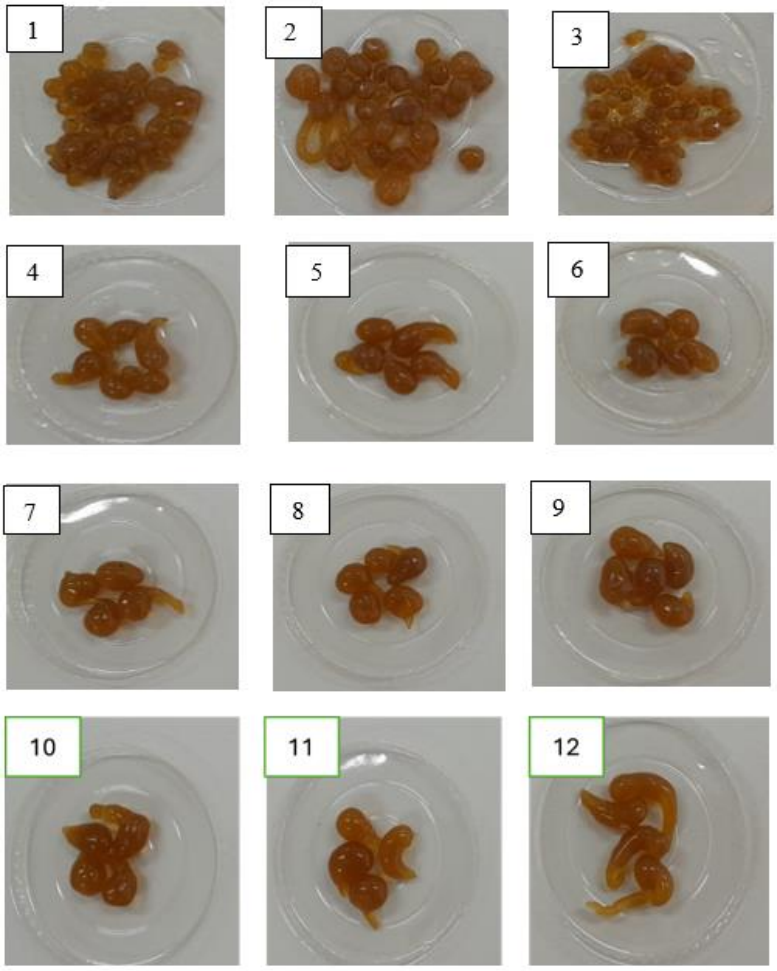


Figure 1 Morphology and size of kombucha beads obtained with (A) 1% of sodium alginate at exposure time of (1) 3 min; (2) 6 min; (3) 9 min; (B) 2% of sodium alginate at exposure time of (4) 3 min; (5) 6 min; (6) 9 min; (C) 3% of sodium alginate at exposure time of (7) 3 min; (8) 6 min; (9) 9 min; (D) 4% of sodium alginate at exposure time of (10) 3 min; (11) 6 min; (12) 9 min.

Table 1 The effect of sodium alginate concentration and soaking time on kombucha bead hardnes[s]

Concentration of sodium alginate (%)	Soaking time (min)		
	3	6	9
1	21.33 ± 0.39 N	26.67±3.95 N	35.14±3.14 N
2	35.655±7.83 N	41.029±4.11 N	45.76±10.92 N
3	26.57±4.79 N	41.03±4.11 N	45.75±10.02 N
4	38.64±2.14 N	50.94±8.04 N	57.72±1.57 N

The hardness of kombucha beads increased at higher concentrations of sodium alginate and longer soaking times (Table 1). The hardness of the kombucha beads was in the range of 21.33 ± 0.39 N to 80.82 ± 1.60 N. The mixture of kombucha and sodium alginate exhibited high viscosity and displayed a strong gel-like behaviour due to the strong covalent bond (Farrés et al. 2013, Yang et al. 2020). This result is higher than that reported by Silveira et al. (2023). According to Quemeneur et al. (2024), the acceptable hardness for a chewable capsule ranged from 26.97 to 49.76 N.

Dundar et al. (2022) reported that increasing the hydrocolloid concentration increases encapsulation efficiency, thereby decreasing the release of total phenolic content from kombucha beads. Gholamian et al. (2021) also reported that high entrapment efficiency was achieved with alginate beads at high concentrations. However,

the total flavonoid content released from kombucha beads fluctuated at different concentrations of sodium alginate and exposure times.

Figure 2 shows the total phenolic content released from kombucha beads. Total phenolic content released from kombucha beads was 0.098 ± 0.01 mg GAE/100 g beads. It seems that the release of total phenolic content from beads decreases as the concentration of sodium alginate increases. Moreover, total phenolic content released from kombucha beads decreases as the exposure time increases.

Figure 3 shows antioxidant capacity released from kombucha beads reported as µM Trolox equivalent/ g beads. The release of antioxidant capacity also fluctuated at different concentrations of sodium alginate and exposure time. The release of antioxidant capacity from kombucha beads was 16.31 ± 4,71 µM Trolox equivalent/ g of beads. The total phenolic content and total flavonoid content released from kombucha beads contributed to the antioxidant capacity (Cardoso et al. 2020).

The release of reducing sugar, reported as glucose, was not different at varying concentrations of sodium alginate and soaking times. The release of reducing sugar was in the range of 20-80 mg glucose/g of beads (Figure 6).

However, total phenolic content released from kombucha beads fluctuated at different exposure times, depending on the concentration of sodium alginate at 1% and 2%. Viable bacteria were released from the kombucha beads (Figure 5). The results show that increasing the concentration of sodium alginate led to an increased number of viable bacteria released at the exposure times of 3 and 9. On the contrary, the number of viable bacteria decreased at the exposure time of 6 min when the concentration of sodium alginate was increased.

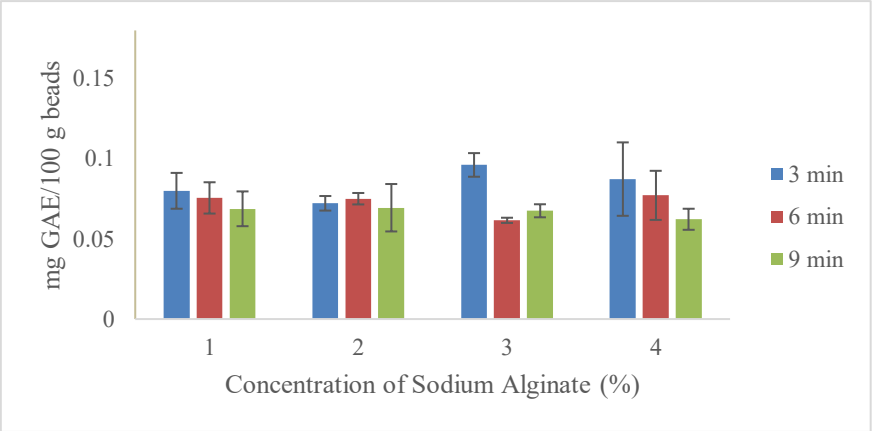


Figure 2 Effect of Concentration of Sodium Alginate and exposure-time on the release of phenolic content from kombucha beads

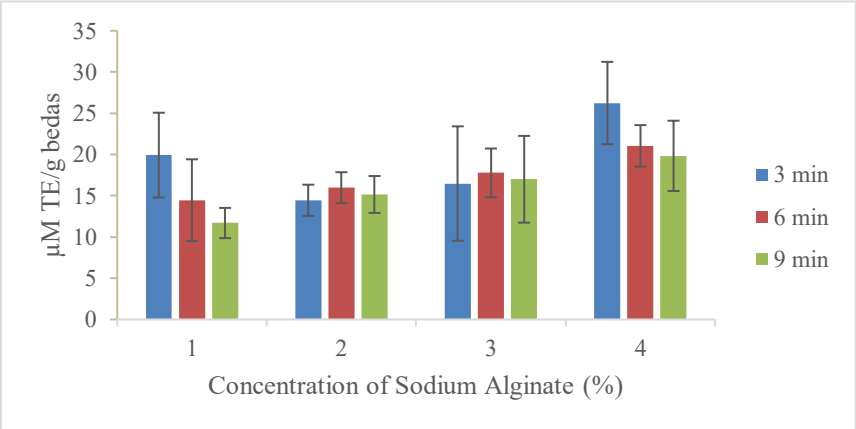


Figure 3 Effect of Concentration of Sodium Alginate and exposure-time on the release of antioxidant capacity from kombucha beads.

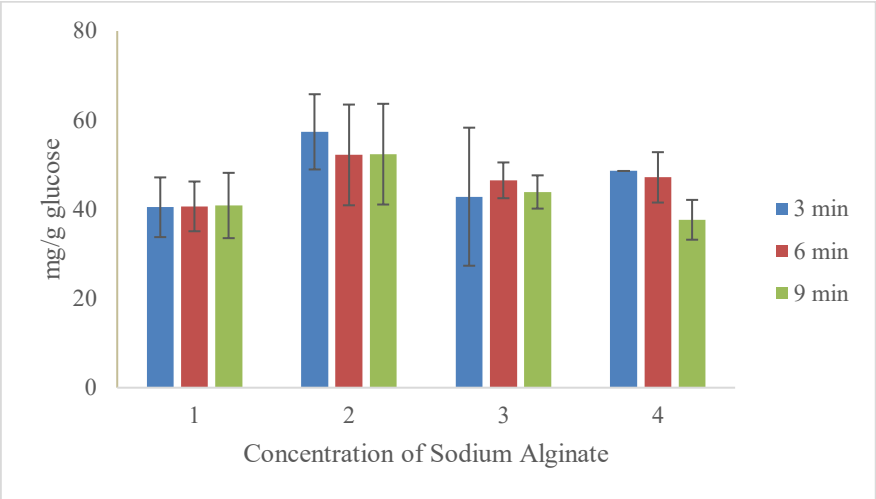


Figure 4 Effect of Concentration of Sodium Alginate and exposure-time on the release of reducing sugar from kombucha beads.

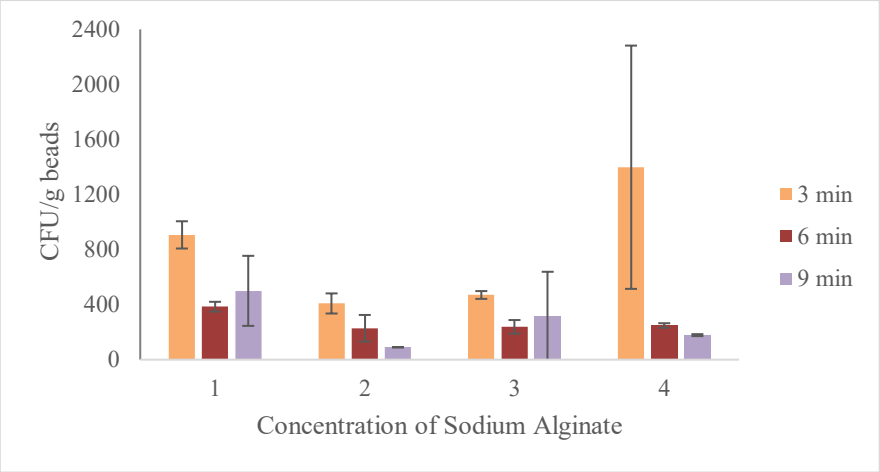


Figure 5 Effect of Concentration of Sodium Alginate and exposure-time on the release of viable bacteria from kombucha beads

Kombucha has an initial pH of 3.6, which falls within the range of the pKa values of galacturonic acid and mannuronic acid, the monomers of alginate, at 3.38 and 3.65, respectively. It appears that the low pH of kombucha produces a denser and more interconnected microstructure of kombucha beads, likely due to stronger chain interactions (Bennacef et al. 2021). These phenomena lead to a low total phenolic content and antioxidant capacity released from kombucha beads. Moreover, the diffusion of polyphenolic compounds from the beads to the medium was influenced by the environment, primarily pH (Bevan et al. 2023). Therefore, soaking kombucha beads in sodium citrate at pH 7 resulted in the low release of bioactive compounds as well as viable bacteria.

This research represents the first attempt at kombucha diversification, aiming to provide consumers with a unique experience of consuming kombucha, not only as a beverage but also as chewy beads. This research represents the first attempt to investigate the stability of phenolic compounds and microorganisms in kombucha during the simulated gastric phase at low pH. Further research is needed to investigate the stability of phenolic compounds and microorganisms during simulated digestion, taking into account the availability of enzymes and bile salts.

CONCLUSION

The findings of this study highlighted the potential of kombucha beads as a preservative tool for polyphenolic compounds found in kombucha. The encapsulation using spherification techniques

relies on a calcium alginate matrix, which yields favourable attributes including suitable size and satisfactory release behaviour of polyphenolic compounds. These kombucha beads are expected to be added to drink products and desserts, conveniently.

ACKNOWLEDGEMENT

The research was funded by a grant from Universitas Pendidikan Indonesia, No. 342/UN40.LP/PT.01.03/2024.

REFERENCES

Akarca, G. (2021). Determination of potential antimicrobial activities of some local berry fruits in kombucha tea production. *Brazilian Archives of Biology and Technology, 64*, e21210023.

Barakat, N., S. Beaufort, Z. Rizk, J. Bouajila, P. Taillandier, and Y. El Rayess. 2023. Kombucha analogues around the world: A review. *Critical Reviews in Food Science and Nutrition 63*(29):10105-10129.

Bennacef, C., S. Desobry-Banon, L. Probst, and S. Desobry. 2021. Advances on alginate use for spherification to encapsulate biomolecules. *Food Hydrocolloids 118*:106782.

Bevan, P., M. V. Pastor, M. P. Almajano, and I. Codina-Torrella. 2023. Antioxidant and antiradical activities of Hibiscus sabdariffa L. extracts encapsulated in calcium alginate spheres. *Polymers 15*(7):1740.

Bortolomedi, B. M., C. S. Paglarini, and F. C. A. Brod. 2022. Bioactive compounds in kombucha: A review of substrate effect and fermentation conditions. *Food Chemistry 385*:132719.

Cardoso, R. R., R. O. Neto, C. T. dos Santos D'Almeida, T. P. do Nascimento, C. G. Pressete, L. Azevedo, H. S. D. Martino, L. C. Cameron, M. S. L. Ferreira, and F. A. R. de Barros. 2020. Kombuchas from green and black teas have different phenolic profile, which impacts their antioxidant capacities, antibacterial and antiproliferative activities. *Food Research International 128*:108782.

Dewanto, V., X. Wu, K. K. Adom, and R. H. Liu. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry* 50(10):3010-3014.

Dundar, A. N., K. Uzuner, M. E. Parlak, O. I. Sahin, F. T. Saricaoglu, and S. Simsek. 2022. Enhanced functionality and bio-accessibility of composite pomegranate peel extract-enriched “boba balls”. *Foods 11*(23):3785.

Farrés, I. F., M. Douaire, and I. Norton. 2013. Rheology and tribological properties of Ca-alginate fluid gels produced by diffusion-controlled method. *Food Hydrocolloids 32*(1):115-122.

Gaikwad, S., A. Kulthe, and T. Suthar. 2019. Characterisation of flavoured sweet water balls prepared by basic spherification technique. *International Journal of Chemical Studies 7*(1):1714-1718.

Gholamian, S., M. Nourani, and N. Bakhshi. 2021. Formation and characterisation of calcium alginate hydrogel beads filled with cumin seeds essential oil. *Food Chemistry 338*:128143.

Khajehie, N., M. Niakousari, M. H. Eskandari, M. Majdinasab, and S. S. Shekarforoush. 2023. Microencapsulation of Lacticaseibacillus rhamnosus GG and L. plantarum 299 V by reverse spherification: A promising method to improve the survival of probiotics. *Letters in Applied Microbiology 76*(1):ovac025.

Kim, J., and K. Adhikari. 2020. Current trends in kombucha: Marketing perspectives and the need for improved sensory research. *Beverages 6*(1):15.

Mohsin, A. Z., N. A. M. Nor, B. J. Muhialdin, B. H. M. Roby, M. M. Abadl, A. A. Marzlan, N. Hussain, and A. S. M. Hussin. 2022. The effects of encapsulation process involving arabic gum on the metabolites, antioxidant and antibacterial activity of kombucha (fermented sugared tea). *Food Hydrocolloids for Health 2*:100072.

Nicholas, D., H. Chua, A. Teresa, M. Hazijah, and A. Zakaria. 2022. Transforming liquids into pops of flavours by using Juice Pearl Kit. *Food Research 6*(2):91-96.

Quemeneur, F., A. Igonin, C. Herry, and S. Girod-Fullana. 2024. Shell materials for chewable capsules, from design of experiments to optimal dosage form manufacturing. *Advanced Materials Technologies* 2400808.

Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans. 1999. Antioxidant activity applying an improved ABTS radical cation decolorisation assay. *Free Radical Biology and Medicine 26*(9-10):1231-1237.

Samard, S., P. Jiranitsarawit, and G.-H. Ryu. 2023. Development of vitamin C butterfly pea juice beads by reverse spherification technique. The 13th National and the 9th International PIM Conference, July 7, Bangkok, Thailand.

Sharifudin, S. A., W. Y. Ho, S. K. Yeap, R. Abdullah, and S. P. Koh. 2021. Fermentation and characterisation of potential kombucha cultures on papaya-based substrates. *LWT 151*:112060.

Silveira, M. P., F. L. C. Almeida, I. D. Alvim, and A. S. Prata. 2023. Encapsulation of pomegranate polyphenols by ionic gelation: Strategies for improved retention and controlled release. *Food Research International 174*:113590.

Tsai, F.-H., P.-Y. Chiang, Y. Kitamura, M. Kokawa, and M. Islam. 2017. Producing liquid-core hydrogel beads by reverse spherification: Effect of secondary gelation on physical properties and release characteristics. *Food Hydrocolloids 62*:140-148.

Yang, J., J. Steck, and Z. Suo. 2020. Gelation kinetics of alginate chains through covalent bonds. *Extreme Mechanics Letters 40*:100898.

Zubaidah, E., Afgani, C. A., Kalsum, U., Srianta, I., & Blanc, P. J. (2019). Comparison of in vivo antidiabetes activity of snake fruit Kombucha, black tea Kombucha and metformin. *Biocatalysis and Agricultural Biotechnology, 17*, 465-469.