BIO Web of Conferences **117**, 01043 (2024) https://doi.org/10.1051/bioconf/202411701043
ICoLiST 2023

Rheological and Antioxidant Properties of Purple Yam Yoghurt Enhanced with Alginate-Chitosan Microcapsule Bacteria

Abdul Gofur[1*], *Siti Nur* Arifah[1], *Mochammad Fitri* Atho'illah[3], *Shofiyatul* Mas'udah[2], *Muhammad Imam* Surya[2], *Fauziah* Fauziah [2], *Elok Rifqi* Firdiana[2], *Esha* Ardiansyah[3], *Nur Alfi Maghfirotus* Sa'adah[3], and *Mardiana* Lelitawati[1]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Malang, 65145, Indonesia
[2]Purwodadi Botanic Garden-Research Center for Plant Conservation and Botanic Gardens, National Research and Innovation Agency (BRIN), Pasuruan, East Java, 67163, Indonesia
[3]Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, 65145, Indonesia.

Abstract. Yoghurt is a diary product that contain with probiotics such as *Lactobacillus bulgaricus, Streptococcus thermophilus,* and *Bifidobacterium longum.* The microencapsulation process in bacteria increased the nutritional value of yoghurt. Fortification of purple yam contains rich anthocyanins could increase the health benefit of yoghurt. The aimed of this study are to determine the rheological and antioxidant properties of purple yam yoghurt. Microencapsulation on *B.longum* used the extrusion method. Water holding capacity (WHC), syneresis, pH, viscosity, color, and antioxidant were measured to determine the quality of yoghurt. Based on the analysis, the addition of purple yam in yoghurt increased WHC and decreased syneresis. Antioxidants assay showed that anthocyanins in purple yam increased the antioxidant activity. Purple yam yoghurt with microencapsulated bacteria has best quality of yoghurt based on the WHC, syneresis, pH, and antioxidant properties. This result suggested that microencapsulated bacteria and fortification of purple yam could increase the health benefit of yoghurt.

1 INTRODUCTION

The modern lifestyle associated with processed foods, exposure to various chemicals, and lack of exercise play an essential role in the induction of oxidative stress [1]. The effects of fast food produce harmful compounds called free radicals. The endogenous antioxidant system normally normalizes free radicalsm. Unfortunately, imbalance conditions between free radicals and endogenous antioxidants trigger an adverse chain reaction in the body which can induce cellular damage through oxidative stress such as oxidizing cellular proteins,

*Corresponding author: abdul.gofur.fmipa@um.ac.id

enzymes, lipid membranes, and DNA. These mechanisms arrest cellular respiration [2-4]. Long-term exposure to elevated levels of pro-oxidant factors can lead to structural defects at the level of mitochondrial DNA, as well as functional changes in some enzymes and cellular structures leading to aberrations in gene expression. Oxidative stress plays an important role in the pathogenesis of chronic diseases such as cardiovascular disease, diabetes, neurodegenerative diseases, and cancer [1].

Nowadays, yoghurt consumption has grown rapidly and meets many nutritional needs. The presence of lactic acid bacteria (LAB) with proteolytic activity allows them to grow well in milk and potentially release many bioactive peptides[5]. It has been reported that the high oxidative stability of yoghurt is due to the peptides released during fermentation by LAB [6]. In previous studies, *Lactobacilli* and *Bifidobacteria* species can reduce oxidative stress through increasing antioxidant enzyme expression, lowering proinflammatory cytokine levels, lowering cholesterol, deconjugating bile salts, and lowering lipid profiles [7-9]. According to the Food and Drug Administration and the World Health Organisation, to obtain maximum results, the viable cell count of probiotics should not be lower than 10^6-10^7 CFU/mL when ingested. Unfortunately, the amount of probiotics ingested is affected by many physiological factors, including low pH, gastrointestinal conditions, food matrix properties, processing, storage conditions, and physical factors that affect the adhesion and colonization of probiotics in the gut. Researchers are finding strategies to improve probiotic viability, including nanoparticles, polymer gels, and microencapsulation to protect probiotic cells that are sensitive to harsh conditions in the gastrointestinal tract and during storage[10].

Encapsulation techniques have been applied to maintain the viability of probiotics. Encapsulation protects the encapsulated active compounds (core material) against unfavorable or adverse environments (such as light, moisture, temperature, and oxygen) [11]. A wide variety of products, including probiotics, antioxidants, antimicrobials, enzymes, and nucleic acids, are encapsulated to prevent the core material from degrading, slow down the rate of evaporation, separate components that will react with each other, improve stability, mask undesirable taste, color, and odor, control the rate of release, control oxidative reactions, and extend shelf life [12,13].

The medicinal uses of plants with antioxidant properties have been explored for their ability to treat or prevent several human pathologies caused by oxidative stress [1]. Numerous studies have reported the purple yam (*Diocorea alata*) has high nutritional value, especially as an alternative starch source and several important micronutrients and anthocyanin compounds. *D.alata* has antioxidant properties that can neutralize free radicals. Saponins, steroids, phenanthrene, and anthocyanins are essential constituents in the tuber [14]. Anthocyanin compounds have been confirmed to play a pre-protective role in oxidative damage, inhibiting lipid peroxidation and counteracting free radicals:

2 MATERIAL AND METHODS

2.1 Probiotic Bacteria Culture and Microencapsulation Procedures

Lactobacillus bulgaricus (FNCC 0041), *Streptococcus thermophilus* (FNCC 0040) and *Bifidobacterium longum* (FNCC 0463) was obtained from the Center for Food and Nutrition Studies, Gajah Mada University, Yogyakarta, Indonesia. Each strain from stock cultures of *L. bulgaricus, S. thermophilus* and *B. longum* were cultured separately in de Man, Rogosa, and Sharpe (MRS) broth (HiMedia Laboratories, USA) then incubated at 37°C for 24 h under anaerobic conditions. The cultures were then harvested by centrifuging at 4000 rpm for 10 min at 4°C and washed in 0.85% (w/v) sodium chloride (NaCl) solution then the cell pellets were resuspended in distilled water to achieve a cell concentration of 10^9 CFU/mL.

B. longum were encapsulated in a sodium alginate-chitosan as described by Pupa et al [15]. First, 10^9 CFU/mL *B. longum* was mixed with 1.5% alginate solution (Sigma-Aldrich, Missouri, USA) at ratio 1:5 (v/v). Drop-wised the alginate-bacteria mixture in 100 mL of 1 mol/L calcium chloride ($CaCl_2$) (Merck KGaA, Darmstadt, Germany) using 18G needle and left for 30 min for gelation to achieve the alginate beads. The encapsulated probiotic bacteria were separated and washed with 0.85% (w/v) of NaCl solution and then kept in 0.1% (w/v) peptone solutions (Becton, Dickinson and Company, Maryland, USA) at 4°C. Chitosan solution (0.5%) (Sigma-Aldrich, Missouri, USA) was dissolved in 1% (v/v) acetic acid solution. The alginate beads obtained from the extrusion method were immersed into the chitosan solution, and shaken at 100 rpm for 40 min. The alginate-chitosan coated beads were then washed with 0.85% NaCl and stored in 0.1% (w/v) peptone solution at room temperature for further analysis.

2.2 Preparation of Yoghurt

Purple yam (*Dioscorea alata*) was purchased from a local market located in Malang. Purple yam was washed and boiled for 10 minutes. 5% of purple yam (w/v) and 6% sucrose (w/v) were blended with ultra-high temperature milk using a blender. Purple yam milk was pasteurized for at 85°C 30 minutes. Pasteurized purple yam milk was then cooled until 40ºC then added with bacteria culture. The yoghurt were divided into 4 types, namely standard yoghurt yoghurt contains *L. bulgaricus* and *S. thermophilus,* standard yoghurt added with *B.longum*, yoghurt enriched with encapsulated *B. longum,* purple yam yoghurt enriched with encapsulated *B. longum.*

2.3 Extraction of yoghurt water

Extraction of yoghurt water followed protocols by Muniandy et al[16] with slight modification. Briefly, 5 grams of yoghurt were homogenized with 1.25 mL of sterile distilled water, adjusting pH to 4.0 by adding 0.1 M HCl, incubated at 45ºC for 10 minutes. After incubation, the mixture was then centrifuged at 3500 rpm 10ºC for 15 minutes, the clear supernatant was collected and adjusted into pH 7.0 by adding 0.1 M of sodium hydroxide (NaOH). Then, centrifuged at 3500 rpm 10ºC for 15 minutes. The clear supernatant was collected as yoghurt water extract and stored at -20ºC for further analysis.

2.4 Rheological Analysis

Rheological analysis included pH, water holding capacity (WHC), syneresis, viscosity, and colour. The pH of yoghurt was measured using digital pH meter. The WHC and synaeresis were measured using centrifugation of yoghurt at 3500 rpm 10ºC for 15 minutes[16]. WHC and synaeresis were calculated by following Equation 1 and 2:

$$WHC\ (\%) = \left[1 - \left(\frac{whey\ weight}{initial\ weight}\right)\right] \times 100\% \quad (1)$$

$$Syneresis = \frac{whey\ weight}{initial\ weight} \text{ x } 100\% \quad (2)$$

the viscosity of yoghurt was measured using viscometer (NDJ-8S, spindle number 3), while colour of yoghurt was measured using digital handled colorimeter (CS-10).

2.5 Antioxidant assay

The antioxidant activity measurement used 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. Briefly, the yoghurt water extract was mixed with 50µM of DPPH solution in methanol with ratio 1:5. The mixture was incubated in the darkness for 30 min. After incubation, the absorbance of mixture was measured at 517 nm. Methanol was used as blank and DPPH solution as control. The percent of DPPH radical inhibition of the samples was calculated according to the following Equation 3.

$$\%inhibition = \frac{Ac - As}{Ac} \times 100\% \quad (3)$$

where: Ac for absorbance of control ; As for absorbance of samples.

3 Results and Discussion

Consumption of yoghurt as a dairy product is associated with improved wellness. Yoghurt as fermented milk products are made by adding various live LAB. Furthermore, the human gastrointestinal tract is colonized by various and complex microbiome populations, commonly called gut microbiome diversity. The diversity of gut microbiomes regulated many critical functions in metabolism, including extracting nutrients, modulating digestive and immune system homeostasis, and protecting against pathogens[17]. Conversely, the popularity of yoghurt consumption also affected by its sensory properties, including texture, color, and flavor.

Texture of yoghurt depends on the percentage of WHC and syneresis. High-level syneresis is indicated as a low-quality yoghurt product in set yogurt. Meanwhile, high-level of WHC is generally regarded as high-quality of seet yoghurt. The syneresis and WHC in 4 types of yoghurt resulted that the syneresis and WHC of standard yoghurt was 6.19% and 93.81%, yoghurt added with *B.longum* was 6.73% and 93.27%, yoghurt with encapsulated *B.longum* was 10.83% and 89.17%, and purple yam yoghurt with encapsulated *B.longum* was 5.61% and 94.39% (Table 1). These result indicated that addition of *B.longum* standard yoghurt increased the sensory properties according to WHC, syneresis, also supported with viscosity value which up to 17.60 cPa, meanwhile standard yoghurt only reached 9.00 cPa of viscosity value. Bifidobacteria has been reported as its ability to exhibit a characteristic aroma and slightly acidic flavour [18].

Moreover, Bifidobacteria also reported that more stable than Lactobacillus plantarum, L. acidophilus, and L. casei [19]. On the other hand, purple yam yoghurt enriched with encapsulated B.longum has the highest WHC and lowest syneresis. The texture of yoghurt in purple yam yoghurt is the best among other yoghurt types caused by inulin that found in yam tuber. Inulin is a prebiotic which can support probiotic bacteria growth [20]. Fortification purple yam tuber could increase the sensory properties, especially in texture.

Antioxidant activity of various yoghurt show that purple yam yoghurt enriched with encapsulated *B.longum* has the highest percentage inhibition of DPPH activity (58.83%), while standard yoghurt only reached 44.09%. Purple yam (*D. alata*) contains rich source of anthocyanins including cyanidin-3-gentiobioside, alatanins 1 and 2, alantanin A-C, petunidin-3-gentiobiosides which has high antioxidant activity [21], [22]. Lubag et al. also reported that the antioxidant activity of purple yam (*D.alata*) had higher antioxidant activity compared with white yam, α-tocopherol, and butylhydrixyanisole (BHA).

Table 1. Rheology and antioxidant activity of various yoghurt

Sample of yoghurt	pH	Syneresis (%)	WHC (%)	Colour	Viscosity (cPa)	DPPH (% inhibition)
Standard yoghurt	4.98	6.19	93.81	0.00	9.00	44.09
Yoghurt added with B.longum	4.54	6.73	93.27	2.05	17.60	43.47
Yoghurt with encapsulated B.longum	5.21	10.83	89.17	1.56	6.00	45.79
Purple yam yoghurt with encapsulated B.longum	4.46	5.61	94.39	6.68	23.00	58.83

4 CONCLUSION

In conclusion, fortification of purple yam enriched with encapsulated *B. longum* increased the sensory properties based on WHC, syneresis, viscosity and also antioxidant activity of yoghurt. Purple yam-rich anthocyanin can be used as a novel taste of yoghurt, which has greater health benefits than only plain standard yoghurt.

We would like to thank the Ministry of Education, Culture, Research and Technology for supporting this research with grant funding number 3.4.36/UN32.20.1/LT/2023.

References

1. M. Sharifi-Rad et al., “Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases,” Front. Physiol., vol. 11, no. July, pp. 1–21, 2020, doi: 10.3389/fphys.2020.00694.
2. Y. Ali Moustafa Elkhateeb, “Effects of Fast Foods in Relation to Free Radicals and Antioxidants,” Am. J. Lab. Med., vol. 2, no. 6, p. 156, 2017, doi: 10.11648/j.ajlm.20170206.17.
3. M. L. Urso and P. M. Clarkson, “Oxidative stress, exercise, and antioxidant supplementation,” Toxicology, vol. 189, no. 1–2, pp. 41–54, 2003, doi: 10.1016/S0300-483X(03)00151-3.
4. J. Cadet and J. Richard Wagner, “DNA base damage by reactive oxygen species, oxidizing agents, and UV radiation,” Cold Spring Harb. Perspect. Biol., vol. 5, no. 2, pp. 1–18, 2013, doi: 10.1101/cshperspect.a012559.
5. B. N. P. Sah, T. Vasiljevic, S. McKechnie, and O. N. Donkor, “Effect of probiotics on antioxidant and antimutagenic activities of crude peptide extract from yogurt,” Food Chem., vol. 156, pp. 264–270, 2014, doi: 10.1016/j.foodchem.2014.01.105.
6. K. H. Sabeena Farvin, C. P. Baron, N. S. Nielsen, J. Otte, and C. Jacobsen, “Antioxidant activity of yoghurt peptides: Part 2 - Characterisation of peptide fractions,” Food Chem., vol. 123, no. 4, pp. 1090–1097, 2010, doi: 10.1016/j.foodchem.2010.05.029.
7. P. Vitheejongjaroen, A. Kasorn, N. Puttarat, F. Loison, and M. Taweechotipatr, “Bifidobacterium animalis MSMC83 Improves Oxidative Stress and Gut Microbiota in D-Galactose-Induced Rats,” Antioxidants, vol. 11, no. 11, 2022, doi: 10.3390/antiox11112146.

8. L. Zhao et al., “Identification, Characterization, and Antioxidant Potential of Bifidobacterium longum subsp. longum Strains Isolated From Feces of Healthy Infants,” Front. Microbiol., vol. 12, no. November, 2021, doi: 10.3389/fmicb.2021.756519.
9. S. J. Kim et al., “Hypocholesterolemic effects of probiotic mixture on diet-induced hypercholesterolemic rats,” Nutrients, vol. 9, no. 3, pp. 1–10, 2017, doi: 10.3390/nu9030293.
10. [M. Sbehat, G. Mauriello, and M. Altamimi, “Microencapsulation of Probiotics for Food Functionalization: An Update on Literature Reviews,” Microorganisms, vol. 10, no. 10, pp. 1–17, 2022, doi: 10.3390/microorganisms10101948.
11. W. Y. Koh, X. X. Lim, T. C. Tan, R. Kobun, and B. Rasti, “Encapsulated Probiotics: Potential Techniques and Coating Materials for Non-Dairy Food Applications,” Appl. Sci. Switz., vol. 12, no. 19, 2022, doi: 10.3390/app121910005.
12. M. Yao, J. Xie, H. Du, D. J. McClements, H. Xiao, and L. Li, “Progress in microencapsulation of probiotics: A review,” Compr. Rev. Food Sci. Food Saf., vol. 19, no. 2, pp. 857–874, 2020, doi: 10.1111/1541-4337.12532.
13. A. D. C. Pech-canul, D. Ortega, A. Garcia-Triana, N. Gonzalez-Silva, and R. L. Solis-Oviedo, “A Brief Review of Edible Coating Materials for the,” Coating, vol. 10, no. 197, pp. 1–34, 2020.
14. X. Qi, S. Simsek, J. B. Ohm, B. Chen, and J. Rao, “Viability of: Lactobacillus rhamnosus GG microencapsulated in alginate/chitosan hydrogel particles during storage and simulated gastrointestinal digestion: Role of chitosan molecular weight,” Soft Matter, vol. 16, no. 7, pp. 1877–1887, 2020, doi: 10.1039/c9sm02387a.
15. [P. Pupa et al., “The efficacy of three double-microencapsulation methods for preservation of probiotic bacteria,” Sci. Rep., vol. 11, no. 1, pp. 1–9, 2021, doi: 10.1038/s41598-021-93263-z.
16. [P. Muniandy, A. B. Shori, and A. S. Baba, “Influence of green, white and black tea addition on the antioxidant activity of probiotic yogurt during refrigerated storage,” Food Packag. Shelf Life, vol. 8, pp. 1–8, Jun. 2016, doi: 10.1016/j.fpsl.2016.02.002.
17. D. A. Savaiano and R. W. Hutkins, “Yogurt, cultured fermented milk, and health: a systematic review,” Nutr. Rev., vol. 79, no. 5, pp. 599–614, May 2020, doi: 10.1093/nutrit/nuaa013.
18. C. Chen, S. Zhao, G. Hao, H. Yu, H. Tian, and G. Zhao, “Role of lactic acid bacteria on the yogurt flavour: A review,” Int. J. Food Prop., vol. 20, no. sup1, pp. S316–S330, Dec. 2017, doi: 10.1080/10942912.2017.1295988.
19. R. Soni, N. K. Jain, V. Shah, J. Soni, D. Suthar, and P. Gohel, “Development of probiotic yogurt: effect of strain combination on nutritional, rheological, organoleptic and probiotic properties,” J. Food Sci. Technol., vol. 57, no. 6, pp. 2038–2050, Jun. 2020, doi: 10.1007/s13197-020-04238-3.
20. E. Zubaidah and W. Akhadiana, “Comparative Study of Inulin Extracts from Dahlia, Yam, and Gembili Tubers as Prebiotic,” Food Nutr. Sci., vol. 04, no. 11, pp. 8–12, 2013, doi: 10.4236/fns.2013.411A002.
21. S. Srivichai and P. Hongsprabhas, “Profiling Anthocyanins in Thai Purple Yams (Dioscorea alata L.),” Int. J. Food Sci., vol. 2020, p. e1594291, Jul. 2020, doi: 10.1155/2020/1594291.
22. C. Moriya et al., “New acylated anthocyanins from purple yam and their antioxidant activity,” Biosci. Biotechnol. Biochem., vol. 79, no. 9, pp. 1484–1492, Sep. 2015, doi: 10.1080/09168451.2015.1027652.