

Functional Food Formulation Of Spinach (*Amaranthus*) With Pineapple (*Ananas Comosus*) Which Is High In Antioxidants As A Potential Antidepressant

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Abstract

Probiotics, such as *Lactobacillus paracasei*, are living microorganisms that, if administered sufficiently, may provide beneficial health properties to the host through balancing the gastroenteric microbiota. There is growing evidence that probiotics may have a beneficial effect on depression, stress, and mental health as an antidepressant which are known as psychobiotics. A recent review also highlighted the health benefits of pineapple, such as hypocholesterolemic, antiatherogenic, anti-inflammatory, and affecting nervous system function while spinach is also known for its many beneficial health properties, such as antiviral, anti-stress and anti-depressive, anticancer, anti-obesity, and hypoglycemic effects. This research aims to process pineapple and spinach into a probiotic drink and determine the best formula with the highest antioxidant activity and vitamin C content. There was 3 samples formulation based on spinach, pineapple, and water concentration (S1 = 1:1:0.5; S2 = 2:1:1; and S3 = 3:2:2). All ingredients are mixed and stirred using a blender-type mixing machine for 15 minutes. Next, all samples were inoculated with *Lactobacillus paracasei* 5% b/v for 14 days under anaerobic conditions. Sample variation was carried out to determine the best formulation based on average antioxidant activity and vitamin C content in it. The vitamin C content was determined using the Titration Iodometric Method while antioxidant activity was determined with 2,2-diphenyl-1-picrylhydrazyl (DPPH), each with a triplicate. The results of vitamin C content and antioxidant activity in each sample of probiotic drinks were as follow; S1 contains 110.50mg/100g vitamin C with 30.31% antioxidant activity, S2 contains 111.67mg/100 g vitamin C with 33.74% antioxidant activity, and S3 contains 127.66mg/100g vitamin C with 37.42% antioxidant activity. A significant difference ($p < 0.05$) was found in both the vitamin C content and antioxidant activity between samples. This shows that the formulation of probiotic drinks from pineapple and spinach fermented with *Lactobacillus paracasei* contains high vitamin C content and antioxidant activity with the potential as an antidepressant functional food.

Keywords: Probiotic, Pineapple (*Ananas comosus*), Spinach (*Amaranthus*), Antioxidant, Antidepressant

Introduction

Probiotics, such as *Lactobacillus paracasei*, are living microorganisms that, if administered sufficiently, may provide beneficial health properties to the host through balancing the gastroenteric microbiota (Margiotta et al., 2021). Probiotic is categorized as functional food, a type of food that contains immune boosters, bioactive compounds, vitamins, and minerals that help fight viral infections (Alkhatib, 2020). Probiotics also

have many roles in combating respiratory infections, controlling gut microbiota, mucosal immune response, and immune enhancers (Sundararaman et al., 2020). Furthermore, there is growing evidence that probiotics may have a beneficial effect on depression, stress, and mental health as an antidepressant (Ansari et al., 2020; Liu et al., 2019) which are known as psychobiotics (Cheng et al., 2019).

Pineapple (*Ananas comosus*) is a high-nutritive tropical fruit with several bioactive compounds, mainly phenolics and flavonoids (Sayago-Ayerdi et al., 2021), and rich in vitamins and minerals (Mohd Ali et al., 2020). Flavonoids contribute to high-antioxidant activities (Londok et al., 2017) and improve immunity (Yang et al., 2020). A recent review also highlighted the health benefits of pineapple, such as hypocholesterolemic, antiatherogenic, anti-inflammatory, and affecting nervous system function (Mohd Ali et al., 2020; Sayago-Ayerdi et al., 2021). Spinach (*Amaranthus*) is also known for its many beneficial health properties, such as antiviral (Chang et al., 2020), anti-stress and anti-depressive (Son et al., 2018), management of hepatic steatosis (Inés Elvira-Torales et al., 2020), non-alcoholic fatty liver disease (NAFLD) (Mokhtari et al., 2021), anticancer, anti-obesity, and hypoglycemic effects (Roberts & Moreau, 2016).

Looking at all those health-beneficial properties, pineapple and spinach can be utilized as a functional probiotic drink with high antioxidant activity and a potential antidepressant property. This research aims to process pineapple and spinach into a probiotic drink and determine the best formula with the highest antioxidant activity and vitamin C content.

Methods

There was 3 samples formulation based on spinach, pineapple, and water concentration (S1 = 1:1:0.5; S2 = 2:1:1; and S3 = 3:2:2). All ingredients are mixed and stirred using a blender-type mixing machine for 15 minutes. Next, all samples were inoculated with *Lactobacillus paracasei* 5% b/v for 14 days under anaerobic conditions. Sample variation was carried out to determine the best formulation based on average antioxidant activity and vitamin C content in it. The vitamin C content was determined using the Titration Iodometric Method while antioxidant activity was determined with 2,2-diphenyl-1-picrylhydrazyl (DPPH), each with a triplicate.

$$\text{Vitamin C} \left(\frac{\text{mg}}{100\text{g}} \right) = \frac{\text{VI2} \times 0,88 \times \text{Fp} \times 100}{\text{Ws gram}}$$

Results and Discussion

The results of vitamin C content and antioxidant activity in each sample of probiotic drinks were as follow; S1 contains 110.50mg/100g vitamin C with 30.31% antioxidant activity, S2 contains 111.67mg/100 g vitamin C with 33.74% antioxidant activity, and S3 contains 127.66mg/100g vitamin C with 37.42% antioxidant activity. A significant difference ($p < 0.05$) was found in both the vitamin C content and antioxidant activity between samples. This result highlighted the high-antioxidant activity in the probiotic drinks from spinach and pineapples fermented with *L. paracasei*. The average vitamin C content in the three probiotic drink samples was 116.61 ± 9.59 mg/100g. S3 showed the best antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) at 37.42%. The addition of pineapple contributed to increasing vitamin C content and antioxidant activity of the probiotic drink (Kusumawati et al., 2019).

The fermented spinach and pineapples have the potential to be developed into a functional probiotic drink. The antioxidant activity and vitamin C content may contribute to lowering the risk for non-communicable diseases, improving the oxidant balance (Barchitta et al., 2019; Mohammed et al., 2016), and enhancing immunity (Biesalski et al., 2010). Higher intake of fruit and vegetable, both raw and processed, also predicted higher positive mood and reduced depressive symptoms (Brookie et al., 2018). Serotonin content in both

spinach and pineapples proved their potential as an antidepressant since pineapple is categorized as food with high serotonin content ($17.0 \pm 5.1 \mu\text{g/g}$) and spinach has a moderate serotonin content ($0.1 \mu\text{g/g}$) (Feldman & Lee, 1985). Spinach also worked as an antidepressant by reducing blood corticosterone levels and increasing glutamate and glutamine levels (Son et al., 2018). This shows that the formulation of probiotic drinks from pineapple and spinach fermented with *Lactobacillus paracasei* contains high vitamin C content and antioxidant activity with the potential as an antidepressant functional food.

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Reference

- Alkhatib, A. (2020). Antiviral functional foods and exercise lifestyle prevention of coronavirus. *Nutrients*, 12(9), 1–17. <https://doi.org/10.3390/nu12092633>
- Ansari, F., Pourjafar, H., Tabrizi, A., & Homayouni, A. (2020). The Effects of Probiotics and Prebiotics on Mental Disorders: A Review on Depression, Anxiety, Alzheimer, and Autism Spectrum Disorders. *Current Pharmaceutical Biotechnology*, 21(7), 555–565. <https://doi.org/10.2174/1389201021666200107113812>
- Barchitta, M., Maugeri, A., Favara, G., San Lio, R. M., Evola, G., Agodi, A., & Basile, G. (2019). Nutrition and wound healing: An overview focusing on the beneficial effects of curcumin. *International Journal of Molecular Sciences*, 20(5). <https://doi.org/10.3390/ijms20051119>
- Biesalski, H. K., Grune, T., Tinz, J., Zöllner, I., & Blumberg, J. B. (2010). Reexamination of a meta-analysis of the effect of antioxidant supplementation on mortality and health in randomized trials. *Nutrients*, 2(9), 929–949. <https://doi.org/10.3390/nu2090929>
- Brookie, K. L., Best, G. I., & Conner, T. S. (2018). Intake of raw fruits and vegetables is associated with better mental health than intake of processed fruits and vegetables. *Frontiers in Psychology*, 9(APR), 1–14. <https://doi.org/10.3389/fpsyg.2018.00487>
- Chang, Y. J., Pong, L. Y., Hassan, S. S., & Choo, W. S. (2020). Antiviral activity of betacyanins from red pitahaya (*Hylocereus polyrhizus*) and red spinach (*Amaranthus dubius*) against dengue virus type 2 (GenBank accession no. MH488959). *Access Microbiology*, 2(1). <https://doi.org/10.1099/acmi.0.000073>
- Cheng, L. H., Liu, Y. W., Wu, C. C., Wang, S., & Tsai, Y. C. (2019). Psychobiotics in mental health, neurodegenerative and neurodevelopmental disorders. *Journal of Food and Drug Analysis*, 27(3), 632–648. <https://doi.org/10.1016/j.jfda.2019.01.002>
- Feldman, M., & Lee, M. (1985). Serotonin excretion content of foods : effect of 5-hydroxyindoleacetic. *Clinical Nutrition*, February, 639–643.
- Inés Elvira-Torales, L., Navarro-González, I., Rodrigo-García, J., Seva, J., García-Alonso, J., & Jesús Periago-Castón, M. (2020). Consumption of spinach and tomato modifies lipid metabolism, reducing hepatic steatosis in rats. *Antioxidants*, 9(11), 1–21. <https://doi.org/10.3390/antiox9111041>
- Kusumawati, I., Purwanti, R., & Afifah, D. N. (2019). ANALISIS KANDUNGAN GIZI DAN AKTIVITAS ANTIOKSIDAN PADA YOGHURT DENGAN PENAMBAHAN NANAS MADU (*Ananas Comosus* Mer.) DAN EKSTRAK KAYU MANIS (*Cinnamomum Burmanni*). *Journal of Nutrition College*, 8(4), 196–206. <https://doi.org/10.14710/jnc.v8i4.25833>

Liu, R. T., Walsh, R. F. L., & Sheehan, A. E. (2019). Prebiotics and probiotics for depression and anxiety: A systematic review and meta-analysis of controlled clinical trials. *Neurosci Biobehav*, 102, 13–23. <https://doi.org/10.1016/j.neubiorev.2019.03.023>

Londok, J. J. M. R., Manalu, W., Wiryawan, K. G., & Sumiati. (2017). Growth performance, carcass characteristics and fatty acids profile of broilers supplemented with lauric acid and natural antioxidant from *Areca vestiaria giseke*. *Pakistan Journal of Nutrition*, 16(9), 719–730. <https://doi.org/10.3923/pjn.2017.719.730>

Margiotta, G., Ferretti, S., Graglia, B., Gatto, A., Capossela, L., Bersani, G., Curatola, A., & Chiaretti, A. (2021). Effect of *Lactobacillus reuteri* LRE02-*Lactobacillus rhamnosus* LR04 combination and gastrointestinal functional disorders in an Emergency Department pediatric population. *European Review for Medical and Pharmacological Sciences*, 25(7), 3097–3104. https://doi.org/10.26355/eurev_202104_25564

Mohammed, B. M., Fisher, B. J., Kraskauskas, D., Ward, S., Wayne, J. S., Brophy, D. F., Fowler, A. A., Yager, D. R., & Natarajan, R. (2016). Vitamin C promotes wound healing through novel pleiotropic mechanisms. *International Wound Journal*, 13(4), 572–584. <https://doi.org/10.1111/iwj.12484>

Mohd Ali, M., Hashim, N., Abd Aziz, S., & Lasekan, O. (2020). Pineapple (*Ananas comosus*): A comprehensive review of nutritional values, volatile compounds, health benefits, and potential food products. *Food Research International*, 137(September), 109675. <https://doi.org/10.1016/j.foodres.2020.109675>

Mokhtari, E., Farhadnejad, H., Salehi-Sahlabadi, A., Najibi, N., Azadi, M., Teymoori, F., & Mirmiran, P. (2021). Spinach consumption and nonalcoholic fatty liver disease among adults: a case-control study. *BMC Gastroenterology*, 21(1), 1–9. <https://doi.org/10.1186/s12876-021-01784-8>

Roberts, J. L., & Moreau, R. (2016). Functional properties of spinach (*Spinacia oleracea* L.) phytochemicals and bioactives. *Food and Function*, 7(8), 3337–3353. <https://doi.org/10.1039/c6fo00051g>

Sayago-Ayerdi, S., García-Martínez, D. L., Ramírez-Castillo, A. C., Ramírez-Concepción, H. R., & Viuda-Martos, M. (2021). Tropical Fruits and Their Co-Products as Bioactive Compounds and Their Health Effects: A Review. *Foods*, 10(8), 1952. <https://doi.org/10.3390/foods10081952>

Son, H., Jung, S., Shin, J., Kang, M., & Kim, H. (2018). Anti-Stress and Anti-Depressive Effects of Spinach Extracts on a Chronic Stress-Induced Depression Mouse Model through Lowering Blood Corticosterone and Increasing Brain Glutamate and Glutamine Levels. *Journal of Clinical Medicine*, 7(11), 406. <https://doi.org/10.3390/jcm7110406>

Sundararaman, A., Ray, M., Ravindra, P. V., & Halami, P. M. (2020). Role of probiotics to combat viral infections with emphasis on COVID-19. *Applied Microbiology and Biotechnology*, 104(19), 8089–8104. <https://doi.org/10.1007/s00253-020-10832-4>

Yang, F., Zhang, Y., Tariq, A., Jiang, X., Ahmed, Z., Zhihao, Z., Idrees, M., Azizullah, A., Adnan, M., & Bussmann, R. W. (2020). Food as medicine: A possible preventive measure against coronavirus disease (COVID-19). *Phytotherapy Research : PTR*, 34(12), 3124–3136. <https://doi.org/10.1002/ptr.6770>

Sample	Anti-Oxidant Activity towards DPPH (%)	Vitamin C (mg/100g)
S1	30.31	110.50

S2	33.74	111.67
S3	37.42	127.66
Mean	33.82 ± 3.56	116.61 ± 9.59