**Effect of Adding Encapsulated Amaranth Microgreens Extract on Antioxidant, Microbiological, Physicochemical and Sensory Properties of Yogurt**

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**Abstract**

In this study, encapsulation of amaranth microgreens extract was carried out using complex coacervation method with bovine gelatin and gum arabic in order to increase the stability and bioavailability of its bioactive components. FTIR and XRD analyses of the obtained capsules confirmed a successful encapsulation process and the amorphous structure of the extract. The pH, acidity, antioxidant activity, microbiological properties, color and sensory properties of yogurt samples to which capsules were added at 0.1% and 0.5% levels were examined during 4 weeks of storage. Yogurts with capsule addition showed higher performance than the control group in terms of antioxidant activity. E. coli and S. aureus were not detected in microbiological analyses. The yogurt sample with 0.1% capsule addition was appreciated more than the control group in sensory evaluation. The results show that encapsulation of amaranth microgreens extract preserves the stability of bioactive components and increases the nutritional value of yogurt, providing a functional product.

Keywords: Amaranth microgreens, antioxidant, encapsulation, yogurt

1. **INTRODUCTION**

Healthy nutrition is a determining factor in preventing diseases and maintaining well-being throughout life, leading the society to healthy nutrition. With the increasing interest in healthy nutrition, the trend towards fresh, nutraceutical and functional foods is also increasing [1], [2]. The stability of bioactive compounds is of critical importance for their proper inclusion in food systems.

Bioactive components such as vitamins, minerals, polyphenols and probiotics are sensitive to environmental factors such as light, heat, water and oxygen, which limits their nutritional value and shelf life. In addition, changes in the chemical structures of bioactive compounds during processes in the gastrointestinal tract can also negatively affect their stability. The encapsulation method protects bioactive compounds from environmental factors by trapping them in a protective matrix, thus helping to overcome these problems. This method also offers the advantage of not significantly changing the rheological and sensory properties of food [3].

Recent research to increase the efficacy and health benefits of bioactive ingredients focuses on the encapsulation of various enzymes, coenzymes, therapeutic agents or bioactive peptides [4]. The protection of bioactive ingredients from environmental stress factors such as temperature, humidity, oxygen, radiation, light and adverse pH conditions and the control of the release of active ingredients from food matrices at the desired rate, at the right time and in the right place are among the main objectives. In addition, recent trends in encapsulation technology aim to increase the bioavailability of a nutrient, facilitate the addition of bioactive ingredients to foods without changing their sensory properties in foods and thus improve product use [4]. Encapsulation is defined as the encapsulation of an active ingredient in a matrix consisting of an inert material. Encapsulation technology also acts as a barrier to protect bioactive compounds such as polyphenols, antioxidants, micronutrients, and enzymes against heat, light, moisture, pH, oxygen, or other adverse conditions [5]. Nanoencapsulation protects the bioactive compounds to be used (nutraceuticals, polyphenols, antioxidants, micronutrients, and enzymes) from environmental conditions in the capsule and provides controlled release of these compounds in targeted areas. Thus, targeted distribution and controlled release of bioactive compounds are improved and their bioavailability is also increased [6],[7].

Many encapsulation materials can be used for encapsulation [4], [7]. The most commonly used encapsulation materials in the food industry include amylose, amylopectin, starch and its derivatives, maltodextrins, dextrins, cellulose and polydextrin and their derivatives, polysaccharides. In addition, polysaccharides such as alginate, carrageenan, dextran, xanthan, chitosan and gellan are also used. Lipid materials used in food applications are fatty acids, glycerides, waxes (carnauba, candelilla, beeswax), phospholipids and paraffin [7]. One of the most widely used biopolymers for drug and food delivery applications is gelatin [8]. It is a good candidate for use in food systems due to its biodegradability, biocompatibility, easy modification and low cost [9]. High value-added food products can be produced in the food industry with nanoencapsulation technology [10].

Microgreens produced from the seeds of many crops such as legumes, cereals, pseudo-cereals, vegetables, plants and oilseeds have an increasing popularity due to their strong aroma and high vitamin, mineral and phytochemical contents [11], [12]. Amaranth microgreens also contain compounds with high antioxidant and radical scavenging properties such as phenolic compounds, flavonoids, tocopherols and betalain. It is suggested that its overall eating quality is high compared to many different microgreens [13]. As a fermented dairy product, yogurt is an excellent source of protein, calcium, phosphorus, magnesium, zinc, riboflavin, niacin, folate and vitamin B12. On the other hand, since fermented dairy products are among the most consumed foods in the world, it is reported that fortification of fermented dairy products such as yogurt is a good way to improve the intake of nutrients in daily nutrition [14].

In this study, the physicochemical, microbiological and biochemical properties of yogurt enriched with encapsulated amaranth microgreens extract were investigated.

1. **MATERIALS AND METHODS**

* 1. **Production and extraction of Amaranth Microgreens**

Amaranth microgreens seeds were planted in a suitable container in a fertile soil, moistened with water and then left to germinate for 1-2 days by covering in the dark. Then, 25 g were weighed and distilled water was added and blended and the mixture was mixed with a magnetic stirrer at 500 rpm for 24 hours. Afterwards, microgreens extraction was obtained by filtering with filter paper.



Figure 1. Amaranth microgreens

* 1. **Preparation of Nanocapsules**

Amaranth microgreens extract was encapsulated by complex coacervation method using bovine gelatin and gum Arabic. Characterization of capsule samples prepared with amaranth microgreens extract was performed by FTIR analysis and XRD measurements.

* 1. **Preparation of Yogurt Samples**

Boxed milks containing 1.5% milk fat were heated to 90°C and cooled to 45°C for incubation. Probiotic-containing lyophilized yogurt starter culture was added to 300 mL of milk at 45°C to activate it. Then, this mixture was added to milk cooled to 45°C, mixed, and filled into 150 mL sterile jars and incubated at 45°C for fermentation. After incubation, yogurt samples were cooled to 4°C and stored overnight. One group was separated as control yogurt and the other two groups were mixed with capsules at 0.1% and 0.5%. Samples were stored at 4°C for analyses to be performed in weeks 1, 2, 3 and 4.

**2.4 Antioxidant Activity Analyses**

Antioxidant activity in yogurt samples was determined using KUPRAK determination, iron (Fe3+) ion reducing antioxidant power (FRAP) determination and DPPH● free radical scavenging activity determination methods.

* 1. **Microbiological analyses**

Antimicrobial analysis of yogurt samples was performed on Mueller Hinton Agar (MHA) plates using the agar well diffusion method.

* 1. **Sensory Analyses**

Yoghurt samples were prepared as 3 samples. Prepared yogurt samples were presented to the panelists in PET cups as coded.

K: Control yoğurt

Sample 1: Sample mixed with 0.5% capsules

Sample 2: Sample mixed with 0.1% capsules

Then, the panelists were asked to evaluate the given yogurt samples in terms of color, odor, consistency, taste and general acceptability by filling out the sensory evaluation form from 1 to 10 (very bad to very good).

* 1. **Statistical Analyses**

The statistical analysis of the data obtained as a result of the studies was evaluated using the GraphPad Prism 5.00 Software (GraphPad Software, La Jolla, CA) statistical program and p values ​​were determined by performing a two-way ANOVA test. Significance level p>0.05 = ns was evaluated as insignificant, \*p<0.05 as significant, \*\*p<0.01 as very significant, \*\*\*p<0.001 as highly significant.

1. **RESULTS AND DISCUSSION**

The capsules prepared with amaranth microgreens extract were characterized by FTIR and XRD analyses. According to the XRD analysis results, it was determined that the amaranth microgreens extract had a largely amorphous structure and contained high levels of organic compounds. According to the FTIR analysis findings, it was determined that the capsules were successfully formed. With the obtained capsules, 2 different yogurt samples with 0.1% and 0.5% capsule addition were developed and plain yogurt was used as a control. All developed yogurt samples were stored for 4 weeks and pH, acidity, antioxidant (Cuprak, FRAP, DPPH•), microbiological (*E. coli and S. aureus*), colorimetric and sensory analyses of the samples were performed. According to the antioxidant activity results, it was determined that the yogurt samples with capsule addition exhibited the highest antioxidant activity in all weeks. It was also observed that the antioxidant capacity of yoghurt increased in a study enriched with encapsulation with olive leaf extract [15]. According to the microbiological analyses, *E. coli* and *S. aureus* were not detected in the yogurt samples. Similarly, the effect of carrot waste extract capsule on the microbiological profiles of yoghurt was tested and no values ​​were found for *Salmonella*, *Listeria monocytogenes* and *Staphylococcus aureus* [16]. According to the colorimetric analysis results, it was determined that the capsule addition did not affect the whiteness and greenness of the yogurt but affected the yellowness. According to the sensory analysis results, the yogurt sample with 0.1% capsule added received higher scores in terms of taste parameters and was liked more compared to the control yogurt. In a previous study, it was shown that Doogh samples, a traditional Iranian yogurt enriched with nanoemulsion, received higher scores than samples with free nettle extract, and it was stated that the nanocapsules of nettle extract in Doogh could mask the intense taste and that this could facilitate its use as a functional ingredient for yogurt [17].

1. **CONCLUSIONS**

As a result of the research, by adding amaranth microgreens extract to yogurt by encapsulating it, the stability of the bioactive components contained in amaranth microgreens against environmental conditions was maintained and thus their beneficial health effects were utilized. In addition, the nutritional value of the yogurt was increased and a functional food with high antioxidant activity, microbiological safety and generally accepted by the panelists was obtained.

**References**

1. Corzo, L., Fernández-Novoa, L., Carrera, I., Martínez, O., Rodríguez, S., Alejo, R., & Cacabelos, R. (2020). Nutrition, health, and disease: Role of selected marine and vegetal nutraceuticals. *Nutrients,* 12(3), 747.
2. Gul, K., Singh, A., & Jabeen, R. (2016). Nutraceuticals and functional foods: the foods for the future world. *Critical Reviews in Food Science and Nutrition*, 56(16), 2617-2627.
3. Đorđević, V., Balanč, B., Belščak-Cvitanović, A., Lević, S., Trifković, K., Kalušević, A., Kostić, I., Komes, D., Bugarski, B., & Nedović, V. (2015). Trends in encapsulation technologies for delivery of food bioactive compounds. Food Engineering Reviews, 7, 452-490. https://doi.org/10.1007/s12393-014-9106-7
4. Timilsena, Y. P., Vongsvivut, J., Tobin, M. J., Adhikari, R., Barrow, C., & Adhikari, B. (2019). Investigation of oil distribution in spray-dried chia seed oil microcapsules using synchrotron-FTIR microspectroscopy. Food Chemistry, 275, 457-466.
5. Rahaiee, S., Assadpour, E., Esfanjani, A. F., Silva, A. S., & Jafari, S. M. (2020). Application of nano/microencapsulated phenolic compounds against cancer. Advances in Colloid And Interface Science, 279, 102153.
6. Ezhilarasi, P., Karthik, P., Chhanwal, N., & Anandharamakrishnan, C. (2013). Nanoencapsulation techniques for food bioactive components: a review. *Food and Bioprocess Technology*, 6, 628-647.
7. Tutun, S., & Yurdakul, O. (2022). Enkapsülasyon ve gıda teknolojisinde kullanımı. Veteriner Farmakoloji ve Toksikoloji Derneği Bülteni, 13(2), 99-119.
8. Jiang, X., Du, Z., Zhang, X., Zaman, F., Song, Z., Guan, Y., ... & Huang, Y. (2023). Gelatin-based anticancer drug delivery nanosystems: A mini review. Frontiers in Bioengineering and Biotechnology, 11, 1158749.
9. Omnia M. A., Hashem, Y., Bekhit, A. A., Khattab, S. N., Elkhodairy, K. A., Freag, M. S., ... & Elzoghby, A. O. (2019). Nanostructures of gelatin for encapsulation of food ingredients. In Biopolymer Nanostructures for Food Encapsulation Purposes (pp. 189-216). *Academic Press.*
10. Matalanis, A., Jones, O. G., & McClements, D. J. (2011). Structured biopolymer-based delivery systems for encapsulation, protection, and release of lipophilic compounds. Food Hydrocolloids, 25(8), 1865-1880.
11. Kyriacou, M. C., Rouphael, Y., Di Gioia, F., Kyratzis, A., Serio, F., Renna, M., De Pascale, S., & Santamaria, P. (2016). Micro-scale vegetable production and the rise of microgreens. Trends in Food Science & Technology, 57, 103-115.
12. Bhaswant, M., Shanmugam, D. K., Miyazawa, T., Abe, C., & Miyazawa, T. (2023). Microgreens: a comprehensive review of bioactive molecules and health benefits. Molecules, 28(2), 867.
13. Xiao, Z., Lester, G. E., Park, E., Saftner, R. A., Luo, Y., & Wang, Q. (2015). Evaluation and correlation of sensory attributes and chemical compositions of emerging fresh produce: Microgreens. Postharvest Biology and Technology, 110, 140-148.
14. Gahruie, H. H., Eskandari, M. H., Mesbahi, G., & Hanifpour, M. A. (2015). Scientific and technical aspects of yogurt fortification: A review. *Food Science and Human Wellness,* 4(1), 1-8.
15. Tavakoli, H., Hosseini, O., Jafari, S. M., & Katouzian, I. (2018). Evaluation of physicochemical and antioxidant properties of yogurt enriched by olive leaf phenolics within nanoliposomes. *Journal of Agricultural and food chemistry*, 66(35), 9231-9240.
16. Šeregelj, V., Pezo, L., Šovljanski, O., Lević, S., Nedović, V., Markov, S., Tomić, A., Čanadanović-Brunet, J., Vulić, J., & Šaponjac, V. T. (2021). New concept of fortified yogurt formulation with encapsulated carrot waste extract. *Lwt*, 138, 110732.
17. Amiri, Z. R., Nemati, A., Tirgarian, B., Dehghan, B., & Nasiri, H. (2021). Influence of stinging nettle *Urtica dioica* L.) extract-loaded nano-emulsion on the storage stability and antioxidant attributes of Doogh (Traditional Iranian yoghurt beverage). *Journal of Food Measurement and Characterization*, 15, 437-448.