RESEARCH SCIENTIFIC PAPER
DOI: 10.63395/STEDConf14022025932K259

Submitted: 21.08.2025.
Accepted: 08.11.2025.

# CIRCULAR ECONOMY: IMMOBILIZATION OF *LACTOBACILLUS ACIDOPHILUS* ON FLAXSEED CAKE

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## ABSTRACT

*Lactobacillus acidophilus* has been increasingly studied as a probiotic for allergy treatment. For probiotics to deliver their beneficial effects, they need to be present in adequate numbers, which is often achieved by immobilizing them within protective matrices. A flaxseed cake is rich in fibers, proteins, and bioactive components, which makes it a potential good carrier for probiotics.

Flaxseed cake, obtained from the cold-pressed oils industry, was ground, sieved to a diameter of less than 600μm, sterilized, and diluted in distilled water at 10 % (w/v). After *L. acidophilus* inoculation, one sample was fermented, incubated at 4 °C for 1 h, frozen, and freeze-dried, while the other was subjected to every step but fermentation. The lyophilized probiotic powder was examined for probiotic viability and antioxidant capacity.

The probiotic culture showed a high survival rate in both samples. The fermentation decreased probiotic viability during the lyophilization procedure by 13 % but significantly increased the antioxidant activity of probiotic powder. The results indicate that flaxseed cake is a good probiotic carrier.

**Keywords:** probiotics, freeze-dry, immobilization, flaxseed residue, antioxidant

## INTRODUCTION

The circular economy promotes sustainable practices by reusing agricultural by-products in value-added applications. One such by-product, flaxseed cake, the material remaining after cold pressing flaxseed (*Linum usitatissimum L.*) oil, is increasingly recognized for its high protein and fiber content, along with bioactive compounds such as lignans and polyphenols, which make it suitable as a functional food ingredient and a carrier for probiotic bacteria (Gutiérrez, et al., 2010). The integration of such by-products supports not only nutritional innovations but also environmental sustainability through waste valorization.

The global demand for cold-pressed flaxseed oil has grown significantly in recent years, driven by its health-promoting properties, particularly its high omega-3 fatty acid content, and consumer interest in plant-based and clean-label products. This market expansion has increased the availability of flaxseed cake, enhancing its potential for further functional applications, including in non-dairy probiotic formulations (Łopusiewicz, et al., 2021).

Immobilization is a well-established technique increasingly utilized in the food industry, particularly for enhancing the stability and functionality of probiotics. This method offers several advantages, primarily by protecting probiotic cells within a carrier matrix from external stressors. As a result, immobilized probiotics exhibit improved survival during processing, storage, and under adverse environmental conditions (Krunic, et al., 2016).

Notably, immobilization enhances the viability of probiotic bacteria by protecting them from harsh conditions, such as low pH, digestive enzymes, and bile salts, in the human gastrointestinal tract (Krunic, et al., 2016; Krunic, et al., 2019). A fundamental criterion for successful immobilization is maintaining the viability of bacterial cells, which has guided the selection of appropriate immobilization techniques.

Among these, emulsification and extrusion are two commonly employed encapsulation methods that effectively preserve probiotic viability. Emulsification offers the flexibility of producing microcapsules across a broad particle size range (0.2–5000 μm), whereas extrusion yields more uniform beads within a narrower size distribution (Burgain, et al., 2011; Krunic, et al., 2019). However, the extrusion method is limited by its low throughput and difficulty in scaling up due to the slow formation rate of encapsulated carriers.

Spray drying is one of the most widely adopted microencapsulation techniques, particularly for industrial applications, owing to its rapid processing time, cost-efficiency, and ability to produce stable, high-quality end products. Nonetheless, the exposure of probiotic cells to high temperatures and dry air during spray drying can compromise cell viability. Therefore, careful optimization of processing parameters is essential to mitigate thermal stress and ensure maximal survival (Barbosa, et al., 2015).

Among probiotic delivery strategies, lyophilization (freeze-drying) stands out as one of the most effective methods for immobilizing probiotic microorganisms. Compared to other techniques such as gel entrapment or spray-drying, lyophilization provides superior bacterial viability during storage and gastrointestinal transit, while maintaining functionality and scalability for industrial use (Łopusiewicz, et al., 2021).

The probiotic *Lactobacillus acidophilus* is widely recognized for its health benefits. It supports gut microbiota balance, improves immune response, and has been linked to alleviation of lactose intolerance and the inhibition of pathogens. It is commonly used in fermented dairy products, probiotic supplements, and plant-based functional foods. Its ability to adhere to intestinal cells, produce antimicrobial substances, and modulate host responses makes it a model organism for probiotic studies (Łopusiewicz, et al., 2021).

Immobilizing probiotics serves several important purposes: it enhances survivability during processing and storage, protects against acidic and enzymatic degradation in the gastrointestinal tract, and enables controlled release of the bacteria at the site of action. These advantages are critical for ensuring probiotic efficacy in real-world applications (Łopusiewicz, et al., 2021).

Historically, both flaxseed and fermented probiotics have been valued in traditional medicine and diets. Flaxseed has been cultivated for millennia, not only for oil production but also for its digestive and anti-inflammatory properties, while probiotic use dates back to ancient fermented foods like yogurt. The modern scientific validation of these traditional practices now supports their integration into functional and sustainable food systems.

This study explores the immobilization of *Lactobacillus acidophilus* on flaxseed cake using freeze-drying, as a novel application aligned with circular economy principles, aiming to valorize agricultural waste while enhancing probiotic delivery

## MATERIALS AND METHODS

### Materials

In this work was used flaxseed cake obtained from Linum d.o.o., Serbia, and *Lactobacillus acidophilus*, Now foods, USA. DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), and other analytical grade reagents were purchased from Sigma-Aldrich Chemie Gmbh, USA.

### Flaxseed cake pre-treatment

Flaxseed cake, obtained from the cold-pressed oils industry, was used. The composition of the raw material was determined before immobilization. Flaxseed cake was ground in a ball mill and sieved through a sieve with a pore size of 0.6 mm. The flaxseed powder obtained in this way was sterilized in an autoclave at 120 °C, with a pressure of 1.5 bar for 30 minutes. After sterilization, the flaxseed powder was diluted in distilled water to a 10 % (w/v) concentration.

### Culture activation and lyophilization

*L. acidophilus* from the frozen stock was incubated under anaerobic conditions in De Man, Rogosa and Sharpe (MRS) broth (Torlak, Serbia) at 37 °C for 18 h two times consecutively and then washed in 10 mL of NS 3 times. The obtained culture was centrifuged at 4000 rpm for 5 min, and bacterial pellets were further re-suspended in 1 mL of NS and then added to two Erlenmeyer flasks with 50 mL of 10 % flaxseed powder dilution. One sample was poured onto a plastic plate for lyophilisation, frozen at -80 °C, and then freeze-dried for 24 h. The second sample was incubated at 37 °C for 3.5 h, then poured onto a plastic plate for lyophilisation, frozen at -80 °C, and freeze-dried for 24 h.

**Viable cell number determination**

Viable cell numbers were determined before and after immobilization by the pure plate method. The cell number was expressed as CFU/g and calculated percentage of viability after lyophilisation, which is the immobilization efficiency (IE).

$$IE\ \% = \frac{Ni}{N} x100$$

Ni is the total number of bacteria immobilized on the carrier (CFU/g) calculated relative to the weight of the carrier; N is the total number of bacteria in the suspension before the immobilization procedure (CFU/g).

The obtained values for the number of cells are recalculated in relation to the logarithmic value of the obtained number of cells.

**Antioxidant capacity**

***Determination of inhibition of DPPH free radicals***

The flaxseed cake powder before and after probiotic immobilization were taken in 1 % dilution and mixed with 0.1 mM–thanolic DPPH free radical, in ratio 1 : 18. Mixtures were homogenized and left in the dark for 30 min. The absorbance was measured at 517 nm using a UV-visible spectrophotometer (Ultrospec 3300 pro, Amerischam Bioscienc). For control, ethanol was used instead of the sample. The antioxidant activity was expressed as a percentage of DPPH activity calculated as:

$$DPPH\ capacity\ (\%) = \frac{Absorbance\ of\ blank - Absorbance\ of\ sample}{Absorbance\ of\ blank} \times 100$$

***ABTS free radical scavenging activity assay***

The ability of samples to scavenge ABTS radical was tested before and after the immobilization process using method described by Re, et al., (1999). ABTS radical cation was produced in the reaction between ABTS (7 mM) and potassium persulfate (2.45 mM final concentration). The reaction mixture was kept in the dark at room temperature for 16 h before use. Further, ABTS solution was diluted with 5 mM phosphate-buffered saline (pH 7.4) until the absorbance of 0.700 (± 0.030) was reached. Diluted ABTS solution was mixed with samples (prepared in different dilutions) in a ratio 100 : 1; absorbance was measured at 734 nm after 5 min. The antioxidant activity was expressed as a percentage of free radicals inhibition calculated as:

$$ABTS\ capacity\ (\%) = \frac{Absorbance\ of\ blank - Absorbance\ of\ sample}{Absorbance\ of\ blank} \times 100$$

**Statistical analysis**

All experiments were performed in triplicate and the results are presented as means with standard deviations. The Tukey test was used to determine significant differences between samples

($p < 0.05$). Data analysis was conducted using Microsoft Excel (Microsoft Office 2013 Edition, USA) and OriginPro 8 (Origin Lab Co., Northampton, USA).

## RESULTS AND DISCUSSION

This study investigates the potential use of flaxseed cake, a by-product generated in large quantities during the production of cold-pressed flaxseed oil, as a carrier for probiotics. The experimental workflow is illustrated in Figure 1.

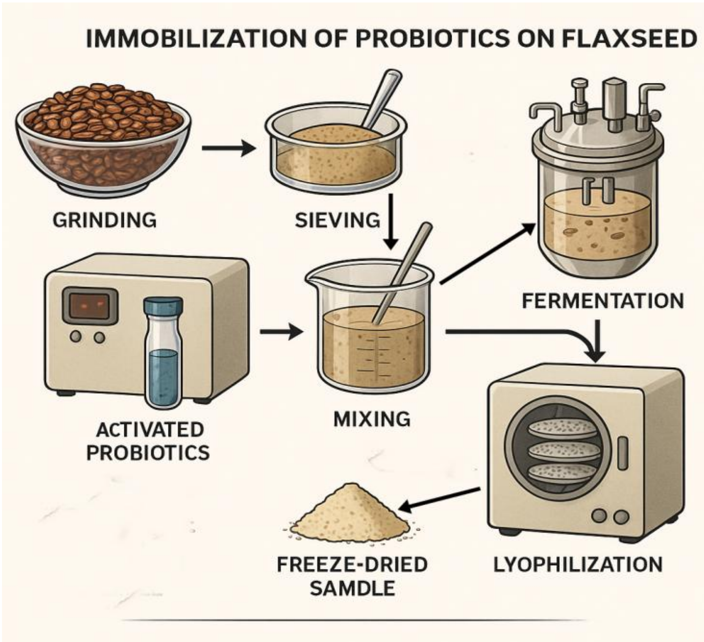


Figure 1. Experimental scheme

The flaxseed cake, which is produced in approximately double the quantity of the final product (flaxseed oil), was a huge pollutant because it has not found a way to an appropriate number of consumers. Using it as row material is a circular economy. Flaxseed cake was first ground and then sieved to obtain powder particles smaller than 600 μm. These particles are sufficiently small to be incorporated into food products without significantly altering their rheological properties, and large enough to be carriers for bacteria.

Prior to further experimentation, the composition of the flaxseed powder was determined and is presented in Table 1. The observed ratio of fiber to protein indicates a promising potential as a probiotic carrier. Dietary fibers not only serve as a nutrient source for probiotics but also provide protection against environmental stress by forming a network or gel-like structure in aqueous solutions. In addition to their protective capacity, proteins possess inherent bioactive potential. Carbohydrates are also food for used probiotic bacteria.

Table 1. Flaxseed cake composition

| | Protein, % | Oil, % | Fiber, % | Carbohydrate, % | Moister, % |
|---|---|---|---|---|---|
| **Flaxseed cake** | 37.1 ± 2.5 | 20.7 ± 1.2 | 28.0 ± 3.5 | 7.1 ± 0.8 | 8.1 ± 0.9 |

Figure 2. shows viable cell numbers before and after immobilization for non-fermented and fermented flaxseed probiotic powder. The number of viable probiotic bacteria was significantly higher before the lyophilization process, which is in accordance with previously published data

(Krunic, & Osmokrovic, 2025). During lyophilization, a substantial loss of viable cells occurs due to the formation of damaging ice crystals during the freezing phase. The use of various cryoprotectants can significantly enhance probiotic survival. In our study, a survival rate of 80 % was achieved, which is notably higher compared to the survival of the culture in physiological saline (67 %, data not shown). These findings suggest that the flaxseed cake exhibits a protective effect during the lyophilization process. Soltani, Sadeghi, & Shirvani (2025) show improved *L. acidophilus* viability and metabolic activity by the addition of flaxseed gum.

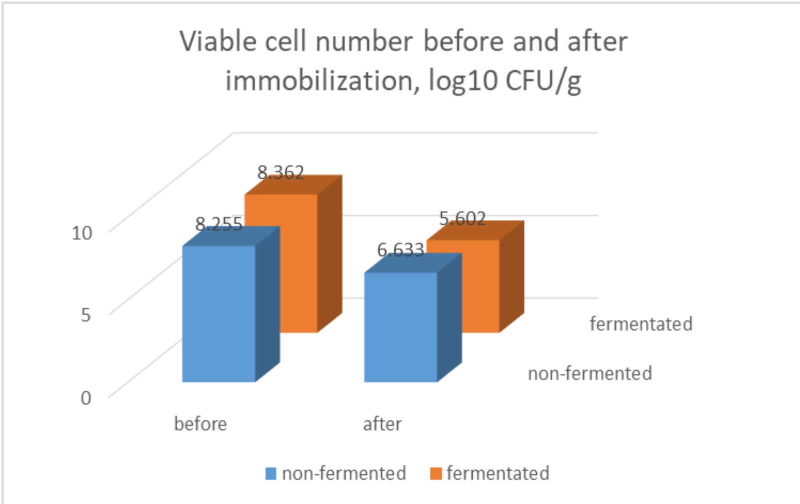


Figure 2. Viable cell numbers before and after 24h of lyophilization for non-fermented and fermented flaxseed probiotic powder

Fermentation of flaxseed was introduced with the aim of modifying the carrier structure through microbial hydrolysis of proteins and fibers, thereby enhancing probiotic binding. Fermentation by *L. acidophilus* altered the flaxseed gum to be more porous and improved its functional properties, such as swelling, water-holding, and oil-holding capacities (Wang, et al., 2024). However, the results demonstrated a 13% lower probiotic survival rate following this treatment compared to the non-fermented sample (Table 2). This can be explained by the additional activation of probiotics during fermentation, which brings them into a more sensitive phase. Although fermentation did not show a positive influence on the survival of probiotics during lyophilization, Theegala, et al. (2021) demonstrated that flaxseed provides a high protective capacity toward probiotics under simulated gastrointestinal conditions.

Table 2 Immobilization efficiency of fermented and non-fermented flaxseed powder

| | **Non-fermented** | **Fermented** |
|---|---|---|
| **Immobilization efficiency (IE), %** | 80.1 ± 2.2 | 67.2 ± 2.1 |

Based on these findings, it can be concluded that non-fermented flaxseed serves as a more effective cryoprotectant and carrier for the applied probiotic strain during the lyophilization process. Microbial hydrolysis, i.e., fermentation of flaxseed, does not increase probiotic viability and immobilization efficiency but may lead to an increase in the bioactivity of the raw material. This is primarily a result of the microbial hydrolysis of flaxseed proteins, as well as the formation of new bioactive peptides and molecules by the probiotic culture. As shown in Figure 3, the antioxidant capacity of the fermented flaxseed probiotic powder was significantly higher, in a statistically significant manner, compared to the non-fermented sample. This effect is likely due to the biotransformation or release of phenolic compounds and other bioactive substances during fermentation. Research supports that *L. acidophilus* and other lactic acid bacteria improve the antioxidant potential of plant-based substrates, including flaxseed, by modifying polysaccharide structure, increasing polyphenol content, and producing metabolites with antioxidant activity. Results from this paper are in accordance with the literature, which shows that fermentation with *L. acidophilus* leads to increased antioxidant activity in flaxseed (Wang et al., 2024). In soy and almond milk systems, *L. acidophilus* significantly improved antioxidant capacity and polyphenol content during fermentation (Zahrani, & Shori, 2023). Also, *L. acidophilus* enhanced the antioxidant activity of fermented apple pulp by biotransforming polyphenols into more bioavailable compounds (Ran, et al., 2023). Waszkowiak, et al. (2025) show that fermenting bacteria caused changes in important functional and technological properties of flaxseed cake, such as the increase in the antiradical activity and total phenolic content, and the decrease in the water holding capacity and viscosity. These changes can be particularly beneficial for food applications. Since *L. acidophilus* is not considered a metabolically highly active bacterium and fermentation takes only 3.5 h, the observed antioxidant capacity of probiotic powder is more likely a result of flaxseed protein hydrolysis rather than the production of various antioxidant-active compounds during fermentation.

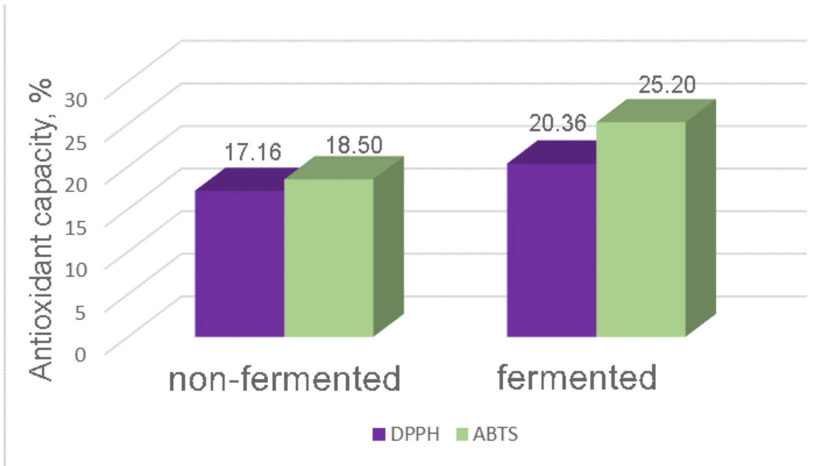


Figure 3. Antioxidant capacity of flaxseed probiotic powder after immobilization. One sample was fermented for 3.5 h before the immobilization process.

## CONCLUSION

The high fiber content of about 28 % and protein of about 37 % in flaxseed cake makes it a material with good potential for use as a probiotic carrier. The probiotic culture showed a high survival rate in both samples. The non-fermented sample showed a survival rate of *L. acidophilus* of 80 %, while fermentation decreased probiotic viability during the lyophilization procedure by 13 %. These results show that flaxseed cake powder is a good material for immobilizing probiotics, providing them with a high percentage of viability. Although fermentation is often a good method for improving the properties of materials and products. In this research, fermentation of flaxseed significantly increased the antioxidant activity of probiotic powder. The reason for the reduced number of viable cells in the fermented compared to the non-fermented sample may be the adaptation of the culture to the fermentation temperature, as well as the introduction of the culture into the accelerated growth phase when it is highly sensitive to external factors. This can be remedied by extending the time of storing the samples in the refrigerator before freezing and lyophilization.

## ACKNOWLEDGMENT

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-136/2025-03/200287) and UNDP in

Krunić, T. (2025). Circular economy: immobilization of *lactobacillus acidophilus* on flaxseed cake. *STED Conference 14*(2), 259-266.

Serbia through the Circular voucher (Contract No.00131890/1138320/2024/01-03). Sincere thanks to Linum d.o.o. for their trust and for providing the raw materials used in this work.

**DECLARATIONS OF INTEREST STATEMENT**

The authors affirm that there are no conflicts of interest to declare in relation to the research presented in this paper.

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