*ID: 707*

# Effect of Plant Protein-Based Microencapsulation on Antioxidant Capacity in Probiotic Systems

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## Abstract

The protection of bioactive compounds against environmental stress factors is of paramount importance in the development of functional food ingredients. This study was conducted to determine the effectiveness of microencapsulation systems based on rice protein, a plant-derived protein, and inulin, a prebiotic polysaccharide, using conjugation and physical mixing methods, and to evaluate their impact on the antioxidant capacity of systems containing different probiotic strains (*Lactobacillus reuteri* and *Lactobacillus acidophilus*). In the research, conjugate structures prepared through covalent modification of rice protein and inulin, as well as traditional physical mixtures, were utilized as wall materials. The total phenolic content of the microcapsules was analyzed using the Folin-Ciocalteu method, and their radical scavenging activities were determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The findings indicated that the total phenolic content of the microcapsules ranged from 275.94 to 291.56 milligrams of gallic acid equivalent per gram (mg GAE/g), while radical scavenging activities ranged between 157.36 and 227.54 milligrams of ascorbic acid equivalent per gram (mg AAE/g). The highest phenolic content and antioxidant potential were identified in the formulation containing *Lactobacillus acidophilus* prepared with the rice protein-inulin conjugate matrix (LAPC). The results of the study revealed that the covalent conjugation of rice protein and inulin strengthened molecular interactions with phenolic compounds through modifications induced in the protein structure and created a more resilient protective barrier compared to physical mixtures. These findings demonstrate that plant protein-based conjugate systems offer a superior encapsulation strategy for enhancing the oxidative stability of bioactive components in food formulations containing probiotics.

***Keywords:*** *Plant protein, Rice protein, Microencapsulation, Probiotic, Antioxidant capacity.*

## Introduction

In recent years, microencapsulation has proven effective in protecting bioactive compounds when exposed to an unfavorable food processing environment and the human gut. Among the different coating matrices, food-grade polymers such as milk proteins, plant proteins, and polysaccharides have been extensively investigated as wall materials for the encapsulation of bioactive substances. However, protein-based delivery systems, in addition to their precipitation and aggregation disadvantages (pH and ionic strength effects), show limited stability against digestive enzymes in the gastrointestinal tract and undergo degradation, leading to the burst release of encapsulated bioactive compounds in the gastric environment instead of the intestine (Marson et al., 2020; Nooshkam & Varidi, 2020). Moreover, the application of chemicals as crosslinkers is a limiting factor due to health concerns (Li & Huang, 2015).

To overcome these challenges, protein–carbohydrate conjugation/complexation based on the Maillard reaction has rapidly expanded and proved to be excellent candidates for the encapsulation and delivery of bioactive components in food and pharmaceutical fields (Cao et al., 2022; Sun et al., 2022). This is mainly because of their outstanding characteristics, such as good solubility and emulsifying capacity, stability over a wide range of pH values, temperatures, and ionic strengths, and the ability to promote a thicker, continuous, viscoelastic, and shear-resistant layer surrounding bioactive components (Kan et al., 2021). In this study, the total phenolic content (TPC) and DPPH radical scavenging activities of the obtained encapsulated samples were comparatively analyzed across different combinations including physical mixtures (LRPP and LAPP) and conjugate formulations (LRPC and LAPC) prepared with *Lactobacillus reuteri* and *Lactobacillus acidophilus*. This study aims to pave the way for increased utilization of plant proteins not only to provide a protective barrier in food formulations but also as functional ingredients that optimize antioxidant capacity.

## Materials and Methods

### *Determination of DPPH (2,2-Diphenyl-1-picrylhydrazyl) Scavenging Activity*

The DPPH free radical scavenging activity was determined by modifying the method reported by Du et al. (2023). The method is based on the principle of lightening the dark purple color as a result of the reduction of the DPPH radical by antioxidant substances. The decrease in absorbance is measured at 517 nm. Within the scope of the analysis, 3.9 mL of DPPH solution (0.05 mM prepared in 70% ethanol) was added onto 0.1 mL of extract, and

the mixture was vortexed for 30 s. After 30 min of incubation in the dark and at room temperature, the samples were centrifuged (4 °C, 8965 × g, 5 min). After collecting the supernatant, absorbance values were measured at 517 nm with a spectrophotometer. The % DPPH scavenging activity of the samples was determined according to Formula 1:

$$\% \text{ DPPH scavenging} = (1- \frac{A\ sample}{A\ control})x100 \quad (1)$$

*($A_{control}$: expresses DPPH solution + solvent; $A_{sample}$: expresses DPPH solution + extract.)*

Ascorbic acid was used as the antioxidant standard, and a standard curve was prepared in the concentration range of 20–500 µg/mL. Using Equation 2 created over the % inhibition values, the results were expressed as mg ascorbic acid equivalent (AAE) / g sample.

$$y = 0{,}0513x + 2{,}1835 \quad R^2=0{,}9643 \quad (2)$$

***Determination of Total Phenolic Content***

The total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent (FCR) according to the method reported by Singleton and Rossi (1965). In the analytical procedure, pre-diluted Folin-Ciocalteu reagent (2.5 mL) was added onto 0.5 mL of extract, and the mixture was kept at room temperature for 5 min. Then, 2 mL of sodium carbonate ($Na_2CO_3$) solution (20% w/v) was added to the mixture, and the samples were incubated for 60 min at room temperature in the dark. The absorbance values of the samples were measured at 765 nm using a UV-Vis spectrophotometer. Gallic acid was used as the phenolic compound standard, and serial dilutions of gallic acid (20–100 µg/mL) were prepared for this purpose. Using the standard curve equation (Equation 3), the TPC of the samples was expressed as mg gallic acid equivalent (GAE) / g sample.

$$y = 0{,}0016x + 0{,}2707 \quad R^2=0{,}9606 \quad (3)$$

**Results**

***DPPH Radical Scavenging Activity***

The DPPH analysis results, conducted to determine the free radical scavenging capacities of the microcapsule samples, were calculated in terms of mg AAE/g. The antioxidant activities of the samples ranged between 157.36 and 227.54 mg AAE/g (Table 1), and the differences among the formulations were found to be statistically significant ($p < 0.05$, Table 1).

In parallel with the TPC results, the highest radical scavenging activity was observed in the LAPC sample (227.54 ± 4.05 mg AAE/g), which was significantly different from all other samples ($p < 0.05$). This demonstrates that the LAPC formulation is the most effective combination, not only in terms of total phenolic content but also regarding the biological activity capacity (radical scavenging) of these phenolics. The prebiotic effect of inulin and the structural integrity of rice protein enhanced the stability of antioxidant metabolites, thereby enabling the achievement of higher activity values.

***Total Phenolic Content (TPC)***

The total phenolic contents of the samples are presented in Table 1. According to the analysis results, the TPC values of the microcapsules varied between 275.94 and 291.56 mg GAE/g. The highest phenolic content was detected in the sample coded LAPC (291.56 ± 2.64 mg GAE/g), followed by LAPP (288.44 ± 1.98 mg GAE/g), showing no statistically significant difference between these two specific formulations ($p > 0.05$). However, both conjugate structures (LAPC and LRPC) exhibited significantly higher TPC values compared to their respective physical mixture counterparts (LRPP and LAPP) ($p < 0.05$). The high phenolic content in the samples prepared with the combination of rice protein and inulin is associated with the capacity of the wall materials to protect antioxidant components and the release efficiency during methanol extraction.

Table 1. Total phenolic content (TPC) and DPPH radical scavenging activities of the encapsulated probiotic microcapsules.

| Sample Code | DPPH (mg AAE/g) | TPC (mg GAE / g sample) |
|---|---|---|
| LRPP | $157{,}36 \pm 2.14^{d}$ | $275{,}94 \pm 3.12^{c}$ |
| LRPC | $161{,}26 \pm 1.85^{c}$ | $285{,}44 \pm 2.45^{b}$ |
| LAPP | $188{,}56 \pm 3.22^{b}$ | $288{,}44 \pm 1.98^{ab}$ |
| LAPC | $227{,}54 \pm 4.05^{a}$ | $291{,}56 \pm 2.64^{a}$ |

*Values are expressed as mean ± standard deviation. Different superscript letters (a–d) within the same column indicate statistically significant differences ($p < 0.05$).; *LRPP: L. reuteri + Rice Protein + Inulin Physical Mixture; LRPC: L. reuteri + Rice Protein-Inulin Conjugate; LAPP: L. acidophilus + Rice Protein + Inulin Physical Mixture; LAPC: L. acidophilus + Rice Protein-Inulin Conjugate.

## Discussion

When the total phenolic content (TPC) and DPPH radical scavenging activity values of the samples are examined, it is observed that the protein-inulin conjugate matrices (PC) provide a distinct superiority in protecting the antioxidant properties of rutin and related bioactive components compared to physical mixtures (PP). The highest phenolic content and radical scavenging activity were detected in the sample coded LAPC (291.56 mg GAE/g and 227.54 mg AAE/g), whereas the lowest antioxidant and phenolic compound values were determined in the LRPP sample (157.36 mg AAE/g and 275.94 mg GAE/g), which is a physical mixture formulation. This clearly demonstrates that the conjugate structures prepared via the Maillard reaction significantly increase the preservation of antioxidant properties. Statistically, the transition from physical mixtures to conjugate matrices (from LRPP to LRPC, and LAPP to LAPC) resulted in a significant upward shift in both parameters ($p < 0.05$), confirming that the covalent bonding matrix offers a predictable and mathematically validated protective performance over simple blending.

The high success obtained in the conjugate samples (LAPC and LRPC) is directly related to the capacity of covalent modification to enhance antioxidant stability. In the literature, it is reported that the conjugation of proteins with polysaccharides, such as inulin, provides higher protection in preserving the antioxidant capacities of bioactive components compared to native proteins or physical mixtures (Yi et al., 2016). The conjugation process creates a tighter and more resilient protective barrier around phenolic compounds through modifications induced in the protein framework. These structural alterations and the increase in antioxidant power can be attributed to the presence of high molecular weight (HMW) melanoidins formed during the Maillard reaction, which are well-known for their superior antioxidant properties (Nooshkam et al., 2019). Conversely, in physical mixtures (LRPP and LAPP), since this molecular protective network remains weaker, the phenolic components become more open to environmental effects, and subsequent decreases have been observed in both TPC and DPPH values.

Furthermore, the protective role of encapsulation technology on the structural integrity of bioactive substances is of critical importance in preventing the adverse conditions to which free or poorly protected components are exposed (Babazadeh et al., 2017). In our study, the emergence of the LAPC sample, which possesses a conjugate matrix, as the most effective combination proves that the prebiotic effect of inulin, combined with the dense network integrity formed by the conjugated protein structure, synergistically enhances the stability of antioxidant metabolites. The capacity of conjugated systems to hold phenolic compounds more stably within the matrix and manage the release profile more efficiently is in perfect agreement with literature findings showing that conjugated structures (e.g., pectin-chitosan conjugates) are far more efficient in protecting the antioxidant potential of bioactive substances compared to non-conjugated systems (Shishir et al., 2019). Consequently, plant protein and inulin conjugation represents a superior encapsulation strategy for preserving bioactive components and developing functional products with high antioxidant potential in probiotic systems.

## Conclusion

This study demonstrates that the conjugation of rice protein and inulin is a far more effective strategy for enhancing the antioxidant stability of probiotic-containing microcapsules compared to physical mixtures. The obtained findings prove that the conjugated matrices prepared particularly with the *Lactobacillus acidophilus* strain (LAPC) exhibit the highest total phenolic content and radical scavenging activity. It was determined that the protein-polysaccharide network structure, strengthened by the conjugation process, protects bioactive components against oxidative degradation. In future studies, it is recommended to investigate the storage stability of these conjugated systems in different food matrices and their release mechanisms in the gastrointestinal tract using in vivo methods.

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