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Ultrasound-Assisted Extraction of Inulin From Dandelion Leaves Application for Probiotics Spray Drying

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ABSTRACT

Dandelion leaves (*Taraxacum officinale*) are known for their high prebiotic inulin content. Ultrasound-assisted extraction (UAE) is considered one of the most efficient methods for improving extraction performance. This study focused on using an alternative ultrasound-assisted method to extract inulin from dandelion leaves. Additionally, the research explored the use of extracted inulin as a wall material in synbiotic spray drying with *Lactobacillus acidophilus*. Various combinations of hot water (70°C, 80°C, and 90°C) and ultrasonic power (30 W/g, 60 W/g, and 90 W/g) were tested for the extraction process. The material-to-water ratio was maintained at 1:10, with an extraction time of 20 minutes for all trials. The findings revealed that both water temperature and ultrasonic power had a significant impact on inulin concentration. The highest amount of extracted inulin (86.25 mg/g dw) was achieved with 60 W/g ultrasonic power at 80°C. UAE enhanced inulin extraction by 9.48% compared to traditional methods. The *Lactobacillus acidophilus* cells demonstrated satisfactory survival rates at high spray drying temperatures (inlet temperatures of 140°C and 180°C), indicating their heat resistance during spray drying. In conclusion, inulin extracted from dandelion leaves has promising potential as a wall material for spray-drying probiotics. However, further optimization of spray drying conditions is needed for efficient synbiotic production.

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1. Introduction

Inulin is a polysaccharide composed primarily of fructose units with terminal glucose residues, naturally occurring in over 30,000 plant species [1]. It is predominantly found in the roots and tubers of various plants, including banana (*Musa cavendishii*), chicory (*Cichorium intybus*), garlic (*Allium sativum*), Jerusalem artichoke (*Helianthus tuberosus*), onion (*Allium cepa*), and dandelion (*Taraxacum officinale*) [1], [2]. Due to its unique chemical and physical properties, inulin offers a range of technological advantages and health benefits, particularly in human nutrition. As a natural carbohydrate, extracted inulin functions as a starchy dietary component that supports the restoration of gut microbiota, thus classifying it as a prebiotic and a contributor to dietary fiber [3]-[5]. Inulin have been documented in various leaf and shows the prebiotic properties [6], [7]. Moreover, inulin serves as a natural carrier in the development of synbiotic formulations and the encapsulation of probiotics [8].

Dandelion (*Taraxacum officinale*), a member of the Asteraceae (Compositae) family, is native to Europe but is also found in the highland regions of Vietnam, such as Tam Dao, Sapa, and Da Lat. Traditionally recognized as a non-toxic medicinal herb, dandelion has been utilized in the production of beverages and liquors and is valued for its pharmacological properties, including detoxification, diuretic effects, and antibacterial and antitumor activities [9], [10]. Recent studies have highlighted its antioxidant potential and suggested its possible protective effects against obesity, cancer, and various cardiovascular risk factors [11]. In Vietnam, young dandelion leaves are commonly consumed fresh in salads or cooked in soups, while dried leaves are frequently brewed as tea. Notably, the inulin content of both dandelion leaves and roots has been reported to range from 12 to 15 g per 100 g of dry weight [1], [12].

Given the wide-ranging applications and significant physiological functions of inulin, both its extraction and quantification are critical for the valorization of inulin-rich plant sources and the investigation of its functional properties. Among the various extraction techniques available, ultrasound-assisted extraction (UAE) has emerged as an efficient and sustainable method for recovering bioactive compounds from plant materials [13]. Compared to traditional solvent extraction methods, UAE offers several advantages, including higher extraction yields, reduced energy consumption, and improved environmental sustainability and feasible for inulin extraction [14]. Additionally, the UAE has been optimized to enhance process efficiency and significantly shorten extraction times relative to conventional approaches [15], [16].

Spray drying, widely recognized as a microencapsulation technique, has been extensively studied for its ability to stabilize probiotic bacteria within different food matrices. Numerous formulations employing various carrier materials have been developed using this method [17]. Notably, research indicates that the inclusion of prebiotics, such as inulin in spray-drying formulations, can protect probiotics during processing and enhance their viability throughout storage [18], [19].

To date, extensive research has focused on the extraction of inulin from various plant sources, particularly Jerusalem artichoke and chicory, using a range of techniques, including ultrasound-assisted extraction and accelerated solvent extraction. However, the extraction of inulin from dandelion (*Taraxacum officinale*) leaves using ultrasound-assisted methods remains underexplored. Moreover, the potential application of inulin derived from dandelion as a prebiotic in spray-dried probiotic formulations has not been thoroughly investigated. Therefore, the present study aimed to (i) extract inulin from dandelion leaves using ultrasound-assisted extraction and (ii) evaluate the application of the extracted inulin as a prebiotic agent to enhance the viability of probiotics during and after spray drying.

2. Materials and Methods

2.1. Materials

2.1.1. Sample procurement and preparation

Dandelion (*Taraxacum officinale*) leaves were harvested after a four-month cultivation period at the Lam Vien Agricultural Products Joint Stock Company farm, located in Da Lat City, Lam Dong Province, Vietnam. Only fresh, intact leaves were selected for further processing. The leaves were thoroughly washed with tap water to remove dust and other extraneous materials and then allowed to drain. Subsequently, they were air-dried at 55°C for 8 hours. The dried leaves were stored in moisture-resistant zip-lock bags under dry conditions for later use. Before extraction, the dried leaves were milled and passed through a 0.5 mm mesh sieve. The resulting powdered sample had an approximate moisture content of 7.7%.

2.1.2. Chemicals

Analytical-grade chemicals were purchased from local suppliers. D-fructose, MRS broth, peptone (HiMedia, India), agar-agar (Platapiantong, Thailand), resorcinol, and concentrated HCl (37%) (Xi Long, China)

2.2. Methods

2.2.1. Inulin extraction using an ultrasound-assisted method

The objective of this experiment was to determine the most suitable conditions for extracting inulin from dandelion leaf powder (DLP) using the ultrasound-assisted extraction (UAE) method. The independent variables investigated were extraction temperature and ultrasonic power. Each experimental condition was conducted in triplicate to ensure reproducibility. Inulin extraction was performed using hot water in combination with ultrasound-assisted treatment. For each trial, 5 grams of DLP were mixed with hot water at varying temperatures (70°C, 80°C, and 90°C) at a solid-to-liquid ratio of 1:10 (w/w). The mixture was stirred using a magnetic stirrer to ensure uniform dispersion of the powder. The dispersion was then subjected to ultrasonic treatment at different power levels (30, 60, and 90 W/g) for 20 minutes. Following sonication, the solution was allowed to cool to room temperature. The resulting mixture was filtered using filter cloths to remove coarse particulates. The filtrate was

subsequently centrifuged at 4,500 rpm for 10 minutes to further separate insoluble materials. The clear supernatant obtained contained the extracted inulin.

2.2.2. Spray drying process of probiotics in the presence of inulin

Feed solutions were prepared by incorporating extracted inulin (0.5% w/v) and maltodextrin (20% w/v) with *Lactobacillus acidophilus* (2% w/v, approximately 10^8 CFU/g). Before spray drying, the feed solutions were homogenized using a magnetic stirrer to ensure uniform distribution of all components. The solutions were subjected to spray drying (L-8i, Ohkawara Kakohki Co. Ltd., Yokohama, Japan) at two inlet air temperatures: 140°C and 180°C (the outlet air temperatures were 89°C and 116°C, respectively). The homogenized feed was introduced into the spray drying chamber using a peristaltic pump equipped with a 0.1 mm nozzle (centrifugal pressure type), operating at a feed rate of 15 mL/min and an air pressure of 1.6 MPa. The resulting microparticles were collected from the base of the cyclone separator and immediately transferred into sterile vials. The final powders were stored in airtight containers under refrigerated conditions (4 ± 1°C) until further analysis.

2.2.3. Quantification of the viable encapsulated L. acidophilus cells

In this study, the pour plate method was employed to enumerate viable probiotic cells in the microparticle samples. To prepare the initial dilution, 10 g of the microparticles were aseptically transferred into sterile polyethylene bags containing 90 mL of sterile 0.1% peptone water. The mixture was homogenized using a stomacher for 60 seconds. The resulting suspension, representing a 10^{-1} dilution, was aseptically transferred into sterile test tubes. Serial dilutions were subsequently prepared by transferring 0.5 mL of the culture into additional test tubes containing 4.5 mL of sterile 0.1% peptone water. One milliliter of appropriately diluted samples was then poured into sterile MRS agar. The plates were incubated anaerobically in an inverted position at 37°C for 48 hours. After the incubation period, the number of viable *Lactobacillus acidophilus* colonies was counted and expressed as log colony-forming units per gram (log CFU/g), following the Vietnamese national standard TCVN 9716:2013.

The effect of inulin on the viability of *L. acidophilus* in the synbiotic powder was assessed by calculating the survival rate (%) of the microorganisms before and after spray drying, using the following equation (1).

$$\text{Survival rate (\%)} = (N/N_o) \times 100\% \quad (1)$$

In equation (1), N (log CFU/g) represents number of viable cells released from the microparticles after the spray-drying process, N_o (log CFU/g) denotes number of viable cells in the feed solution before the spray-drying process.

2.2.4. Determination of extracted inulin concentration

The method of inulin determination described by Petkova et al. (2018) [20] was followed. Seliwanoff reagent was prepared by dissolving 0.33 g of resorcinol in 100 mL of 37% hydrochloric acid, followed by dilution with distilled water at a 1:1 ratio. For inulin hydrolysis, 3 mL of the prepared Seliwanoff reagent was added to test tubes containing 1 mL of the sample solution. The mixture was vortexed and then incubated in a water bath at 80°C for 10 minutes. The development of a red color within 30 seconds indicated the presence of ketose sugars, particularly fructose. After incubation, the mixture was allowed to cool to room temperature, and the absorbance was measured at 483 nm using a UV-Vis spectrophotometer. The inulin content in the sample was quantified based on a standard curve constructed using known concentrations of fructose. The free fructose in the sample was subtracted from the standard curve as a background control. The concentration of fructose in the sample (x, in mg/mL) was determined using the linear regression equation: y=ax+b where y represents the absorbance at 483 nm, and a and b are constants derived from the standard curve.

2.2.5. Scanning electron microscopy

Spray-dried powders were affixed onto metal stubs using double-sided carbon adhesive tape. Subsequently, they were coated with a thin platinum (Pt) layer to enhance conductivity. Imaging was

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carried out with a scanning electron microscope (SEM, model S-3400, Hitachi, Tokyo, Japan) operated at an accelerating voltage of 10 kV and a working distance of 7.8 mm.

2.3. Statistical analysis

Statistical analysis of the experimental data was performed using SPSS software version 20.0 (SPSS Inc., USA). All experiments were conducted in triplicate, and the results are presented as mean ± standard deviation. Error bars in the graphs represent the standard deviations from the mean values. Analysis of variance (ANOVA) was employed to assess the significance of differences among treatment groups. When significant differences were detected, Tukey's post hoc test was used for pairwise comparisons. Statistical significance was defined as a *P*-value less than 0.05. Graphs illustrating the mean values and associated error bars were generated using Microsoft Excel 2010.

3. Results and Discussion

3.1. Effect of extraction temperature and ultrasonic power on inulin extract concentration

Figure 1 illustrates the effect of varying extraction temperatures (70°C, 80°C, and 90°C) and ultrasonic power levels (30, 60, and 90 W/g) on the concentration of inulin extracted from dandelion leaf powder (DLP). The results demonstrate that ultrasound-assisted extraction significantly enhanced the yield of inulin compared to conventional extraction methods. Increased temperature and ultrasonic power both contributed to improved extraction efficiency, indicating the synergistic effect of thermal and ultrasonic energy on cell wall disruption and solubilization of inulin.

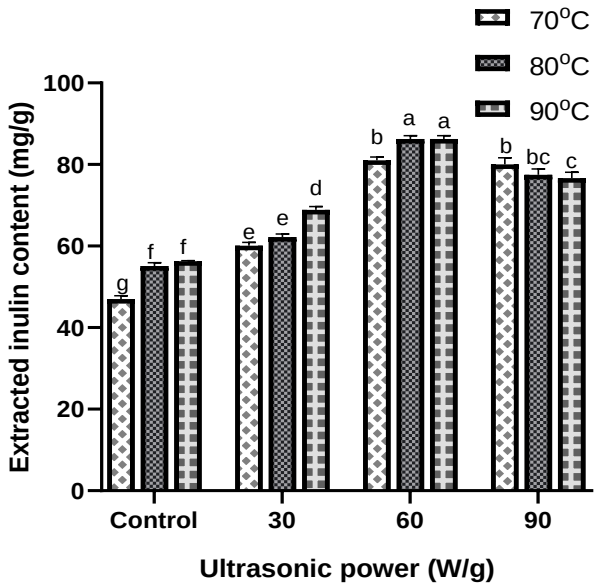

Figure 1. *Effect of extraction temperature and ultrasonic power on extracted inulin content. The data were expressed as mean ± standard deviation (n =3), different letters show statistically significant difference (p <0.05).*

Overall, the concentration of extracted inulin increased significantly ($p < 0.05$) as the extraction temperature rose from 70°C to 80°C. However, a decline in inulin yield was observed at 90°C, suggesting that excessively high temperatures may lead to thermal degradation or reduced extraction efficiency. The highest inulin concentration obtained was 86.25 mg/g dw, recorded at an extraction temperature of 80°C with ultrasonic powers of both 60 W/g and 90 W/g. In contrast, the lowest concentration, 47.02 mg/g dw, was observed at 70°C and 30 W/g, which did not differ significantly ($p \geq 0.05$) from the concentration obtained by conventional extraction at 70°C. These findings indicate that both ultrasonic power and extraction temperature positively influence inulin yield up to an highest point. Beyond this, further increases, particularly to 90°C in temperature or 90 W/g in power, led to a reduction in extracted inulin concentration, possibly due to structural breakdown or saturation effects.

Ultrasonic power plays a critical role in facilitating cell wall disruption during ultrasound-assisted extraction (UAE) through two primary physical mechanisms: acoustic cavitation and enhanced

diffusion. Acoustic cavitation refers to the formation, growth, and violent collapse of microbubbles near the particle surface, which leads to localized high temperatures and pressures that disrupt cellular structures. Concurrently, diffusion through cell walls allows the released inulin to migrate into the surrounding solvent. An increased ultrasonic power promotes more extensive cell wall rupture, thereby enhancing the release and dispersion of inulin from plant tissues [16], [21]. Under UAE, the expansion and implosion of cavitation bubbles near cell surfaces, along with the resulting turbulence at the solvent–solid interface, accelerate the breakdown of cellular structures and significantly improve the mass transfer of target compounds into the solvent medium [22]. These effects are further amplified by elevated extraction temperatures. At higher temperatures, the viscosity and density of the solvent decrease due to vaporization, which enhances mass transfer and improves solvent penetration into plant matrices. As a result, inulin yield increases. Additionally, higher temperatures contribute to an increased number of cavitation events and expanded surface contact areas between the solvent and solid material, further boosting extraction efficiency [21], [23].

The findings of this study are consistent with these observations, as the maximum inulin concentration was recorded at an extraction temperature of 80°C. However, it is important to note that excessively high ultrasonic power or temperature can lead to the degradation of target compounds. Stronger ultrasonic intensity may induce unwanted chemical decomposition due to prolonged or intense acoustic cavitation. Furthermore, prior research indicates that as the temperature approaches the boiling point of the extraction solution, a decrease in surface tension and an increase in vapor pressure promote the formation of microbubbles, which can dampen the propagation of ultrasonic waves [22]. This phenomenon likely contributed to the observed decline in inulin yield at 90°C, as shown in Figure 1.

At an extraction temperature of 80°C, increasing the ultrasonic power from 60 W/g to 90 W/g did not result in a statistically significant ($p \geq 0.05$) increase in the concentration of inulin extracted from dandelion leaf powder (DLP). This suggests that an ultrasonic power of 60 W/g is sufficient for effective ultrasound-assisted extraction (UAE) of inulin from dandelion leaves. Based on these findings, the most suitable extraction conditions were determined to be 80°C and 60 W/g. The extraction efficiency improved markedly, with an 8.27% increase in inulin yield as the temperature rose from 70°C to 80°C. This increase was statistically significant ($p < 0.05$). However, a further increase in temperature to 90°C led to a reduction in the ultrasound effect, reaching a decrease of approximately 12.11%, although the difference between 80°C and 90°C was not statistically significant ($p \geq 0.05$). This decline may be attributed to the damping of ultrasonic waves at elevated temperatures due to increased vapor pressure and bubble formation, which reduces cavitation effectiveness.

Ultrasound enhances extraction through the generation of cavitation bubbles, which undergo rapid adiabatic compression and expansion. This phenomenon causes localized increases in temperature and pressure, resulting in cell swelling, increased solvent uptake, and pore enlargement in the cell walls. These effects facilitate greater diffusivity and enhance the release of soluble compounds, such as inulin, into the extraction medium [24], [25]. A comparison of inulin yields with and without ultrasound confirmed a significant positive effect of ultrasound on the extraction process, reinforcing its value as an efficient, non-thermal extraction technique.

3.2. Properties of spray-dried inulin and L. acidophilus powder

Table 1. *Properties of spray-dried microcapsules produced with different materials without probiotics and with probiotics.*

Parameters	**Microcapsules**	
	Without probiotics	**With probiotics**
TSS (°Brix) of spray drying material	19.40	21.00
MC (% wb)	3.80	2.91
Particle size range (μm)	2.11 - 24.32	3.72 - 25.14

** Data were expressed as the mean (n=3); wb: wet basis*

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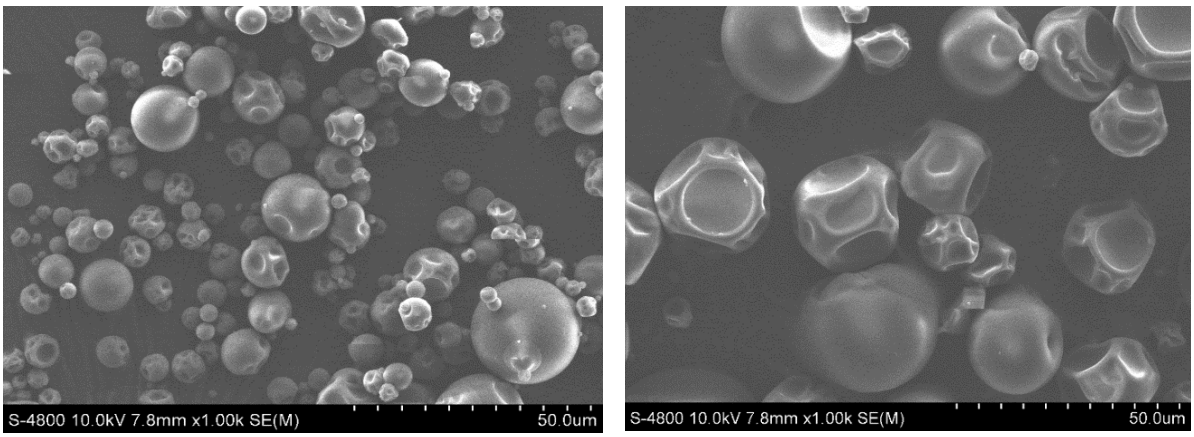
Table 1 presents the properties of spray-dried microcapsules produced with different encapsulating materials, both with and without probiotics. The microcapsules exhibited varied sizes, with microparticle size of the sample without probiotics ranging from 2.11 to 24.32 μm and that of the sample with probiotics ranging from 3.72 to 25.14 μm. The moisture content of the sample containing probiotics was 2.91% (wet basis), which is favorable for the stability of the powder. According to Simpson (2005) [26], powders with a moisture content of 4% or less are considered microbiologically stable, as this low moisture level limits the available free water for biochemical reactions, thereby extending shelf life. In addition, the water activity of the obtained powders were lower than 0.4, confirming for their microbial stability.

Figure 2 displays Scanning Electron Microscopy (SEM) images of the spray-dried inulin powder with various encapsulating agents. The SEM images did not reveal any visible presence of *Lactobacillus acidophilus* (*L. acidophilus*), likely due to the limitations in magnification used in this study, which may not have been sufficient to capture the bacteria in the synbiotic powder. However, the SEM images confirmed the formation of microcapsules. Specifically, the microcapsules containing *L. acidophilus*, encapsulated with a single layer of inulin, exhibited irregularly spherical shapes with some surface concavities. These morphological features were consistent with those typically produced by spray drying.

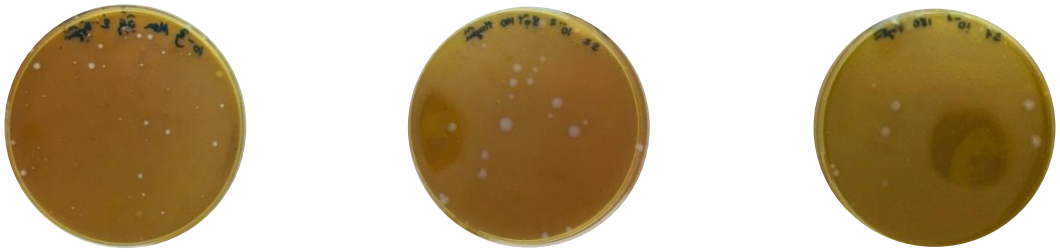


(a) *(b)*

Figure 2. *SEM photographs of the spray-dried inulin powder at a magnification of 1000X with different materials: (a) inulin and maltodextrin; (b) inulin, maltodextrin and L. acidophilus.*

According to [27], the formation of concavities on the surface of atomized particles can be attributed to the shrinkage of the particles during the drying process, which occurs due to the rapid evaporation of the liquid droplets. The external surfaces of the microcapsules were found to be free of fissures or disruptions, a feature that is crucial for ensuring higher protection and lower permeability to gases. This structural integrity is fundamental for maintaining the stability and viability of encapsulated probiotics during storage [17].

3.3. Viability of encapsulated microorganisms after the spray drying process



S_1 S_2 S_3

Figure 3. *Growth of L. acidophilus from three samples: pre-spray-dried samples (S_1) and two spray-dried samples at different temperatures of 140°C (S_2) and 180°C (S_3) in MRS agar after 48h of incubation.*

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After 48-hour incubation period, *L. acidophilus* colonies from three samples of pre-spray-dried samples (S_1) and two spray-dried samples at different temperatures of 140°C (S_2) and 180°C (S_3) were grown shown in Figure 3. On MRS agar, *L. acidophilus* formed small, round, white colonies that measured from 0.1 to 0.5 mm or oval brown colonies that formed approximately 1.0 mm.

Table 2 and Figure 4 present the number of cultured cells and the survival rate (%) of *Lactobacillus acidophilus* from three pre-spray-dried samples (S1) and two spray-dried samples processed at different temperatures (140°C for S2 and 180°C for S3). The initial concentration of *L. acidophilus* cells was 6.03 log CFU/g, and significant differences ($p < 0.05$) were observed following the spray-drying process. The viability of *L. acidophilus* cells in the spray-dried microparticles produced at 140°C and 180°C was 4.26 ± 0.01 log CFU/g and 2.94 ± 0.02 log CFU/g, respectively. The survival rate of probiotics decreased significantly by 29.41% when the inlet temperature of the spray-drying process was 140°C, and further plummeted to 48.67% when the inlet temperature was raised to 180°C. Notably, the spray-dried microparticles produced at 140°C exhibited higher viability of *L. acidophilus* compared to those produced at 180°C.

Table 2. *The number of cultured L. acidophilus cells from three samples of pre-spray-dried samples (S_1) and two spray-dried samples at different temperatures of 140°C (S_2) and 180°C (S_3) in MRS agar after 48h of incubation.*

Sample	Viable cell count (Log CFU/g)
S_1	6.03 ± 0.01^{a}
S_2	4.26 ± 0.01^{b}
S_3	2.94 ± 0.02^{c}

Data were expressed as the mean ± standard deviation (n = 6).

Mean values in the same column with different letters are significantly different at $p < 0.05$.

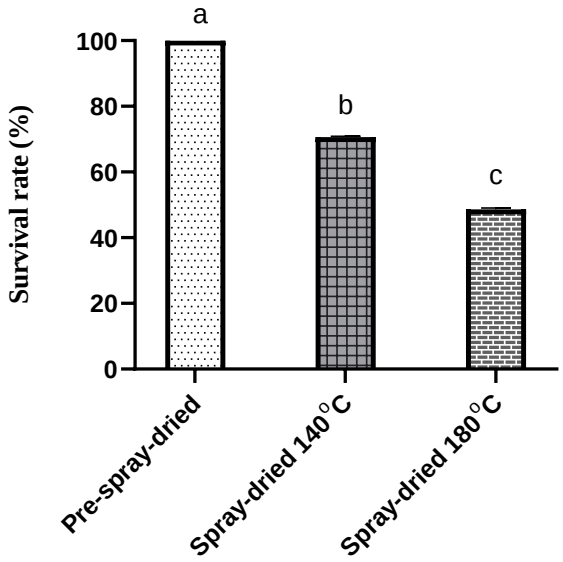

Figure 4. *Survival rate (%) of L. acidophilus from three samples of pre-spray-dried samples (S_1) and two spray-dried samples at different temperatures of 140°C (S_2) and 180°C (S_3). The values are means ± standard deviation (n=6). Different letters show statistical difference at the significant level of $p<0.05$.*

An increase in the air inlet temperature of the spray dryer leads to a decrease in the viability of microorganisms. This is because the rise in inlet temperature results in a corresponding increase in the outlet temperature, which directly elevates the temperature to which the microparticles are exposed. Conversely, a reduction in the outlet temperature results in longer drying times. The loss of viability during the spray-drying process can be attributed to both dehydration and exposure to high temperatures. These two concurrent mechanisms negatively affect the survival of probiotic microorganisms, as they contribute to cellular stress and reduced viability [18], [28].

4. Conclusion

Inulin was successfully extracted from dandelion leaves using water and ultrasound-assisted treatment. The results of this study demonstrate that both water temperature and ultrasonic power significantly influenced the yield of inulin. The most appropriate extraction conditions were identified as 60 W/g ultrasonic power at 80°C, which proved to be the most suitable for extracting inulin from dandelion leaves. An ultrasound power of 60 W/g was deemed adequate for this treatment. Compared to conventional extraction methods, ultrasound-assisted extraction resulted in a 9.48% increase in inulin concentration. Therefore, ultrasound-assisted extraction is an effective alternative for improving the extraction of inulin from dandelion leaves. The initial application of extracted inulin for synbiotic production was successfully carried out, with inulin being incorporated with maltodextrin for the spray drying of *Lactobacillus acidophilus*. The synbiotic powders exhibited intact surfaces with no cracks visible under Scanning Electron Microscopy (SEM), indicating a compact encapsulation of probiotics. The survival rates of *L. acidophilus* were found to be 70% and 50% when processed at inlet temperatures of 140°C and 180°C, respectively. Further studies should focus on optimizing spray-drying conditions to achieve a higher survival rate of probiotics in synbiotic powders.

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Conflict of Interest

The authors declare no conflict of interest.

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