

Enhancing Nutritional and Organoleptic Properties of Jerusalem Artichoke Tuber through Probiotic Fermentation

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Abstract

Fermented plant-based products are rapidly gaining popularity worldwide. Factors influencing the consumers' choice of such products include not only a desire to improve their health but also to reduce the environmental impact associated with the high consumption of animal products such as meat and dairy. Numerous studies demonstrate that fermentation enhances the bioavailability of nutrients such as amino acids, vitamins, and minerals in plant-based products. Consuming cultures of probiotic microorganisms as part of fermented plant-based products can have a beneficial impact on the digestive and immune systems. This article focuses on the use of different pre-treatment methods on Jerusalem artichoke tubers and the possibility of using fermentation to create new plant-based products with unique organoleptic properties and health benefits. Due to the unfavorable influence on quality indicators of plant dispersions, such as pH level, percentage of dry matter, content of proteins, fats, carbohydrates, fiber, and polyphenols, an increase in minerals, among ultrasound-treated, pre-frozen and untreated samples, a decision was made to carry out fermentation process using untreated raw material. Our study shows that among the various samples of Jerusalem artichoke tuber dispersions fermented with selected probiotic strains, the one with *L. bulgaricus* provides the most efficient fermentation process: the highest antioxidant activity with a maximum value of 67.5% DPPH activity on day 5 after fermentation; the highest increase of microbial cells during fermentation was recorded to be $\log_{10}(\text{CFU/ml}) = 2.3$; the fastest acid accumulation and the best results of sensory estimation were also achieved.

Introduction

The European out-of-home food sector is witnessing a surge in the availability of plant-based products, reflecting the increasing popularity of vegetarian diets. The adoption of vegetarian diets is driven by various factors, including ecological concerns, economic considerations, religious beliefs, and ethical motivations. The latter category encompasses individuals' aspirations to manage their weight, enhance their physical fitness, and adhere to medical recommendations advocating the avoidance of meat consumption. Extensive research has demonstrated the manifold effects of vegetarian diets on body mass, plasma lipid profiles, blood pressure levels, and the prevention of heart diseases and atherosclerosis [1–3].

Fermentation can be applied to a wide array of staple foods consumed globally, many of which are deeply rooted in the culinary traditions of various regions. Fermented products constitute a substantial part of diets worldwide and are perceived as natural and health-promoting [4, 5].

The probiotic and biogenic effects of fermented products are among the reasons for their recognized benefits. Fermented plant-based products provide an alternative source of non-dairy probiotics that align with modern dietary trends, including low cholesterol, lactose-free, dairy-free, vegetarian, and vegan preferences [6].

Scientific research has unequivocally affirmed the positive influence of fermented products on health. A recent study investigating the effects of fermented plant-based foods on microbial and metabolomic variations revealed significant differences in beta diversity between individuals who consume fermented products and those who do not [7].

During the fermentation process, proteins are partially broken down, rendering them more digestible. Furthermore, fermented products may contain enhanced levels of vitamins and antioxidants. These observations support the recommendation for the fermented products inclusion in international dietary guidelines due to the presence of beneficial components [8].

The plant-derived sources present a novel avenue for probiotic delivery, particularly since most commercial probiotic strains originate from the gastrointestinal tract [9].

A diverse array of plants, including legumes, seeds, nuts, and grains like rice, serve as the foundation for alternative dairy products [10]. Currently, research is underway to explore the health advantages of *Helianthus tuberosus*, commonly known as Jerusalem artichoke. Jerusalem artichoke is a valuable crop with traits such as disease resistance, adaptability to poor soil conditions, and rapid growth. Additionally, it exhibits high resistance to plant diseases [11]. Numerous studies have established that Jerusalem artichoke tubers can serve as biologically active ingredients in various food products, encompassing dairy items, beverages, etc. [12, 13].

Nonetheless, there is a dearth of research on the creation of fermented plant-based beverages using Jerusalem artichoke tubers, and notably, no studies have addressed the influence of different pretreatment methods on the physicochemical properties, organoleptic characteristics, and mineral content alterations in Jerusalem artichoke dispersions.

Consequently, the objectives of this study are twofold: first, to assess the impact of various plant material pretreatments on the diverse characteristics of Jerusalem artichoke dispersions, and secondly, to explore the technology and evaluate the potential of using probiotic cultures in the development of fermented Jerusalem artichoke tuber-based beverages.

Materials and methods

The materials and detailed procedures are described in the Supplementary online resource.

Results and discussion

Preliminary research on pretreatment methods and analysis of Jerusalem tuber dispersions made from untreated and treated raw materials

Pre-treatment of plant raw materials can contribute to the release of low molecular weight and water-soluble compounds through the cell membranes that were damaged [14–16]. Physico-chemical

indicators and mineral composition are subject to changes depending on the type and duration of processing of raw materials (**Table S1**).

Pre-treatment had the greatest effect on the content of dry matter, protein, polyphenols, and carbohydrate contents in the Jerusalem artichoke dispersion. The highest content of dry matter, protein, and carbohydrates was in the control sample that was obtained from untreated raw materials. Any type of pretreatment led to a decrease in the content of protein, polyphenols, dry substances, and carbohydrates. Freezing at -80°C had the most negative effect on carbohydrate, and dry matter content decreasing the value from 5.99 ± 0.89 and $6.91 \pm 0.11\%$ to $4.08 \pm 0.52\%$ and $4.86 \pm 0.11\%$ and $16.58 \pm 0.96\%$, respectively. During ultrasonic treatment, the hydrophobicity of proteins increases due to their partial denaturation in their secondary and tertiary structures. These changes can affect the dispersion of protein molecules and lead to a decrease in their distribution, which facilitates the movement of the protein phase into the solution [17–19]. The decrease in protein content was maximum when ultrasound treatment for 6 min was used or the raw material was pre-frozen before processing and reached the value from 0.66–0.42%.

It is known that pretreatment can modify plant material in such a way as to stimulate the release of bioactive components weakly bound to cell wall polymers [20]. Damaging the membrane can be sufficient to release the active compounds and can improve the extraction efficiency of bioactive compounds [21, 22, 14]. But at the same time, cell disruption methods can negatively affect the biological activity of the resulting product. It is known that pre-freezing, commonly used to preserve the quality of frozen foods, can degrade cell structure, resulting in discoloration, loss of moisture, softening, and loss of nutrients and biologically active compounds [23, 24]. Polyphenolic compounds constitute a diverse array of secondary metabolites characterized by multiple phenol functional groups. Found in a range of dietary sources like fruits, vegetables, and beverages such as tea and wine, polyphenols have been extensively studied for their bioactive properties. One of the primary biological activities ascribed to polyphenolic compounds is their capacity for free radical scavenging. Studies have demonstrated that polyphenols possess robust antioxidant properties, effective in neutralizing reactive oxygen species (ROS) and thereby ameliorating oxidative stress [25]. According to data obtained, it can be seen that the preliminary freezing of Jerusalem artichoke tubers decreases the polyphenol content, perhaps, due to the fact associated with the native composition of plant cells and the destruction of cells that occurs during freezing that led to the release of biologically active substances and their degradation due to chemical and enzymatic oxidation reactions. [26–28].

Different ultrasound and freezing treatments can affect the increase or decrease in the content of various mineral components [29, 30]. We have studied the effect of preliminary ultrasonic treatment and freezing methods of Jerusalem artichoke tubers on the mineral composition of plant dispersions (**Table S2**). There was a tendency to decrease the iron content after freezing and especially after sonication over time compared to the control sample. As the sonication time increased and decreased the freezing temperature, the iron content decreased by an average of 23–45% for the samples subjected to ultrasound treatment and 7–8% for the frozen samples. About the pre-freezing, there was no consistent pattern. When compared with the untreated Jerusalem artichoke tubers, frozen at -80°C tubers contained

significantly more Ca, Si, Mg, Sr, and P. There was also an increase in Al, Ba, K, Cu, and Cr in the samples that were frozen at -17°C. An increase and decrease in the content of microelements may be due to damage to the cellular structures of vegetable raw materials during the preliminary freezing at a temperature regime of -17°C and -85°C as cell membranes are disrupted by ice crystals during the freezing process [31].

Organoleptic evaluation of samples of Jerusalem artichoke tuber dispersions (**Fig. S1**) showed that pre-treatment of raw materials affects their sensory properties. None of the pre-treatment methods had significant changes in the viscosity of the samples, as well as bitter aftertaste, sour taste, homogeneity, putrid aroma, and fungus/earthy flavor of plant dispersions. Prolongation of ultrasound treatment led to the tendency of bitter taste. The 2-minute ultrasound treatment increased the fungus/earth aroma but then reduced this intensity with the expansion of ultrasound treatment time. The ultrasound pre-treatment also increased the Jerusalem artichoke tuber aftertaste but slightly decreased Jerusalem artichoke tuber flavor and aroma when 4 min ultrasound and longer were used. Ultrasonic treatment had a slight effect on wateriness, sweet taste, and pea flavor. As for freezing treatment, the -17°C influenced a greater decrease of wateriness, Jerusalem artichoke tuber flavor and aroma, and pea flavor, but an increase in sweet taste descriptor. Due to the formation of small crystals of ice and the least extent of cell disruption the freezing at -80°C led to insignificant changes in sweet taste compared to the control sample and pre-frozen at -17°C.

According to the results obtained the pre-treatment decreased the overall acceptability attribute to a different extent and the preference was for the control sample without pre-treatment. Therefore, for the next studies, the control sample was chosen.

Impact of probiotic strain fermentation on quality of Jerusalem artichoke tuber dispersions

Acid accumulation

Based on the results presented in **Fig. S2** we can conclude that changes in microorganism strain of starter cultures significantly affect the product fermentation time. The most rapid acid accumulation process among all selected *L. acidophilus* strains was performed by *L. acidophilus* H9, which attained a pH value of 4.72 and titratable acidity of 0.301% in 7 h. The most rapid fermentation till 4.75 was for *S. thermophilus* for 5 h. The use of *B. longum* B379M and *B. bifidum* led to a decrease in pH value which dropped between 8 h and 10 h to 4.68 and 4.73, respectively. The slowest acid accumulation was when using *B. coagulans* MTCC 5856 and *P. shermani* KM-186 for 13 h. Generally, all selected probiotic bacterial strains were able to adapt to plant dispersion from Jerusalem artichoke tubers.

The maximum acidification rate (V_{max} , pH/min) was for *L. acidophilus* AT-41 and is equal to 0.007, for *L. acidophilus* 57S and *L. acidophilus* 8 reached 0.005. During fermentation by *L. acidophilus* H9 and *L. bulgaricus*, the maximum acidification rates were 0.006, and 0.009, respectively. For the samples

fermented with *B. longum* B379M the maximum acidification rate was 0.009, with *B. bifidum* the rate was 0.006, with *B. coagulans* MTCC 5856 was 0.003, with *S. thermophilus* was 0.007, and with *P. shermanii* KM-186 was 0.003.

Antioxidant activity

Resultant values of antioxidant activities assayed (**Fig. S3**) during storage when using different starter cultures showed, that changes in DPPH radical scavenging activity depend on the probiotic microorganism used. Results showed that fermentation by *B. bifidum* and strains *L. acidophilus* AT-41, 57S, and H9 allowed to reach maximum values (51.34%, 51.54%, 54.85% and 55.39%, respectively) after the fermentation process was completed. While results indicated that there was no significant difference between fermented samples by *B. coagulans* MTCC 5856 and *B. longum* B379M compared to the same samples before the fermentation process. As for *L. bulgaricus* the chosen strain could inhibit 43.37% of DPPH radicals. The minimum value for the percentage of radical scavenging activity was for samples fermented with *L. acidophilus* 8 (38.23%), *P. shermanii* KM-186 (38.12%), and *S. thermophilus* (41.11%). Refrigerated storage increased DPPH inhibition in samples fermented with *L. acidophilus* AT-41, 57S, 8, H9, and *B. coagulans* MTCC 5856 on the 5 days and the maximum values for the samples fermented with *B. longum* B379M, *B. bifidum*, *P. shermanii* KM-186, *S. thermophilus* and *L. bulgaricus* were on 3 days of storage with the subsequent decrease till the 11th day. Some studies have shown that fermentation and post-acidification products can improve the antioxidative activity by increasing the release, for instance, organic acids and other phenolic compounds, produced during storage and hence could be possible sources of DPPH inhibitors [32, 33].

The greatest decrease in antioxidant activity on day 11 of storage occurred when *L. acidophilus* 57S was used for fermentation, and was 44.23%, which was 10.62% down from the antioxidant activity values after fermentation on day 0. The samples fermented with strains *L. acidophilus* H9 reduced DPPH scavenging activity on day 11 by 2.12% compared to day 0. The values of antioxidant activity in samples fermented by *L. acidophilus* AT-41 on day 11 of storage returned to the initial value, which was on day 0. The use of *B. longum* B379M, *P. shermanii* KM-186, *L. bulgaricus*, *S. thermophilus*, and *L. acidophilus* 8 as starter cultures showed an increase in antioxidant activity on day 11 compared to day 0 by 3.13%, 9.34%, 7.24%, 2.78% and 5.87%, respectively. As for the strains *B. bifidum* and *B. coagulans* MTCC 5856, the antioxidant activity on day 11 decreased by 3.35% and 4.62%, respectively, compared to day 0.

Impact of fermentation on viability of probiotic bacteria

Data shown in **Fig. S4(a)** demonstrated the viability of probiotic bacterial cultures on fermented Jerusalem artichoke tubers dispersion. The viability microorganisms of fermented dispersions increased significantly from zero time before fermentation to the end of fermentation, with viable counts biomass increase 2.3, 2.27, and 2.12 log₁₀CFU/ml during 9, 10, and 13 h for *L. bulgaricus*, *L. acidophilus* AT-41 and *B. coagulans* MTCC 5856, respectively and these were the highest viability among all selected probiotic microorganisms. *L. acidophilus* H9, *S. thermophilus*, and *L. acidophilus* 8 also showed significant increases in viability, but less than the former microorganisms and were 1.55, 1.46, and 1.58 log₁₀CFU/ml

to 7, 5, and 9 h, respectively. In contrast, the lowest viability showed *B. longum* B379M to 8h of fermentation, *B. bifidum* to 10 h, and *P. shermanii* KM-186 to 13 h (0.41, 0.21 and 0.37 log₁₀CFU/ml increase, respectively).

L. acidophilus 57S showed no changes in biomass increase and had a value of 7.03 log₁₀CFU/ml. So, it can be concluded that Jerusalem dispersion might be deficient in nutrients essential for their growth.

According to the data in **Fig. S4(b, c)** the specific growth rate (μ_G) among the *L. acidophilus* strains were 0.0226, 0.0281, 0.028, and 0.00039 h⁻¹, the doubling time of microbial cell concentration (t_d , h) was 30.65, 24.68, 24.26, 1782.89 h, multiplication rate (MR) was 0.033, 0.041, 0.041, 0.0005 and for Jerusalem artichoke dispersions fermented by *L. acidophilus* 8, *L. acidophilus* AT-41, *L. acidophilus* H9 and *L. acidophilus* 57S, respectively. Data also revealed that the specific growth rate (μ_G) was 0.0071, 0.0378, 0.0316, 0.0030, 0.0039, and 0.0204 h⁻¹, the doubling time (t_d) was 97.42, 18.29, 21.96, 234.50, 174.94, and 34.06 h, multiplication rate (MR) was 0.010, 0.055, 0.045, 0.004, 0.005, and 0.029 for the fermented samples of Jerusalem artichoke dispersions with *B. longum* B379M, *S. thermophilus*, *L. bulgaricus*, *B. bifidum*, *P. shermanii* KM-186, *B. coagulans* MTCC 5856, respectively.

Viscosity values

After fermentation, the presence of a structure (clot) that could be destroyed under the action of shear deformation was not visually detected in the studied samples. The same was confirmed when measuring rheological properties on a rotational rheometer. It can be seen from data in **Fig. S5** that the viscosity of the studied samples does not depend on the shear rate and is on average only 2.16 times greater than the dynamic viscosity of water at the same temperature (4°C). The sample fermented by *B. longum* B379M sample has the highest viscosity. The increase in viscosity compared to the control sample before the fermentation was 8.56%. However, in general, the viscosity of Jerusalem artichoke dispersions after fermentation by various bacterial cultures does not differ significantly. Fermented vegetable dispersions of Jerusalem artichoke tubers can be consumed as a beverage itself, somewhat like resembling whey-based beverages. It is also possible to further increase the viscosity by adding various fillers and thickeners to obtain a product similar to yogurt-like beverage.

Impact of fermentation on the organoleptic liking

When making Jerusalem artichoke dispersion the dark color is appears due to the oxidation of polyphenolic compounds by peroxidase and polyphenol oxidase. However, during fermentation process, released acids suppress the action of enzymes. During the fermentation process, all samples change color from dark brown to light beige. According to the data presented in **Fig. S6** using hybrid hedonic scale, depending on the starter culture added, the organoleptic properties of the fermented dispersions of Jerusalem artichoke tuber varied greatly, due to the fact that each strain performs a unique organoleptic profile of the product.

In terms of taste liking attribute, the least scores gained the sample fermented with *L. acidophilus* AT-41 due to the most pronounced sour taste in comparison to other samples. The samples *L. bulgaricus* gained the highest scores as they had the moderate sweetness and there is no bitterness in taste. The samples fermented by *B. coagulans* MTCC 5856, *S. thermophilus*, and *B. longum* B379M had the lowest scores from assessors for flavor, as the former had the flavor of rotten eggs, the two latter samples had the unusual off-flavors, despite that the sample fermented with *B. longum* B379M had the pleasant strawberry flavor. Sample with *L. acidophilus* 57S and *L. bulgaricus* achieved the highest scores due to the similarity of the former samples with brine of pickled cucumbers with honey, and the latter with diluted sauce BBQ. Assessors also noted the similarity of the samples fermented with *L. acidophilus* H9 to carrot and potato puree in flavor attribute. These samples as well as the samples fermented with *L. bulgaricus* received a commensurately high rating in terms of appearance liking. The lowest results for appearance liking parameter were demonstrated by *B. coagulans* MTCC 5856 and *B. longum* B379M, since flakes and fractionation were formed the fermentation process. In terms of texture liking the samples fermented with *B. coagulans* MTCC 5856 gained the least scores. The samples with *S. thermophilus*, *L. bulgaricus* and *P. shermanii* KM-186, on the contrary, received high marks from assessors. Ultimately, the sample fermented with *L. bulgaricus* received the highest overall liking scores and the lowest were for *B. coagulans* MTCC 5856.

Conclusion

It was found that Jerusalem artichoke tubers subjected to different pre-treatment methods led to the changes in the qualitative and quantitative indicators of the resulting dispersions. Despite the increase in microelements content after pre-treatment methods, other quality characteristics, such as percentage of dry matter, content of proteins, fats, carbohydrates, fiber and polyphenols and organoleptic properties decreased to different extent. Therefore, a decision was made to continue working with untreated samples.

Almost all fermented samples showed an increase in the number of living cells and antioxidant activity during fermentation, except for the sample fermented with *L. acidophilus* 57S. The samples fermented by *L. acidophilus* H9 and *L. acidophilus* 57S have the highest antioxidant activity in the end of fermentation. The most active cell growth was demonstrated by *L. bulgaricus*, *B. coagulans* MTCC 5856, and *L. acidophilus* AT-41. Results in terms of organoleptic assessment showed that *L. bulgaricus* demonstrated the highest values for all assessed sensory attributes. Based on the general estimation of quality indicators, the most advantageous as a future commercial product is a dispersion fermented with *L. bulgaricus*. However, considering the rest of the strains for future studies is relevant to use other strains, which demonstrated high rates of antioxidant activity or cell growth or organoleptic properties. Combining them and applying these cultures in combination to ferment Jerusalem artichoke tuber dispersions might be another step in obtaining new products with unique properties and with their niche and buyer.

Declarations

Data Availability

All data generated or analyzed during this study are included in this published article (and its supplementary information files). Any additional data or information can be made available on request to the corresponding author.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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