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Biochemical, Microstructural, and Probiotic Bacterial Patterns of Innovative Fresh Cheese Fortified with *Helianthus tuberosus* Tubers

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Abstract: Recently, functional foods have become the aim of customers and food producers. Integrating vegetable ingredients in the food industry is a productive goal to reduce costs while maintaining quality. Dried Jerusalem artichoke tubers powder (DJATP) was used as a novel approach in cheese manufacturing. Innovatively, its holding capacity features and impact on probiotic development were evaluated. The SEM microstructure image and biochemical analysis of DJATP confirmed higher water holding (5.31 g/g), oil absorption (1.99 g/g), and swelling (1.79 g/g) capacities than casein. DJATP (3%) supported the probiotic bacterial growth (*Streptococcus thermophilus*, *Bifidobacterium bifidum*, and *Lactobacillus acidophilus*) and accelerated the fermentation of skimmed milk more than pure inulin. When fortified with DJATP (3% or 6%), the cheese yield increased (24.66% and 27.85%, respectively) compared with 17.55% for control after storage (14 days). Besides the high levels of amino acids, minerals, flavonoids, phenols, and antioxidants, the probiotic bacteria in the DJATP-fortified cheese were better active, with better sensory features, recording the highest judging score (87.67) against the control (79.00). To our knowledge, no preceding studies used DJATP in fresh cheese manufacturing followed the probiotic behavior in DJATP or compared the microstructure of DJATP and casein. Instead of inulin, our novel approach suggests using DJATP as a prebiotic and an enhancer for fresh cheese quality and yield, all while being cost-effective. Future studies are encouraged to explore the potential use of DJATP in other functional cheese products.

Keywords: functional fresh cheese; probiotics; lactic acid bacteria; scanning electron microscopy; amino acids; sensory; texture



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1. Introduction

The perennial earth apple, or Jerusalem artichoke plant (*Helianthus tuberosus* L.), is grown for its edible tubers. Jerusalem artichoke (JA) is a relatively low-maintenance crop, and it is resistant to many pests and diseases. As a result, it is a popular choice for commercial cultivation worldwide, yielding 50–60 tons of tubers per hectare [1].

Owing to their high nutritional value, tubers can be cooked in a variety of ways but the prime use of JA is the production of fructose syrups, inulin, fodder yeast, alcohol, concentrated tablets or capsules, and sweeteners for diabetes mellitus patients; furthermore, latterly, there has been a great affirmation of JA used in the food industry, pharmaceutical

trends, canning processes, and feed production [2,3]. Tubers are a significant source of nutrients, especially carbohydrates, and fibers, mainly fructooligosaccharides and inulin, which reach 80% of dry matter [1].

Tubers contain a unique complex mixture of poly- and oligosaccharides (namely inulin), a polymer of D-fructose that relates to each other through β (2→1) linkages [1,2,4]. In addition, JA tubers contain protein (rich in essential amino acids: valine, arginine, histidine, leucine, isoleucine, lysine, threonine, and methionine), ash, crude fat, and a balanced amount of macro and microelements such as phosphorus, potassium, iron, zinc, and calcium [1,3]. Tubers also contain organic and fatty acids as well as vitamins, i.e., B complex, C, and E [4,5].

Inulin serves as biological stimuli for beneficial bacteria that inhibit the pathogenic and undesirable intestinal microflora, improve intestinal movement, mend the bioavailability of minerals (magnesium, calcium, and iron), retard fat absorption, assist the digestion of protein diets, reduce blood cholesterol, enhances glucose tolerance and triglycerides, and exerts an anti-diabetic effect [6–8].

As a result of the sweet taste of tubers, they can replace sugar, cereal flour, and fats in food products; what is more, it is not required to extract ingredients from tubers before being applied in the manufacturing of flavorsome functional foods [8]. This makes JA tubers more suitable for the preparation of functional foods.

Many researchers have developed various dairy products by adding JA tubers (grated or extract); these products have biological, therapeutic, and prophylactic properties. For instance, in a previously developed yogurt formula with the addition of JA extract or juice, the resulting products contained inulin (129.78 mg/g) and decreased blood sugar to 146.44 mg/g [9].

Furthermore, many bakery products (cake, biscuits, and bread), sausages, dairy products (yogurt and cheese), and beverages have been improved by adding powder of JA tubers [10,11]. Despite these works, no previous work was deeply performed to investigate the variation in the physicochemical and biological properties that occur when fortifying the cheese with JA tubes.

To our knowledge, no studies used dried Jerusalem artichoke tuber powder (DJATP) in cheese manufacturing; furthermore, the effect of DJATP on the development of probiotics, in comparison with pure inulin, was not studied, shedding light on the potential of DJATP to support probiotic growth. Innovatively, the direct application of DJATP in cheese manufacturing represents a pioneering approach with the potential to significantly enhance both the nutritional profile and overall quality of cheese products.

The powder of JA, rich in inulin and functional compounds like antioxidants and flavonoids, not only boosts the cheese's nutritional value but also imparts beneficial functional qualities, surpassing the ordinary use of pure inulin alone [9]. Moreover, this approach offers cost-saving advantages by avoiding the need for traditional inulin extraction from JA tubers, making it applicable even to small-scale cheese producers. Additionally, dairy products—traditionally lacking in dietary fibers—can be fortified with Jerusalem artichoke to create fiber-enriched alternatives that also support probiotic bacteria in the gut [9]. A novel aspect of this research involves evaluating the water and oil holding capacities of DJATP and comparing its microstructure with casein using scanning electron microscopy, highlighting its unique role in the yield and quality of cheese.

This work revolves around two focal aspects. The preliminary phase involves evaluating the characteristics of prepared DJATP in contrast to the commonly used prebiotic, inulin. This phase also encompasses SEM-based microstructure (water-holding capacity and oil absorption capacity) comparisons between DJATP and standard milk casein. In addition, the behavior of probiotic bacteria when exposed to DJATP is studied in comparison with inulin. The subsequent phase depends on the findings from the preliminary study to facilitate fresh cheese production to set up a suitable protocol for manufacturing functional DJATP-fortified cheese (Karish). The investigation was extended to assessing the physicochemical and biological attributes of the resulting cheese.

2. Materials and Methods

Dried milk casein was obtained from El-Gomhouria Company for trading drugs, chemicals, and medical Supplies in Cairo, Egypt.

The medium-chain inulin (Raftilin[®]GR with an average chain length DP of 10) was provided by Mandurah Australia Pty. Ltd. (Dandenong, VIC, Australia).

Helianthus tuberosus tubers (cultivar; Fuseau) were provided from Al-Serw Research Station, Agricultural Research Center, Giza, Egypt.

The freeze-dried yogurt starter commercial product (ABT-5) was provided by Chr. Hansen Laboratories, Copenhagen, Denmark. ABT-5 was used during fresh cheese preparation as a source of probiotic bacteria (*Streptococcus thermophilus*, *Bifidobacterium bifidum*, and *Lactobacillus acidophilus*).

Buffalo defatted milk (10.49% total solids, 0.2% fat, 4.3% protein, 5.2% lactose, 0.79% ash, and 6.69 pH), which was used for manufacturing the fresh cheese, was analyzed [12] and provided by the Dairy Technology Unit, Food Science Department, Faculty of Agriculture, Zagazig University, Egypt.

2.1. Preparation of JA Tubers

The dried powder of JA tubers was prepared as described earlier [13]. Briefly, the fresh tubers of JA were cleaned and washed with tap water, then brunched with 5% sodium hydroxide solution at 90 °C for 1.5 min, followed by rinsing five times with cool water, until cooled to room temperature, before being peeled and submerged in citric acid solution (3%, 15 min) and then cut into pieces of 1.5 cm thickness. The pieces were left for 10 min at room temperature before being dried by a rotary vacuum microwave oven (vacuum microwave dryer, March Cool Co., Ltd., Bangkok, Thailand) (2500 g/cycle, 90 min) up to a moisture content of 15%. This was followed by further drying by a hot air dryer Memmert, Germany) at 50 °C for 4 h. The dried pieces were ground using a blender and hammer mill machine (FW-200 A, Beijing Zhongxing Weiye Instruments Co., Ltd., Beijing, China) and then sieved to a size particle of 300 µm (Sieve shaker, Restex, Loughborough, UK). A set of sieves was used with a mechanical sieve shaker (Model M200) to determine the particle sizes of DJATP. The resulting milled DJATP were packed in a vacuum aluminum foil bag and stored under freezing until use. The recovered yield of DJATP (%) after preparation was calculated following the next Equation (1):

$$\text{Yield\%} = \frac{\text{Weight of dry powder}}{\text{Weight of raw material}} \times 100 \quad (1)$$

2.2. The Physical Properties of DJATP

The color of DJATP powder was assessed to determine its suitability for addition during cheese making. The color was rated using Hunter Lab (Color Flex Model 45/0, Reston, Virginia, USA), which measured color attributes as L*, a*, and b* color parameters, where L is the lightness from white (100) to black (0); a* is the redness, ranging from green (−) to red (+); and b* is the yellowness color range from yellow (+) to blue (−) [12].

The apparent viscosity of DJATP was measured [14] in a solution of 4 gm in 100 mL distilled water at 20 °C by Brookfield Engineering Labs DV III ultra rheometer, Inc. Stoughton, MA, USA. The measurement unit of viscosity was expressed in millipascal seconds (mPa·s).

2.3. The Chemical Properties of DJATP

The chemical composition of DJATP was determined. The content of moisture, total solids, lipids, total protein, ash, titratable acidity, pH, and the percentages of soluble, insoluble, and total dietary fibers were assayed as described by AOAC [12]. Carbohydrates were estimated by subtracting the protein, total solids, fat, and ash from 100. The total dietary fibers were calculated as the sum of insoluble and soluble dietary fibers. Inulin was determined according to Saengkanuk et al. [15].

The DJATP contents of iron, zinc, calcium, phosphorus, and magnesium were detected with atomic absorption spectrophotometry (Varian Model Spectra AA 100 and 200). Potassium and manganese were detected by the flame photometer (Jenway PF7 Flame Photometer, Essex, UK) AOAC [12].

2.3.1. Flavonoids, Phenols, and Antioxidant Activity

The sample (5.0 g) was extracted with 50 mL ethanol (50%) with continuous stirring at room temperature for 1 h, followed by filtration by Whatman No. 1 filter paper. The obtained ethanolic extract was used for the determination of total flavonoid content, total phenols, and antioxidant action.

The total flavonoid content was detected following the method by Bayba et al. [16]. Briefly, 0.2 mL of ethanolic extract was dissolved in distilled water (3.5 mL). Then, 0.3 mL of sodium nitrite (5%) was mixed and kept for 5 min followed by the addition of 0.3 mL of aluminum chloride (10%) and 2 mL of sodium hydroxide (1 M NaOH). After 5 min, the flavonoid content was measured spectrophotometrically (6505 UV/Vis, Jenway Ltd., Felsted, Dunmow, UK) at 510 nm, utilizing *d*-Catechin as a reference standard.

For the determination of total phenols, 0.3 mL of ethanolic extract was mixed with 1.5 mL Folin–Ciocalteu reagent (10-fold diluted) and 1.2 mL sodium carbonate (7.5% *w/v*). After 30 min, the absorbance of the mixture was measured spectrophotometrically (6505 UV/Vis, Jenway Ltd., Felsted, Dunmow, UK) at 765 nm using gallic acid as a reference standard [17].

The radical scavenging (antioxidant) activity was measured using a 2,3-diphenyl-1-picrylhydrazyl (DPPH*) assay by mixing 100 μ L of the ethanolic extract with 5 mL of DPPH* (0.004% *w/v* in methanol) and vortexing for 15 s. The mixture was then left to stand. After 30 min at room temperature, the decrease in the solution absorbance due to proton-donating activity was measured spectrophotometrically (6505 UV/Vis Jenway Ltd., Felsted, Dunmow, UK) at 517 nm against a blank of ethanol [18]. The control was equipped without extract. The DPPH* scavenging activity was estimated by Equation (2):

$$\text{DPPH}^* (\%) = (1 - A_1) / A_0 \times 100 \quad (2)$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

2.3.2. Capacity Properties of DJATP

The water-holding capacity (WHC) was measured by mixing 1.0 g of DJATP (W_0) with 10 mL of distilled water for 5 min at 5 °C. The residue was weighed (W_2) and dried (30 min) before being re-weighed (W_1) again and WHC was calculated [19] using Equation (3):

$$\text{WHC} = (W_2 - W_1) / W_0 \quad (3)$$

For the determination of the oil absorption capacity (OAC), 1.0 g of sample was mixed with 10 mL of vegetable oil (V_1) and left for 30 min; after that, the mixture was centrifuged (3–30 KS, Sigma, Taufkirchen, Germany) at 20,000 rpm for 5 min at 5 °C. The volume of the supernatant was recorded (V_2) and the OAC was calculated [20] by Equation (4):

$$\text{OAC} = (V_1 - V_2) / W_0 \quad (4)$$

The swelling capacity (SC) was measured [21] by hydrating 100 mg of the sample in a 10-mL cylinder, the sample was dispersed by gently stirring before being covered and left undistributed at room temperature for 18 h to be hydrated and equilibrated. The settled volume occupied by the sample was recorded and the SC was calculated (Equation (5)) as follows:

$$\text{SC} = \frac{\text{The volume occupied by the sample}}{\text{Original sample dry weigh}} \quad (5)$$

2.4. Scanning Electron Microscopy (SEM) Investigation

Samples of SEM were fixed as described by Omar et al. [22]. The samples were investigated by SEM (FEI Company, Eindhoven, The Netherlands, Model Quanta 250 FEG (Field Emission Gun) with an accelerating voltage of 10 kV. The samples underwent freeze-fracturing in liquid nitrogen to create approximately 1 mm-sized pieces. These pieces were then affixed to aluminum stubs using silver paint, dried to a critical point, and coated with a layer of gold for 300 s utilizing a sputter-coater (SCD 005 Sputter Coater) and scanned at 60 pascals. Images were captured at various magnifications.

2.5. Growth of Probiotic Bacterial Culture on DJATP

2.5.1. Preparation of Probiotic Bacterial Culture

This trial was performed to inspect the ability of probiotic bacteria to ferment DJATP when used as supplementation during cheese manufacturing. To prepare the probiotic culture, 2% (*w/v*) from the freeze-dried culture (ABT-5 starter) was added to 100 mL of sterile reconstituted skimmed milk (spray dried skimmed milk, low heat, France origin) at 40 °C and the starter was incubated until the onset of gelation. In total, 100 mL of sterile skimmed milk was inoculated with 2 mL of the starter culture and incubated at 40 °C until the creation of the gel [23].

To test the impact of inulin or DJATP on the bioactivity of probiotics, three flasks containing 100 mL of sterilized skimmed milk (13% TS) were supported with sterilized inulin or DJATP at a ratio (3%, *w/v*) of skimmed milk. A third flask of skimmed milk was left without additives (control). All flasks were inoculated with 2% of activated starter ATB-5. The content of each flask was mixed well and then incubated at 40 °C for 15 h. The pH values of fermented milk and the counts of *S. thermophilus*, *B. bifidum*, and *L. acidophilus* were evaluated at time zero and at time intervals.

2.5.2. Enumeration of Probiotics

The enumeration of *S. thermophilus* counts was performed according to Dave and Shah [24] on the M17 agar (Biolife, Bolzano, Italy) and incubated at 37 °C for 48 h under aerobic conditions. *Bifidobacterium bifidum* was enumerated on the MRS-NNLP medium. While *L. acidophilus* numbers were determined on MRS-sorbitol agar. The plates of *B. bifidum* and *L. acidophilus* were anaerobically (Anerocult A system; Merck, Darmstadt, Germany) incubated at 37 °C for 72 ± 1 h [25,26]. The data were introduced as the logarithmic number of colony-forming units (log CFU/g).

2.6. Fresh Cheese Manufacturing

Fresh cheese was prepared using ABT-5 culture according to Ahmed et al. [27] with slight modifications. Fresh buffalo skimmed milk (30 Kg) was heated at 75 °C for 15 s, directly cooled to 40 °C, and then divided into 4 portions. The first was left as a control. DJATP was added to the other three portions at 1, 3, or 6% (these ratios were chosen according to some initial experiments and the addition of more than 6% of DJATP was excluded, taking into account the economic and technological aspects) and mixed well. The culture of ABT-5 was added (3%, *v/v*) and all four treatments were incubated at 40 °C until full coagulation. The curd was scooped in hoops of a suitable size and then placed on a porous mat of cloth to expel more whey. After that, it was cut into pieces. The resultant fresh cheese was packed in plastic containers and kept in the refrigerator (5 ± 1 °C). Usually, this kind of soft and unsalted cheese has a short shelf-life; therefore, all tests were performed on fresh cheeses and after 7 and 14 days of cold storage. The cheese yield (%) was estimated according to the following Equation (6) [28]:

$$\text{Yield, \%} = \frac{\text{Amount of cheese, kg}}{\text{Amount of skimmed milk, kg}} \times 100 \quad (6)$$

2.7. Evaluation of Fresh Cheese

2.7.1. General Chemical Composition

Cheese samples were investigated for the content of total solids, total protein, ash, titratable acidity, pH, and minerals as described by AOAC [12]. Total flavonoids, total phenols, and antioxidant activity were determined as described above.

2.7.2. Amino Acids Profile

The profile of amino acids was determined in cheese samples according to Pellett and Young [29]. Briefly, acid hydrolysis was performed on the sample and then a cation-exchange separation column of 4.6×150 mm was used (Sykam GmbH, LCA K06/Na, Herzogenaurach, Germany) with a post-column ninhydrin derivation using an amino acid analyzer (Sykam GmbH, Herzogenaurach, Germany).

2.7.3. Microbiological Properties of Cheese

Various cheese samples were biologically tested for the content of probiotic bacteria (*S. thermophilus*, *B. bifidum*, and *L. acidophilus*), at time zero and after 7 and 14 days of storage. The previously mentioned methods were applied for the enumeration of the three bacteria [24–26]. The results were expressed as log CFU/g of the sample and the viability of each bacterium in various samples was estimated [30] as follows (Equation (7)):

$$\text{Viability, \%} = \frac{\text{CFU/g after 14 days of storage}}{\text{initial CFU pergram}} \times 100 \quad (7)$$

2.7.4. Sensory Evaluation

Sensory evaluation was conducted humanly by twelve highly trained panelists, without using any machine to evaluate experimental cheese. Sensory evaluation of the cheese samples was performed according to the scheme described by the International Dairy Federation [31], which consists of 50 points for flavor, 35 points for body and texture, and 15 points for appearance, with 100 points for the total score.

2.7.5. Texture Profile

The texture features of fresh cheese were measured at 23 °C using the Instron Universal Tasting Machine model 1195, Stable Microsystem. (SMS) LTD., Godalming, UK, loaded with dimension software SMS program. The penetration value was also measured based on the method of Stewart et al. [32].

2.8. Statistical Analysis

Experiments were performed and introduced as the average \pm standard deviation of means. To compare treatments, the analysis of variance was performed using IBM SPSS statistics version 21 software (SPSS Inc., Chicago, IL, USA) to examine the statistical differences among means at $\alpha < 0.05$.

3. Results and Discussion

This research introduces an innovative approach by utilizing DJATP in cheese manufacturing, a practice not previously explored in the literature. The reason behind this is that DJATP is rich in inulin, antioxidants, and flavonoids [9], which are expected to enhance the nutritional profile and functional qualities of cheese more than using pure inulin. Importantly, this approach offers cost-saving benefits by bypassing traditional inulin extraction methods.

Additionally, traditional dairy products lack dietary fiber, which can be faced through integrating vegetable ingredients, i.e., DJATP, creating fiber-enriched dairy product alternatives that support gut probiotics. The next preliminary phase of the study dealt with preparation and studying the chemical, microstructure, and probiotic behavior of DJATP.

3.1. Physicochemical Properties of DJATP

The prepared DJATP was investigated for physicochemical properties to provide important information about its composition, stability, safety, quality, and sensory attributes. These properties provide valuable information about the DJATP's physical state, stability, and reactivity.

3.1.1. Physical Properties of DJATP

As shown in Table 1, a total of 28.72 g of DJATP was recovered from each 100 g of fresh tubers. Physically, the color is characterized by values of a^* , b^* , and L^* , corresponding to 7.13, 18.31, and 53.21, respectively. The brown color did not appear due to the use of an appropriate temperature of 50 °C during the drying process, which explains the absence of the Maillard reaction which occurs at 140 °C. Assessment of DJATP aimed to determine its suitability for adding during cheese making. These values indicate that the color of the powder is white-light gray and suitable for adding to dairy products without negatively affecting its appearance and quality. These are similar to previously reported properties [33].

Table 1. Physicochemical properties of the prepared DJATP.

Physical Properties		Chemical Composition		Minerals (mg/100 g)	
Yield (dry matter, %)	28.72	Moisture %	7.93 ± 0.25	Fe	15.59 ± 0.37
Color	a^* 7.13 ± 0.07	Protein %	8.99 ± 0.19	Ca	34.72 ± 0.57
	b^* 18.31 ± 0.08	Dry mass %	20.690 ± 0.017	Mg	83.23 ± 0.31
	L^* 53.21 ± 0.04	Lipid %	1.53 ± 0.41	Zn	1.58 ± 0.27
Viscosity	2.391 ± 0.04 mPa.s	TDF %	33.02 ± 0.32	P	329.32 ± 0.21
Size of particles	300 ± 2.15 µm	SDF %	20.13 ± 0.15	K	245.19 ± 0.23
		IDF %	12.89 ± 0.27	Mn	0.13 ± 0.12
		TC %	72.13 ± 0.67		
		Inulin (g/100 g)	56.89 ± 0.53		
		TP (mg/gm)	7.71 ± 0.51		
		AO (mg/gm)	92.61 ± 0.38		
		TF (mg/gm)	81 ± 0.12		
		Ash %	4.73 ± 0.12		

Means of three replications ± standard deviation. Color measurement: where L^* is the lightness from white (100) to black (0); a^* is the redness, ranging from green (−) to red (+); and b^* is the yellowness color range from yellow (+) to blue (−). TDF = total dietary fibers, SDF = soluble dietary fibers, IDF = insoluble dietary fibers, TC = total carbohydrate, TP = total phenols, AO = antioxidant, TF = total flavonoid.

Regarding the viscosity, it was 2.391 mPa.s, whereas the viscosity of water under the same conditions was 1.0016 mPa.s. The huge difference is very clear and is due to the presence of DJATP particles, which swell and increase the viscosity, thus the texture becomes sticky [34].

The particle size of DJATP was around 300 µm; this size increased the surface exposed to the liquid, resulting in high bonding water with the particles which have a significant amount of inulin and soluble dietary fibers, leading to an increase in viscosity. Oladunmoye et al. [35] confirmed that the fine particles of DJATP have a high capacity to absorb water. Additionally, the inulin of JA is an amphiphilic particle that forms hydrogen bonds with protein fragments and thus augments the gel networking [36].

3.1.2. Chemical Composition of DJATP

The chemical configuration of DJATP was analyzed. The moisture, dry mass, protein, lipid, total dietary fiber, insoluble fiber, soluble fiber, total carbohydrate, inulin, total

phenols, antioxidant, flavonoid compounds, and ash were found to be 7.93, 20.69, 8.99, 1.53, 33.02, 20.13, 12.89, 72.13, 56.89, 7.71, 92.61, 81, and 4.73, respectively. Such a composition makes the DJATP suitable for cheese manufacturing. Therefore, it is expected that the addition of DJATP increases the yield of the dairy product and improves its rheological and technological properties as well as raising their nutritional value.

Previous studies on proteome analysis of DJATP have found the existence of functional health-related proteins, which are reported to improve human metabolism and manage Parkinson's, Huntington's, and Alzheimer's diseases. DJATP contains two Kunitz-type and serine hydroxy-methyltransferase proteins that have a role as antifungal and anticancer, antimicrobial, and antineoplastic agents [37,38]. The high crude protein (5.82–13.36%) content in fresh JA makes it a candidate for healthy feeding [37,38]. Moreover, the lipids were low (1.53%) as vegetable lipids that are classified as healthy fats [39].

The DJATP contents of phosphorus (329.32 mg/100 g) and potassium (245.19 mg/100 g) were in pioneer amounts, whereas iron, calcium, and magnesium were found in medium values (15.59, 34.72, and 83.23 mg/100 g, respectively). Whereas, zinc and manganese were at the lowest values (1.58 and 0.13 mg/100 g, respectively). These findings are consistent with those obtained by Taleb [40] who reported that the high ratio of ash gives this tuber special importance in human nutrition. These minerals are a protection shield from many diseases and act on several biological processes in the human body.

Principally, the main carbohydrates of DJATP are the inulin polysaccharide [41–43]. In consistency with the current results, Taleb [40] found that the level of inulin was 57.95%. However, higher inulin content was stated to reach up to 73% [44]. The wide range in the inulin ratio is due to the genetic traits of the cultivar, climate, soil, agricultural transactions, time of harvest, storage of tubers, method of preparing the powder, and time of analysis [45,46].

Chemically, inulin is categorized as a soluble fiber polymer, consisting of from 2 to 60 units of fructose, thus having obvious health profits such as functional dietary fibers, a reduction in gastrointestinal disease risks, a bifidogenic effect, and stimulation of the immune system [47]. Fructooligosaccharides and inulin are known to stimulate prebiotic bacteria, reduce bowel transit time, improve immune responses, increase mineral absorption, and prevent diseases, i.e., colorectal cancers, intestinal infections, obesity, type II diabetes, and cardiovascular diseases [2].

The previous benefits are due to the unique composition of inulin, which is a linear polymer fructose molecule linked together by β -(2-1) and terminated by an α -(1-2) bond connected to a glucose molecule [8,46]. Several factors, such as growth, storage conditions, plant variety, and maturity harvesting affect the inulin content of JA tubers [48]. The blanching process of JA tubers inhibits the activity of the inulinase enzyme, which is naturally found in the tubers of JA and can also break down inulin into sugars. Another study found 53.00 g inulin and 54.86 g dry mass per 100 g [39].

Dietary fibers and inulin play a vital role in colon and intestine health; moreover, the hydration properties of the dietary fibers regulate their colonic function and physiological effects [39]. DJATP contained 33.02 g/100 g, including insoluble and soluble fibers, which affect the technological and rheological features. Nutritionally, insoluble fibers reduce constipation and reduce the risk of colon/rectal cancer; furthermore, soluble fibers are less common in foods than insoluble fibers but they are believed to have important effects on the digestive and absorptive processes [49].

3.1.3. Capacity Analysis of DJATP

The capacity analysis provides valuable information about the functional properties of cheese and can impact the quality and characteristics of the final product. The results in Table 2 show that the WHC was 3.56 g/g and 5.31 g/g, OAC was 1.71 1.99 g/g, and SC was 0.97 and 1.79 mL/g at pH 5.2 and 6.67 for dried milk casein and DJATP, respectively.

Table 2. Capacity analysis of DJADP in comparison with dried milk casein.

Type	pH	WHC (g/g)	OAC (g/g)	SC (g/g)
Dried milk casein	5.2 b	3.56 ± 0.07 b	1.71 ± 0.13 b	0.97 ± 0.11 b
DJADP	6.67 a	5.31 ± 0.14 a	1.99 ± 0.27 a	1.79 ± 0.01 a

For each of the tested criteria within a column, the means with different letters indicate significant differences ($p < 0.05$). WHC; water holding capacity, OAC; oil absorption capacity, SC: swelling capacity.

There are distinct variations among the three tested capacity criteria. WHC refers to the ability of cheese to retain moisture that affects the texture, yield, and shelf life. This information is crucial for ensuring the desired texture, moisture content, and meltability of the cheese; also, the higher WHC indicates better moisture retention and can result in a more desirable and consistent product [50,51].

Like WHC, OAC refers to the ability of cheese to absorb or retain oil. It is particularly important in the production of cheese analogs to enhance flavor, texture, and meltability [52]. Determining the OAC helps manufacturers optimize the amount of oil or fat required to achieve the desired characteristics in the final product [53]. It ensures that the cheese has the appropriate fat content, which affects aspects like creaminess and mouthfeel.

The SC is another capacity parameter that refers to the ability of the food product to expand or swell when subjected to heat or moisture. Determining the SC helps assess the stretchiness and meltability to ensure that the product has the desired elasticity for specific applications like a cheese pull, which contributes to the overall sensory experience and consumer satisfaction [53].

However, as previously stated, DJATP has high-capacity behavior, which forms a gel network to retain liquids [35]. When designing protein or dietary fiber-rich food products, WHC, OAC, and SC of the ingredients must be considered because these properties affect the chemical, microbial, and rheological properties as well as the yield and quality of the food product, particularly the shelf-life products [53]. The water-holding capacity results from the interaction of proteins or fibers with water; bound water is the water that is not freezable at $-40\text{ }^{\circ}\text{C}$ and not separable by centrifugation. This interaction usually occurs in food systems and enables the protein matrix to retain and absorb bound hydrodynamic, capillary, and physically entrapped water against gravity [51].

Polar amino groups are responsible for hydrogen bonding with water molecules [51] as well as the high content of carbohydrates, especially complex ones, such as inulin. Knudsen [54] reported that there are many reactions between dietary fiber and water to bind water involving the polar effect, strong hydrogen bonding, weak hydrogen bonding, and ionic interactions involving strongly held and oriented water. Since the majority of DJATP carbohydrates are inulin, and casein is also the main protein of milk, it was necessary to estimate the previously mentioned properties of DJATP and casein.

The mechanism of oil retention is a vital functional feature required by food products. Protein is the main responsible component for the physical capture of oil [52]. However, the OAC varies according to the composition of amino acids, protein structure, and hydrophobicity or surface polarity. The number of protein-exposed hydrophobic groups and the non-polar side chains of proteins have an affinity towards the hydrophobic chains of the oil grain, thus improving the OAC [55,56].

3.1.4. SEM Investigation

A microstructure investigation was carried out to discover how the net of protein, polysaccharides, and fibers could hold the free water and absorb oil, thus affecting the capacity feature of the DJATP particles. SEM images can provide valuable insights into the structure and surface morphology as well as provide details of the porosity of DJATP and casein (as control).

The SEM images showed that the particle fibers of DJATP (Figure 1A,B) were able to bond the free water more than casein particles (Figure 1C,D) at a magnification capacity of

250 \times ; this is more obvious in magnification 1000 \times . Furthermore, the adhesion rate of oil on DJATP particles (Figure 2A,B) was higher than on casein particles (Figure 2C,D). Generally, the holding capacity of the DJATP fibers can be inferred from that observed in casein by a lower fiber size and more pore distribution. This confirms the efficiency of DJATP particles to hold water and oil absorption. These SEM images confirm and come in line with the previous investigation data of WHC and OAC. To the best of our knowledge, no preceding studies compared the microstructure variation in DJATP and casein.

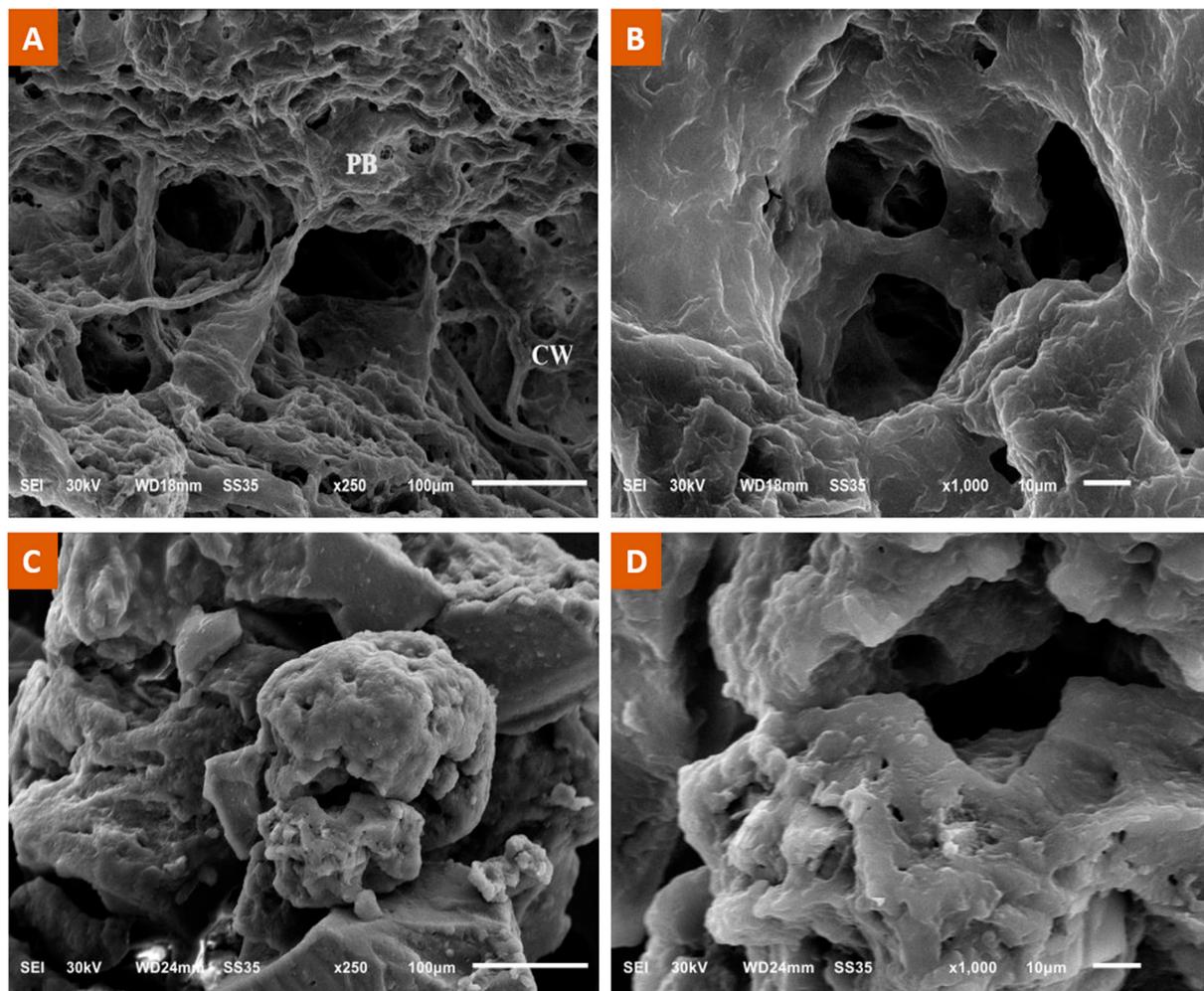


Figure 1. SEM micrograph at 250 \times and 1000 \times magnitude of the water holding capacity of DJATP (A,B) and milk casein (C,D). CW—cell wall; PB—protein body.

However, the cell wall structure and the preparation of plant materials greatly affect the capacity features of the product. The properties and functions of dietary fibers are affected by the structure of the cell wall and the content of each substance. Thus, dietary fibers affect the final quality of the product involving WHC, OAC, SC, gelling properties, binder or adhesive properties, film forming, and emulsifying properties. Furthermore, during the drying process, plant cell wall compositions and the modification of microstructure likely occur, where the high temperatures may cause a partial degradation of some plant cell components and may alter the hydration properties and fat adsorption capacity of the fiber [57,58].

It was observed that the OAC increased with the decrease in particle size. The current particle size of DJATP (300 μm) was small enough to increase OAC. The reduction in particle size as much as possible likely affects the structure of the fiber matrix, leading to an enlargement in the surface area and breaking of pores in the fiber matrix, thereby affecting the hydration features [59]. Lessening of particle size of insoluble dietary fibers increased

the WHC owing to the increase in surface area, resulting in extra exposed polar groups and other water binding sites, thus increasing WHC [60].

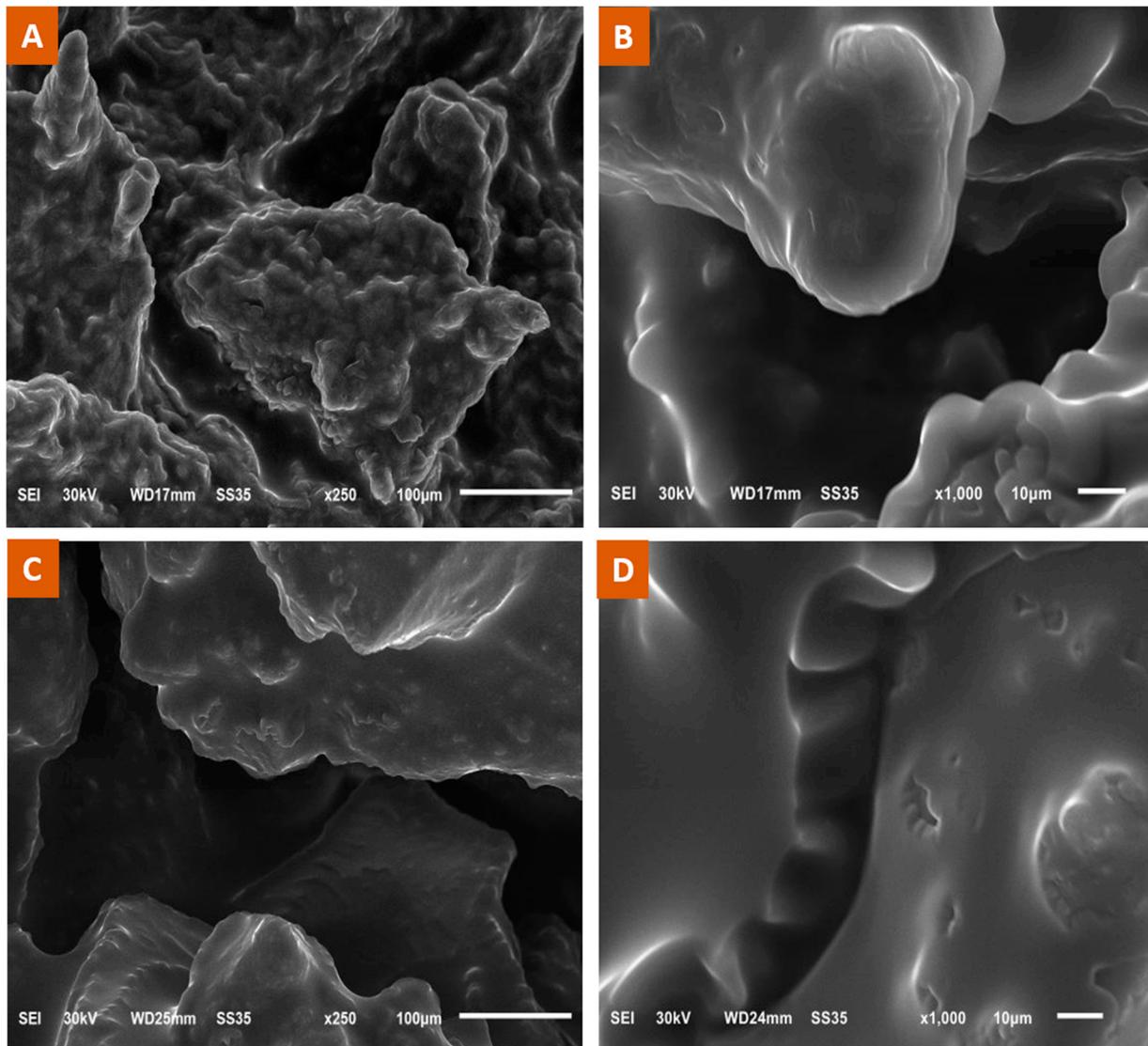


Figure 2. SEM micrograph at 250 \times and 1000 \times magnitude of the oil absorption capacity of DJATP (A,B) and milk casein (C,D).

3.1.5. Performance of Probiotic Bacteria in Inulin and DJATP

The growth pattern of *S. thermophilus*, *B. bifidum*, and *L. acidophilus* on a medium containing inulin, or DJATP, was explored (Table 3). Inulin was used for comparison because it is considered an important prebiotic and represents the main constituent of carbohydrates in DJATP. Therefore, it was better to explore the bacterial growth nature in the presence of inulin or DJATP in comparison to the control.

Compared to skimmed milk (control), inulin and DJATP accelerated the fermentation time and acted as growth enrichment for the three tested probiotic bacteria: *S. thermophilus*, *B. bifidum*, and *L. acidophilus*. This was indicated by the lowering pH value when the fermentation was carried out in the presence of inulin or DJATP than that occurred in skimmed milk. However, the growth pattern and pH value were correlated positively and negatively with the 15 h fermentation time.

Table 3. The growth pattern of probiotic bacteria in the presence of inulin or DJATP (3%, w/v) in comparison to skimmed milk medium.

Time, h	Skimmed Milk (Control)				Inulin				DJATP			
	pH	Log CFU/g			pH	Log CFU/g			pH	Log CFU/g		
		<i>S. thermophilus</i>	<i>B. bifidum</i>	<i>L. acidophilus</i>		<i>S. thermophilus</i>	<i>B. bifidum</i>	<i>L. acidophilus</i>		<i>S. thermophilus</i>	<i>B. bifidum</i>	<i>L. acidophilus</i>
0	6.5 ± 0.02	6.15 ± 0.11	6.13 ± 0.12	6.27 ± 0.06	6.5 ± 0.01	6.15 ± 0.05	6.11 ± 0.09	6.26 ± 0.13	6.5 ± 0.02	6.14 ± 0.11	6.11 ± 0.14	6.26 ± 0.06
3	6.1 ± 0.01	6.18 ± 0.12	6.12 ± 0.15	6.28 ± 0.10	5.8 ± 0.01	6.19 ± 0.03	6.16 ± 0.11	6.30 ± 0.08	5.7 ± 0.01	6.21 ± 0.05	6.22 ± 0.07	6.34 ± 0.20
6	5.4 ± 0.03	6.25 ± 0.04	6.18 ± 0.03	6.30 ± 0.11	5.1 ± 0.02	6.27 ± 0.04	6.35 ± 0.04	6.37 ± 0.14	5.3 ± 0.01	6.30 ± 0.02	6.41 ± 0.03	6.39 ± 0.12
9	5.1 ± 0.03	6.35 ± 0.01	6.22 ± 0.11	6.32 ± 0.06	4.7 ± 0.03	6.33 ± 0.12	6.36 ± 0.08	6.43 ± 0.12	4.6 ± 0.02	6.35 ± 0.05	6.43 ± 0.15	6.46 ± 0.18
12	4.8 ± 0.02	6.39 ± 0.05	6.24 ± 0.09	6.31 ± 0.18	4.5 ± 0.01	6.39 ± 0.13	6.43 ± 0.10	6.45 ± 0.23	4.2 ± 0.03	6.39 ± 0.70	6.48 ± 0.02	6.51 ± 0.11
15	4.5 ± 0.01	6.43 ± 0.04	6.25 ± 0.14	6.32 ± 0.12	4.3 ± 0.02	6.46 ± 0.07	6.44 ± 0.05	6.51 ± 0.19	4.0 ± 0.02	6.42 ± 0.12	6.53 ± 0.18	6.59 ± 0.02

Furthermore, DJATP was more influential than inulin whether on pH or the growth of bacteria. This may have occurred because of the reduction in the generation time of bacteria [61]. After 15 h of bacterial inoculation, the pH of the medium decreased to 4.5, 4.3, and 4.0 in control, inulin, and DJATP, respectively. At the same time, the population of *S. thermophilus*, *B. bifidum*, and *L. acidophilus* recorded 6.42, 6.53, and 6.59 log CFU/g, respectively, in the presence of DJATP, whereas, the populations were recorded as being 6.46, 6.44, and 6.51 log CFU/g, respectively, in the presence of inulin. In any case, the control skimmed milk was relatively lower (6.43, 6.25, and 6.32 log CFU/g, respectively).

The high bacterial count in the presence of inulin and DJATP and lower pH in the presence of DJATP may be due to its rich content of minerals, total phenols, antioxidants, and flavonoid compounds as stated in the current study (Table 1). To the best of our knowledge, no extensive work compared the probiotic behavior in DJATP and inulin-containing medium neither before nor during storage of the bioprocessed dairy products.

A previous study reported that the addition of inulin to a culture of lactic acid bacteria lowered the generation time of *S. thermophilus*, *L. acidophilus*, and *B. lactis* whether in single or co-cultures [61]. Moreover, the powder of JA tubers stimulated the growth of *L. acidophilus* LA-5 and lowered the pH value during bio-labneh manufacturing [23]. Therefore, the prepared DJATP can be used as a decent substitute for inulin or polysaccharides as a prebiotic in the manufacturing of functional fresh cheese.

3.2. Manufacturing and Evaluation of DJATP-Fortified Fresh Cheese

The previous preliminary investigations encouraged the further application of DJATP in the manufacturing of cheese. Moreover, before further processing, the microbiological safety test was carried out on the DJATP (Supplementary Table S1), which revealed the absence of pathogenic microbes. Therefore, trials were carried out for processing DJATP-fortified cheese.

The subsequent phase utilizes the findings of the previous preliminary study to innovatively manufacture functional DJATP-fortified cheese and evaluate its physicochemical and biological attributes. After manufacturing of DJATP-fortified fresh cheese, the yield, physicochemical features, and functional properties tests were evaluated in fresh status and after the storage period. The textural profile and sensory were assessed as well.

3.2.1. Yield and Chemical Composition of DJATP-Fortified Cheese

Economically, the increment of yield and high quality is the major goal of all producers. The addition of DJATP at levels 1, 3, or 6% significantly increased cheese yield and moisture content as compared to the control cheese sample (Table 4). Moreover, no signs of spoilage appeared.

In a previous experiment in this work, WHC, OAC, and SC of casein and DJATP were tested and the results of that trial explained the reason for the increment of yield fresh cheese made by adding 1, 3, or 6% of DJATP. The increment of cheese yield in the presence of DJATP happened because of the high-capacity behavior of DJATP, which formed a gel network to retain whey [35]. This was confirmed by practical experiments and SEM

investigation. These results are due to the high level of dietary fiber in DJTP added. The fine particles of DJATP have a better capacity to absorb water rapidly and improve their technological properties [35].

Table 4. Yield and chemical composition of fortified fresh cheese with DJATP during a storage period at 5 ± 1 °C for 14 days.

Criterion	Storage (Day)	Control	DJATP, %					
			1%		3%		6%	
Yield (%)	0	17.84 ± 1.05 d	19.21 ± 0.55 c	7.69 *	25.15 ± 0.27 b	40.97 *	28.27 ± 0.77 a	58.46 *
	7	17.77 ± 0.09 d	19.11 ± 0.38 c	7.54	24.87 ± 0.47 b	39.95	28.02 ± 1.05 a	57.58
	14	17.55 ± 0.28 d	18.89 ± 0.61 c	7.63	24.66 ± 0.27 b	40.5	27.85 ± 1.12 a	58.68
Titratable acidity (%)	0	1.45 ± 0.04 b	1.46 ± 0.05 c		1.31 ± 0.03 c		1.16 ± 0.03 d	
	7	1.51 ± 0.03 b	1.48 ± 0.02 b		1.38 ± 0.02 c		1.21 ± 0.03 d	
	14	1.62 ± 0.02 a	1.52 ± 0.03 a		1.42 ± 0.02 b		1.28 ± 0.02 d	
pH value	0	4.42 ± 0.03 d	4.71 ± 0.02 b		4.75 ± 0.04 a		4.82 ± 0.06 a	
	7	4.38 ± 0.03 d	4.73 ± 0.03 b		4.71 ± 0.03 b		4.79 ± 0.02 a	
	14	4.32 ± 0.05 d	4.64 ± 0.03 c		4.63 ± 0.04 b		4.80 ± 0.05 a	
Total solids, %	0	25.13 ± 0.08 c	25.82 ± 0.1 c		26.56 ± 0.33 b		27.21 ± 0.1 a	
	7	25.59 ± 0.33 c	26.51 ± 0.35 b		27.21 ± 0.15 a		27.58 ± 0.13 a	
	14	26.12 ± 0.47 b	26.63 ± 0.28 b		27.78 ± 0.15 a		27.97 ± 0.36 a	
Total protein, %	0	16.65 ± 0.16 c	17.05 ± 0.05 b		17.69 ± 0.05 a		18.05 ± 0.04 a	
	7	16.79 ± 0.09 c	17.15 ± 0.03 b		17.75 ± 0.05 a		17.93 ± 0.02 a	
	14	16.84 ± 0.05 c	17.22 ± 0.07 b		17.77 ± 0.05 a		17.75 ± 0.05 a	
Ash, %	0	1.31 ± 0.13 c	1.55 ± 0.07 b		1.71 ± 0.05 b		1.97 ± 0.21 a	
	7	1.33 ± 0.05 c	1.57 ± 0.09 b		1.75 ± 0.07 b		1.99 ± 0.15 a	
	14	1.38 ± 0.17 c	1.89 ± 0.01 b		1.92 ± 0.12 b		2.09 ± 0.20 a	

* The short column under each DJATP concentration indicates the percentage of yield increase in cheese. For each of the tested criteria, means with different letters indicate significant differences ($p < 0.05$).

The increment of yield was around 7, 40, and 58% when adding 1, 3, or 6% of DJATP, respectively, at 0, 7, and 14 days of stored cheese. That could achieve an abundant economic return. Similarly, Alnemr et al. [62] and Mohamed [63] reported improvements when adding inulin to cheese processing. These results may be due to the inulin content [64]. Our results stated 56.89% of inulin in DJATP, which supports the higher cheese yield obtained through the current study.

The titratable acidity of all treatments and the control increased during the storage period. When the curd's acidity increases, its shrinkage increases, which expels more whey and thus makes the cheese harder. It is clear from the data that the acidity decreases and pH values increase with increasing the percentage of DJATP compared to the control cheese.

The low acidity observed in all treatments may be due to the increase in inulin, which is difficult to hydrolyze and takes a long time to degrade. However, the acidity gradually increased with the progression of the storage. This is of course due to the presence of fermentable carbohydrates, e.g., lactose, and the monosaccharides that are released gradually from inulin by the probiotic and lactic acid bacteria that have acclimatized under these conditions [62,63].

Concerning the total protein and total solids content of DJATP-fortified cheese, both increased significantly with increasing DJATP levels. Contrary to DJATP-fortified cheese at 3 and 6%, which contained the highest levels of total protein and total solids, the control sample contained the lowest concentrations. Data show that there were no significant differences between the addition of 3 and 6% of DJATP during the storage period. These increments are probably related to the addition of DJATP, which contains an appropriate

amount of protein (8.99%), and the total dry mass (20.69%) (Table 1). In this connection, protein content and total solids significantly increased when 1% DJATP was added to the prepared labneh. The increments of the total protein and total solid content became more obvious at higher levels of DJATP [23].

Regarding ash analysis, again, a significant increase was found when adding DJATP; however, there were no significant differences between concentrations at 1% and 3% during the storage period but both concentrations differed significantly in comparison with 6% of DJATP. This is expected since ash naturally increases with the extra DJATP, which contains a high percentage of ash of 4.73% (Table 1). Similar results were observed on the DJATP-supported labneh [23].

3.2.2. Functional Compounds

The functional properties of the different treatments of cheese were evaluated. The analysis of the functional compounds in DJATP-fortified cheese (Table 5) clearly indicated that the total flavonoid contents and phenolic compounds significantly increased in all treatments against control cheese. Furthermore, cheese fortified with 6% of DJATP came the highest followed by the lower concentrations and then the control treatment.

Table 5. Total flavonoid, and phenolic compounds content, as well as total antioxidant activity of DJATP-fortified fresh cheese during a storage period at 5 ± 1 °C for 14 days.

Criterion	Storage (Day)	Control	DJATP, %		
			1%	3%	6%
Flavonoids (mg/g)	0	20.6 ± 0.17 d	27.33 ± 0.15 c	33.45 ± 0.28 b	40.5 ± 0.11 a
	7	20.3 ± 0.27 d	27.03 ± 0.06 c	33.14 ± 0.06 b	40.3 ± 0.11 a
	14	19.1 ± 0.11 d	26.49 ± 0.31 c	33.05 ± 0.15 b	39.92 ± 0.21 a
Phenolic compounds (mg/g)	0	36.56 ± 0.14 d	45.61 ± 0.31 c	58.62 ± 0.37 b	70.65 ± 0.22 a
	7	36.3 ± 0.24 d	45.1 ± 0.15 c	58.35 ± 0.22 b	70.13 ± 0.12 a
	14	36.2 ± 0.26 d	44.94 ± 0.14 c	58.05 ± 0.15 b	69.83 ± 0.31 a
Antioxidant activity (%)	0	30.35 ± 0.22 c	40.58 ± 0.18 b	48.41 ± 0.25 a	52.52 ± 0.13 a
	7	30.62 ± 0.21 c	40.8 ± 0.02 b	49.43 ± 0.21 a	52.95 ± 0.05 a
	14	31.8 ± 0.26 c	41.48 ± 0.24 b	50.49 ± 0.03 a	54.4 ± 0.43 a

For each of the tested criteria, means with different letters indicate significant differences ($p < 0.05$).

The same trend was observed in antioxidant activity; the only exception was the non-significant differences between 3% and 6%, which both recorded the greatest concentrations of total antioxidant activity. However, there was a significant difference between the 1% and control cheese. The control contains the lowest antioxidant activity; the reason for the difference between the treatments and control is likely the addition of DJATP or the activity of the ABT-5 starter and/or the difference in the ratio of DJATP. Moreover, no signs of spoilage appeared; this may be due to the increased content of cheese in flavonoids, phenolic compounds, and antioxidants (Table 5).

These results confirmed that DJATP improved the functional properties of fresh cheese, especially at 6%. This comes in line with our previous analysis that confirmed that DJATP is a good source of antioxidants, flavonoids, and phenolic compounds (Table 1).

There was some evidence that JA improves the functional properties of dairy products. For instance, whey beverages prepared supported with the juice of JA contained high concentrations of antioxidant, total flavonoid, and phenolic compounds compared with the non-supported beverage with the juice of JA [65]. Nonetheless, the richness of JA tubers with these functional compounds increases the rate of consumer demand for those products [66], representing another importance of the functional compounds.

3.2.3. Profile of Amino Acids

Cheese is known as a protein-rich food, with protein being a primary component. When DJATP is introduced during cheese preparation, a blending of vegetable and animal proteins takes place. Consequently, it is crucial to analyze and assess the amino acid content in the experimental cheese and compare it to the control samples.

Data in the amino acid profile (Table 6 and Supplementary Figures S1–S4) of fortified fresh cheese with DJATP revealed differences among the content of amino acids of the control and all other treatments. In general, little difference was noticed between the control and DJATP at 1%. The differences became clearer in the higher concentrations of DJATP. The DJATP-fortified cheese content of amino acids greatly increased in the highest concentrations (6%), particularly isoleucine, methionine, threonine, valine, glutamic acid, and arginine. The total amino acids (TAA), total essential amino acids (EAA), total non-EAA, EAA/TAA, total branched-chain (leucine, isoleucine, and valine) amino acids (BCAA), and the total BCAA/TAA recorded the greatest concentrations at 6% DJATP, then the control sample at 1 and 3% DJATP. The results are like those reported on using dried mushrooms [67] but contrary to the results on processed cheese [68].

Table 6. Amino acid profile in fresh cheese fortified with DJATP after 14 days of storage at 5 ± 1 °C.

Amino Acid (mg/g)	Control	DJATP, %		
		1%	3%	6%
Leucine *	10.94	11.70	18.20	18.43
Isoleucine *	6.93	6.64	10.79	12.32
Methionine *	2.55	4.32	6.68	9.15
Phenylalanine *	6.79	7.32	11.45	10.78
Lysine *	11.05	12.51	18.54	17.50
Threonine *	4.26	3.69	4.00	5.77
Tyrosine *	5.03	6.33	8.76	9.16
Valine *	10.03	8.12	12.90	14.05
Aspartic	7.90	9.77	12.05	13.23
Glutamic acid	2.61	3.07	4.42	5.19
Serine	4.22	2.51	6.48	4.29
Proline	22.38	33.16	35.23	32.96
Glycine	2.45	2.36	3.45	3.65
Alanine	4.11	4.58	6.28	5.12
Histidine	4.95	4.87	9.17	6.73
Arginine	2.10	4.64	5.42	7.72
Cystine	1.43	0.62	3.57	2.68
Total amino acids	109.72	126.21	177.39	178.73
Total EAA *	57.58	60.63	91.32	97.16
Total non-EAA	52.15	60.58	86.07	81.57
Total BCAA	27.90	26.46	41.89	44.80
BCAA, %	25.42%	20.96%	23.61%	25.07%
EAA/TAA, %	52.47%	48.04%	51.48%	54.36

* Essential amino acids, EAA; essential amino acids, TAA; total amino acids, BCAA; total branched-chain amino acids (leucine, isoleucine, and valine).

Several amino acids were detected; some are regarded as essential for humans. Some amino acids are required in specific growth stages, like sulfur-containing cysteine, aromatic

tyrosine), and arginine which are required during the growth of infants and children [69]. Other amino acids (conditionally essential) must be supplied exogenously to those who do not synthesize them in sufficient quantities [70].

BCAAs are proteinogenic essential amino acids for humans and represent about 35% of the required essential amino acids for building and reducing the breakdown of muscles. BCAAs prevent fatigue, improve mental functions, and cure some diseases [69–71].

Aside from their high inulin content, JA tubers are rich in protein with approximately 18 amino acids, including 9 essential ones (arginine, valine, gliadin, isoleucine, leucine, lysine, methionine, tryptophan, and phenylalanine), in favorable proportions and nearly ideal ratios for human consumption [34,45].

These tubers boast an EAA/TAA ratio of around 48% and an EAA/non-EAA ratio of about 91% [72]. These ratios align with ideal standards set by organizations like FAO and WHO for both animal and plant-based foods, confirming JA tubers as a valuable source of high-quality protein [34]. As a result of the protein richness, JA tubers are recommended for both children and adults [73]. The wide variation in the amino acids makes the cheese rich and complementary in functional nutrition.

3.2.4. Minerals Content

Regarding mineral contents in DJATP-fortified fresh cheese (Table 7), there was a significant increase in all determined minerals in fortified cheese with DJATP compared to the control. The increase in minerals in fortified cheese indicated that the loss of minerals was at the lowest possible level during the manufacturing process with the addition of the DJATP.

Table 7. Mineral contents of the fortified fresh cheese with DJATP during a storage period at 5 ± 1 °C for 14 days.

Mineral, ppm	Control	DJATP, %		
		1%	3%	6%
Ca	3957.5 ± 0.01 d	4657 ± 0.17 c	5855 ± 0.15 b	7432.25 ± 0.11 a
Fe	411.25 ± 0.05 d	568.75 ± 0.12 c	790.75 ± 0.07 b	838.25 ± 0.15 a
Zn	492.92 ± 0.18 d	585.91 ± 0.35 c	1180.2 ± 0.42 b	4901.9 ± 0.25 a
P	31.16 ± 0.11 d	36.70 ± 0.01 c	38.94 ± 0.02 b	40.02 ± 0.05 a
K	4.86 ± 0.01 d	9.78 ± 0.07 c	24.86 ± 0.05 b	30.98 ± 0.02 a
Mg	2052.25 ± 0.02 d	2722.25 ± 0.01 c	4377.2 ± 0.12 b	6029.5 ± 0.02 a
Mn	12.22 ± 0.01 d	18.12 ± 0.01 c	22.20 ± 0.03 b	29.11 ± 0.01 a

For each of the tested minerals, means with designated different letters indicate significant differences ($p < 0.05$).

The gel network that was formed by the added DJATP helped to hold and bind the minerals. This led to the addition of functional value to the cheese. Dietary fiber has a high capacity to bind minerals, which may be released during the fermentation of these fibers in the gastrointestinal tract [74]. Inulin-type fructans and many kinds of oligosaccharides have been studied widely for their potential beneficial effects on mineral retaining and metabolism, especially calcium and manganese, and others; they further facilitate the digestion of high protein diets, retard fat absorption, and lower blood cholesterol and triglycerides [6].

3.3. Bacterial Pattern

The pattern of the three probiotic bacteria was followed up during the storage period to explore the survivability of the probiotic bacteria in the suggested DJATP-fortified cheese (Table 8). *S. thermophilus*, *B. bifidum*, and *L. acidophilus* were able to grow and survive in fresh cheese even under acidic conditions during the storage period; therefore, consumption of DJATP-fortified cheese even after storage allows these bacteria to arrive to the human gut in a viable form, thus playing their therapeutic action.

Table 8. The viability (log CFU/g) of probiotic bacteria during storage of fresh cheese fortified with DJATP for 14 days at 5 ± 1 °C.

Bacterium	Storage (Day)	Control	DJATP, %		
			1%	3%	6%
<i>S. thermophilus</i>	0	7.75 ± 0.06	7.77 ± 0.03	7.76 ± 0.03	7.75 ± 0.09
	7	7.94 ± 0.03	7.99 ± 0.05	8.18 ± 0.04	8.25 ± 0.04
	14	6.87 ± 0.04	7.18 ± 0.10	7.34 ± 0.05	7.41 ± 0.06
	Viability *	88.64%	92.41%	94.59%	95.61%
<i>B. bifidum</i>	0	7.69 ± 0.05	7.71 ± 0.04	7.69 ± 0.09	7.71 ± 0.04
	7	7.78 ± 0.04	8.05 ± 0.03	8.11 ± 0.06	8.15 ± 0.05
	14	6.62 ± 0.07	7.05 ± 0.03	7.10 ± 0.03	7.24 ± 0.05
	Viability *	86.09%	91.44%	92.33%	93.90%
<i>L. acidophilus</i>	0	7.54 ± 0.04	7.55 ± 0.06	7.51 ± 0.03	7.56 ± 0.05
	7	7.62 ± 0.03	7.85 ± 0.04	8.03 ± 0.03	8.21 ± 0.03
	14	6.58 ± 0.18	7.08 ± 0.06	7.12 ± 0.04	7.31 ± 0.04
	Viability *	87.26%	93.77%	94.81%	96.69%

* Remaining viability cells after 14 days of storage in relation to the zero time.

Moreover, our findings indicated that the survivability of bacteria was positively affected by the addition of DJATP, which supported the growth and viability of the three tested probiotic bacteria. Additionally, *L. acidophilus* had the greatest growth and survivability followed by *S. thermophilus* and then *B. bifidum* after 14 days of storage; these results may be due to different nutritional needs and the availability for each bacterial species.

These results may be due to the rich nutritional value of crude DJATP (Table 1) since it is already known that the abundance of nutrients, suitable temperature, appropriate pH, and osmotic pressure, as well as other conditions, are necessities for the survival and viability of microorganisms. However, the ability of bacteria to consume inulin depends on the purity and degree of polymerization of fructose-oligomeric chains [75]. Moreover, the susceptibility of saccharides to be fermented depends mainly on the water solubility, polymerization degree, chain length, branched or linear structure, and composition of monomer units [76]. During the preparation of DJATP, monosaccharides and polysaccharides as fructose-oligomeric may be released and their presence encourages bacteria to grow and keep viable.

3.4. Textural Profile

The texture profile analysis includes adhesiveness, chewiness, cohesiveness, gumminess, hardness, and springiness. Generally, there was a connection between the chemical structure and texture profile; for example, hardness and chewiness increased after 14 days of storage due to the rise in protein content and total solids with decreasing moisture content associated with increased acidity. In the current study, the addition of DJATP at different levels affected the texture profile; the results revealed that DJATP caused weakness in the cheese hardness (Table 9).

Cheese hardness decreased when the level of DJATP was increased. The main reason for that is the increased moisture content of cheese, which results from the high efficiency of the DJATP in binding and absorbing water and fat. Chewiness decreased with the increasing level of DJATP. On the other side, adhesiveness, cohesiveness, and springiness were not affected by storage whether in the control or treatments but it was slightly affected by the rate of adding DJATP, as they decreased with the increased level of DJATP. Gumminess and chewiness parameters decreased with the increment of DJATP, especially after 14 days of storage.

Table 9. Textural profile of DJATP-fortified fresh cheese during a storage period at 5 ± 1 °C for 14 days.

Criterion	Storage (Day)	Control	DJATP, %		
			1%	3%	6%
Hardness	0	3.800 ± 0.11 ^a	2.800 ± 0.02 ^a	2.600 ± 0.11 ^b	2.500 ± 0.02 ^c
	14	3.920 ± 0.08 ^a	2.860 ± 0.13 ^a	2.670 ± 0.04 ^b	2.560 ± 0.07 ^c
Adhesiveness	0	0.282 ± 0.01 ^a	0.272 ± 0.08 ^b	0.269 ± 0.01 ^b	0.220 ± 0.02 ^d
	14	0.280 ± 0.05 ^a	0.261 ± 0.05 ^c	0.268 ± 0.02 ^b	0.218 ± 0.08 ^d
Cohesiveness	0	0.820 ± 0.11 ^a	0.710 ± 0.10 ^b	0.700 ± 0.14 ^b	0.690 ± 0.02 ^c
	14	0.800 ± 0.12 ^a	0.700 ± 0.07 ^b	0.720 ± 0.02 ^b	0.680 ± 0.12 ^c
Springiness	0	5.230 ± 0.07 ^a	5.180 ± 0.01 ^b	5.210 ± 0.05 ^a	5.210 ± 0.02 ^a
	14	5.210 ± 0.02 ^a	5.110 ± 0.02 ^b	5.070 ± 0.15 ^c	5.170 ± 0.05 ^b
Gumminess	0	3.120 ± 0.02 ^a	1.980 ± 0.05 ^b	1.820 ± 0.05 ^c	1.720 ± 0.02 ^d
	14	3.140 ± 0.05 ^a	2.020 ± 0.06 ^b	1.920 ± 0.08 ^c	1.740 ± 0.01 ^d
Chewiness	0	16.320 ± 0.11 ^a	10.260 ± 0.04 ^b	9.480 ± 0.11 ^c	8.960 ± 0.02 ^d
	14	16.370 ± 0.14 ^a	10.320 ± 0.02 ^b	9.730 ± 0.02 ^c	9.000 ± 0.15 ^d

For each of the tested criteria, the means with different letters indicate significant differences ($p < 0.05$).

Several factors affect the texture profile of fresh cheese, including the curd moisture content (coagulation temperature and whey drain), cheese composition, acidity, interactions between casein or dietary fiber and serum proteins, ionic strength, Ca and salt contents, starter used, manufacturing technique, and acid development. Furthermore, moisture and fat contents are responsible for many desirable functions and textures. This was evident in our work when DJATP was added during cheese making, which gave the cheese a distinctive texture profile. These findings agree with those reported by Mohamed [63]. Moreover, Kaya [77] reported that the inulin gel network leads to firm cheese; this is obvious with inulin and whey protein during soft cheese manufacturing.

3.5. Sensory Evaluation

The improvement in DJATP-fortified fresh cheese not only occurred in the yield or functional properties but it also appeared during sensory evaluation compared to the control sample during the storage period (Table 10). The arbitrators acknowledged that the treatment of fresh cheese fortified with 3% DJATP was the best compared to the plain control and other treatments in all sensory parameters, followed by 6% and 1% and then the control. However, all the treatments, as well as the control, were acceptable.

It was clear that DJATP at different levels enhances the sensory evaluation of fresh cheeses that are linked to a reduction in the properties of the creaminess score. This may be owing to the lower fat content in the control samples that leads to a real loss of textural and sensorial parameters [47]. The results of sensory evaluation showed that the DJATP increases creaminess, due to the higher WHC and OAC as well as lower solubility. All values decreased during the storage period due to the lowering of moisture. However, all the cheeses kept their quality until the end of storage.

Therefore, DJATP could also be used as a fresh cheese improver and as a replacement for milk fat without an off-flavor or changing color, as well as improving the appearance characteristics. Finally, incorporating DJATP into cheese enhances the texture and sensory properties, extends the shelf life, and boosts the nutritional value, health benefits, and industrial usability. Thus, this work could be used to guide the development of health-promoting cheese in a new form, which is more suitable for the present consumer lifestyle.

Table 10. Evaluation of sensory of DJATP-fortified fresh cheese after a storage period at 5 ± 1 °C for 14 days.

Criterion	Storage (Day)	Control	DJATP, %		
			1%	3%	6%
Appearance (15 points)	0	12.00 ± 1 ^a	12.33 ± 0.58 ^a	13.67 ± 0.58 ^a	13.33 ± 0.58 ^a
	7	11.33 ± 0.58 ^b	12.00 ± 1.00 ^b	13.00 ± 1.00 ^b	13.00 ± 0.01 ^b
	14	11.00 ± 1.00 ^c	11.67 ± 1.53 ^c	12.67 ± 0.58 ^c	12.67 ± 0.58 ^c
Body and texture (35 points)	0	29.33 ± 0.58 ^a	30.67 ± 0.57 ^a	32.67 ± 0.57 ^a	32.33 ± 0.57 ^a
	7	28.33 ± 0.57 ^b	29.00 ± 1.00 ^b	32.00 ± 1.00 ^b	31.00 ± 1.00 ^b
	14	28.00 ± 1.00 ^c	28.33 ± 0.57 ^c	31.00 ± 1.00 ^c	30.67 ± 0.57 ^c
Flavor (50 points)	0	40.33 ± 0.58 ^a	41.67 ± 1.53 ^a	46.33 ± 1.53 ^a	45.67 ± 2.52 ^a
	7	40.67 ± 0.57 ^a	41.00 ± 1.00 ^b	44.33 ± 2.08 ^b	44.00 ± 3.60 ^b
	14	40.00 ± 2.00 ^b	40.67 ± 0.57 ^c	44.00 ± 2.64 ^c	43.67 ± 2.51 ^c
Total (100 points)	0	81.66 ± 2.52 ^a	84.67 ± 2.51 ^a	92.67 ± 2.08 ^a	91.33 ± 2.52 ^a
	7	80.33 ± 1.15 ^b	82.00 ± 1.00 ^b	89.33 ± 3.78 ^b	88.00 ± 4.00 ^b
	14	79.00 ± 2.00 ^c	80.67 ± 1.53 ^c	87.67 ± 2.31 ^c	87.00 ± 2.00 ^c

For each of the tested individual criteria and total points, the means with different letters indicate significant differences ($p < 0.05$).

4. Conclusions

This study explored the novel application of DJATP in cheese production, investigating its effects on the biochemical properties, microstructure, and probiotic development, suggesting its suitability as an additive for DJATP-fortified cheese production. DJATP preparation revealed improved microstructure properties (WHC, OAC, and SC) and demonstrated support for probiotic bacterial growth. It also exhibited a high content of bioactive compounds, including inulin, protein, minerals, antioxidants, phenolics, and flavonoids. Consequently, DJATP proved effective when incorporated into cheese manufacturing at levels of 3% or 6%, offering guidance for producers seeking to enhance product quality and nutritional value. The resulting DJATP-fortified cheese displayed superior biochemical characteristics (amino acids, minerals, flavonoids, phenols, and antioxidants), functional attributes (probiotic bacterial pattern), and sensory qualities for up to 14 days, surpassing traditional cheese production methods. Due to its capacity to stimulate probiotic growth, along with its high WHC and OAC, as evidenced by SEM analysis, DJATP emerges as a viable substitute for inulin as a prebiotic ingredient in cheese production.

The current suggested paradigm can potentially reduce costs, enhance the shelf life, and extend the marketability of the product. Furthermore, this study suggests further research on using DJATP to positively modify the microstructure of cheese products, which can provide valuable insights into its functional properties. In addition, future researchers need to continue exploring the potential applications of DJATP in other products to expand its utilization as a cost-effective and functional ingredient instead of other prebiotics.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pr11102854/s1>, Table S1: Microbiological evaluation of the DJATP. Figure S1: Amino acid contents of fresh cheese made from buffalo skimmed milk using ABT-5 starter. Figure S2: Amino acid contents of DJATP-fortified fresh cheese at 1% made from buffalo skimmed milk using ABT-5 starter. Figure S3: Amino acid contents of DJATP-fortified fresh cheese at 3% made from buffalo skimmed milk using ABT-5 starter. Figure S4: Amino acid contents of DJATP-fortified fresh cheese at 6% made from buffalo skimmed milk using ABT-5 starter. References [78,79] are cited in the supplementary materials.

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