applied sciences

MDPI

Article

The Mulberry Juice Fermented by *Lactiplantibacillus plantarum* O21: The Functional Ingredient in the Formulations of Fruity Jellies Based on Different Gelling Agents

Aleksandra Szydłowska [1,*], Dorota Zielińska [1], Barbara Sionek [1] and Danuta Kołożyn-Krajewska [1,2]

[1] Department of Food Gastronomy and Food Hygiene, Institute of Human Nutrition Sciences, Warsaw University of Life Sciences (WULS), Nowoursynowska St. 159C, 02-776 Warsaw, Poland; dorota_zielinska@sggw.edu.pl (D.Z.); barbara_sionek@sggw.edu.pl (B.S.); danuta_kolozyn_krajewska@sggw.edu.pl (D.K.-K.)

[2] Department of Dietetics and Food Studies, Faculty of Science and Technology, Jan Dlugosz University in Czestochowa, Al. Armii Krajowej 13/15, 42-200 Częstochowa, Poland

* Correspondence: aleksandra_szydlowska@sggw.edu.pl

Abstract: This study aimed to investigate the effects of adding probiotics, prebiotics, and different types of jelly agents on a few key quality attributes of potentially functional mulberry jellies throughout a 10-day storage period at 4 °C. Mullbery juice was separately fermented at 37 °C for 24 h using *Lactiplantibacillus plantarum* O21; it was a favorable matrix for the proliferation of probiotics. Lactic acid fermentation positively affected the total anthocyanin concentration of investigated products. Also, antioxidant capacities of mulberry juices were improved by *L. plantarum* O21 fermentation. The results showed that the applied prebiotic–inulin addition and agar–agar addition, as a gelling agent in recipes of potentially functional mulberry jellies, were proved to be beneficial technological solutions, both in fresh and stored products, and obtained an appropriate, high number of LAB bacteria, good sensory quality, and beneficial antioxidant properties.

Keywords: mulberry juice; probiotics; lactic acid fermentation; antioxidant activity; gelatin; agar–agar; sensory properties

Citation: Szydłowska, A.; Zielińska, D.; Sionek, B.; Kołożyn-Krajewska, D. The Mulberry Juice Fermented by *Lactiplantibacillus plantarum* O21: The Functional Ingredient in the Formulations of Fruity Jellies Based on Different Gelling Agents. *Appl. Sci.* **2023**, *13*, 12780. https://doi.org/10.3390/app132312780

Academic Editors: Theodoros Varzakas and Maria Antoniadou

Received: 2 November 2023
Revised: 21 November 2023
Accepted: 27 November 2023
Published: 28 November 2023

1. Introduction

Customers' appreciation of the relationship between food and wellness has resulted in a significant shift in eating habits and lifestyle changes. The rise in consumer knowledge has been one of the main forces behind the development of functional food products that may satisfy both basic nutritional needs and offer health advantages. The term "functional food" refers to natural and industrially processed foods, which "when regularly consumed within a diverse diet at efficacious levels have potentially positive effects on health beyond basic nutrition" [1]. One of the functional food segments is the so-called "probiotic food".

It is generally known that lactic acid bacteria (LAB) fermentation has a considerable potential to enhance the functional, nutritional, and sensory qualities of both plant-based and animal feeds [2,3]. The definition of probiotic bacteria is "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". This definition captures the essence of probiotics (microbial, viable, and health-promoting) while embracing a wide range of microorganisms and uses [4]. As of right now, probiotics for human usage are limited to lactic acid bacteria that have been isolated from the gastrointestinal system.

However, the International Scientific Association of Probiotics and Prebiotics (ISAPP) in a position statement [5] agreed with the definition that was offered [4]. The term "probiotic" in this context does not refer only to traditional probiotics. Innovation will surely lead to the isolation of promising probiotics from novel sources with intriguing new health benefits and hitherto undiscovered functions [6]. Moreover, some authors report

that strains considered probiotics can be extracted from fermented products of animal origin [7–9] and plant origin [10–13]. Traditional fermented products are a rich source of microorganisms, some of which may have probiotic properties [14]. On the other hand, prebiotics set an example of food ingredients, which are not digested by endogenous enzymes of the human gastrointestinal tract, but they end up intact in the colon, where they ferment, providing food for probiotic microorganisms. In addition, prebiotics are broken down by sucrose bacteria present in the lower gastrointestinal tract and have the ability to stimulate their growth. Inulin and oligofructose are the most effective and most commonly used prebiotics [15].

Growth can be noted in in the popularity of using plant diets among consumers in the world, due to the growing level of knowledge regarding its health-promoting aspects and the availability of products on the food market that enables the incorporation of such a diet [16,17]. It has recently appeared that there is a modern tendency in the development of vegan probiotic food products. The failure to comply with the criterion of "plant origin" in the case of the probiotic bacterial strain used may deprive the final product of the status of vegan food [18]. Considering the biochemical composition of fruits and vegetables, it has been discovered that they are excellent starting materials for the fermentation of probiotics. In addition to their phytochemical content, which promotes health, fruits and vegetables also provide a number of benefits (such as being high in sugar and nutrients) for the growth and survival of probiotics [19]. Fruits and vegetables have a limited shelf life, thus it is critical to perform probiotic fermentation to add value and extend the shelf life of processed foods [20]. The black mulberry (*Morus nigra* L.) belongs to the *Moraceae* family. It is a multipurpose plant that can be used for a variety of purposes including fuel, fodder, fiber, and fruit. It is abundant in bioactive substances, such as bioflavonoids and non-anthocyanin, which are responsible for its medicinal properties and has earned it a "superfood" status in European countries [21–23]. Recently, the mulberry has gained prominence because of its phytochemical makeup and positive health impacts on humans, including its immunomodulatory, antidiabetic, antioxidant, and anticancer qualities [24]. This plant has also been reported to be used in Chinese conventional medicine for fever treatment, diabetes, obesity, blood pressure, urinal disorders, liver damage prevention, atherosclerosis, inflammation, and body joint strengthening, among other things [25,26]. On the other hand, Bong et al. 2019 [27] reported that LAB-biotransformed mulberry fruit extract showed antibacterial action by inhibiting *S. Typhimurium* growth and biofilm formation, and showed an anti-inflammatory effect in the bacterially infected intestinal epithelial cells.

Fruits of the black mulberry (*Morus nigra* L.) have a distinct flavor with juicy and acidic qualities and high staining activity, and are therefore attractive for usage in the food processing industry. Mulberry-based products such as fruit juice [28], liquor [29] ice cream, jam or muffins have been developed and manufactured [30]. Mulberry fruits, due to their mouth-watering taste, are a plant used both fresh and added to food products such as yoghurt, vegetables or muesli [31,32]; they also have a culinary use [33]. By the relatively high water activity (A_w) and low acidity, mulberry fruits are difficult to preserve. Due to this, there is a great need to use technologies to extend the mulberry fruit's shelf life and improve its nutritional, organoleptic, and health benefits in order to increase the commercial production for benefits to both the economy and public health. In recent years, researchers have demonstrated that mulberry fruit can be also a healthy food matrix for probiotic delivery [34–37].

In light of this, the objective of this study was to estimate the possibility of developing potentially functional mulberry jellies based on a probiotic bacterial strain isolated from traditional plant food and impact of the type of jelly agent used for the selected quality properties of these products during 10 days of refrigerated storage.

2. Materials and Methods

2.1. Materials

Mulberry (*Morus nigra* L.) pasteurized juice (Eka Medica Co., Kozy, Poland), inulin (Frutafit® Tex, Roosendaal, The Netherlands), agar–agar (Agnex, Białystok, Poland), and Sucrose (Diamant, Gostyń, Poland) were purchased at the local market in the city (Warsaw), Poland. Distilled water was used in the preparation of the products.

2.2. Preparing of Bacterial Cultures

In this study, one bacterial strain with probiotic properties—*Lactiplantibacillus plantarum* O21 (GenBank accession KM 186159)—was applied. It was obtained from a pure culture maintained in the collection of the Department of Food Gastronomy and Food Hygiene, Institute of Human Nutrition Sciences at Warsaw University of Life Sciences. Some probiotic properties of the used strain were previously described [38].

The bacterial strains were cultured by a two-fold passage in modified Vegitone MRS broth (Sigma–Aldrich Co., Darmstadt, Germany) with 2% (v/v) of inoculum after being kept at −80 °C in 20% (m/w) glycerol.

For 5 min, the bacterial culture was centrifuged at 10,000× g. After obtaining the cell pellets, they were cleaned and resuspended to their original volume in 10 mL of sterile 0.85% saline solution. The ready bacterial culture was then added to 400 mL of mulberry juice that contained 5% v/v sucrose.

In the prepared starting culture, there were roughly 9.75 log CFU (colony forming units) mL^{-1} of probiotic bacteria. A volume of mulberry juice containing 2% (v/v) of the bacterial strain was added (Table 1).

Table 1. Mulberry jelly formulations.

Formulation	Ingredient [% w/v]						
	Inulin	**Agar–Agar**	**Gelatin**	**Mulberry Juice**	**Distilled Water**	**Inoculum**	**Sucrose**
G 0P	0	0	2	70	21.0	2	5
G I-1	1	0	2	70	20.0	2	5
G I-3	3	0	2	70	18.0	2	5
A 0P	0	2	0	70	21.0	2	5
A I-1	1	2	0	70	20.0	2	5
A I-3	3	2	0	70	18.0	2	5

Explanatory notes: G—product based on gelatin; A—product based on agar–agar; 0P—no addition of prebiotics; I-1—with 1% w/v of inulin addition; I-3—with 3% w/v of inulin addition.

Every determination was made three times. The results that were collected were presented as mean ± standard deviation (SD).

2.3. Preparing of Jelly Formulations

For the study, a total of 6 formulations of jellies were produced: 3 samples with a gelatin addition and 3 samples with an agar–agar addition (I-1 with 1% of inulin addition; I-3 with 3% of inulin addition; 0P—no addition of prebiotics). All manufactured samples are shown in Table 1. All mixtures contained mulberry juice (70% v/v), sucrose (5% w/v), 1% (w/v) or 3% (w/v) of a prebiotic addition or without a prebiotic addition, and 2% (v/v) of an inoculum addition, respectively. It was fermented using the potentially probiotic bacterial strain *L. plantarum* O21 strain, at 37 °C for 24 h. Gelatin and agar–agar were used as the gelling agents.

The fermentation process was carried out in sterile, glass vessels of a 500 mL capacity. The fermented juices were then combined with the other recipe ingredients; gelatin (2% w/v) or agar–agar (2% w/v) prepared with distilled water, with a final volume of 400 mL.

The samples were prepared as follows:

Gelatin: gelatin was thoroughly dissolved in a suitable portion of hot water, cooled and added to fermented juice. Then, 200 mL were placed in plastic vessels and cooled. After 3 h, it was placed at 4 °C.

Agar–agar version: a large portion of agar–agar was poured into a suitable portion of cold water and left until it swelled (approximately, 10 min.). Then, it was transferred to a small pot and heated until boiling (90–100 °C), dissolving the agar–agar. Then, it was cooled and other ingredients were added. Finally, the mixtures were divided into 200 mL pieces, each of which were put into a plastic container.

The product remained at room temperature (22 °C to concentration; after 3 h, it was placed in the above-refrigerated conditions, at 4 °C).

The investigated samples were stored at refrigerated conditions for 24 h. Next, the particular determinations were performed.

All determinations were performed in triplicate. The obtained results were expressed as a mean ± standard deviation (SD).

2.4. Microbiological Analysis

The TEMPO® System, an automated quality indicator system (used by BioMérieux, Mercy Etoile, France), performed the analyses. The TEMPO® System was used to calculate the amount of LAB bacteria present in investigated samples (log CFU g^{-1}). The most probable number (MPN) algorithm was used to create this system. The samples were diluted by 1/400 in a single vial. The Tempo Filler moved the inoculated medium into the Tempo card.

The cards were incubated at 37 °C for 48 h before the Tempo LAB test was able to obtain performance levels that were comparable to the NF ISO15214: 1998 standard [39]. The data were automatically processed by the software system that determines which of the wells tested positive. The volume of wells and sample dilution used to count the positive wells allowed for an automatic conversion of the results to log CFU g^{-1}.

All determinations were performed in triplicate. The obtained results were expressed as a mean ± standard deviation (SD).

2.5. Acidity Analysis (pH)

Using a pH meter, the studied jellies' pH values were determined three times. (Elmetron, CP551, Zabrze, Poland). A total of 20 g of the product sample was utilized for each measurement. The acquired results were interpreted with 0.001 accuracy.

All determinations were performed in triplicate. The obtained results were expressed as a mean ± standard deviation (SD).

2.6. Total Anthocyanin Concentration

The total anthocyanin concentration (TAC) was determined by a pH differential method, which was adapted from the methods described by Tchabo et al., 2017 [40]. Two buffer solutions were prepared: CH_3COONa (0.4 mol/L) at pH 4.5; KCl (0.025 mol/L) at pH 1. Two sets of trials were prepared. A total of 100 µL was measured. All determinations were performed in triplicate. The obtained results were expressed as a mean ± standard deviation (SD).

2.7. Extract Preparation

The extraction of gels was conducted before the total anthocyanin content and antioxidant activity were assessed. Then, 2 g of gels with 20 mL of acidified methanol (the HCl: methanol ratio was 1:99) were mixed and homogenized. After the homogenized mixture was filtered and allowed to stand for 24 h, the extracted mixture was utilized for the aforementioned analyses.

Cyanidin-3-glucoside chloride was used to express the results because it is the most common anthocyanin in plants. A 2 mL buffer with pH = 1 (KCl/HCl) was added to the sample, and the absorbance of the solution at 520 nm and 700 nm was measured by using

the spectrophotometer Genesys TM 20 (Thermo Scientific Co., Waltham, MA, USA). The 2 mL buffer with a pH (4.5) CH_3COONa/CH_3 COOH = was added to the second portion of the sample.

The TAC was expressed as milligram equivalent of cyanidin 3-gucoside per mL of juice. It was calculated according to the following Equation (1):

$$\text{TAC} = [(A_1 - A_2) - (A_3 - A_4)] \times \frac{MW \times DF \times 10^2}{\varepsilon \times \text{L}} \quad (1)$$

where A_1 stands for the absorbance at 520 nm with pH 1.0; A_2 stands for the absorbance at 700 nm with pH 1.0; A_3 stands for the absorbance at 520 nm with pH 4.5; A_4 stands for the absorbance at 700 nm with pH 4.5; *MW*—molecular weight of cyanidin-3-glucoside (449.2 g/mol); *DF*—dilution factor (100); L—path length (1 cm); ε—molar extinction coefficient for cyanidin-3-glucoside (26.900 L/mol·cm).

All determinations were performed in triplicate. The obtained results were expressed as a mean ± standard deviation (SD).

2.8. Determination of Antioxidant Activity

2.8.1. DPPH Radical Scavenging Assay

The antioxidant activity was determined by updated existing methods for measuring Alothman, Bhat, and Karim (2009) [41] and Brand–Wiliams et al. (1995) [42]. 2-diphenyl-1-picrylhydrazyl (DPPH), a synthetic radical, was used in the procedure (Sigma–Aldrich, Darmstadt, Germany). A total of 0.012 g of DPPH (M = 394.32 g/mol), produced as a DPPH solution, was dissolved in 100 cm^3 of ethanol. Crushed bar samples weighing 25 g were added to 100 mL of ethanol to create extracts. The solution was shaken. Then, after 20 h, it was filtered.

The solution was kept in a dark place. By mixing up to 0.2 cm^3 of DPPH solution with 0.8 cm^3 of ethanol, the absorbance A0 was calculated. A total of 0.2 cm^3 of DPPH solution, 0.6 cm^3 of ethanol, and 0.2 cm^3 of the test extract were all present in the test sample. The absorbance was measured at 517 nm using a spectrophotometer GenesysTM 20 (Thermo Scientific Co., Waltham, MA, USA). A measurement of absorbance (A) was made 5 and 30 min. after the process started. The solution's mean absorbance value (AR) was computed after three repetitions of each measurement. When the DPPH solution was lowered by test samples of bars, the violet color vanished.

The spectrophotometer at wavelength = 517 nm captured the reduction in absorbance. Ethanol was used to calibrate the utilized machinery. Applying the subsequent Equation (2), the *inhibition* % of the DPPH radical discoloration was determined:

$$\%\ Inhibition = 100\ (A_0 - Ar)/A_0 \quad (2)$$

where A_0 is the absorbance of the control; *Ar* is the absorbance of the extract.

All determinations were performed in triplicate. The obtained results were expressed as a mean ± standard deviation (SD).

2.8.2. ABTS Radical Scavenging Assay

Following Re et al., 1999 [43], the radical scavenging assay for ABTS (2,2′-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid, Sigma Aldrich, Darmstadt, Germany) was carried out. After 6 min of incubation with the extracts, fluctuations in the concentration of the ABTS●+ radical cations were assessed using a spectrophotometer, the GenesysTM 20 (Thermo Scientific Co., Waltham, MA, USA). The decrease in the absorbance of the solution at a 734 nm wavelength indicates that the antioxidative characteristics of these extracts have lowered the levels of ABTS●+. Equation (2) is used to calculate the extracts' capacity to thwart the oxidation reaction based on the formula.

All determinations were performed in triplicate. The obtained results were expressed as a mean ± standard deviation (SD).

2.9. Color Measurement

The Konica Minolta CM-2300d spectrophotometer (Konica Minolta Business Technologies, Inc., Osaka, Japan) was used to measure the CIE color parameters (L*, a* and b*) according to the method described by Fazaeli et al., 2013 [44]. A white reference tile was used to calibrate the spectrophotometer.

- The relative color difference index (ΔE) and the index of the hue angle (H^0) were calculated according to the following Equation (3):

$$\Delta E = \left[L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2 \right]^{0.5} \quad (3)$$

where:

Parameter L*—lightness coefficient (L* = 0 indicates black and L* = 100 indicates white [dimensionless value]);
Parameter a*—red color coefficient (dimensionless value);
Parameter b*—yellow color coefficient (dimensionless value);
L*0, a*0, b*0—color coefficients related to for unfermented juice (dimensionless value).

- The index of hue angle (H^0), Equation (4):

$$H^0 = \tan^{-1}(b*/a*) \quad (4)$$

- Chroma (C), Equation (5):

$$C = (a^2 + b^2)^{1/2} \quad (5)$$

All determinations were performed in triplicate. The obtained results were expressed as a mean ± standard deviation (SD).

2.10. Sensory Analysis

The quantitative descriptive analysis (QDA) following the ISO procedure [45] was performed to evaluate the sensory characteristics of the investigated jellies. The sensory estimation of the products was carried out, 1 day after the manufacturing process and 10 days after the refrigerated storage. Ten panelists of WULS's Department of Food Gastronomy and Food Hygiene took part in the sensory evaluation. The panelists received instruction in the fundamentals of sensory evaluation methodology [46]. Experts in an age range from 22 to 55 who possessed strong expertise in sensory assessment techniques, such as estimating the profiles of frozen delicacies such as fruit jellies, were included.

The chosen sensory attributes of mulberry jellies fermented by *Lactiplantibacillus plantarum* O21 were as follows: color, compactness, smoothness, watery, sweet odor, bitter odour, acid odor, other odor, sweet taste, bitter taste, acid flavor, and other flavors as well as overall quality.

The panelists' task was to mark the intensity of each of the quality attributes and conduct their assessment on an appropriate scale (linear graphical scale of 0 (low)–10 (high) conventional units (c.u.).

All determinations were performed in triplicate. The obtained results were expressed as a mean ± standard deviation (SD).

2.11. Statistical Analysis

All measurements were performed in triplicates. A one-way analysis of variance (ANOVA) test was followed by Fisher NIR test, with the overall significance level set to 0.05.

Pearson's correlation was used to identify the correlation of TAC and different color values (L* a* b* H^0 C ΔE) in investigated samples.

Principal component analysis (PCA) is a statistical method of reducing the dimensionalities of high-dimensional datasets in a variety of research areas [47]. In this study, the PCA

method was used to estimate the changes in the sensory quality of potentially functional mulberry jellies during storage and to interpret the overall sensory quality, color evaluation, antioxidant activity, and the total anthocyanin content results of investigated products.

All statistical analyses were performed using STATISTICA 13.3 PL software (StatSoft, Kraków, Poland).

3. Results

3.1. Microbiological Analysis

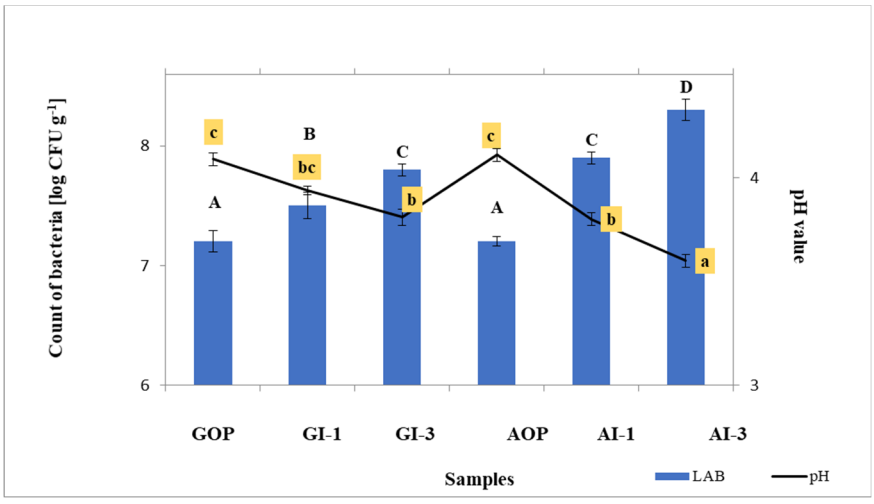
At the first stage of the study, during the fermentation process and as a result of the metabolic activity of *Lactiplantibacillus plantarum* O21, a decrease in pH values in each sample was noted. The pH of fermented mulberry juice dropped from the initial 4.8 to 3.94 (juice with 3% of inulin addition), and then to 4.09 (control sample, without prebiotic addition), respectively.

The results of the study indicate that the highest significant count of LAB bacteria ($p < 0.05$) among all fermented samples of fruit juice was observed in juice with a 3% of inulin addition at 9.35 log CFU g^{-1}, respectively. On the other hand, in the case of the control sample, the lowest level of the count of bacteria was noted at the level of 9.05 log CFU g^{-1}, respectively. These determinations were the initial stages necessary to select fermentation conditions and to determine additive levels. However, they were not critical to the aim of this study.

The results of changes in the pH value and the count of LAB bacteria in potentially functional mulberry jellies fermented by *Lactiplantibacillus plantarum* O21, based on different gelling agents during 10 days of storage at 4 °C, are shown in Figure 1a,b.

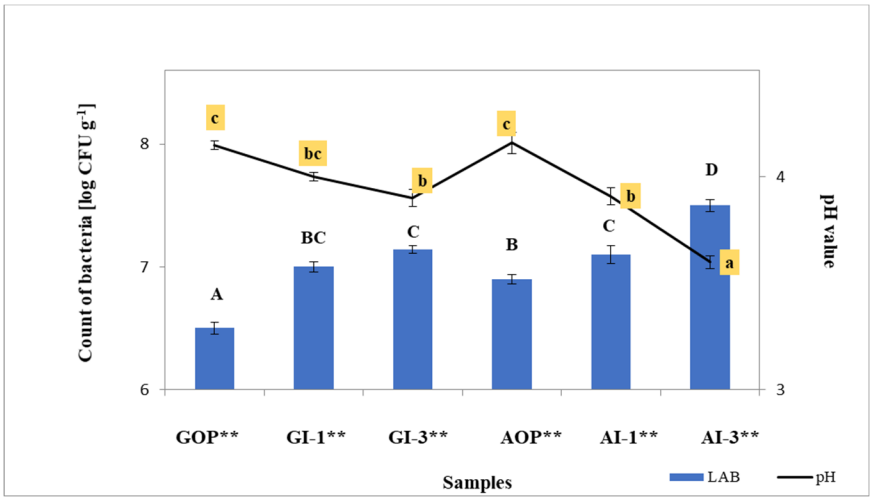
As a result of the fermentation process and the addition of fermented mulberry juice into the jelly recipes, the pH decrement was accompanied by culture growth in the investigated products (Figure 1a). As illustrated in Figure 1, the statistically significant highest count of LAB bacteria was obtained in sample AI-3 (with 3% *w*/*v* of inulin addition; 2% *w*/*v* agar–agar; 7.5 log CFUg^{-1}), respectively. In addition, this results in a pH value that is noticeably lower (3.6), respectively (Figure 1a), when compared to other investigated products. A similar trend in the pH value and bacterial count was also observed in the stored products (Figure 1b).

In summary, regardless of the recipe composition and storage time, each of the evaluated products had a sufficient amount of LAB probiotic bacteria in their concentration (higher than 6.5 log CFU g^{-1}, respectively) at a minimum level of 6 log CFU g^{-1}.



(**a**)

Figure 1. *Cont.*



(**b**)

Figure 1. Changes in pH value and the count of LAB bacteria in investigated mulberry jellies fermented by *Lactiplantibacillus plantarum* O21, based on different gelling agents during 10 days of storage at 4 °C ((**a**)—fresh products; (**b**)—stored products). ** Explanatory notes: The presented samples are coded according to Section 2, Table 1. The results are expressed as the mean ± standard deviation (n = 3). Mean values denoted by the same letters (upper case letters—in regards to the LAB count, and lower case letters—in regards to the pH value) do not differ significantly ($p > 0.05$).

3.2. Color Measurement and Total Anthocyanin Concentration

The changes in total anthocyanin content and color values (L* a* b* H^0 C ΔE) in the investigated samples of fruity jellies are shown in Table 2. The effect of fermentation on color properties of investigated samples was examined along with the following color features (L*, a* and b*) and the total color difference (ΔE).

Table 2. Changes in total anthocyanin concentration and color values (L* a* b* H^0 C ΔE) in investigated mulberry jellies fermented by *Lactiplantibacillus plantarum* O21, based on different gelling agents during 10 days of storage at 4 °C.

Sample Code	Total Anthocyanin Concentration (TAC) (mg/mL)	L*	a*	b*	H^0	C*	ΔE
UMJ	0.90 ± 0.24	10.5 ± 0.10	31.50 ± 0.13	9.80 ± 0.16	17.28 ± 0.16	32.90 ± 0.11	-
FMJ	1.70 ± 0.12	10.00 ± 0.14	31.82 ± 0.10	9.45 ± 0.17	16.54 ± 0.13	33.19 ± 0.12	0.72 ± 0.16
GOP	1.20 ± 0.55 ^{Ab}	9.50 ± 0.10 ^{Ca}	32.30 ± 1.14 ^{Aa}	9.10 ± 0.15 ^{Eb}	15.75 ± 0.09 ^{Fb}	33.5 ± 0.90 ^{Ab}	1.46 ± 0.85 ^{Aa}
GOP **	0.95 ± 0.03 ^{aba}	10.45 ± 0.14 ^{bb}	31.80 ± 0.14 ^{ab}	8.15 ± 0.10 ^{aa}	14.57 ± 0.08 ^{aa}	32.82 ± 0.09 ^{Aa}	2.35 ± 0.15 ^{cdb}
GI1	1.40 ± 0.41 ^{Bb}	9.31 ± 0.06 ^{Ba}	32.80 ± 0.09 ^{Fb}	8.80 ± 0.12 ^{Cb}	15.11 ± 0.13 ^{Da}	33.96 ± 0.90 ^{Fab}	1.83 ± 0.08 ^{Ca}
GI1 **	1.25 ± 0.21 ^{ca}	9.71 ± 0.11 ^{cb}	32.40 ± 0.09 ^{bca}	9.20 ± 0.09 ^{da}	15.80 ± 0.09 ^{db}	33.68 ± 0.90 ^{ba}	1.86 ± 0.32 ^{ba}
GI3	1.55 ± 0.11 ^{Cab}	9.26 ± 0.23 ^{Aa}	33.58 ± 0.19 ^{Db}	8.65 ± 1.23 ^{Ba}	14.57 ± 0.11 ^{Ca}	32.26 ± 0.90 ^{Aa}	2.35 ± 0.10 ^{Da}
GI3 **	1.50 ± 0.25 ^{ea}	9.75 ± 0.22 ^{cdb}	32.80 ± 0.0 ^{da}	8.95 ± 0.19 ^{ca}	15.21 ± 0.09 ^{bb}	34.00 ± 0.09 ^{cb}	2.37 ± 0.12 ^{cda}
AOP	1.25 ± 0.06 ^{ABb}	9.35 ± 0.21 ^{Ba}	33.21 ± 0.09 ^{Ca}	8.95 ± 0.28 ^{Da}	15.59 ± 0.09 ^{Ea}	33.32 ± 0.22 ^{Ba}	1.55 ± 0.10 ^{Bb}
AOP **	0.90 ± 0.18 ^{aa}	10.22 ± 0.21 ^{ebc}	32.60 ± 0.18 ^{cb}	9.55 ± 0.21 ^{eab}	16.17 ± 0.12 ^{eb}	33.94 ± 0.11 ^{bb}	1.14 ± 0.11 ^{aa}
AI1	1.40 ± 0.09 ^{Bb}	9.25 ± 0.26 ^{Aa}	33.50 ± 0.19 ^{Db}	8.60 ±0.61 ^{Ba}	14.41 ± 0.10 ^{Ba}	34.60 ± 0.03 ^{Da}	2.65 ± 0.15 ^{Eb}
AI1 **	1.15 ± 0.19 ^{ba}	9.55 ± 0.09 ^{bb}	33.35 ± 0.08 ^{ea}	8.95 ± 0.13 ^{cab}	15.37 ± 0.11 ^{cb}	34.50 ± 0.09 ^{da}	2.22 ± 0.09 ^{ca}

Table 2. *Cont.*

Sample Code	Total Anthocyanin Concentration (TAC) (mg/mL)	L*	a*	b*	H^0	C*	ΔE
AI3	1.63 ± 0.03^{Db}	9.31 ± 0.15^{Ba}	33.60 ± 0.11^{DEb}	8.50 ± 0.10^{Aa}	14.20 ± 0.11^{Aa}	34.65 ± 0.11^{DEb}	2.74 ± 0.07^{Fb}
AI3 **	1.45 ± 0.06^{da}	9.36 ± 0.09^{aab}	$33.40 \pm 0.0\,5^{fa}$	8.70 ± 0.21^{bb}	14.57 ± 0.13^{ab}	34.51 ± 0.05^{da}	2.47 ± 0.11^{ea}

Explanatory notes: The presented samples are coded according to Section 2, Table 1. **—product stored during 10 days storage at 4 °C. The results are expressed as the mean ± standard deviation ($n = 3$). Values denoted by different letters differ significantly ($p < 0.05$). UMJ—unfermented mulberry juice; FMJ—fermented mulberry juice; TAC—Total anthocyanin concentration; L*—Lightness; a*—Redness; b*—Yellowness; C*—Chroma; H^0—Hue angle; ΔE—Relative color difference index. Values denoted by different capital letters in the fresh products, differ significantly ($p < 0.05$). Values are denoted by different lowercase letters in the stored products, significantly ($p < 0.05$). Values are denoted by different color lowercase letters in the same fresh and stored batch products, significantly ($p < 0.05$).

Results posted in Table 2 allow for an estimation of the impact of the fermentation process on color properties of investigated products. The recorded color parameters in the case of fruity jellies based on fermented mulberry juice, testify to the greater saturation of the red component—the value of the parameter a* (+31.8–+33.6) and lower yellow saturation (parameter value b* (+8.15–+9.55) (Table 2).

Due to the process of fermentation, there was an increase in the content of the total anthocyanin concentration (TAC) in mulberry juice, from 0.90 mg/mL to 1.70 mg/mL, respectively. Despite a slight decrease in the TAC content following the combination of all the recipe's ingredients, in fresh jelly samples, significantly higher ($p < 0.05$) (1.20–1.55 mg/mL) TAC compared to the sample of unfermented juice (slightly higher values were obtained for agar-concentrated samples—AI 1 and AI 3). Regardless of the gelling agent used, the inulin addition statistically and significantly influenced ($p < 0.05$) the increase in TAC content in fresh products, which, at the same time, corresponded to the increased number of bacteria *Lactiplantibacillus plantarum* O21 in these samples (Figure 1a). As a result of the refrigerated storage, TAC content significantly decreased ($p < 0.05$), regardless of the product type (prebiotic addition; used gelling agent). Also note that the used gelling agent and prebiotic addition affect TAC content after the refrigerated storage. In products based on gelatin, with the addition of a prebiotic (regardless of the added level), a smaller loss of TAC content (Table 2) was observed as compared to agar–agar-based tests.

In the finished goods, a slight decrease in L * was observed in fermented mulberry juice ($p < 0.05$), which indicated the darkening of the color after combining all recipes. The values of the L* and b* parameter decreased during juice fermentation and during the production of potentially functional jellies. An increase in the color parameter—a* was also observed in finished products compared to the fermented intermediate—mulberry juice (Table 2).

There was an increase in the value of the ΔE parameter as a result of combining all components and obtaining mulberry jelly tests. Fresh products based on both agar and gelatin (GO1, GI3, AI1, and AI3), with the addition of a prebiotic, displayed noticeably greater ΔE values ($p < 0.05$) in relation to control tests (G0P and A0P). However, significantly higher values of the ΔE parameter were recorded for the jelly agar-agar-based tests. As a result of the refrigerated storage, there was a slight but statistically significant ($p < 0.05$) reduction in these values in the case of the agar–agar product-based and the control sample, G0P (Table 2).

Results shown in Table 2 are verified by a noteworthy inverse relationship between parameters L* and TAC (R2 = −0.763; $p < 0.05$), and b* and TAC (R2 = −0.353; $p < 0.01$), as well as between H^0 and TAC (R2 = −0.649; $p < 0.05$) (Table 3). Nevertheless, the parameters b* and L are positively related to the color value H^0, with coefficients (R2 = +0.896; $p < 0.05$; R2 = +0.412; $p < 0.01$), respectively.

Table 3. Pearson's correlation coefficients of total anthocyanin concentration and color attributes of investigated mulberry jellies fermented by *Lactiplantibacillus plantarum* O21, including refrigerated storage.

	TAC	L*	a*	b*	H^0	C*
TAC	1					
L*	−0.763 *	1				
a*	0.282	−0.121	1			
b*	−0.358 **	0.232	0.353	1		
H^0	−0.649 *	0.412 **	0.017	0.896 *	1	
C*	0.216	−0.206	0.192	0.136	−0.097	1

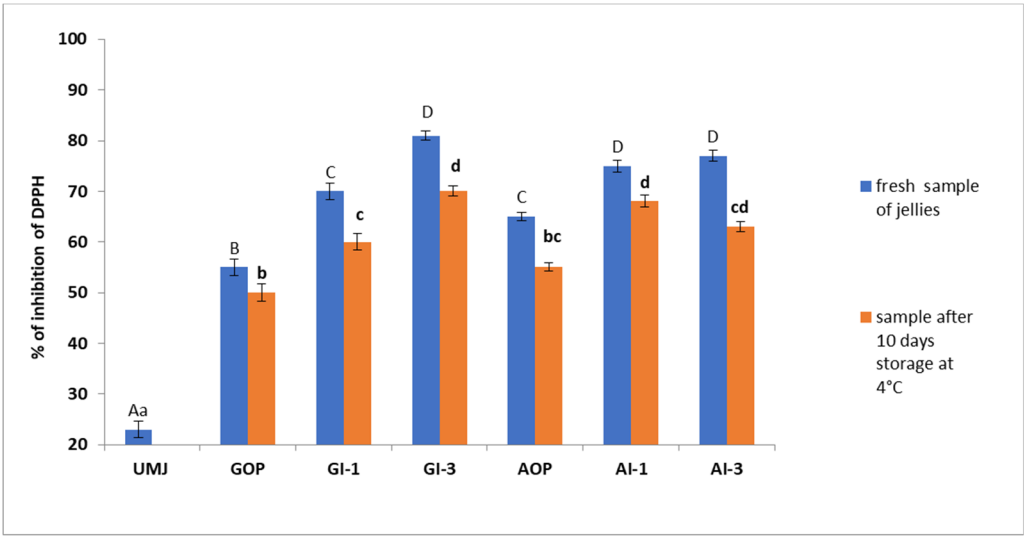
Explanatory notes: TAC—Total anthocyanin concentration; L*—Lightness; a*—Redness; b*—Yellowness; C*—Chroma; H^0—Hue angle; ΔE—Relative color difference index. * Correlation is significant at $p < 0.05$. ** Correlation is significant at $p < 0.01$.

3.3. Determination of Antioxidant Activity

The changes in antioxidant activity of the investigated functional mulberry jellies fermented by *Lactiplantibacillus plantarum* O21 are presented in Figure 1a,b.

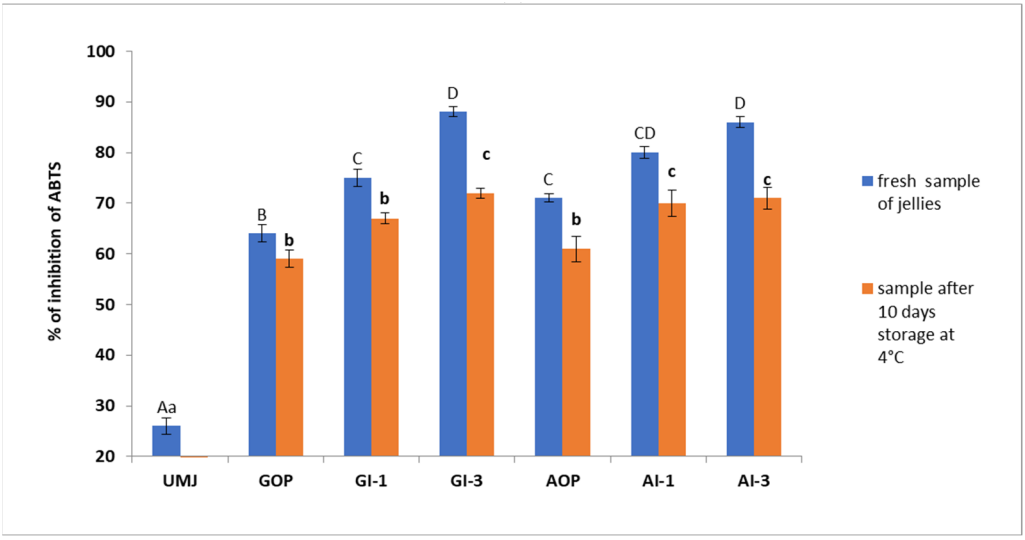
The obtained results illustrate the positive effect that lactic acid fermentation had on the scavenging activities of the DPPH and ABTS radical. Regardless of the gelling agent utilized, the lactic fermentation procedure produced fermented mulberry jellies with antioxidant activity that was roughly two to three times higher than unfermented mulberry juice. Regardless of the level used, fresh and inulin samples after storage had a higher antioxidant activity value on the DPPH and ABTS assay.

The DPPH activity of fresh fruity jellies, referred to as % DPPH inhibition, ranged from 55.0% (sample G0P) to 81.0% in the sample GI3, respectively (Figure 2a). In stored products, regardless of the gelling agent used, these values have changed significantly ($p < 0.05$) and have decreased. In both gelatin and agar–agar-based trials, the trend in assessing fresh products in the antioxidant capacity detected by the DPPH and ABTS assay continued. For stored samples with a 1% and 3% inulin addition, statistically significant higher values of antioxidant activity ($p < 0.05$) were reported.



(**a**)

Figure 2. *Cont.*



(**b**)

Figure 2. Changes in antioxidant activity of functional mulberry jellies fermented by *Lactiplantibacillus plantarum* O21, based on different gelling agents stored at 4 °C during 10 days: (**a**) % inhibition of DPPH; (**b**) % inhibition of ABTS. Explanatory notes: Data are expressed as mean values ± standard deviations. Refer to Table 1 for identification of test samples. Values denoted by different capital letters in the fresh samples differ significantly ($p < 0.05$); $n = 3$. Values denoted by different lowercase letters in the samples after 10 days of storage at 4 °C differ significantly ($p < 0.05$); $n = 3$.

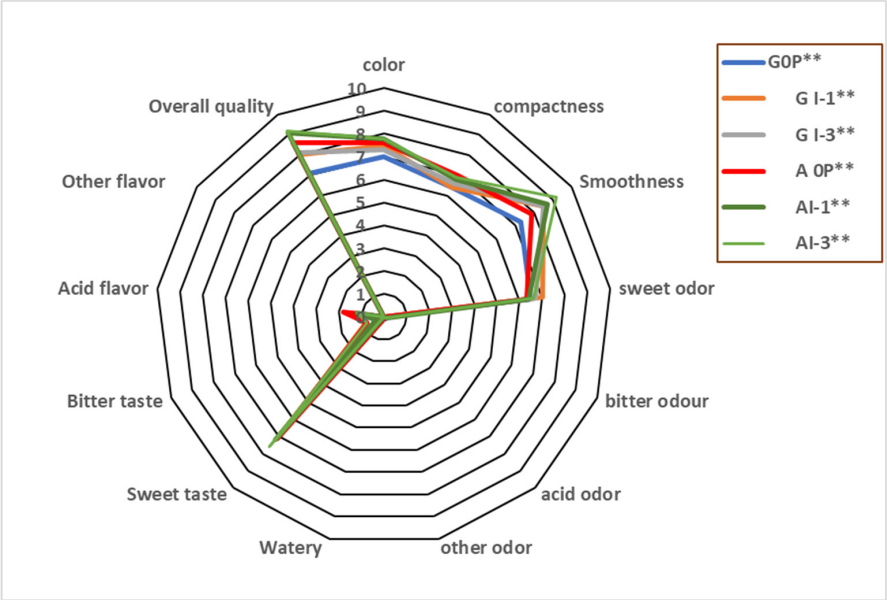
When comparing the results of the DPPH assay to the results of the ABTS assay, the antioxidant capacity of the examined unfermented mulberry juice, and fresh and preserved functional jellies, was slightly greater (Figure 2a,b).

3.4. Sensory Characteristics of Functional Mulberry Jellies

The changes in sensory profiles of investigated mulberry jellies fermented by *Lactiplantibacillus plantarum* O21 are shown in Figure 3a,b.

(**a**)

Figure 3. *Cont.*



(**b**)

Figure 3. Sensory profile of functional mulberry jellies fermented by *Lactiplantibacillus plantarum* O21, based on different gelling agents: (**a**) fresh mulberry jellies; (**b**) products stored at 4 °C during 10 days. ** Explanatory notes: The presented samples are coded according to Section 2, Table 1.

The highest value was the smoothness (8.60–9.23 c.u.), and intensity of the sweet taste sensation (6.0–7.5 c.u.) of functional jellies with the addition of inulin (I-1; AI-3) (Section 2, Table 1), regardless of the level of the additive and gelling agent used. In contrast, in the control tests of the G (G0P) (Section 2, Table 1), a gelatin-based series, the highest level of intensity of the consistency distinguishing feature, i.e., "watery", was recorded at 0.1–0.15, respectively (Figure 3a,b).

Gelatin-based samples (G0P; GI-1, GI-3) (Section 2, Table 1) obtained slightly higher color distinguishing values (Figure 3a). However, these differences were not statistically significant ($p > 0.01$).

In agar–agar-based trials (A0P; AI-1; AI-3) (Section 2, Table 1), the highest intensity of the sour taste sensation was noted. All treatments of investigated products were characterized by a good overall quality (meaning higher than 7.5 c.u.). All of the samples in the A series (agar–agar-based) and products that were gelatin-based with the prebiotic addition (GI-1, GI-3) (Section 2, Table 1) showed the highest level of overall quality intensity (8.0—9.2 c.u.), which can be attributed to the high intensity of positive sensory factors such as sweet taste and smoothness (Figure 3a).

During storage, the value of the assessed sensory quality characteristics of innovative mulberry jellies has not changed significantly ($p > 0.01$), and therefore the sensory overall quality has not significantly deteriorated either. However, the overall quality of stored products was rated at 7.1–9.2 c.u., respectively (Figure 3b).

The results of the sensory evaluation of potentially functional mulberry jellies fermented by *Lactiplantibacillus plantarum* O21 during refrigerated storage were also elaborated by the PCA method. The PCA graph of the selected sensory attributes in investigated products is shown in Figure 4.

The analysis of the study's collected results employed the sum of the first two principal components. The first two main components accounted for 42.2% of the difference between the samples, according to a multivariate analysis of the data. The investigated products were grouped into four clusters with their substance gell and inulin addition (Figure 4).

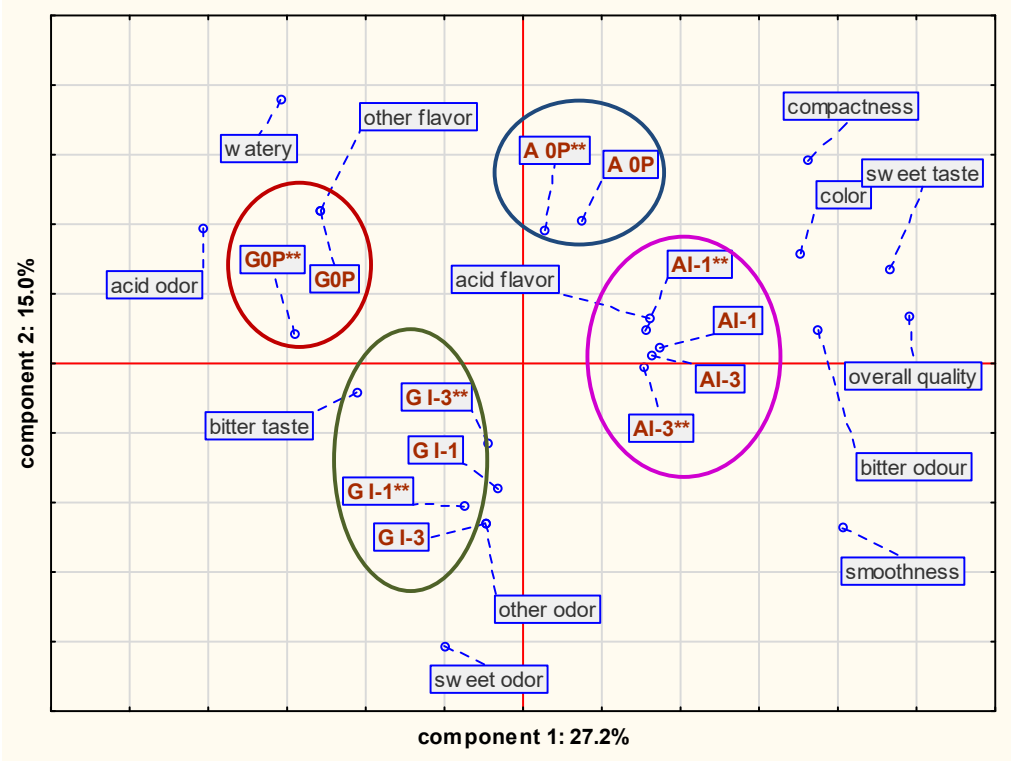


Figure 4. Principal Component Analysis (PCA) graph of the selected sensory attributes and overall quality in investigated samples of mulberry jellies fermented by *Lactiplantibacillus plantarum* O21, based on different gelling agents. ** Explanatory notes: The presented samples are coded according to Section 2, Table 1.

The first component explained 27.2% of the total variability and was mainly related to "color", "compactness", "smoothness", "bitter odor", "sweet taste", "acid flavor", and "overall quality". However, the second component explained 15.0% of the total variability and was mainly related to for such distinguishing features as "watery", "other flavor", or "acid flavor" (Figure 4).

Analyzing the arrangement of tested samples in the space of the plot, it can be concluded that the gelling agents differentiated the products located on the right side (A series; Section 2, Table 1) and the left side (G series; (Section 2, Table 1) of the plot (Figure 4).

The first homogeneous group, in terms of quality, were fresh and stored jelly samples based on gelatin (G 0P; G0P**) (Section 2, Table 1). These samples were characterized by a high intensity of "*watery*" and low intensity of "acid odor" (Figure 4).

On the other hand, the second group consisted of fresh and stored jellies that are agar–agar based (A 0P; A0P**) (Section 2, Table 1). These samples received the highest marks among the products evaluated, in the case of the "compactness" distinguishing feature.

The third cluster is constituted of fresh and stored samples of agar–agar-based jellies (Section 2, Table 1). This region of the plot's properties shows that the goods under investigation share similar qualities, including the strongest overall quality intensity, sweetest flavor, and strongest color notes.

The fourth indicated cluster was fresh and stored gelatin-based products, seria G (Section 2, Table 1). These goods are distinguished by their strong "*sweet odor*". Also in the GI3 sample, the highest value for the "other odor" distinguishing feature was recorded, which was described by the evaluators as "meaty".

4. Discussion

New carriers introduce beneficial bacteria into the diet, which makes us enrich and rebuild our microbiome. Bioactive ingredients contained in food support the survival and

growth of microorganisms in the intestines. Currently, there is a high demand for this type of food.

The probiotic features and health benefits conferred are known to be LAB-strain-specific [48]. Probiotic food products must have a high concentration of microorganism cells [$\geq 10^6$ CFU mL^{-1}], or between 10^8 and 10^{11} CFU per day, to have the required positive impact [49]. In our study, all investigated, fresh, and stored samples of developed mulberry jellies contained an appropriate and high number of LAB bacteria *Lactiplantibacillus plantarum* O21, which was higher than 6.5 log CFU g^{-1} of product. The addition of inulin significantly affected $p < 0.05$ (Figure 1a,b) to increase this number. This is in accordance with Farinha et al., 2015 [50], who reports that to improve probiotic survival, prebiotic ingredients can be added to food preparations containing probiotic microbiota. Moreover, our results are in agreement with the report of other authors, who confirmed that the addition of agar–agar into fermented coconut jelly can improve probiotics' survivability, and phenolic and antioxidant compounds during in vitro digestion [51].

Plants are valuable raw materials for the fermentation of food, due to them being rich in bioactive ingredients that can increase the survival rate of probiotics. On the other hand, probiotic bacteria can affect the higher content of polyphenols and antioxidant activity.

An increase in the antioxidant activity of plant-based foods as a result of lactic fermentation occurs due to an increase in the release of antioxidant compounds, mainly due to an increase in phenolic compounds and flavonoids via microbial hydrolysis [52]. Changes in the level of antioxidant activity compounds' production during lactic acid fermentation may be at different levels, which depends on used microorganisms, cultivation medium, pH, temperature, inhibitors, stimulators, or the atmosphere. Rodríguez et al., 2009 noted that *L. plantarum* was able to break down several phenolic compounds found in food, producing molecules that affect the aroma of the meal as well as compounds with higher antioxidant activity [53]. In turn, it was also found that a prebiotic addition can affect the antioxidant activity of plant-based food. For example, Michalska et al., 2019 [54] suggested that inulin is a better protection agent for anthocyanins than maltodextrin, except for vacuum drying at 90 °C, which probably causes inulin to form 5-(Hydroxymethyl)furfural (HMF), thus limiting its ability to protect anthocyanins. The authors suggest that the higher pace of the process meant a stronger anthocyanin degradation. In our study, regardless of the gelling agent used, significantly higher ($p < 0.05$) antioxidant activity values were observed in samples with the addition of a prebiotic assessed by two methods, DPPH and ABTS, while maintaining this trend after refrigerated storage.

Phenolic compounds are closely related to the sensory quality of food, through the impact on such distinguishing features as astringency, color, and flavor [55]. The color of mulberry fruit-based products is mainly dependent on the level of anthocyanins [56]. In all fruity jellies based on fermented juice, a slight increase in (b) and a decrease in (L) and (b) was observed compared to unfermented mulberry juice [57] (Table 2). Our results were in agreement with Kwaw et al. 2016 [34], who also demonstrated that an effect of lactic fermentation on mulberry juices' quality includes a color assessment. The reason for such changes may be the increase in the monomeric anthocyanin concentration in attempts based on fermented juice. As reported by Boranbayeva et al. 2014 [58], antioxidant activity in black mulberry juice is correlated with total monomeric anthocyanins.

It is well known that variations in the products' sensory profiles are caused by the fermentation technique used to produce fermented goods, in addition to the ingredients in the recipe [59]. According to Tkacz et al. 2021 [59], in assessing the sensory quality of novel functional products, the differentiating factor was the intensity of the sour taste sensation. In our study, in agar–agar-based trials, especially in (AI-1; AI-3) (Section 2, Table 1) with the addition of inulin, the highest intensity of the sour taste sensation was observed, which at the same time was correlated with a significantly ($p < 0.05$) higher number of bacteria *Lactiplantibacillus plantarum* O21 in these products (Figure 1a,b). Inulin as a prebiotic is used by probiotic bacteria as a source of carbon. Because probiotics selectively break down inulin, they can increase their population in the environment. A negative consequence

of this process from the point of view of product quality is the overproduction of organic acids by LAB bacteria, mainly lactic acid, and their accumulation in food. The metabolic activity of bacteria can be reduced, for example, by storing food in refrigerated conditions.

Because of its technological qualities, inulin is also employed in food technology as a low-calorie sweetener, fat substitute, and texture adjuster [60]. In the present study, the highest values of the texture distinguishing feature—smoothness and the intensity of the sweet taste sensation—was noted in the case of mulberry jellies with the addition of inulin (GO-1, GI-3; AI-1; AI-3) (Section 2, Table 1), regardless of the level of this additive used and the gelling agent used. Moreover, the results showed that agar–agar-based samples of jellies were best evaluated in terms of the texture-distinguishing feature, "compactness" (Figure 3a,b). Domínguez-Murillo and Urías-Silvas, 2023, suggested that the addition of 1% of agar to samples of coconut jellies presented the maximum hardness ($p < 0.05$), due to more bonding points and intermolecular interactions [51]. It can be concluded that inulin can be a good alternative for sucrose, which is in line with current food design trends. In contrast, the addition of agar is both texture-forming and protective against probiotic bacteria at the same time. The above properties of these additives are conducive to their use in the production of jellies.

5. Conclusions

This study demonstrated the use of a half-product in the form of mulberry juice fermented with the *Lactiplantibacillus plantarum* O21 strain with probiotic properties, including such additives as: the gelling agent (gelatin; agar–agar), prebiotic (inulin), impact on the color parameters, TAC content, antioxidant activities, and sensory quality of final products, such as functional jellies.

Results generated in this study suggest that the agar–agar-based products with the addition of inulin were characterized by high parameters in the field of microbiological assessment (significantly higher numbers of LAB), antioxidant activity values, and good notes in sensory evaluation—"overall sensory quality". However, the use of gelatin and a higher 3% level of an inulin supplement as the gelling agent means that the sensory evaluation of this product variant could taste a bit "meaty".

It should be further noted that the functional mulberry jellies in the agar–agar-based version can be beneficial alternatives for a wide range of consumers, for example: people with milk protein allergy, lactose intolerance, and vegans, as well children. In conclusion, our research will continue in order to refine production technology, as well as optimize parameters to obtain a symbiotic product.

Author Contributions: A.S., Conceptualization, Investigation, Supervision, Methodology, Software, Validation, Formal analysis, Investigation, Writing—review and editing, Visualization, Writing—original draft, and Data Curation. D.Z., Writing—review and editing. B.S., Investigation. D.K.-K., Formal analysis. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki, and approved by the Rector's Committee for the Ethics of Scientific Research Involving Humans at WULS-SGGW (Resolution No. 28/RKE/2023/U of 6 July 2023).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Granato, D.; Barba, F.J.; Kovačević, D.B.; Lorenzo, J.M.; Cruz, A.G.; Putnik, P. Functional Foods: Product Development, Technological Trends, Efficacy Testing, and Safety. *Annu. Rev. Food Sci. Technol.* **2020**, *11*, 93–118. [CrossRef] [PubMed]
2. Kumar, B.V.; Vijayendra, S.V.N.; Reddy, O.V.S. Trends in dairy and non-dairy probiotic products - a review. *J. Food Sci. Technol.* **2015**, *52*, 6112–6124. [CrossRef] [PubMed]

3. Zhang, Y.; Hu, P.; Lou, L.; Zhan, J.; Fan, M.; Li, D.; Liao, Q. Antioxidant activities of lactic acid bacteria for quality improvement of fermented sausage. *J. Food Sci.* **2017**, *82*, 2960–2967. [CrossRef] [PubMed]
4. Hill, C.; Guarner, F.; Reid, G.; Gibson, G.R.; Merenstein, D.J.; Pot, B.; Morelli, L.; Canani, R.B.; Flint, H.J.; Salminen, S.; et al. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol.* **2014**, *11*, 506–514. [CrossRef] [PubMed]
5. ISAPP. *Minimum Criteria for Probiotics*; International Scientific Association for Probiotics and Prebiotics: Sacramento, CA, USA, 2018.
6. Johansen, E. Future access and improvement of industrial lactic acid bacteria cultures. *Microb. Cell Factories* **2017**, *16*, 230. [CrossRef] [PubMed]
7. Łepecka, A.; Szymański, P.; Okoń, A.; Zielińska, D. Antioxidant activity of environmental lactic acid bacteria strains isolated from organic raw fermented meat products. *LWT* **2023**, *174*, 114440. [CrossRef]
8. Javed, G.A.; Arshad, N.; Munir, A.; Khan, S.Y.; Rasheed, S.; Hussain, I. Signature probiotic and pharmacological attributes of lactic acid bacteria isolated from human breast milk. *Int. Dairy J.* **2022**, *127*, 105297. [CrossRef]
9. Falfán-Cortés, R.N.; Mora-Peñaflor, N.; Gómez-Aldapa, C.A.; Rangel-Vargas, E.; Acevedo-Sandoval, O.A.; Franco-Fernández, M.J.; Castro-Rosas, J. Characterization and Evaluation of the Probiotic Potential In Vitro and In Situ of Lacticaseibacillus paracasei Isolated from Tenate Cheese. *J. Food Prot.* **2022**, *85*, 112–121. [CrossRef]
10. Trindade, D.P.d.A.; Barbosa, J.P.; Martins, E.M.F.; Tette, P.A.S. Isolation and identification of lactic acid bacteria in fruit processing residues from the Brazilian Cerrado and its probiotic potential. *Food Biosci.* **2022**, *48*, 101739. [CrossRef]
11. Sampaio, K.B.; de Albuquerque, T.M.R.; Rodrigues, N.P.A.; de Oliveira, M.E.G.; de Souza, E.L. Selection of Lactic Acid Bacteria with In Vitro Probiotic-Related Characteristics from the Cactus *Pilosocereus gounellei* (A. Weber ex. K. Schum.) Bly. ex Rowl. *Foods* **2021**, *10*, 2960. [CrossRef]
12. Argyri, A.A.; Zoumpopoulou, G.; Karatzas, K.A.G.; Tsakalidou, E.; Nychas, G.-J.E.; Panagou, E.Z.; Tassou, C.C. Selection of potential probiotic lactic acid bacteria from fermented olives by in vitro tests. *Food Microbiol.* **2013**, *33*, 282–291. [CrossRef] [PubMed]
13. Gupta, A.; Sharma, N. Probiotic Potential of Lactic Acid Bacteria Ch-2 Isolated from Chuli Characterization of Potential Probiotic Lactic Acid Bacteria- Pediococcus acidilactici Ch-2 Isolated from Chuli- A Traditional Apricot Product of Himalayan Region for the Production of Novel Bioactive Compounds with Special Therapeutic Properties. *J. Food Microbiol. Saf. Hyg.* **2017**, *2*, 1–11. [CrossRef]
14. Lactic Acid Bacteria Isolated from Food Can Have Probiotic Properties. Available online: https://foodfakty.pl/bakterie-mlekowe-wyizolowane-z-zywnosci-moga-miec-wlasciwosci-probiotyczne (accessed on 20 January 2023).
15. Markowiak, P.; Śliżewska, K. Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients* **2017**, *9*, 1021. [CrossRef] [PubMed]
16. Wu, J.Y. Everyone vegetarian, world enriching. *Open J. Philos.* **2014**, *4*, 160–165. [CrossRef]
17. Kessler, C.S.; Holler, S.; Joy, S.; Dhruva, A.; Michalsen, A.; Dobos, G.; Cramer, H. Personality profiles, values and empathy: Differences between lacto-Ovo-vegetarians and vegans. *Forsch. Komplementärmedizin* **2016**, *23*, 95–102. [CrossRef]
18. Pimentel, T.C.; da Costa, W.K.A.; Barão, C.E.; Rosset, M.; Magnani, M. Vegan probiotic products: A modern tendency or the newest challenge in functional foods. *Food Res. Int.* **2021**, *140*, 110033. [CrossRef]
19. Tamang, J.P.; Tamang, B.; Schillinger, U.; Guigas, C.; Holzapfel, W.H. Functional properties of lactic acid bacteria isolated from ethnic fermented vegetables of the Himalayas. *Int. J. Food Microbiol.* **2009**, *135*, 28–33. [CrossRef]
20. Panghal, A.; Janghu, S.; Virkar, K.; Gat, Y.; Kumar, V.; Chhikara, N. Potential non-dairy probiotic products—A healthy approach. *Food Biosci.* **2018**, *2*, 80–89. [CrossRef]
21. Ercisli, S.; Orhan, E. Some physico-chemical characteristics of black mulberry (*Morus nigra* L.) genotypes from Northeast Anatolia region of Turkey. *Sci. Hortic.* **2008**, *116*, 41–46. [CrossRef]
22. Hojjatpanah, G.; Fazaeli, M.; Emam-Djomeh, Z. Effects of heating method and conditions on the quality attributes of black mulberry (*Morus nigra*) juice concentrate. *Int. J. Food Sci. Technol.* **2007**, *46*, 956–962. [CrossRef]
23. Krishna, H.; Singh, D.; Singh, R.S.; Kumar, L.; Sharma, B.D.; Saroj, P.L. Morphological and antioxidant characteristics of mulberry (*Morus* spp.) genotypes. *J. Saudi Soc. Agric. Sci.* **2018**, *19*, 136–145. [CrossRef]
24. Memete, A.R.; Timar, A.V.; Vuscan, A.N.; Miere, F.; Venter, A.C.; Vicas, S.I. Phytochemical Composition of Different Botanical Parts of *Morus* Species, Health Benefits and Application in Food Industry. *Plants* **2022**, *11*, 152. [CrossRef] [PubMed]
25. Wang, Y.; Xiang, L.; Wang, C.; Tang, C.; He, X. Antidiabetic and antioxidant effects and phytochemicals of mulberry fruit (*Morus alba* L.) polyphenol enhanced extract. *PLoS ONE* **2013**, *8*, e71144. [CrossRef] [PubMed]
26. Bhattacharjya, D.; Sadat, A.; Dam, P.; Buccini, D.F.; Mondal, R.; Biswas, T.; Biswas, K.; Sarkar, H.; Bhuimali, A.; Kati, A.; et al. Current concepts and prospects of mulberry fruits for nutraceutical and medicinal benefits. *Curr. Opin. Food Sci.* **2021**, *40*, 121–135. [CrossRef]
27. Kim, B.S.; Kim, H.; Kang, S.-S. In vitro anti-bacterial and anti-inflammatory activities of lactic acid bacteria-biotransformed mulberry (*Morus alba* Linnaeus) fruit extract against Salmonella Typhimurium. *Food Control.* **2019**, *106*, 106758. [CrossRef]
28. Wang, F.; Du, B.-L.; Cui, Z.-W.; Xu, L.-P.; Li, C.-Y. Effects of high hydrostatic pressure and thermal processing on bioactive compounds, antioxidant activity, and volatile profile of mulberry juice. *Food Sci. Technol. Int.* **2017**, *23*, 119–127. [CrossRef] [PubMed]

29. Tang, C.; Wu, J.; Luo, G.; Wu, F.; Yang, Q.; Xiao, G. Wine-making experiment using mulberry fruits from different fruit mulberry varieties. *Acta Sericologica Sin.* **2008**, *34*, 24–27.
30. Lee, J.; Choi, S. Quality characteristics of muffins added with mulberry concentrate. *Korean J. Culin. Res.* **2011**, *17*, 285–294. [CrossRef]
31. Jan, B.; Parveen, R.; Zahiruddin, S.; Khan, M.U.; Mohapatra, S.; Ahmad, S. Nutritional constituents of mulberry and their potential applications in food and pharmaceuticals: A review. *Saudi J. Biol. Sci.* **2021**, *28*, 3909–3921. [CrossRef]
32. Zhang, H.; Ma, Z.F.; Luo, X.; Li, X. Effects of Mulberry Fruit (*Morus alba* L.) Consumption on Health Outcomes: A Mini-Review. *Antioxidants* **2018**, *7*, 69. [CrossRef]
33. Kobus-Cisowska, J.; Gramza-Michalowska, A.; Kmiecik, D.; Flaczyk, E.; Korczak, J. Mulberry fruit as an antioxidant component in muesli. *Agric. Sci.* **2013**, *4*, 130–135. [CrossRef]
34. Kwaw, E.; Ma, Y.; Tchabo, W.; Apaliya, M.T.; Wu, M.; Sackey, A.S.; Xiao, L.; Tahir, H.E. Effect of lactobacillus strains on phenolic profile, color attributes and antioxidant activities of lactic-acid-fermented mulberry juice. *Food Chem.* **2018**, *250*, 148–154. [CrossRef] [PubMed]
35. Chaudhary, A.; Sharma, V.; Saharan, B.S. Probiotic Potential of Noni and Mulberry Juice Fermented with Lactic Acid Bacteria. *Asian J. Dairy Food Res.* **2019**, *38*, 114–120. [CrossRef]
36. Chuah, H.Q.; Tang, P.L.; Ang, N.J.; Tan, H.Y. Submerged fermentation improves bioactivity of mulberry fruits and leaves. *Chin. Herb. Med.* **2021**, *13*, 565–572. [CrossRef] [PubMed]
37. Lv, M.; Aihaiti, A.; Liu, X.; Tuerhong, N.; Yang, J.; Chen, K.; Wang, L. Development of Probiotic-Fermented Black Mulberry (*Morus nigra* L.) Juice and Its Antioxidant Activity in C2C12 Cells. *Fermentation* **2022**, *8*, 697. [CrossRef]
38. Zielińska, D.; Rzepkowska, A.; Radawska, A.; Zieliński, K. In Vitro Screening of Selected Probiotic Properties of Lactobacillus Strains Isolated from Traditional Fermented Cabbage and Cucumber. *Curr. Microbiol.* **2015**, *70*, 183–194. [CrossRef] [PubMed]
39. *NF ISO 15214*; Microbiology of Food and Animal Feeding Stuffs. Horizontal Method for the Enumeration of Mesophilic Lactic Acid Bacteria. Colony Count Technique at 30 °C. ISO: Geneva, Switzerland, 1998.
40. Tchabo, W.; Ma, E.; Kwaw, Y.; Zhang, X.; Li, H.; Afoakwah, N.A. Effects of ultrasound, high pressure, and manosonication processes on phenolic profile and antioxidant properties of a sulfur dioxide-free mulberry (*Morus nigra*) wine. *Food Bioprocess Technol.* **2017**, *10*, 1210–1223. [CrossRef]
41. Alothman, M.; Bhat, R.; Karim, A. UV radiation-induced changes of antioxidant capacity of fresh-cut tropical fruits. *Innov. Food Sci. Emerg. Technol.* **2009**, *10*, 512–516. [CrossRef]
42. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci. Technol.* **1995**, *28*, 25–30. [CrossRef]
43. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
44. Fazaeli, M.; Hojjatpanah, G.; Emam-Djomeh, Z. Effects of Heating Method and Conditions on the Evaporation Rate and Quality Attributes of Black Mulberry (*Morus nigra*) Juice Concentrate. *J. Food Sci. Technol.* **2013**, *50*, 35–43. [CrossRef] [PubMed]
45. Sensory Analysis—Methodology—General Guidance for Establishing a Sensory Profile. Available online: https://www.iso.org/standard/58042.html (accessed on 22 March 2023).
46. *ISO 8586-2:1994*; Sensory Analysis. General Guidance for the Selection, Training and Monitoring of Assessors—Part 2: Experts. ISO: Geneva, Switzerland, 1994.
47. Kitao, A. Principal Component Analysis and Related Methods for Investigating the Dynamics of Biological Macromolecules. *Multidiscip. Sci. J.* **2022**, *5*, 298–317. [CrossRef]
48. Jampaphaeng, K.; Cocolin, L.; Maneerat, S. Selection and evaluation of functional characteristics of autochthonous lactic acid bacteria isolated from traditional fermented stinky bean (Sataw-Dong). *Ann. Microbiol.* **2016**, *67*, 25–36. [CrossRef]
49. Uriot, O.; Denis SJuniua, M.; Roussel, Y.; Dary-Mourot, A.; Blanquet-Diot, S. *Streptococcus thermophilus:* From yogurt starter to a new promising probiotic candidate? *J. Funct. Food.* **2017**, *37*, 74–89. [CrossRef]
50. Farinha, L.R.L.; Sabo, S.S.; Porto, M.C.; Souza, E.C.; Oliveira, M.N.; Oliveira, R.P.S. Influence of prebiotic ingredients on the growth kinetics and bacteriocin production of *Lactococcus lactis*. *Chem. Eng. J.* **2015**, *43*, 313–318.
51. Domínguez-Murillo, A.; Urías-Silvas, J. Fermented coconut jelly as a probiotic vehicle, physicochemical and microbiology characterisation during an in vitro digestion. *Int. J. Food Sci. Technol.* **2022**, *58*, 45–52. [CrossRef]
52. Hur, S.J.; Lee, S.Y.; Kim, Y.-C.; Choi, I.; Kim, G.-B. Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chem.* **2014**, *160*, 346–356. [CrossRef]
53. Rodríguez, H.; Curiel, J.A.; Landete, J.M.; De las Rivas, B.; de Felipe, F.L.; Gómez-Cordovés, C.; Mancheño, J.M.; Muñoz, R. Food phenolics and lactic acid bacteria. *Int. J. Food Microbiol.* **2009**, *132*, 79–90. [CrossRef]
54. Michalska, A.; Wojdyło, A.; Brzezowska, J.; Majerska, J.; Ciska, E. The Influence of Inulin on the Retention of Polyphenolic Compounds during the Drying of Blackcurrant Juice. *Molecules* **2019**, *24*, 4167. [CrossRef]
55. Natić, M.M.; Dabić, D.C.; Papetti, A.; Fotirić Akšić, M.; Ognjanov, V.; Ljubojević, M.; Tešić, Ž. Analysis and characterisation of phytochemicals in mulberry (*Morus alba* L.) fruits grown in Vojvodina, North Serbia. *Food Chem.* **2015**, *171*, 128–136. [CrossRef]
56. Tchabo, W.; Ma, Y.; Kwaw, E.; Zhang, H.; Xiao, L.; Apaliya, M.T. Statistical interpretation of chromatic indicators in correlation to phytochemical profile of a sulphur dioxide-free mulberry (*Morus nigra*) wine submitted to non-thermal maturation processes. *Food Chem.* **2018**, *239*, 470–477. [CrossRef] [PubMed]

57. Chen, L.; Xin, X.L.; Yuan, Q.P.; Su, D.H.; Liu, W. Phytochemical properties and antioxidant capacities of various colored berries. *J. Sci. Food Agric.* **2014**, *94*, 180–188. [CrossRef] [PubMed]
58. Boranbayeva, T.; Karadeniz, F.; Yılmaz, E. Effect of Storage on Anthocyanin Degradation in Black Mulberry Juice and Concentrates. *Food Bioprocess Technol.* **2014**, *7*, 1894–1902. [CrossRef]
59. Tkacz, K.; Wojdyło, A.; Turkiewicz, I.P.; Nowicka, P. Anti-diabetic, anti-cholinesterase, and antioxidant potential, chemical composition and sensory evaluation of novel sea buckthorn-based smoothies. *Food Chem.* **2021**, *338*, 128105. [CrossRef]
60. Canbulat, Z.; Ozcan, T. Effects of short-chain and long-chain inulin on the quality of probiotic yogurt containing Lactobacillus rhamnosus. *J. Food Process. Preserv.* **2015**, *39*, 1251–1260. [CrossRef]