The Impact of the Fermentation Method on the Pigment Content in Pickled Beetroot and Red Bell Pepper Juices and Freeze-Dried Powders

Emilia Janiszewska-Turak 1,*, Kacper Tracz 1, Patrycja Bielińska 1, Katarzyna Rybak 1, Katarzyna Pobiega 2*, Małgorzata Gniewosz 2*, Łukasz Woźniak 3 and Anna Gramza-Michałowska 4,*

1 Department of Food Engineering and Process Management, Institute of Food Sciences, Warsaw University of Life Sciences—SGGW, 02-787 Warsaw, Poland; 193068@sggw.edu.pl (K.T.); b.patrycja60@gmail.com (P.B.); katarzyna.rybak@sggw.edu.pl (K.R.)
2 Department of Food Biotechnology and Microbiology, Institute of Food Sciences, Warsaw University of Life Sciences—SGGW, 02-787 Warsaw, Poland; katarzyna.pobiega@sggw.edu.pl (K.P.); malgorzata.gniewosz@sggw.edu.pl (M.G.)
3 Department of Food Safety and Chemical Analysis, Institute of Agricultural and Food Biotechnology, 36 Rakowiecka Street, 02-532 Warsaw, Poland; lukasz.wozniak@ibprs.pl
4 Department of Gastronomy Science and Functional Foods, Faculty of Food Science and Nutrition, Poznań University of Life Sciences, Wojska Polskiego 31, 60-624 Poznań, Poland
* Correspondence: emilia.janiszewska_turak@sggw.edu.pl (E.J.-T.); anna.gramza@up.poznan.pl (A.G.-M.); Tel.: +48-22-593-7366 (E.J.-T.); +48-61-848-7327 (A.G.-M.)

Abstract: The beetroot and red bell pepper are vegetables rich in active ingredients, and their potential for health benefits are crucial. Both presented raw materials are rich in natural pigments, but are unstable and seasonal; thus, it was decided to take steps to extend their durability. Lactic fermentation has been recognized as a food preservation method, requiring minimal resources. The activities undertaken were also aimed at creating a new product with a coloring and probiotic potential. For this reason, the study aimed to evaluate the impact of the method of fermentation on the content of active compounds (pigments) in pickled juices and freeze-dried powders. The lactic acid fermentation guided in two ways. The second step of the research was to obtain powders in the freeze-drying process. For fermentation, Levilactobacillus brevis and Limosilactobacillus fermentum were used. In juices and powders, pigments, color, and dry matter were tested. In this research, no differences in fermented juice pigment contents were seen; however, the color coefficient differed in raw juices. The freeze-drying process resulted in lowering the pigment content, and increasing dry matter and good storage conditions (glass transition temperatures 48–66 °C). The selection of vegetable methods suggested the use of fermentation and mixing it with a marinade (higher pigments and lactic acid bacteria content). All powders were stable and can be used as a colorant source, whereas for probiotic properties, a higher number of bacteria is needed.

Keywords: beetroot; red bell pepper; pigments; freeze-drying; LAB; color; betalain; carotenoids

1. Introduction

Lactic acid fermentation has been recognized for centuries as a safe technique of food preservation which requires minimum of resources. The fermentation, with lactic acid bacteria (LAB) application, leads to a decrease in pH at the end of the process. Processing vegetables, such as by fermentation or heating, increases the bioavailability of nutrients and pigments, most likely by disrupting the plant tissues cell walls. The strains of Levilactobacillus brevis, Limosilactobacillus fermentum, and Lactiplantibacillus plantarum are most often used in the fermentation of vegetables [1–3]. Pickled foods, because of low pH ensuring the long shelf-life of products, can be preserved for many months. However, it depends mainly on having good storage conditions, such as a low temperature and the
lack of UV radiation [4–6]. Fermented products have unique taste properties. The taste of silage depends on both the type of raw material and the method and the parameters of the ensilage process [7]. The desired changes in taste, consistency, and color take place over time, ultimately creating products with a completely different taste impression from the initial ingredients. This is mainly due to microorganisms such as lactic acid bacteria, yeast, and filamentous fungi, which contribute to the unique taste of the silage and ensure the safety and required quality of the resulting product.

Many pickled foods have been shown to be a valuable source of protein, carbohydrates, minerals, vitamins, and fiber [8]. As a result of lactic acid bacteria presence, fermented foods are classified as foods that have health-promoting properties. However, to be permanently classified as this type of food, it must have a high (over $1 \times 10^7$ log CFU/g) number of lactic acid bacteria [9]. In addition, vegetables, due to their composition and the increasingly popular trend of a healthy life-style, have become a valuable processed raw material [8]. The combination of the health-promoting properties of vegetables and lactic acid bacteria in fermented products is an excellent replacement for dairy products containing LAB. Natural pigments include betalains, carotenoids, chlorophylls, and anthocyanins. The natural pigments found in vegetables and fruits are now progressively being used as a source for coloring food. They are increasingly replacing the use of artificial pigments, which nowadays are mentioned as “not-safe” and causing undesirable health effects [6]. The disadvantage of using natural pigments is their sensitivity to the influence of the environment through the action of UV rays, oxygen, temperature, pH or the presence of metal ions, enzymes, or sugars [4,5,10,11].

In Western Europe the most popular fermented vegetables are cucumbers, cabbage, beetroot, carrots, and red bell peppers. Of these, the highest amount of pigments are in beetroot, carrots and red bell peppers [12–14]. Moreover, beetroot is rich in carotenoids, a protein with unsaturated fatty acids [15]. The betalains isolated from the beetroot were vulgaxanthin I, vulgaxanthin II, indicaxanthin, betanin, neobetanin, prebetanin, and isobetanin [3,16]. Betalains are soluble in water N-acylated pigments divided into yellow-orange-colored betaxanthins and red-violet-colored betacyanins. They are stable in a low pH range (3–7), their stability increases with decreasing oxygen concentration, and they are resistant to fermentation temperatures [11,17]. However, the thermal stability of betacyanin and betaxanthins is still unclear, as mentioned in the beetroot pigments analysis made by Lombardelli et al. [4] Despite the process conditions, yellow or red beetroot pigments could be stable.

Red bell peppers are rich in carotenoids and are botanically included in fruits, whereas a carrot is a root vegetable. Carrots have been recognized as an excellent source of flavonoids, quercetin, vitamin C, and carotenoids [18]. Carotenoids are tetraterpenoid compounds with a conjugated double-bond system; they are mainly lipophilic pigments whose structure is based on the number of carbons linked in chains. Their cyclic structure can be modified by hydrogenation, dehydrogenation, cyclization, and oxidation [19].

However, fermented vegetables can be difficult to store, mainly due to their subsequent refrigerated storage and space requirements. Moreover, the lactic acid bacteria, after using the carbon source, is not able to multiply further, which is disadvantageous from the consumer’s perspective. Therefore, it was decided to use the lyophilization process in order to protect the active ingredients (pigments) and bacteria (LAB). The freeze-drying process can also be called low-thermal drying. After this process, dried substances are obtained. However, after the completion of the process of freeze-drying vegetable juices, water can be absorbed very quickly from the environment and the powder structure broken down. Freeze-drying can be a solution to the problem of protecting sensitive substances from juices containing high amounts of low molecular weight sugars. This is caused by the low glass transition temperatures of the juice itself. When the glass transition temperature is exceeded, the product becomes compact with a glassy sheen on the surface of the agglomerated powder [20,21]. This phenomenon can be prevented by adding a high
molecular weight carrier to the juice prior to the freeze-drying process. Maltodextrin is most often added [22,23].

Both presented raw materials are rich in natural pigments, but are unstable and seasonal; thus, it was decided to take steps to extend their durability. The activities undertaken were also aimed at creating a new potential product with coloring and probiotic potential. For this reason, the study aimed at the determination of the fermentation method influence on the content of active compounds (pigments) in pickled juices and freeze-dried powders.

2. Materials and Methods

2.1. Materials

Materials were purchased at the Bronisze Market (Warsaw, Poland) Beetroot (Beta vulgaris) and red bell pepper (Capsicum annuum L.) and cold-stored (4 to 6 °C) before use. As an inoculum for fermentation two bacterial strains: Levilactobacillus brevis KKP 804 (LB) and Limosilactobacillus fermentum KKP 811 (LF) were applied. The strains originated from the Collection of Industrial Microorganisms (KKP, Warsaw, Poland). Maltodextrin DE10 was supplied by Pepees S.A. (Łomża, Poland).

2.2. Technological Treatment

2.2.1. Fermentation Process

Two different kinds of fermentation were used. The first type was the fermentation of juices obtained from the beetroot and red bell pepper. The second type was the fermentation of sliced vegetables/fruits in 200 mL jars. In the first type of juice fermentation, NaCl was directly added to the juice with 2% of juice volume. In the second type of fermentation, NaCl was dissolved in water in a concentration of 2% and then was added to the jars with vegetable slices. Inoculum of 1% to the water/juice volume, which matched to the $1 \times 10^7$ CFU/mL of bacterial content, was added. For spontaneous fermentation (SF) no inoculum was added. For anaerobic conditions, jars were closed and kept in an incubator at a stable temperature of 28 °C. Fermentation process was carried out for 7 days after addition of the inoculum. All experiments were carried out in duplicate and in parallel.

2.2.2. Juice Pressing

The juice was obtained from raw and fermented vegetables. The process was carried out with an NS-621CES juicer model (Kuvings, Daegu, Korea). Separately, juice and pomace were collected. Juice was used in this research.

In the second method, juice was obtained from fermented vegetables. This juice was mixed in a proportion 1:1 with brine (S). In previous research, only pressed juices were tested separately from the post-fermentation solution [3,9] for comparison with the post-fermentation solution, and as a result a high amount of LAB was observed. This is the reason why we mixed it in this research.

Before freeze-drying, the addition of a carrier material was needed. In the presented research, 15% v/v maltodextrin with a low dextrose equivalent (DE = 10) was added to the juices. Fermented juices without a carrier addition were highly hygroscopic, and after being taken from the freeze-dryer their structure collapsed.

2.2.3. Freeze-Drying

The freeze-dried protocol was used in accordance to methodology presented by [24] with some changes. Obtained from both types of fermentation were frozen at −40 °C (Shock Freezer HCM 51.20, Irinox, Treviso, Italy) for 10 h on a petri dish. Freeze-drying was carried out in an ALPHA 1–4 freeze-dryer (Christ, Osterode, Germany) for 24 h at a heating shelf temperature of 30 °C and the constant pressure of 63 Pa; a safety pressure was set up at 103 Pa. The experiments were carried out in duplicate.
2.3. Analytical Method

2.3.1. Dry Matter

Gravimetric method was used to dry matter determination. For juice approximately 0.6–1 g was placed on filter paper in a dish, while 1 g was used for powders. Drying was made in vacuum-dryer (Memmert VO400, Schwabach, Germany) under the pressure of 10 mPa at 75 °C for 24 h until constant weight. Measurements were performed in triplicate.

2.3.2. Total Acidity

For the measurement of total acidity, the titration method with 0.1 M NaOH was used. A known mass of sample juice/powder (m) was taken and diluted with distilled water to a volume of 50 mL (V₁), then 25 mL of diluted juice (V₂) was taken for testing. Titration with the NaOH solution ended when the pH reached 8.1, and the amount of NaOH solution used (V_{NaOH}) was used for calculations. The measurement was conducted in triplicate for each sample.

\[
\text{Total acidity} = \frac{(V_{NaOH} \cdot 0.1 \cdot V_1 - 0.09 \cdot 100)}{(V_2 \cdot m)} \text{ (g lactic acid/100 g product)}
\]  
(1)

where 0.1 is the molarity of NaOH and 0.09 is the index for correction for lactic acid in the sample.

2.3.3. Color Parameters

The color of powders was analysed with the colorimeter CR-5 (Konica Minolta Sensing Inc., Osaka, Japan) in the CIE L*a*b* system. Parameters used: Illuminant D65, an angle of 2°, and calibration with white plate. All measurements were made in 3 repetitions. In addition, the ΔE* factor of the color differences between juices before (parameters L*a*b* with index raw) and after fermentation was calculated from formula [5,25]:

\[
\Delta E = \sqrt{(L* - L_{raw}*)^2 + (a* - a_{raw}*)^2 + (b* - b_{raw}*)^2}
\]

(2)

2.3.4. Thermal Properties

The weight loss upon heating was measured using a TGA/DSC 3+ thermogravimeter (Mettler-Toledo, Greifensee, Switzerland). Ground material in amounts of 5–8 mg were placed in 70 µL alumina crucibles and pyrolyzed from 30 to 600 °C with a heating rate of 5 °C/min under nitrogen atmosphere (50 mL/min). The maximum temperatures of the energy effects were determined from the DTG curves [26]. Two measurements of each sample were made.

A differential scanning calorimeter (DSC 3+ STAR, Mettler-Toledo, Greifensee, Switzerland) with liquid nitrogen cooling was used to determine the glass transition temperature (Tg). Prior to analysis, the samples were dried in a vacuum oven (30 °C, 10 mb, 48 h) and stored over anhydrous P₂O₅. An amount of 3–6 mg of the sample was weighed in 40 µL aluminum pans and subjected to a three-step analysis. In the first stage, the sample was cooled from room temperature to −50 °C, then kept at this temperature for 5 min and heated to 150 °C at the rate of 5 °C/min with a nitrogen flow of 50 mL/min. The obtained DSC curves were analyzed using dedicated STARe software v.16.0.

2.3.5. ATR-FTIR Spectroscopic Analysis

Infrared absorption spectra of the dried samples were recorded using a Cary 630 spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) with a diamond crystal ATR system. The analysis was carried out in the wavenumber range of 4000–650 cm⁻¹ for 32 scans with a resolution of 4 cm⁻¹ [27]. A background measurement was performed before each sample. Each sample was scanned in triplicate.
2.3.6. Determination of the Number of Lactic Acid Bacteria

For enumeration of viable cells a total count by plate method was made. Juices and freeze-dried juice samples were serially diluted using sterile saline (0.85% NaCl, Biomaxima, Lublin, Poland). On the de Man, Rogosa, and Sharpe agar plates samples were placed (MRS, Biomaxima, Lublin, Poland) and incubated at 28 ± 1 °C for 48 ± 4 h. The number of grown colonies was counted and recorded as log CFU per g d.m by ProtoCOL 3, (Synbiosis, Frederick, MD, USA). The samples were analyzed in triplicates.

2.3.7. Betalain Content

Betalain content was measured by two methods. For the calculation of the amount, the spectrophotometric method was used, and for the identification of betalain, the HPLC method was used.

(A) HPLC method

The HPLC method is based on research of Janiszewska-Turak et al. [3]. For freeze-dried samples, approximately 1 g was extracted with 20 mL of a mixture of 0.2% formic acid and acetonitrile (4:1, v/v). The samples were stirred for 5 min, centrifuged at 5000 × g for 5 min. The supernatants were filtered through 0.45 µm syringe PTFE filters (Macherey-Nagel, Duren, Germany) to chromatographic vials.

The analyses were conducted using an equipment from Waters (Milford, MA, USA): the 2695 Separations Module connected with 2995 PDA detector, and 2475 Multi-Wavelength Fluorescence Detector (Waters, Milford, MA, USA). The samples of 10 µL were injected on a Sunfire C8 column (5 µm, 4.6 × 250 mm, Waters) with a precolumn with identical chemistry, which were kept at the temperature of 30 °C and rinsed at 1.0 mL/min with a gradient of 0.2% formic acid and acetonitrile as described by Janiszewska-Turak et al. [3]. Betacyanins were quantified at a wavelength of 538 nm, while betaxanthins at 480 nm. Two independent analyses were performed for each sample.

The analytes were identified using their retention times, UV/VIS spectra, and previous results from our team [16,28–31].

(B) Spectrophotometric method

The spectrophotometric method was based on the methodology presented by Janiszewska-Turak et al. [32]. The measurement was conducted on the Evolution 220 (Evolution 220, Thermo Fisher Scientific Inc., Waltham, MA, USA). The 0.5 g of sample (powder or juice) was mixed with phosphate buffer (5000 mg). Betanin (mg betanin/100 g of dm) and vulgaxanthin I (mg vulgaxanthin I/100 g of dm) determination was based on the red and yellow pigments, respectively. The samples were analyzed in triplicates.

2.3.8. Carotenoids Analysis

The total carotenoids content (TCC) was measured according to a methodology [33,34] based on spectrophotometric measurements. The absorbance of the colored solutions was determined at 450 nm (Spectronic 200; Thermo Fisher Scientific Inc., Waltham, MA, USA). The analysis was conducted in triplicate.

2.4. Statistical Treatment

The results obtained were statistically analysed with use of Statistica 13 software (StatSoft, Warsaw, Poland). A one-way ANOVA (analysis of variance) with the identification of homogenous groups with Tukey’s HSD test at a significance level of α = 0.05 was made. Medium and standard deviation parameters, were determined using MS Excel 16.

3. Results and Discussion

The paper presents the results of this research on juices obtained due to lactic acid fermentation with the use of dedicated fermentation strains (Levilactobacillus brevis KKP 804 (LB) and Limosilactobacillus fermentum KKP 811 (LF)) and spontaneous fermentation. Two raw materials significantly different from each other were used for the research: red
beetroot (B), which is a root vegetable with a compact structure, and red bell pepper (RBP), which is botanically classified as a fruit and has a different structure of tissues than beetroot.

The fermented juices obtained in two ways were tested for the content of active compounds, which include betalains, carotenoids, and the presence of lactic acid bacteria. Moreover, the physiochemical properties of juices such as dry weight, viscosity, total acidity, and color were determined. The obtained fermented beetroot and red pepper juices were then subjected to the freeze-drying process in order to extend their shelf-life and create a new product with coloring and probiotic potential. In the obtained powders, apart from the determinations made for juices, the physical transformation temperatures (TGA, DSC) were tested in order to determine the storage conditions.

3.1. Juices

The process of lactic acid fermentation of the juices alone increased the viscosity of the juices obtained regardless of the type of raw material, with the only exception being beetroot juice with the use of Limosilactobacillus fermentum (Table 1). In the second method of fermentation, the juice was obtained from fermented raw materials and then mixed with a marinade into which water-soluble substances released in the fermentation product could pass during the fermentation process, with no statistically significant changes in the lightness value of the final juices. The lightness of non-fermented juices differed between the analyzed raw materials; higher values were observed for beetroot juice, which may be related to the composition of the vegetable itself and the presence of pigments dissolved in water, which could pass into the juice during the pressing process (Table 1). However, the analysis of the fermented juices did not show any difference in the lightness value, despite of the type of raw material and the fermentation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Viscosity (mPAs)</th>
<th>Dry Matter (g/g)</th>
<th>Total Acidity (g Lactic Acid/100 g Product)</th>
<th>Color Coefficients</th>
<th>ΔE</th>
<th>Number of Lactic Acid Bacteria (log CFU/g d.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B_raw</td>
<td>1.51 ± 0.09</td>
<td>0.054 ± 0.008</td>
<td>0.82 ± 0.13</td>
<td>6.33 ± 0.06</td>
<td>1.15 ± 0.08</td>
<td>2.60 ± 0.03</td>
</tr>
<tr>
<td>B_J_LF</td>
<td>1.15 ± 0.10</td>
<td>0.070 ± 0.006</td>
<td>0.73 ± 0.02</td>
<td>6.47 ± 0.21</td>
<td>0.35 ± 0.06</td>
<td>4.73 ± 0.40</td>
</tr>
<tr>
<td>B_J_LB</td>
<td>1.63 ± 0.27</td>
<td>0.072 ± 0.001</td>
<td>0.68 ± 0.04</td>
<td>5.68 ± 0.05</td>
<td>0.31 ± 0.13</td>
<td>9.23 ± 0.04</td>
</tr>
<tr>
<td>B_J_SF</td>
<td>1.62 ± 0.07</td>
<td>0.052 ± 0.002</td>
<td>0.35 ± 0.01</td>
<td>4.15 ± 0.06</td>
<td>1.20 ± 0.12</td>
<td>6.42 ± 0.10</td>
</tr>
<tr>
<td>B_PJ+S_LF</td>
<td>1.21 ± 0.11</td>
<td>0.033 ± 0.000</td>
<td>0.61 ± 0.01</td>
<td>6.84 ± 0.06</td>
<td>2.57 ± 0.08</td>
<td>10.42 ± 0.08</td>
</tr>
<tr>
<td>B_PJ+S_LB</td>
<td>1.22 ± 0.11</td>
<td>0.025 ± 0.001</td>
<td>0.70 ± 0.00</td>
<td>10.23 ± 0.05</td>
<td>1.72 ± 0.06</td>
<td>10.73 ± 0.06</td>
</tr>
<tr>
<td>B_PJ+S_SF</td>
<td>1.40 ± 0.13</td>
<td>0.032 ± 0.001</td>
<td>0.70 ± 0.01</td>
<td>9.68 ± 0.06</td>
<td>2.81 ± 0.02</td>
<td>8.38 ± 0.06</td>
</tr>
<tr>
<td>RBP_raw</td>
<td>1.25 ± 0.05</td>
<td>0.018 ± 0.003</td>
<td>0.73 ± 0.03</td>
<td>14.96 ± 0.03</td>
<td>14.16 ± 0.06</td>
<td>2.21 ± 0.09</td>
</tr>
<tr>
<td>RBP_J_LF</td>
<td>1.51 ± 0.14</td>
<td>0.047 ± 0.006</td>
<td>1.96 ± 0.08</td>
<td>24.39 ± 0.01</td>
<td>31.40 ± 0.09</td>
<td>21.09 ± 0.07</td>
</tr>
<tr>
<td>RBP_J_LB</td>
<td>1.31 ± 0.08</td>
<td>0.035 ± 0.000</td>
<td>1.60 ± 0.16</td>
<td>24.00 ± 0.01</td>
<td>28.28 ± 0.04</td>
<td>7.91 ± 0.04</td>
</tr>
<tr>
<td>RBP_J_SF</td>
<td>1.46 ± 0.01</td>
<td>0.038 ± 0.002</td>
<td>1.33 ± 0.10</td>
<td>21.96 ± 0.01</td>
<td>31.38 ± 0.12</td>
<td>7.06 ± 0.03</td>
</tr>
<tr>
<td>RBP_PJ+S_LF</td>
<td>1.27 ± 0.09</td>
<td>0.013 ± 0.002</td>
<td>0.94 ± 0.04</td>
<td>17.18 ± 0.03</td>
<td>10.74 ± 0.04</td>
<td>8.37 ± 0.09</td>
</tr>
<tr>
<td>RBP_PJ+S_LB</td>
<td>1.36 ± 0.06</td>
<td>0.015 ± 0.000</td>
<td>0.77 ± 0.04</td>
<td>16.97 ± 0.01</td>
<td>11.05 ± 0.15</td>
<td>7.71 ± 0.07</td>
</tr>
<tr>
<td>RBP_PJ+S_SF</td>
<td>1.33 ± 0.01</td>
<td>0.076 ± 0.001</td>
<td>0.63 ± 0.04</td>
<td>17.06 ± 0.00</td>
<td>10.46 ± 0.04</td>
<td>7.26 ± 0.09</td>
</tr>
</tbody>
</table>

B—beetroot; RBP—red bell pepper; LF—Limosilactobacillus fermentum KKP 811; LB—Levulisactobacillus brevis KKP 804; SF—spontaneous fermentation; J—juice pressed before fermentation; PJ+S—juice pressed from fermented vegetable and mixed with post-fermentation solution. a, b, c and specific letters—different indexes for the beetroot columns mean statistically significant differences for given values at the level of p < 0.05; A, B, C and specific letters—different indexes for red bell pepper columns (RBP) mean statistically significant differences for given values at the level of p < 0.05.

The viscosity of the juices depends on the content of the solute. This is confirmed by the dry matter obtained from the juices. The higher the dry matter content and, therefore, the higher the content of soluble and insoluble substances in the juice, the higher the observed viscosity value (Table 1).

Higher values of dry matter were observed for beet juice, regardless of the fermentation method used and the starter cultures used. For both raw materials, the second fermentation method resulted in the achievement of lower values of dry matter, which is strictly related to the addition of a 1:1 water marinade to the juice. The exception was fermented red bell
pepper with the addition of a water marinade, for which the highest dry matter values were obtained as compared to other pepper juices.

Similar viscosity was observed for beetroot juices (1.56 mPas) by Janiszewska [35]; however, higher values were observed for red bell pepper juices (1.99 mPas) by Rybak et al. [36]. Differences in viscosity and dry matter in presented juices are connected to the ingredients that are present in raw materials; in the beetroot water 87.6 g/100 g product, carbohydrates accounted for approximately 9.5–10 g/100 g of the product, protein 1.6–1.7 g/100 g of the product, and fat 0.17–0.18 g/100 g of the product [12,37], whereas for the red bell pepper water 92.2 g/100 g product, carbohydrates account for approximately 6.03 g/100 g of the product, protein 0.99 g/100 g of the product, and fat 0.3 g/100 g of the product [13]. In the research of Hallmann et al. [38], the dry matter for fermented beetroot was 0.0814–0.0747 g/g, which was a little higher than in the presented research for beetroot juices obtained through the first type of fermentation; however, it could be related to the used cultivar.

The total acidity for both juices tested was similar, as it was 0.82 for beetroot juice and 0.73 for red pepper juice. It was observed that for beet juice after the fermentation process, regardless of the method of fermentation, no changes in acidity values were visible, which is related to the low pH of the beetroot juice itself (pH about 5) before the fermentation process. Similar results were presented by Czyżowska et al. [39] for the lactic acid content in fermented beetroot juices (0.5 g/100 mL).

In the case of fermented red pepper juice, the total acidity was twice as high as for the juice before fermentation and for the juice obtained by squeezing and mixing with the marinade.

The content of lactic acid bacteria is a substantial parameter that determines the quality of fermented juices. The presence of LAB at the level of two log cycles was demonstrated in raw juices. The presence of 6.5–8.0 log CFU/g d.m. of LAB was found in fermented beetroot juices, whereas in juices squeezed from beetroot after fermentation and combined with marinade, the presence of LAB was demonstrated at a comparable level for the respective types of fermentation. Similar dependencies were shown in the case of pepper juices, but when it comes to spontaneous fermentation, the number of lactic acid bacteria after fermentation was higher than in the case of fermentation with the use of one type of bacteria. Both L. brevis and L. fermentum grew in red bell pepper and beetroot juices.

The color of juices differed depending on the raw material tested. The red beet juices were redder and darker compared to the red bell pepper juices, which is related to the content of specific pigments in those raw materials (Table 1).

It was observed that the color components decreased after the fermentation process of the red beet juice, which means that the juices after the fermentation process were darker (L* decrease), less red (a* decrease), and much greener (b* decrease). The color components of the red bell pepper juice after fermentation increased, which means that the juices after the fermentation process were lighter (L* increase), blacker (a* increase), and definitely more yellow (b* increase).

Changing the fermentation method into vegetable/fruit fermentation and then squeezing it and mixing it with water brine resulted in a statistically significant reduction in the value of redness (+a*) and yellowness/greenness (b*) for both juices, in addition to an increase in brightness (+L*).

The color difference factor (ΔE*) is a combination of all color coefficients related to the reference color coefficients. A comparison was made in the presented research with the raw juices, thus the differences after the fermentation process could be observed. It is stated that values below 5 mean there is no difference in color as seen with the human eye, values between 5 and 12 give information about minor variations of the color, and values above 12 mean that the color is totally different than the reference sample [5,25,40]. For both juice vegetables, the calculated factor of color differences (ΔE*) was higher than 5. For fermented beetroot juices, the color difference factor was in the range from 4.7 to 10.7, which means that slight differences were seen and the juices were similar to the raw beetroot juice, however, the difference can be detected by the human eye. For red bell
pepper, slight differences were observed for fermented juices (values from 6.6–7.3), whereas juices obtained from the fermented vegetable and mixed with marinade differed at first sight (values above 17) (Table 1).

Analysis of the content of color pigments in red beetroot and red bell pepper showed the influence of the fermentation method on the pigment content (Figures 1 and 2). In beetroot juice, an increase in betacyanin content was observed for juices obtained by pressing fermented vegetables and using of marinade after fermentation (approximately from 316 for raw juice to 720–950 mg/100 g dm) and in both pigments (betacyanin and betaxanthin) obtained by spontaneous fermentation of beet juice (680 for betacyanin, 530 mg/100 g dm for betaxanthin) (Figure 1). The increase in the content of betalain pigments in the second method of lactic fermentation may be related to the transition of betalain pigments from the inside of the fermented beetroot tissue to the brine. Betalain pigments are water-soluble compounds, and during the fermentation process they can migrate to the marinade [41,42].

NaCl in fermented vegetables can perform several functions: It is a preservative, i.e., it protects against the development of unfavorable microflora and increases the osmotic pressure that causes the extraction of vegetable juice. Increasing the osmotic pressure during the fermentation process on the pigment content (Figure 2). In fermented juices, a decrease in the carotenoid content was observed, regardless of the type of lactic acid bacteria used. Changing the fermentation method to the fermentation of the raw material caused only a slight decrease in the content of carotenoids compared to juice without fermentation. However, it is not possible to unequivocally establish a relationship between the way the lactic acid fermentation process is carried out and the amount of carotenoids. The highest values achieved in the spontaneous fermentation of the juice (about 130 mg/100 g dm.)

![Figure 1. Pigment content in beetroot juices, betacyanin and betaxanthin; A, B—different indexes for series mean statistically significant differences for given values at the level of p < 0.05.](image-url)
have not been confirmed in the second fermentation method, of which the carotenoid content was about 10 mg/100 g d.m.

![Figure 2. Carotenoids content in juice and its powders; A, B, C, and other letters—different indexes for series mean statistically significant differences for given values at the level of p < 0.05.](image)

On the other hand, the use of LF strains for whole pieces of red bell pepper resulted in obtaining the highest values of carotenoids (170 mg/100 g d.m.) in juices mixed with post-fermentation solution. It could be caused by tissue disturbance during the fermentation process, resulting in the release of larger amounts of carotenoids from the interior of pepper particles during pressing [44].

In their research, Hallmann et al. [38] observed cis-β-carotene, α-carotene, capsorubin, α- and β-cryptoxanthin, lutein, and zeaxanthin in fermented red bell peppers. They observed that the highest values were for cis-β-carotene and zeaxanthin (0.38 mg/100 g fw) [38].

### 3.2. Freeze-Dried Powders

To each juice before freeze-drying, 15% v/v of maltodextrin DE = 10 was added. Dried beetroot and red bell pepper juices without the addition of a carrier agent were very hygroscopic, and after a few minutes the FD powder’s structure collapsed and the glass transition process took place.

Obtained after freeze-drying the fermented juices, beetroot powders were characterized by a high dry matter content (84–98%); beetroot powders had a higher total acidity (TA), approximately 3 g lactic acid/100 g, higher lightness (L*) and redness (a*), and lower yellowness/greenness (b*) than in juices (Table 2). For red bell pepper powders, almost the same correlation was observed as for beetroot powders. The smallest differences were observed for the color coefficients a* and b* with respect to the juices (Tables 1 and 2).

The higher total acidity of red bell pepper compared to beetroot powders could be related to the concentration of salt in the marinade and lactic acid concentration in the powder.

Freeze-drying causes a decrease in microbial survival, but it is less than while spray-drying or convection-drying. The number of lactic acid bacteria in the powders obtained as a result of lyophilization is presented in Table 2. A decrease in approximately one to three cycles of the powders’ log number of LAB in relation to juices was observed. The influence of the lactic acid bacteria used and the fermentation method (fermented
juices or juices obtained as a result of squeezed fermented vegetables) on the LAB number was not observed.

Table 2. Selected physicochemical and microbiological properties of fermented freeze-dried powders.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dry Matter (g/g)</th>
<th>Total Acidity (g Lactic Acid/100 g Product)</th>
<th>Color Coefficients α*</th>
<th>Color Coefficients β*</th>
<th>Tg (°C)</th>
<th>Number of Lactic Acid Bacteria (log CFU/g d.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B_J_LF</td>
<td>0.840 ± 0.201 a</td>
<td>2.78 ± 0.21 a</td>
<td>37.92 ± 0.35 ed</td>
<td>25.51 ± 0.26 ab</td>
<td>-10.38 ± 0.17 b</td>
<td>61.51 ± 0.49 b</td>
</tr>
<tr>
<td>B_J_LB</td>
<td>0.947 ± 0.006 a</td>
<td>2.86 ± 0.09 a</td>
<td>36.09 ± 1.14 bc</td>
<td>26.81 ± 3.22 ab</td>
<td>-10.83 ± 1.17 b</td>
<td>58.68 ± 0.86 a</td>
</tr>
<tr>
<td>B_J_SF</td>
<td>0.982 ± 0.000 a</td>
<td>2.96 ± 0.39 a</td>
<td>35.31 ± 1.35 b</td>
<td>21.82 ± 0.70 a</td>
<td>-9.02 ± 0.66 b</td>
<td>59.60 ± 0.23 a</td>
</tr>
<tr>
<td>B_PJ+S_LF</td>
<td>0.940 ± 0.003 a</td>
<td>2.72 ± 0.00 a</td>
<td>36.05 ± 0.01 bc</td>
<td>36.53 ± 0.01 a</td>
<td>-13.31 ± 0.02 a</td>
<td>66.00 ± 0.49 a</td>
</tr>
<tr>
<td>B_PJ+S_LB</td>
<td>0.931 ± 0.038 a</td>
<td>2.57 ± 0.06 a</td>
<td>32.85 ± 0.00 a</td>
<td>33.03 ± 0.01 ab</td>
<td>-0.29 ± 0.01 c</td>
<td>62.55 ± 0.49 b</td>
</tr>
<tr>
<td>B_PJ+S_SF</td>
<td>0.960 ± 0.000 a</td>
<td>2.57 ± 0.06 a</td>
<td>39.37 ± 0.01 d</td>
<td>29.60 ± 0.04 ad</td>
<td>-10.70 ± 0.02 b</td>
<td>64.12 ± 0.33 a</td>
</tr>
</tbody>
</table>

B—beetroot; RBP—red bell pepper; LF—Limosilactobacillus fermentum KKP 811; LB—Lactobacillus brevis KKP 804; SF—spontaneous fermentation; PJ—juice pressed before fermentation; PJ+S—juice pressed from fermented vegetable and mixed with post-fermentation solution; α, β, and specific letters—different indexes for the beetroot columns mean statistically significant differences for given values at the level of p < 0.05, A, B, C and specific letters—different indexes for red bell pepper columns (RBP) mean statistically significant differences for given values at the level of p < 0.05.

Storage conditions for powders from fermented juices obtained by freeze-drying can be predicted on the basis of thermal analysis methods. The differential scanning calorimetry (DSC) method determines the glass transition temperature (Tg) below which materials should be stored. In the powders obtained, the temperatures ranged from 59 to 66 °C for beetroot and 46 to 61 for red bell pepper (Table 2). The obtained values allow that the obtained powders can be stored at room temperature, as the literature suggests values lower than the Tg by about 20 °C are safe [45–47]. Similar values for dried beetroot powders with maltodextrin were obtained by Flores-Mancha et al. [48] and reached 61 °C. Those values for powders can be related to the addition of maltodextrin to juices before freeze-drying. The glass transition is related to the molecular weight of the compound and water content in the sample. It was measured that for maltodextrin, the Tg is above 140 °C, for sucrose 60 °C, and for glucose 31 °C [49,50]. The mixture of 15% MD with vegetable juices that contained approximately 4–7 g/100 g of sugars could result in a decrease in the Tg to the level mentioned in Table 2.

In addition to the DSC method, thermal analysis predicts changes during storage. The TGA method can show phase transitions or chemical reactions in powders, which can occur when temperature increases. Thermogravimetry shows “changes in the mass of a substance during heating or cooling as a function of time and temperature” [51]. In the TGA analysis, specific regions can be selected by using steps in measurement. In the analysis provided in this research, three steps were used: In the first step, the temperature ranged from 30 to 140, the second step was 140–420, and the third step was 420–600 °C. The first step is associated with the loss of moisture, whereas the second step, above 120 °C, shows decomposition processes of, e.g., proteins and carbohydrates [52–54]. In analysis samples the highest mass loss (41–75%) was determined in the second region (step 2) (Table 3), which can be related to the carbohydrates from vegetables (6–9 g/100 g product) [12,13] as well as maltodextrin (a polysaccharide with glucose links), which is present in all samples. The temperature of maltodextrin decomposition is approximately 130–160 °C and is connected to the water content in the powder sample [55]. The highest water evaporation in the first step is related to the highest water content in that sample (Table 3). No specific differences between fermentation type, bacteria type, and material used were observed.
FTIR analysis showed that similar results were observed for both types of powders (beetroot and red bell pepper) (Figure 3). No differences in region size were observed, independently of the type of fermentation or starter cultures used. The most visible regions were: the region of O-H hydrogen bonds seen at wavenumbers of 3250–3050 cm⁻¹, region of C=O bonds at 1500–2000 cm⁻¹, region of C=O stretch vibrations where alcohols absorbs, e.g., from xanthophyll 1144 C-O; C-C; and C-O-C, and the region of the presence of C-O phenols at 1050 cm⁻¹ and at 800–600 cm⁻¹. In the FTIR figures, fingerprint regions (600–1500 cm⁻¹) of functional groups can be detected. For beetroot powders at the 1314, 1350-methylene C-H bend, between 1450 and 1650 cm⁻¹, the presence of nitrogen was seen. Similar results and conclusions have been observed for beetroot juices and beetroot samples [56–58]. The region linked to β-carotene is at wavenumbers from 1550 to 1600 cm⁻¹, which is related to stretching vibrations of the C=C bonds and the region seen at 960 cm⁻¹ (Figure 3b). Comparable results were observed by Quijano-Ortega et al. [59] for dried pumpkin species and also for dried red pepper by Castañeda-Pérez et al. [60].

In the presented research two methods were used for the identification of betalain and its content. First, a spectrophotometric method was used in juices and in freeze-dried powders to test the total betaxanthin and betacyanin content in the samples. A second method, HPLC analysis, was used to obtain the betalain profile in freeze-dried samples, independently of the type of fermentation or starter cultures used. The most visible regions were: the region of O-H hydrogen bonds seen at wavenumbers of 3250–3050 cm⁻¹, region of C=O bonds at 1500–2000 cm⁻¹, region of C=O stretch vibrations where alcohols absorbs, e.g., from xanthophyll 1144 C-O; C-C; and C-O-C, and the region of the presence of C-O phenols at 1050 cm⁻¹ and at 800–600 cm⁻¹. In the FTIR figures, fingerprint regions (600–1500 cm⁻¹) of functional groups can be detected. For beetroot powders at the 1314, 1350-methylene C-H bend, between 1450 and 1650 cm⁻¹, the presence of nitrogen was seen. Similar results and conclusions have been observed for beetroot juices and beetroot samples [56–58]. The region linked to β-carotene is at wavenumbers from 1550 to 1600 cm⁻¹, which is related to stretching vibrations of the C=C bonds and the region seen at 960 cm⁻¹ (Figure 3b). Comparable results were observed by Quijano-Ortega et al. [59] for dried pumpkin species and also for dried red pepper by Castañeda-Pérez et al. [60].

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Table 5. Pigment content in beetroot samples (comparison of two methods).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Betalain Results from HPLC Method</th>
<th>Betalain Results from the Spectrophotometric Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Betacyanin (mg/100 g dm)</td>
<td>Betaxanthin (mg/100 g dm)</td>
</tr>
<tr>
<td>B_J_LF</td>
<td>72.67 ± 1.94 bc</td>
<td>1.22 ± 0.08 b</td>
</tr>
<tr>
<td>B_J_LB</td>
<td>88.38 ± 1.28 d</td>
<td>1.70 ± 0.08 c</td>
</tr>
<tr>
<td>B_J_SF</td>
<td>63.37 ± 2.02 ab</td>
<td>2.19 ± 0.00 d</td>
</tr>
<tr>
<td>B_PJ+S_LF</td>
<td>65.62 ± 2.54 bc</td>
<td>0.15 ± 0.00 a</td>
</tr>
<tr>
<td>B_PJ+S_LB</td>
<td>79.96 ± 5.14 cd</td>
<td>0.09 ± 0.00 a</td>
</tr>
<tr>
<td>B_PJ+S_SF</td>
<td>49.99 ± 0.37 a</td>
<td>1.71 ± 0.11 c</td>
</tr>
</tbody>
</table>

B—beetroot; LF—Limosilactobacillus fermentum KKP 811; LB—Levilactobacillus brevis KKP 804; SF—spontaneous fermentation; J—juice pressed before fermentation; PJ+S—juice pressed from fermented vegetable and mixed with post-fermentation solution; a, b, c, d—different indexes for the columns mean statistically significant differences for given values at the level of p < 0.05.

Figure 3. FTIR results (a) for beetroot, (b) for red bell pepper. B—beetroot; RBP—red bell pepper; LF—Limosilactobacillus fermentum KKP 811; LB—Levilactobacillus brevis KKP 804; SF—spontaneous fermentation; J—juice pressed before fermentation; PJ+S—juice pressed from fermented vegetable and mixed with post-fermentation solution.
As mentioned by other authors [16,39], the betalains identified in beetroot pigments were: vulgaxanthin I, vulgaxanthin II, indicaxanthin, betanin, neobetanin, prebetanin, and isobetanin. In the presented research, identification showed that in fermented freeze-dried beetroot powders only vulgaxanthin I was observed from the betaxanthin group. Of the betacyanins in all samples, betanin and isobetanin were seen in higher amounts than betanidin, neobetanin, and other betacyanins which were not identified in this research. This lower amount of detected betaxanthins could be connected to the lower pH of the juice, as these compounds are more sensitive to pH changes than betacyanins [41,61,62]. In fermented beetroot juices, other authors have identified similar betacyanin and betaxanthin compounds [3,63]. High degradation of both betacyanins and betaxanthins from fresh beetroot juice during fermentation could be resulted by the low pH of the process, as well as to the active peroxidase enzyme present in beetroot cell walls. After slicing the beetroot tissue this enzyme comes in contact with pigments that can fasten its degradation, as was acknowledged by Czyżowska et al. [64]. In addition, the fermentation process temperature used in this study was 26 °C; when betacyanins are more stable than betaxanthins [63,64].

The content of individual compounds given by other authors and their identification in beetroot show that after the extraction process degradation takes place, and the fermentation and freeze-drying process itself may contribute to the reduction of dye content as a result of the influence of the environmental pH and the drying process, which can influence the degradation of the beads [41,42,63,64].

Analysis of the betalain content in the powders obtained as a result of freeze-drying showed a higher betacyanin content than betaxanthins, regardless of the fermentation method and the type of bacterial strain used (Table 5). The different values for the determination of betaxanthins in the HPLC method and the spectrophotometric method may have resulted from the measurement methods themselves. In the spectrophotometric method, waves of 476, 538, and 600 are used, whereas at wave 450 nm absorption of carotenoids has its maximum peak. That is the reason why the possibility of absorption at wave 476 of carotenoids is present in beetroots. The retention time helps the HPLC method in the separation of those compounds [65,66].

4. Conclusions

Both raw materials, beetroot and red bell pepper, are rich in natural pigments but are unstable and seasonal; thus, it was decided to check the influence of two processes, which can prolong storage time, on the stability of the pigments in beetroot and red bell pepper. The analysis of two methods of fermentation and bacterial strains did not show differences in bacterial number; however, there was an increase in pigment content in fermentation of raw material as compared to juice. Thermal analysis of freeze-dried powders showed that they can be stored under normal temperature conditions (25 °C).

The results showed that the conducted process of lactic acid fermentation carried out should be verified, so that the amounts of lactic acid bacteria, both after the fermentation process and after the process of freeze-drying, constitute the basis for stating that this is a product with potential probiotic properties. Further analysis of the process may include standardization or enrichment of the juice obtained with ingredients that can serve as a carbon source necessary for bacterial growth.

The content of colored compounds in the obtained juices was at a high level; however, the use of juices as a coloring substance is very difficult. On the other hand, the obtained powders, which had high stability, did not contain the appropriate amount of dyes that would allow them to be used in food industry products such as yoghurts or loose products.

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