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The Effect of Yogurt and Kefir Starter Cultures on Bioactivity of Fermented Industrial By-Product from *Cannabis sativa* Production—Hemp Press Cake

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Abstract: *Cannabis sativa* (hemp) is a plant considered to be abundant in bioactive compounds. The increasing production of hemp oil is leaving considerable amounts of hemp press cakes (HPC), which have not been sufficiently managed so far. One of the directions of development of plant-based food is the use of by-products of the agri-food industry in accordance with the idea of zero waste and the circular economy, so the purpose of this study was to determine the possibility of HPC fermentation using yogurt and kefir cultures and to determine the effect of the type of starter on the properties of the products. In the present study, starter cultures of yogurt (YO 122) and kefir (commercial grains) were used for HPC fermentation. Changes in lactic acid bacteria (LAB) and yeast population, pH, acidity, the content of bioactive compounds by spectrophotometric methods (proteins, amino acids, polyphenols, flavonoids, reducing sugars) and antioxidant activity (DDPH, ABTS, FRAP and reducing power) were determined. The results showed that it was possible to develop high-value beverages based on HPC with high fermentation efficiency: survivability of LAB and yeast (>10^6 CFU/g) and acidification (pH in a range of 4.82–6.36 and 5.34–6.49 for yogurt and kefir culture, respectively). Moreover, the stability of hemp protein, with its variable free amino acid composition, antioxidant potential and presented changes in polyphenolic content, was observed during storage. The presented results show a new way to manage HPC as an oil industry residue by using it as a raw material for the development of a bioactive food product and illustrate the relationship between applied starter culture, the direction of fermentation and changes in the content of bioactive compounds.

Keywords: *Cannabis sativa*; dairy alternatives; by-products; bioactivity; fermentation; functional food; hemp; kefir; yogurt

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1. Introduction

The demand for organic and plant-based foods and the emphasis on the circular economy model are putting pressure on the development of new products based on various by-products of plant residues, such as bran, husks, bagasse, and press cakes, which are rich in protein, carbohydrates, fiber and minerals. Several of the listed by-products are classified as edible, but they are only used as animal feed or disposed of. From this point of view, the lack of proper management of these by-products can cause food waste and generate an environmental problem whose solution lies in the social sphere. The Food and Agriculture Organization (FAO) predicts that by 2050, the human population will increase by 30%, with a strong increase in demand for food [1,2]. Consumers have become increasingly interested in the way their diet address health deficits and wellbeing [3]. Nowadays, consumers are often interested in a well-balanced diet enriched with valuable cold-pressed oils, such as flaxseed oil, pumpkin oil, and hemp oil. This trend has led to the acquisition of a large number of pressed oilcakes from various plants, which in many cases, despite their high nutritional value, have not yet found a suitable application. However, pressed oilcakes are gaining attention as valuable raw materials for developing innovative food products and food additives [4–11].

Cannabis sativa is commonly known as hemp. It is an herbaceous plant belonging to the *Cannabaceae* family. Hemp seeds are valued primarily for their nutritional properties, as well as their health benefits—they are a valuable source of polyunsaturated fatty acids (including α-linolenic acid and linoleic acid). In addition to the valuable composition of fatty acids, hemp seeds are also abundant in essential amino acids, as well as insoluble fiber (10-15%), vitamin E, and minerals (phosphorus, potassium, sodium, magnesium, sulfur, calcium, iron, zinc) [12,13]. Numerous health benefits and potential therapies are reported for hemp seeds [3]. Moreover, hemp seeds are also an abundant source of phenolic compounds, tocopherols, and phytosterols. It should also be marked that the natural antioxidants from hemp might play a role in reducing the risk of chronic diseases [3]. In addition to the primary use of hemp seeds as a source of oil, they have been used in ground form as a source of plant protein and dietary fiber, making them easier to incorporate into food products such as energy bars, flavoured yogurts, and baked goods [3].

Hemp press cakes (HPC) are a nutrient-rich material derived from industrial hemp that contains bioactive compounds and is safe. According to Kasula et al. [2], hemp's psychoactive compounds do not pass into the press cake. Halle and Schöne [14] also found that the oilcakes are free of THC. Therefore, hemp press cake can be considered THC-free and can be used as a food ingredient or matrix for new products. Fermentation is an ancient biotechnology method for developing new products using bacteria (including probiotic strains) and yeast [15]. A well-balanced diet should include an adequate intake of fermented foods to balance the gut microflora [16]

Kefir and yogurt are the most popular fermented milk products. These two beverages are characterized by their high lactic acid bacteria (LAB) content [7]. The starter culture of kefir, referred to as "kefir grains," consists of symbiotic lactose-fermenting yeast and lactose-nonfermenting yeast, as well as LAB), embedded in the kefiran exopolysaccharide matrix [17]. However, consumer demand for alternatives to cow's milk has increased due to the rise in diagnoses of lactose intolerance, allergies, and cholesterol problems. Labeled dairy-free products are also being stimulated by new trends such as vegan, vegetarian and flexitarian diets. Similarly, ecological aspects play an important role in the formulation of new plant-based dairy alternatives. Whole seeds, such as soy or almond, are more expensive than by-products. Previous studies have shown the benefits of the fermentation of pressed cakes in the formulation of plant-based dairy alternatives [4,5,7].

Despite the interest in hemp products and new dairy alternatives, there are limited reports on hemp press cake fermentation for the development of new dairy alternatives. The aim of this study was to compare HPC fermentation using starter cultures of yogurt and kefir and to evaluate the microbiological, physicochemical and bioactivity properties of obtained products during refrigerated storage for 28 days.

2. Materials and Methods

2.1. Materials and Chemicals

Hemp press cakes (HPC) were kindly donated by Olejarnia Niwki (Olejarnia Niwki, Niwki, Poland). Commercial kefir grains (Yogurt-Tek, Lactoferm Kefir series, Kefir-31, consisting of *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *Lactis* biotype diacetylactis, *Leuconostoc mesenteroides* subsp. *cremoris*, *Lactobacillus delbrueckii* subsp. *Bulgaricus*, and *Saccharomyces cerevisiae*) and commercial yogurt starter culture YO 122 consisting of two strains, *Streptococcus salivarius* subsp. *Thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus*, were obtained from Biochem Srl (Biochem Srl, Rome, Italy). Sodium hydroxide, methanol, Folin–Ciocalteu reagent, sodium carbonate, sodium chloride, gallic acid, sodium nitrite, aluminum chloride, quercetin, 3,5-dinitrosalicylic acid, sodium tartrate tetrahydrate, ninhydrin, glacial acetic acid, cadmium chloride, and glycine were procured from Merck (Merck, Darmstadt, Germany). Glucose, ammonia, boric acid, Tashiro indicator and hydrochloric acid were delivered by Chempur (Chempur, Piekary Śląskie, Poland). All reagents used in the presented study were of analytical purity. MRS agar (de Man, Rogosa, and Sharpe) and Sabouraud agar with chloramphenicol were procured from Merck (Merck, Darmstadt, Germany). Ferric chloride hexahydrate 2,2-di-phenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,4,6-tripyridyl-s-triazine (TPTZ) were purchased from Merck (Merck, Darmstadt, Germany). Acetonitrile, acetic acid for HPLC, standards for HPLC of hydroquinone, rutin, gallic acid, coumaric acid and epicatechin, ammonium acetate for HPLC, triethylamine [TEA] and methanol for HPLC were purchased from Sigma Aldrich (Sigma Aldrich, Saint Louis, Missouri, USA). Merck (Merck, Darmstadt, Germany) also supplied the following chemicals used for SDS-Page: urea, thiourea, tris HCL, sodium dodecyl sulfate (SDS), acrylamide, glycerol, Tris, ammonium persulfate, N,N,N′,N′-Tetramethylethylenediamine (TEMED) and 2-mercaptoethanol. Dithiothreitol (DTT) and bromophenol blue were bought from Thermo Fisher (Thermo Fisher Scientific, Waltham, MA, USA), and a 2-D Quant Kit was received from GE Healthcare Bio- Sciences (GE Healthcare, Kraków, Poland). Coomassie Brilliant Blue R 250 and norleucine were purchased from Sigma Aldrich (Sigma Aldrich, Saint Louis, Missouri, USA). Kjehdahl tablets (Kjeltabs 3.5), a catalyst, were purchased from Foss (Foss Analytical, Warszawa, Poland).

2.2. The Preparation of Hemp-Based Yogurt-like and Kefir-like Beverages

The preparation of the samples consisted of several steps. Firstly, HPC was milled and mixed with 90 °C distilled water (70:30) *w*/*v*. Then, the mix was boiled for 20 min, and after this process, it was cooled down and homogenized with a domestic mixer. Then, the mixture was pasteurized (60 °C, 30 min). The cooled-down sterile material was divided into two bakers. To obtain a yogurt-like product, the sample was inoculated with 0.5 g of yogurt starter culture YO 122 (the starting LAB level was $1.31 \times 10^7 \pm 0.19$ CFU/g). In order to obtain a kefir-like product, the sample was inoculated with 10% (*w*/*w*) of commercial kefir grains (containing $2.14 \times 10^7 \pm 0.36$ CFU/g of lactic acid bacteria (LAB) and $1.08 \times 10^7 \pm 0.41$ CFU/g of yeast). The freshly inoculated samples were used for the first initial analyses. The rest of the kefir and yogurt-like beverages were poured into sterile, low-density polyethylene cups (50 mL capacity), tightly sealed, and incubated for 24 h at 28 ± 1 °C for kefir culture and 24 h at 42 ± 1 °C for yogurt culture. After incubation, the samples were cooled and stored at 5 ± 1 °C in the refrigerator for 28 days.

2.3. Determination of Total Solid Content, Titratable Acidity, and pH

The total solid content was determined according to the AOAC standard method (No. 968.11) [18]. The pH value of unfermented and fermented kefir and yogurt samples was measured at 25 °C using a pH meter (CP-411, Elmetron, Zabrze, Poland). The determination of TA in the samples (expressed as g of lactic acid per 100 mL of sample) was performed according to Bernat et al. [19], which consisted of mixing 5 g of sample with

20 mL of distilled water and titrating with 0.01 M NaOH solution, using phenolphthalein (0.1%, *w*/*v* in 95% ethanol) as an indicator.

2.4. Microbiological Analyses during Storage Time

On each research day, the samples (10 g) were collected and diluted with 90 mL of sterile physiological saline (0.85% NaCl). After the serial dilution preparation, the lactic acid bacteria counts were determined on MRS (de Man, Rogosa and Sharpe) medium (Merck, Darmstad, Germany) after incubation at 37 °C under anaerobic conditions for 72 h, whereas in the case of kefir-like beverages samples, the yeast counts were determined on Sabouraud agar with chloramphenicol (0.005%) at 25 °C for 72 h. The viable cell counts were expressed as CFU/g of product.

2.5. Preparation of Methanolic Extracts

The samples of kefir and yogurt were freeze-dried for 24 h (chamber pressure 0.190 mbar, shelf temperature T_{min} = −35 °C, T_{max} = 20 °C, condenser temperature −85 °C) in Beta 2–8 LSC plus lyophilizer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). The 8 g of lyophilized sample was dissolved in the methanol/water solution at a ratio of 7:3 *v*/*v* and extracted in an ultrasonic bath (Elmasonic S30H, Elma Schmidbauer GmbH, Singen, Germany) for 10 min, then centrifuged at 14,000 rpm/min for 10 min at 20 °C (Centrifuge 5418 Eppendorf, Warsaw, Poland). The samples were filtered through 0.22-µm nylon membrane filters (Sigma-Aldrich, Darmstadt, Germany), and the obtained clear fluids were prepared for further analyses.

2.6. Determination of the Reducing Sugar, Total Phenolic and Total Flavonoid Contents

The reducing sugar content (RSC) was determined by the DNS (3,5-dinitrosalicylic acid) method according to Łopusiewicz et al. [5]. One milliliter of each supernatant was mixed with 1 mL of 0.05 m acetate buffer (pH 4.8), and 3 mL of DNS reagent was added, then vigorously shaken. The mixtures were incubated in boiled water for 5 min and then cooled at room temperature. The samples were dispensed into a 96-well microplate, and the absorbance values were measured at 540 nm with the microplate reader (Synergy LX, BioTek, Winooski, VT, USA). Glucose in acetate buffer was used for a calibration curve.

The total phenolic and flavonoid contents (TPC and TFC) were also determined with spectrophotometric methods as described by Tong et al. [20] with slight adaption to absorbance measurements with a microplate reader. The TPC was performed with Folin–Ciocalteu's reagent. The absorbance of the mixture was measured at 765 nm, and the concentration of TPC was calculated as mg of gallic acid equivalents (GAE) per g of sample (mg GAE/g). The TFC was determined as described elsewhere, and then the absorbance was measured at 510 nm. The quercetin was used as a standard, and the results were expressed as mg of quercetin equivalents (QE) per g of the sample (mg QE/g).

2.7. HPLC Analyses

The HPLC analyses were conducted with an Agilent 1260 Infinity II with a PDA detector. Separation was carried out on a 250 × 4.6 mm Nucleosil 120-5 C18 reverse phase column at ambient temperature. Elution was performed at a flow rate of 0.5 mL/min, using as mobile phase a mixture of acetonitrile (A) and water with 5% acetic acid (95:5 *v*/*v*) (B). The samples were eluted by the following gradient: 15% A and 85% B for 0–12 min, 0% A and 100% B for 30 min, and 85% A and 15% B in 50 min. Samples were filtered through a 0.45 µm membrane filter before injection. The injection volume was 20 µL. Detection was performed at 270 nm, 280 nm, 290 nm, and 257 nm, and the identification of compounds was achieved by comparing their retention time values with the following standards: hydroquinone, rutin, gallic acid, coumaric acid and epicatechin. The results were expressed in µg/mL of extract.

2.8. Determination of Antioxidant Potential

DPPH and ABTS radical scavenging activities were determined as described elsewhere [17]. In a nutshell, DPPH radical scavenging activity was assayed by stirring 1 mL of supernatant with 1 mL of 0.01 mM methanolic DPPH solution and incubating for 30 min. The absorbance was then measured at 517 nm. A total of 3 mL of $ABTS^+$ was mixed with 50 µL of the supernatants, and the absorbance at 734 nm was subsequently measured after 6 min of incubation.

The FRAP method was performed according to Guo et al. [21]. In order to perform the FRAP evaluation, 25 mL acetate buffer (300 mM), 2.5 mL 2,4,6-tripyridyl-s-triazine (TPTZ) solution (10 mM in 40 mM HCl), and 2.5 mL ferric chloride hexahydrate aqueous solution (20 mM) were mixed (Merck, Darmstadt, Germany). The 10 µL of the supernatants were added to 300 µL FRAP reagent in a microcentrifuge tube and vortexed for 10 s. Then, 100 µL of each mixture was transferred into a microwell plate. Absorbance was measured at 593 nm. Results were expressed as ascorbic acid equivalent (mg AAE/g) using ascorbic acid as standard.

For the measurement of reducing power, the supernatants (500 µL) were transferred to a test tube, to which 1.25 mL of phosphate buffer solution (0.2 M, pH 6.6) and 1.25 mL of 1% potassium ferricyanide solution were added. After the incubation at 50 °C for 20 min, 1.25 mL of trichloroacetic acid solution was added to the tube. Then 1.25 mL of the supernatant obtained by centrifugation at 3000 rpm for 10 min was diluted with 1.25 mL of the trichloroacetic acid solution. Finally, 0.25 mL of 0.1% ferric chloride solution was added to complete the determination. Absorbance was determined at 700 nm.

2.9. Determination of Protein Content, Free Amino Acid Content and Protein Profile by SDS Electrophoresis

The protein content was determined using the Kjeldahl method according to the methodology of AOAC (no. 978.02) [18]. Weights of homogeneous samples (2 g) were poured into 15 mL of concentrated sulfuric acid and combusted in the presence of Kjeltabs 3.5 catalyst (Foss Analytical, Warszawa, Poland) at 420 °C for 60 min. After cooling, the samples were diluted by adding 75 mL of distilled water and alkalized by adding 50 mL of 40% sodium hydroxide, and the ammonia was distilled into a receiver containing 50 mL of 2% boric acid. The distillate was titrated in the presence of the Tashiro indicator with 0.1 N hydrochloric acid. Combustion of the samples was carried out in a mineralization block (Foss Tecator AB, Höganäs, Sweden), and distillation was carried out on a Kjeltec System Distillation Unit 2100 apparatus (Foss Tecator AB, Höganäs, Sweden). The amount of nitrogen determined was converted to protein content, taking into account the conversion factor of 6.25.

The free amino acid profile (FAA) of the samples was determined by an HPLC gradient system with precolumn phenylisothiocyanate (PITC) derivatization. The FAAs from defatted samples were extracted for 30 min in an ultrasonic bath in the ratio of 1:10 (g/v) with a 70% (v/v) aqueous solution of ethanol. After extraction, the samples were centrifuged at 10 700 g for 20 min. For the gradient, two buffers were used: buffer A (0.1 M ammonium acetate, pH 5.14) and buffer B (0.1 M ammonium acetate containing acetonitrile 40:60, v/v). The sample was prepared as follows: 1 mL of FAA extract was dried under vacuum, then 160 µL of coupling reagent was added (methanol, water, triethylamine [TEA] [2:2:1, $v/v/v$]), mixed well and dried under vacuum. Subsequently, 1600 µL of PITC reagent (methanol/PITC/TEA/water (7:1:1:1; $v/v/v/v$)) was added and kept at room temperature for 30 min for reaction. Then the PITC was removed under vacuum, and the derivatized sample was redissolved in 1.5 mL of a mixture of buffer A and methanol 50:50 v/v. The samples were filtered through a syringe filter (0.45 µm), and 10 µm was injected into the HPLC system. The temperature of the column was kept at 39 °C. The measurements were taken at the absorbance of 254 nm. The identification was based on retention times of analytical standards. Norleucine was used as an internal standard. The analysis was performed using an LC Agilent Technologies 1200 Rapid Resolution (Santa Clara, CA,

USA) system equipped with a UV-Vis detector DAD 1260 (Santa Clara, CA, USA) and a reverse-phase column Zorbax Eclipse Plus C18 (4.6 × 150 mm, 5 µm) (Santa Clara, CA, USA). All HPLC analyses were done in duplicates. The results of the free amino acid content HPLC analysis are listed in Supplementary Materials as Table S1.

The test material for SDS page analyses was lyophilized. Before heating (3 min at 98 °C) the sample of 3 mg was mixed with a 97 µL buffer (pH 6.8; 8 M urea, 2 M thiourea, 0.05 M Tris–HCl, 0.075 M DTT, 3% (*w*/*v*) SDS, 0.05% (*w*/*v*) bromophenol blue). The protein concentration was determined using a 2-D Quant Kit (GE Healthcare Bio-Sciences). Eighteen µg of protein from each sample were separated into electrophoresis. The separation of proteins in 15% polyacrylamide gels (SDS-PAGE) was performed. The resolving gel contained 30% (*w*/*v*) acrylamide; 75% (*v*/*v*) glycerol; 3 M Tris, pH 8.8; 10% (*w*/*v*) SDS; 1% (*w*/*v*) ammonium persulfate; and TEMED 16 µL. The stacking gel (10% (*w*/*v*) acrylamide, 5% (*v*/*v*) glycerol, 0.125 M Tris (pH 6.8), distilled water, 10% (*w*/*v*) SDS, 1% (*w*/*v*) ammonium persulfate, staining buffer, TEMED 25 µL) was poured on the layer of resolving gel. The separation was performed with buffer (0.25 M Tris base, 1.92 M glycine, 0.5% SDS (*w*/*v*), 10 mM 2-mercaptoethanol). As a standard (ST) for protein molecular weight calibration, a PageRuler Plus Protein Ladder 10 to 250 kDa (Thermo Scientific) was used. The gels (80 × 100 mm, 0.75 mm spacers) were prepared in triplicate. The separation was conducted using the SE 250 type apparatus (Hoefer Scientific Instruments Company, Holliston, MA, USA). Electrophoresis was run with a constant current of 20 mA per gel. Proteins were visualized by staining with 0.05% (*w*/*v*) Coomassie Brilliant Blue R-250; 50% (*v*/*v*) methanol; 10% (*v*/*v*) acetic acid for 30 min and then destained (10% (*v*/*v*) methanol, 4.5% (*v*/*v*) acetic acid) overnight [22].

All the images of the polyacrylamide gels were acquired using an Image Master VDS (Pharmacia Biotech, Vienna, Austria) imaging system and analyzed using the Image Master 1D Elite v. 4.0 program. Computations were based on the assumption that the area of a single protein band accounts for a percentage ratio in relation to the area of all separated protein bands, which constitutes 100%. The images are presented in Supplementary Materials as Figure S1.

2.10. Statistical Analysis

All data were expressed as mean ± standard deviation (SD). Results were statistically analyzed using Statistica software (StatSoft Poland, Krakow, Poland), using the Tukey test and two-factor analysis of variance with repetition (ANOVA). All experiments were performed in triplicate. All tests were performed at a significance level of $p \leq 0.05$.

3. Results and Discussion

3.1. The Changes in TSC, Protein Content, Acidity and Microbial Survivability

The physicochemical properties of kefir-like (HPC-K) and yogurt-like (HPC-Y) beverages were monitored during 28-day storage. It should be noted that their changes were strongly influenced by the culture used in the fermentation process. Table 1 shows the values of TSC, pH, TA, and PC. After fermentation, the TSC for the HPC-K sample was 28.73 ± 0.51%, while that for the HPC-Y was 28.57 ± 0.36% ($p < 0.05$). During storage, the TSC for HPC-Y increased significantly, while a slight decrease was observed for HPC-K. The pH level is one of the important indicators of fermentation. The HPC-Y sample showed a decrease in pH from an initial value of 6.39 to 4.82 after 28 days. The HPC-K sample showed lower changes in pH values during storage, but all were statistically significant ($p < 0.05$). In addition, a deacidification trend was observed in the last weeks of the experiment. The opposite results were observed in previous studies for kefir-type beverages based on flaxseed oil cake [7]. According to Nissen et al. [23], this may be because hemp seed matrices have buffering capacity. The authors pointed out that the pH for hemp beverages was typically higher than for other plant-based matrices. Szparaga et al. [24] reported that for plant-derived materials, many factors can play an inhibitory role during fermentation, such as soluble biochemical compounds such as phenols and flavonoids. Increased acidity

is strongly correlated with lactic acid accumulation due to microbial activity. The highest TA (1.32 ± 0.05 mg/mL) was noticed for the HPC-K sample on day 14 and for HPC-Y on day 1 (1.23 ± 0.01 mg/mL) and day 28 (1.20 ± 0.04 mg/mL). The production of organic acids, such as lactic acid, and the lowering and raising of pH is a natural result of the metabolism of microorganisms and their vitality during storage. In the case of HPC-K, this is also influenced by yeast activity.

Table 1. Total solids content, pH, titrable acidity (TA) and protein content (PC).

	Time of Storage (Days)						
	0	**1**	**3**	**7**	**14**	**21**	**28**
				TSC (%)			
HPC-K	29.44 ± 0.04 ^{Aa}	28.73 ± 0.51 ^{Ba}	27.85 ± 0.13 ^{Ca}	28.51 ± 0.18 ^{Da}	28.45 ± 0.38 ^{Ea}	26.83 ± 1.50 ^{Fa}	28.79 ± 0.19 ^{Ga}
HPC-Y	28.57 ± 0.36 ^{Ab}	28.81 ± 0.05 ^{Bb}	28.45 ± 0.52 ^{Cb}	28.57 ± 0.52 ^{Db}	28.81 ± 0.17 ^{Eb}	28.99 ± 0.07 ^{Fb}	29.22 ± 0.01 ^{Gb}
				PC (%)			
HPC-K	9.34 ± 0.02 ^{Aa}	9.33 ± 0.05 ^{BCa}	9.23 ± 0.05 ^{Da}	9.29 ± 0.08 ^{ABa}	9.15 ± 0.05 ^{Ea}	9.39 ± 0.04 ^{Ca}	9.34 ± 0.05 ^{Ea}
HPC-Y	9.52 ± 0.02 ^{ABb}	9.38 ± 0.07 ^{ABCb}	9.22 ± 0.04 ^{Da}	9.45 ± 0.05 ^{BCDb}	9.25 ± 0.06 ^{Eb}	9.36 ± 0.01 ^{Aa}	9.52 ± 0.07 ^{Fb}
				pH (-)			
HPC-K	6.13 ± 0.01 ^{Aa}	5.34 ± 0.05 ^{Ba}	5.47 ± 0.01 ^{Ca}	5.91 ± 0.00 ^{Da}	5.38 ± 0.01 ^{Ea}	5.72 ± 0.00 ^{Fa}	6.49 ± 0.00 ^{Ga}
HPC-Y	6.39 ± 0.00 ^{Ab}	5.06 ± 0.01 ^{Bb}	5.08 ± 0.00 ^{Cb}	4.82 ± 0.05 ^{Db}	4.94 ± 0.01 ^{Eb}	4.98 ± 0.01 ^{Fb}	4.82 ± 0.00 ^{Gb}
				TA (mg/mL)			
HPC-K	0.64 ± 0.02 ^{Aa}	1.05 ± 0.02 ^{Ba}	1.07 ± 0.01 ^{Ca}	0.75 ± 0.01 ^{Da}	1.32 ± 0.05 ^{Ea}	0.89 ± 0.02 ^{Fa}	0.64 ± 0.05 ^{Ga}
HPC-Y	0.70 ± 0.00 ^{Ab}	1.23 ± 0.01 ^{Bb}	1.14 ± 0.02 ^{Cb}	1.04 ± 0.03 ^{Db}	1.12 ± 0.02 ^{Eb}	1.09 ± 0.00 ^{Fb}	1.20 ± 0.06 ^{Gb}

Values are means ± standard deviation of triplicate determinations. Means with different lowercase letters in the same column are significantly different at $p < 0.05$. Means with different uppercase letters in the same row are significantly different at $p < 0.05$.

During fermentation processes, the viability of LAB is an important factor affecting the safety of the final product. High acidity in products can protect consumers from food-borne pathogens. Microorganisms can produce a wide spectrum of organic acids; in particular, LAB are capable of producing lactic acid [25]. In the starter culture of kefir, the main group of microorganisms is LAB, which is estimated to be around 80–90%, while the second group is yeast (10–20% CFU in kefir). For both kefir and yogurt, there are recommendations for microbial content. A level of $>10^7$ CFU/g for bacteria and $>10^4$ CFU/g for yeast is recommended, while $>10^6$ CFU/g LAB viability is recommended for yogurt. The microbial viability for HPC-K and HPC-Y is shown in Figure 1. For HPC-K, the CFU before fermentation was $>10^8$ CFU/g for LAB and $>10^5$ CFU/g for yeast, and after the process, the microbial population increased and was $>10^{10}$ CFU/g for LAB and $>10^6$ CFU/g for yeast, which is in line with the recommendations for this type of beverage. The therapeutic minimum of live microorganisms in the form of viable lactic acid bacteria (LAB) in fermented foods should be 10^6 CFU/g, which is considered the therapeutic minimum [25].

As previously observed, fermentation in the HPC-K variant was slower (the pH drop was significantly lower than in yogurt), and the yeast content, despite statistical differences ($p < 0.05$), remained at a level of 10^7 CFU/g. Nguela et al. [26] suggested that phenols (mainly proanthocyanidins, e.g., epicatechin and flavanols) can significantly inhibit the growth of cells and the final population of yeast, as well as prolong fermentation time. A similar trend was observed in the presented study. The authors suggested that the binding of phenolic compounds may have disrupted the integrity of the yeast plasma membrane and suppressed functions significantly associated with cell growth. In addition, it should be taken into account that in the presented study, the hemp cake used is a heterogeneous matrix with limited oxygen diffusion capacity, which consists of protein, carbohydrates, phenols and trace amounts of hemp oil. Another explanation, also suggested by Nguela et al. [26], is that compounds in hemp cake, such as phenols and amino acids or phytosterols, are

able to form complexes during the fermentation process. This results in nutrients for yeast growth being more difficult for them to access.

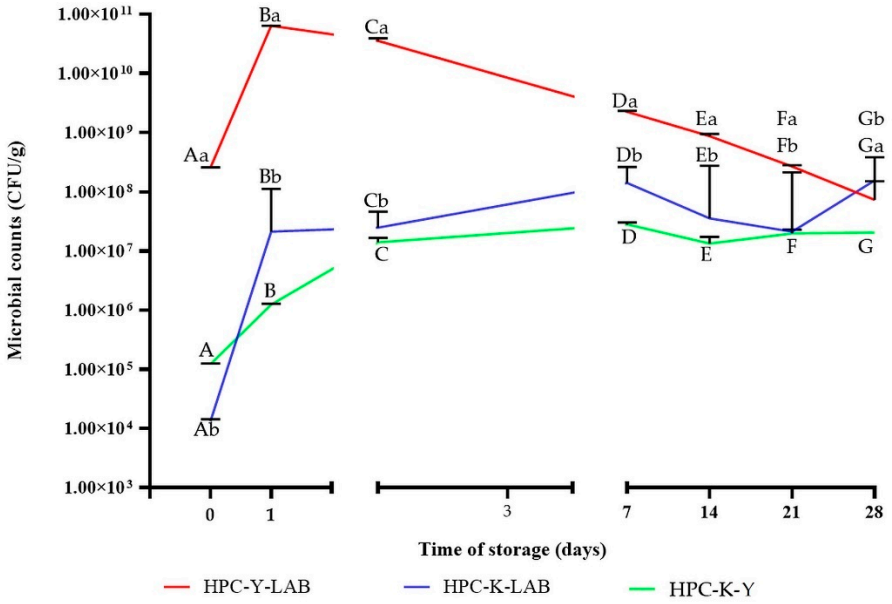

Figure 1. The lactic acid bacteria (LAB) and yeast survivability during storage time. HPC-Y-LAB—hemp press cake-yogurt-lactic acid bacteria; HPC-K-LAB—hemp press cake-kefir-lactic acid bacteria; HPC-K-Y—hemp press cake-kefir-yeast. Values are means ± standard deviation of triplicate determinations. Means with different lowercase letters (a,b) are significantly different at $p < 0.05$. Means with different uppercase (A–G) letters are significantly different at $p < 0.05$.

For HPC-Y, the initial bacterial count was $>10^7$ CFU/g, and after fermentation, it was $>10^9$ CFU/g, which is also in line with recommendations. The final LAB contents were higher than recommended (for yogurt $>10^9$, and for kefir $>10^7$ CFU/g). In both cases, fermentation increases LAB content by approximately two logs. A similarly high LAB content after fermentation was described in fermented hemp paste by Bartikiene et al. [27]. In addition, fermentation can have multiple effects on the antioxidant activity and biotransformation of phenolic compounds.

The fermentation process is often correlated with a cascade of changes in the structure of the protein contained in the matrix [15]. The protein content during storage shown in Table 1 was stable during storage, suggesting that there was no proteolysis of hemp proteins by microorganisms. In studies conducted at each stage of storage, protein profiles were analyzed by the SDS-PAGE technique, the results of which are presented in Supplementary Materials Figure S1. This technique allows the determination of the protein fractions of the tested beverages. The study showed that there were no significant differences between the protein fractions of HPC-based products fermented with yogurt and kefir cultures. Both variants were characterized by two main polypeptide fractions of approximately 20 kDa and 33 kDa. As reported by Tang et al. [28] as well as Wang et al. [29], polypeptides of this mass correspond to the acid and base subunits of edestin. The results obtained are similar to those of Docimo et al. [30], who showed that the main polypeptide fractions in hemp seeds are the acid and base subunits of edestin and the 7S subunit of hemp protein isolate. Similar results were also obtained by Pihlanto et al. [31], who showed that the main polypeptide fractions in hemp protein were characterized by molecular weights of 35 and 22 kDa (edestin). Their study found no significant differences in protein fractions during fermentation.

Despite the stability of hemp protein, changes in the amount of free amino acids and their composition have been observed due to the metabolic activity of microorganisms (Table 2 and Table S1). The amino acids undergo changes such as acylation, decarboxylation

or substituent transfer. One of the objectives of the study on protein profile changes was to describe the fermentation mechanisms in the hemp-based matrix and compare them in two different products. It should also be noted that γ-aminobutyric acid (GABA) was determined in both samples. It is widely described that fermented foods are rich in GABA, such as kimchi, cheese and fermented dairy products. Various microorganisms such as bacteria, yeasts and filamentous fungi could have GABA-producing properties [32]. In addition, many authors have suggested that the enrichment of cereal-based foods in GABA may occur through fermentation by lactic acid bacteria (LAB). In the present study, significant GABA content was also found in both HPC-K and HPC-Y initially. This can be explained based on the matrix processing steps. According to Ma et al. [33], GABA content may have increased under the influence of thorough heat treatment. The authors suggested that processes such as direct cooking methods such as steaming, boiling, baking and microwave treatment increase the content of this amino acid. In the study presented here, HPC was cooked and pasteurized, which may have influenced the high initial GABA content in both samples. The increase in subsequent stages was probably influenced by the metabolic processes of bacteria. It can be concluded that a synergistic effect of pretreatment and fermentation on GABA content was observed in the presented beverages.

Table 2. The total polyphenol content, total flavonoid content, reducing sugars content, and total free amino acids during storage.

	Time of Storage (Days)						
	0	**1**	**3**	**7**	**14**	**21**	**28**
				TPC (mg GAE/g)			
HPC-K	53.36 ± 2.38 ^{Aa}	36.08 ± 5.05 ^{BCb}	30.66 ± 5.11 ^{Da}	34.56 ± 2.86 ^{BCa}	37.96 ± 3.26 ^{BCa}	42.53 ± 6.10 ^{ABCa}	57.76 ± 1.06 ^{Aa}
HPC-Y	56.36 ± 2.11 ^{Aa}	36.05 ± 2.77 ^{Bb}	34.56 ± 4.68 ^{Ba}	38.35 ± 1.78 ^{Bb}	45.24 ± 2.82 ^{Cb}	53.74 ± 1.69 ^{Db}	53.89 ± 0.96 ^{Db}
				TFC (mg QE/g)			
HPC-K	19.26 ± 2.99 ^{ABa}	12.97 ± 7.66 ^{Aa}	13.99 ± 5.11 ^{ABa}	13.31 ± 2.70 ^{ABa}	23.68 ± 3.40 ^{BCa}	24.02 ± 7.85 ^{Ca}	38.47 ± 6.50 ^{Da}
HPC-Y	39.66 ± 1.56 ^{Ab}	27.93 ± 8.17 ^{Bb}	23.00 ± 1.53 ^{Cb}	20.28 ± 0.59 ^{Cb}	24.02 ± 0.75 ^{Db}	29.29 ± 0.29 ^{Eb}	20.79 ± 8.39 ^{Fb}
				RSC (mg/g)			
HPC-K	677.11 ± 7.47 ^{Aa}	233.32 ± 4.32 ^{Ba}	236.91 ± 5.43 ^{Ba}	236.91 ± 3.88 ^{Ba}	258.88 ± 3.11 ^{Ca}	240.50 ± 5.38 ^{Ba}	236.91 ± 3.38 ^{Ba}
HPC-Y	448.94 ± 8.07 ^{Ab}	216.74 ± 2.05 ^{Bb}	247.22 ± 4.58 ^{Cb}	251.70 ± 6.77 ^{Cb}	215.39 ± 1.55 ^{Bb}	194.77 ± 2.69 ^{Db}	188.50 ± 0.78 ^{Db}
				TFAA (mg/g)			
HPC-K	3.57 ± 0.00 ^{Aa}	2.44 ± 0.00 ^{Ba}	2.71 ± 0.01 ^{Ca}	2.22 ± 0.00 ^{Da}	2.42 ± 0.00 ^{Ea}	2.30 ± 0.01 ^{Fa}	2.05 ± 0.01 ^{Ga}
HPC-Y	3.46 ± 0.01 ^{Ab}	3.02 ± 0.01 ^{Bb}	3.20 ± 0.00 ^{Cb}	3.29 ± 0.02 ^{Db}	3.38 ± 0.01 ^{Eb}	4.26 ± 0.01 ^{Fb}	3.12 ± 0.01 ^{Gb}

HPC-K—hemp press cake-kefir; HPC-Y—hemp press cake-yogurt; values are means ± standard deviation of triplicate determinations. Means with different lowercase letters (a,b) in the same column are significantly different at $p < 0.05$. Means with different uppercase (A–G) letters in the same row are significantly different at $p < 0.05$.

Free amino acids (Table 2 and Table S1) also illustrated the timing and specificity of the bacterial and yeast growth model. This trend could be observed especially in the case of arginine. A complete distribution of this amino acid was observed in the HPC-Y sample compared to the HPC-K sample, where 0.36% arginine was eventually observed. Based on literature reports, it can be concluded that *Lactobacillus* bacteria are capable of degrading arginine to citrulline, ornithine and ammonium via the ADI pathway to produce additional energy. It can also be emphasized that based on the report by Huang et al. [34], arginine is most consumed by *Streptococcus thermophilus*. In the study presented here, two other bacterial cultures were used, which suggests that two other mechanisms were involved. Differences between HPC-Y and HPC-K were observed for aspartate and glutamine metabolism. In HPC-Y, a significant decrease in ASP and GLU was observed on the first day of storage, and this is when the lowest content of these amino acids was recorded. In HPC-K, ASP increased, while GLU decreased sharply. In addition, no disturbance in alanine content was observed in both samples. These results correlated with the mechanisms of amino acid decomposition. ASP deamination followed by decarboxylation produces beta-alanine, and an increase in ALA content can be observed as a result of fermentation (days 1 and 3). It is likely that fermentation on day 1 breaks everything down, and oxaloacetate is formed, with a second deamination

process taking place in the meantime. Significant changes in the free amino acid profile were also noted for proline and lysine. The first increase, followed by a decrease in proline, was also a direct effect of LAB metabolism. According to Chourasia et al. [35], the endopeptidases' (PepP, PepX, PepQ, PepR, and PepI) proline-specific activity of LAB is able to release proline-rich peptides during fermentation, which demonstrate antihypertensive activity [36]. Additionally, the authors reported on the release of ACE-inhibitory peptides upon soymilk fermentation by LAB, including *Limosilactobacillus fermentum, L. delbrueckii, and Lacticaseibacillus paracasei* [37]. The increase in lysine is very important for vegan products due to the fact that it is limited in a plant-based vegan diet [38]. In contrast, in milk-based yogurts, some changes coincide. An increase in lysine and leucine content and a decrease in methionine and serine content were observed [39] In conventional yogurt, the first phase of fermentation with *S. thermophilus*, which is part of the yogurt starter culture, is characterized by an increase in lysine, arginine, glutamic acid, threonine, glycine, alanine, valine and leucine when the culture actively produced lactic acid, a decrease in histidine, proline, methionine, phenylalanine, and cystine, and a barely noticeable presence of serine and tyrosine.

Changes in aromatic amino acids were also observed in both samples. Serine content decreased in both samples, while histidine content increased. According to Christensen et al. [40], very rare deviations were observed in HIS. It is likely that the decrease is due to decarboxylation to histamine, regulation of intracellular pH and metabolic energy generation, or deamination to glutamic acid [41]. Additionally, an increase in tyrosine is described for dairy beverages; interestingly, in the work presented here, a decrease was observed for yogurt-type beverages, and an increase was demonstrated for kefir-type beverages for this amino acid.

3.2. Changes in Reducing Sugar, Total Phenolic, Total Flavonoid and Antioxidant Activity

Table 2 shows the results of changes in reducing sugars. The decrease in RSC is influenced by the metabolic processes of microorganisms. In the case of HPC-K, statistically significant changes in RSC levels could be observed after fermentation ($p < 0.05$). However, during storage, the decrease in RSC was not statistically significant, except on day 14, when the lowest RSC level was observed ($p < 0.05$). A different trend was shown for the HPC-Y variant, in which the changes were greater, and a significantly lower reducing sugar content was described after 21 days. This may probably be influenced by different fermentation mechanisms and the fact that yogurt is based on homofermentative cultures, while kefir is a mixture of microorganisms, such as homofermentative and heterofermentative lactic acid bacteria and yeast [42]. According to Szparaga et al. [24], the heterofermentation process results in a low lactic acid content because the bacteria are capable of producing CO_2, acetic acid, acetaldehyde and/or ethanol. Heterofermentative bacteria are also characterized by other pathways of polysaccharide metabolism [24]. In conclusion, the reduction in RSC is related to the consumption of sugars by microorganisms. This behavior has been reported in previous studies with flaxseed oil cake [6]. On the other hand, the consumption of reducing sugars by microorganisms is also strongly correlated with the content of phenolic substances in the fermentation matrix. Leonard et al. [43] suggested that these compounds strongly affect metabolic processes. One of the main phenolic compounds in hemp seeds is substances from the catechin group, e.g., catechin and epicatechin [44]. According to López de Felipe [45], catechin can affect the cell membrane surface and alter the function of proteins and transporters in the microorganism related to the movement of compounds such as sugar. Our results support this finding, as the consumption of reducing sugars during storage is observed to change in a statistically significant manner in the HPC-Y sample, and higher levels of epicatechin are observed in the chromatographic results than in the HPC-K sample. In addition, epicatechin (the cis isomer of catechin) was observed throughout storage, in contrast to the kefir sample. Table 3 summarizes the results of evaluating the content of polyphenols and flavonoids. Changes were observed in the amount of epicatechin, coumaric acid and gallic acid. An increase in the content of gallic

acid could be observed in kefir samples, while a decrease was seen in other polyphenols. Similar effects were observed by Zhao and Shah [46] for fermentations involving *L. brevis* and *L. plantarum* in green and black tea extracts. During fermentation, more complex polyphenols, such as catechin, are converted to gallic acids. As mentioned earlier, different concentrations of polyphenols and flavonoids are strongly correlated with the cascade of biochemical reactions occurring in the samples. Adebo et al. [47] mentioned that the observed decrease in levels of TPC and TFC could also be attributed to the degradation and hydrolysis of the phenolic compounds, which could correspond with the increase in selected compounds like catechin, gallic acid and quercetin. This is attributed to the release of these bioactive compounds after fermentation with *Lactobacillus* strains, such as in the presented study. In the present study, TPC and TFC increased because of HPC-K fermentation. Changes in polyphenols and flavonoids are significantly higher compared to non-fermented samples and between variants during storage ($p < 0.05$). Changes in TPC content between HPC-K and HPC-Y were not significant ($p > 0.05$); for TFC, the same effect was observed after 7 days when the concentration of total flavonoids began to represent a similar level in HPC-Y and HPC-K samples. The maximum TPC content was assessed for the HPC-K sample after 28 days (57.76 ± 1.06 mg GAE/g), while the highest TFC was described for the HPC-Y sample on the initial day. TFC was increased in the HPC-K compared to the HPC-Y, in which flavonoids decreased. The model for the metabolism of polyphenols by LAB in yogurt and kefir during the fermentation process is very complex and may involve the hydrolysis of flavonoid glycosides or cleavage of the C-ring and the generation/release of phenols with simpler structures and lower molecular weight, such as phenolic acids [48]. According to Leonard et al. [43] the loss of flavonoid content during fermentation does not generally translate into a reduction in biological activity. The same phenomenon was observed in the HPC-Y variant. These samples, despite the reduction in flavonoid content, still showed high residual biological activity parameters. The increase in TFC in the HPC-K variant may have resulted from the conversion of flavanols to their aglycone forms. Luksic et al. [49] observed a similar phenomenon during the fermentation of tartar bread on buckwheat sourdough in the presence of *Lactobacillus heilongjiangensis* and *Pediococcus parvulus*. The authors emphasized that the rutin could transform into quercetin. Chromatographic evaluation results showed a decreasing trend of rutin (Table 3). In contrast, the results of spectrophotometric measurements suggested a significant increase in TFC. It can be concluded that this trend could be the result of rutin's transformation into quercetin or other flavonoids, which were not determined.

Table 3. The results of the determination of the polyphenols and flavonoids using the HPLC method.

	Time of Storage (Days)						
	0	**1**	**4**	**7**	**14**	**21**	**28**
				Gallic acid (μg/mL)			
HPC-K	108.00 ± 0.41 ^{Aa}	110.83 ± 0.63 ^{Ba}	112.91 ± 0.03 ^{Ca}	107.58 ± 0.07 ^{Da}	128.67 ± 0.23 ^{Ea}	123.42 ± 0.08 ^{Fa}	127.99 ± 0.23 ^{Ga}
HPC-Y	106.82 ± 0.32 ^{Ab}	106.41 ± 0.01 ^{Bb}	97.86 ± 0.02 ^{Cb}	84.49 ± 0.02 ^{Db}	76.19 ± 0.24 ^{Eb}	79.17 ± 0.01 ^{Fb}	74.07 ± 0.06 ^{Gb}
				Epicatechin (μg/mL)			
HPC-K	189.19 ± 0.84 ^{Aa}	127.24 ± 0.83 ^{Ba}	70.64 ± 0.00 ^{Ca}	-	-	-	-
HPC-Y	180.82 ± 1.27 ^{Ab}	158.84 ± 0.85 ^{Bb}	128.16 ± 0.20 ^{Cb}	116.00 ± 0.05	113.34 ± 0.51	162.52 ± 0.85	157.41 ± 1.04
				Coumaric acid (μg/mL)			
HPC-K	19.68 ± 0.02 ^{Aa}	13.98 ± 0.03 ^{Ba}	8.19 ± 0.00 ^{Ca}	-	-	-	-
HPC-Y	21.14 ± 0.01 ^{Ab}	15.30 ± 0.03 ^{Bb}	13.27 ± 0.00 ^{Cb}	8.57 ± 0.00	-	-	-
				Rutin (μg/mL)			
HPC-K	162.37 ± 0.58 ^{Aa}	108.23 ± 0.00 ^{Ba}	-	-	-	-	-
HPC-Y	175.51 ± 1.13 ^{Ab}	106.91 ± 0.18 ^{Bb}	101.44 ± 0.00	-	-	-	-

HPC-K—hemp press cake-kefir; HPC-Y—hemp press cake-yogurt; values are means ± standard deviation of triplicate determinations. Means with different lowercase letters (a,b) in the same column are significantly different at $p < 0.05$. Means with different uppercase (A–G) letters in the same row are significantly different at $p < 0.05$.

Hemp is rich in phenolic compounds called phenylpropanoids, one of which is flavonoids. Phenolic compounds protect against oxidative stress. Flavonoids and polyphe-

nols play an important role in the human diet. A strong correlation between their intake and reduced incidence of chronic diseases such as neurodegenerative diseases, cancer and cardiovascular disorders has been described by Baron [50].

Biotransformation during fermentation can lead to a cascade of reactions that can activate bioactive compounds that act as natural antioxidants. It should be noted that the antioxidant potential of a compound is related to its reducing capacity. Reducing capacity is one of the methods for determining antioxidant potential. Table 4 shows the results of the determination of reducing power (RP). In both samples, RP increased until day 21. On day 28, a slight decrease was observed. Measuring the scavenging activities of ABTS and DPPH is one of the most widely used spectrophotometric methods to assess the in vitro antioxidant potential of new fermented foods. In the present study, higher levels of DPPH than ABTS were observed. Increased ABTS and DPPH scavenging activity is influenced by fermentation, as also described in previous studies. It was observed that ABTS and DPPH scavenging activity significantly increased after 28 days. After this time, the highest activity of ABTS (56.19 $\pm$ 1.35%) and DPPH (88.85 $\pm$ 0.72%) was found in the HPC-K variant. A similar trend of increased DPPH scavenging capacity during fermentation was also described by Chen et al. [51] for solid fermentation of black soybeans with *Rhizopus oryzae* and *R. oligosporus*. Higher scavenging activities were the result of the fermentation carried out. It should be noted that through the metabolic activity of microorganisms, fermentation also induces structural breakdown of the cell wall, which leads to the synthesis of various bioactive compounds [48]. However, the initial level of scavenging activity is influenced by the natural bioactive compounds contained in the HPC. It can also be noted that fermentation is a key factor that caused an increase in the bioactive potential of the products and the availability of antioxidant compounds contained in HPC. These substances remain in the cake after oil pressing. Fermentation is one way to increase the availability of these compounds. According to Leonard et al. [43], during fermentation, the bioavailability of phenolic compounds increases, and there is a release of bound phenols from the matrix and biotransformation of phenolic compounds to a simpler structure. Fermentation of foods is a pathway to increase the nutritional and functional value of products. Stalmach et al. [52] showed that fermented foods can provide more flavonoids; the authors presented a comparison of the results of recovered flavonoid metabolites in human urine from fermented and non-fermented foods. The results indicated that fermented foods have a lower content of non-digestible flavonoids, indicating higher bioaccessibility. Nevertheless, the higher scavenging abilities of DPPH and ABTS should not be associated with the highest content of TPC. According to Leonard et al. [43], aglycones have higher bioactivity due to their simpler structure. In both cases, the highest FRAP value was observed after 28 days of storage. The results corresponded to the effects of ABTS and DPPH. According to Magro and Castro [53], despite the similarities between all the scavenging activities used, these tests are based on different concentrations of antioxidants and reactivity between the reagents and the target compound. Nevertheless, in the study presented here and in the cited work, it can be observed that the results for ABTS, DPPH and FRAP, all measured together, illustrate the trend of antioxidant changes in the product during storage. It can be concluded that the antioxidant potential of the products is the result of the synergistic action of many components such as polyphenols, flavonoids, amino acids, vitamins, etc. It should also be noted that hemp is rich in amino acids, which have antioxidant functions. However, when considering the levels of sulfur-containing (methionine) as well as negatively charged amino acids with reducing properties (asparagine + glutamine), fluctuations in their levels can be observed. The observed effects require deeper and more extensive studies of the interactions of selected compounds to demonstrate their impact on the total antioxidant potential of the products.

Table 4. The results of the DPPH, ABTS, FRAP and reducing power.

	Time of Storage (Days)						
	0	**1**	**3**	**7**	**14**	**21**	**28**
				DPPH (%)			
HPC-K	84.17 ± 0.79 ^{Aa}	85.31 ± 1.13 ^{Aa}	69.58 ± 0.79 ^{Ca}	70.52 ± 1.26 ^{Ca}	86.98 ± 0.18 ^{ABa}	86.88 ± 0.54 ^{ABa}	88.85 ± 0.72 ^{Ba}
HPC-Y	83.13 ± 0.54 ^{Aa}	88.13 ± 6.56 ^{Ba}	80.10 ± 1.18 ^{Cb}	80.10 ± 0.95 ^{Cb}	82.40 ± 0.48 ^{ACb}	88.13 ± 0.54 ^{Ba}	87.19 ± 1.90 ^{Ba}
				ABTS (%)			
HPC-K	42.10 ± 2.45 ^{Aa}	40.95 ± 2.03 ^{Aa}	37.62 ± 2.07 ^{Ba}	34.95 ± 2.08 ^{Ba}	45.19 ± 1.39 ^{Ca}	48.67 ± 1.57 ^{Da}	56.19 ± 1.35 ^{Ea}
HPC-Y	47.67 ± 1.43 ^{Aa}	45.24 ± 1.54 ^{ABa}	43.95 ± 2.03 ^{Bb}	43.81 ± 5.25 ^{Bb}	45.48 ± 7.79 ^{ABb}	52.95 ± 2.52 ^{Ca}	51.00 ± 1.65 ^{Ca}
				FRAP (mg AAE/g)			
HPC-K	2.21 ± 0.00 ^{Aa}	2.02 ± 0.10 ^{BCa}	2.05 ± 0.12 ^{BDa}	1.86 ± 0.10 ^{Da}	1.88 ± 0.09 ^{Da}	2.27 ± 0.12 ^{Ea}	2.16 ± 0.29 ^{ABEa}
HPC-Y	1.83 ± 0.10 ^{Aa}	1.79 ± 0.03 ^{Aa}	1.58 ± 0.12 ^{Bb}	1.54 ± 0.08 ^{Ba}	2.05 ± 0.14 ^{Ca}	2.05 ± 0.15 ^{Ca}	2.26 ± 0.20 ^{Db}
				RP (-)			
HPC-K	0.522 ± 0.001 ^{Aa}	0.524 ± 0.001 ^{Ba}	0.452 ± 0.000 ^{Ca}	0.503 ± 0.001 ^{Da}	0.589 ± 0.001 ^{Ea}	0.626 ± 0.001 ^{Fa}	0.592 ± 0.001 ^{Ga}
HPC-Y	0.551 ± 0.001 ^{Ab}	0.576 ± 0.001 ^{Bb}	0.438 ± 0.001 ^{Cb}	0.686 ± 0.001 ^{Db}	0.540 ± 0.001 ^{Eb}	0.658 ± 0.001 ^{Fb}	0.617 ± 0.001 ^{Gb}

HPC-K—hemp press cake-kefir; HPC-Y—hemp press cake-yogurt; values are means ± standard deviation of triplicate determinations. Means with different lowercase letters (a,b) in the same column are significantly different at $p < 0.05$. Means with different uppercase (A–G) letters in the same row are significantly different at $p < 0.05$.

4. Conclusions

Kefir and yogurt are popular dairy products that are an important part of the human diet. Current trends are focusing on new plant-based alternatives, but the biochemical reactions occurring in such products are not well described. Hemp is rich in various polyphenols and flavonoids, and this paper presents the relationship between these compounds and the type of fermentation during storage. The use of different microbes in the matrix of HPC initiated further improvements in the conversion of bioactive phenolic compounds and, ultimately, in the nutritional value of the resulting products. The results provided new insights into fermentation mechanisms in hemp cake and illustrated the differences between this process in yogurt-like and kefir-like samples. In addition, the use of valuable by-products of the oil industry has opened a promising avenue for the production of innovative dairy-free beverages, which is in line with the principles of the circular economy and the idea of zero waste, which, due to their content of bioactive components, can be an interesting dietary enrichment.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation8100490/s1, Figure S1: The results of the SDS-Page analyses; Table S1: The Content of Selected Free Amino Acids. Table S2: Content of Free Amino Acids (mg/DM) in HPC Samples.

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Abbreviations

The following abbreviations are used in this manuscript:

HPC	Hemp Press Cake
FAO	Food and Agriculture Organization
THC	Tetrahydrocannabinol
LAB	Lactic Acid Bacteria
DPPH	2,2-diphenyl-1-picrylhydrazyl
ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
TPTZ	Tripyridyl-s-triazine
HPLC	High-Performance Liquid Chromatography
SDS	Sodium Dodecyl Sulfate
TEMED	Tetramethylethylenediamine
DTT	Dithiothreitol
AOAC	Association of Official Agricultural Chemists
RSC	Reducing Sugars Content
DNS	3,5-Dinitrosalicylic Acid
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
GAE	Gallic Acid Equivalents
QE	Quercetin Equivalents
FRAP	Ferric Reducing Antioxidant Power
AAE	Ascorbic Acid Equivalents
FAA	Free Amino Acids
SD	Standard Deviation
TSC	Total Solid Content
PC	Protein Content
TA	Titratable Acidity
HPC-Y	Hemp Press Cake-Yogurt
HPC-K	Hemp Press Cake-Kefir
HPC-Y-LAB	Hemp Press Cake-Yogurt-Lactic Acid Bacteria
HPC-K-LAB	Hemp Press Cake-Kefir-Lactic Acid Bacteria
HPC-K-Y	Hemp Press Cake-Kefir-Yeast
CFU	Colony Forming Unit
TFAA	Total Free Amino Acids
RP	Reducing Power
GABA	Gamma-Aminobutyric Acid
ADI	Acceptable daily intake
ASP	Asparagine
GLU	Glutamine
ALA	Alanine
HIS	Histidine

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