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The Influence of Fermentation Vessels on Yeast Microbiota and Main Parameters of Sauerkraut

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Abstract: The aim of this study was to compare the yeast microbiota occurring during cabbage fermentation, along with selected parameters important for the quality of sauerkraut, depending on the vessel used. For this purpose, glass jars were used, in which anaerobic conditions are quickly created, and stoneware vessels, whose appearance and characteristics are similar to tanks used on an industrial scale. WL nutrient agar with chloramphenicol and 10% NaCl was used to enumerate yeast. Isolates were differentiated using RAPD-PCR and identified by sequencing of the 5.8S-ITS rRNA gene region and PCR-RFLP. Selected organic acids, sugars, and polyols were analyzed using UHPLC-UV-Vis and -RI. Sauerkraut obtained in stoneware vessels was characterized by the presence of a larger amount of yeast, including those considered spoilage, such as *Rhodotorula* and *Wickerhamomyces*. It also contained 50% less lactic acid and a few times more acetic acids than that obtained using glass jars. A pH around 3.8 and 1.5% lactic acid, which are parameters indicating the end of fermentation of sauerkraut, were obtained in glass jars on the tenth day of fermentation. The yeast *Wickerhamomyces anomalus* may be an indicator of the presence of oxygen during sauerkraut fermentation, while *Clavispora lusitaniae* may indicate anaerobic conditions.

Keywords: *Wickerhamomyces anomalus*; *Clavispora lusitaniae*; stoneware vessels; organic acids

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1. Introduction

Sauerkraut is the most widely produced fermented vegetable in the world. It is a food product obtained as a result of chemical changes occurring during spontaneous lactic acid fermentation carried out by the microorganisms originating from the surface of the vegetable leaves [1]. This microbiota occurs in a strictly defined order, determined by the changing conditions during pickling, and is responsible for transforming components from the raw material into substances important for the final product. The final quality of sauerkraut depends on many factors, such as the quality of the raw material, the initial level of contamination (depending on the conditions of growing vegetables), production hygiene, quality of starter cultures, fermentation conditions (time, salinity, and temperature), and the degree of acidity achieved. Maintaining all parameters at an optimal level for the fermentation process may be especially troublesome in the case of small-scale production, which is less automated [2].

The conditions in which fermentation is carried out have a qualitative and quantitative impact on the microbiota present during fermentation and, therefore, also on the quality of the produced sauerkraut. In Poland, the most common addition is NaCl at the level of 2–2.5%, and fermentation is carried out in tanks (often concrete), in which the surface is often covered with a plastic bag filled with water. This is intended to isolate the cabbage during pickling from the external environment, but also by applying pressure, releasing the juice in which fermentation takes place [3]. Unfortunately, because this system is not perfectly tight, it causes oxygen to enter the tank, modifying the conditions during fermentation as well as the microbiota present. At the same time, it contributes to the

intensive development of yeasts, especially film-forming ones, which change the chemical composition of the fermented vegetable. They use organic acids, stabilizing the product, mainly lactic acid, as a carbon source. This also leads to a decrease in acidity and an increase in pH, which can result in the growth of putrefactive bacteria and other spoilage microorganisms [4].

Our previous research has shown that not all yeasts present during sauerkraut fermentation have a negative impact on its quality. We found that *Debaryomyces hansenii*, *Clavispora lusitaniae*, and *Pichia fermentans* cultures are detected during pickling regardless of the cabbage variety used, and may introduce components into the product that lactic acid bacteria (LAB) cannot synthesize. However, the research was conducted in anaerobic conditions, in which there are no yeasts with aerobic metabolism, which most often is a problem in the production of fermented vegetables [5].

This work aims to compare the yeast microbiota that is present during cabbage fermentation depending on the vessel used. For this purpose, glass jars were used, in which anaerobic conditions are quickly created, and stoneware vessels, whose appearance and characteristics are similar to tanks used on an industrial scale. Due to the micropores present in the walls, gases can penetrate the interior of the vessel, similarly to its cover, which is made of a plastic bag with a pressure stone placed on it. In addition to yeast, other basic parameters affecting the quality of the sauerkraut were also analyzed during fermentation and in the finished product, such as the amount of LAB, pH, the content of selected organic acids, sugars, and polyols, and FAN. Sensory analysis was also carried out.

2. Materials and Methods

2.1. Fermentation of Sauerkraut

The experiments included two stages. The first (preliminary research) involved 5 varieties of cabbage (Galaxy, Kilaton, Kronos, Furios, and Zielonor), which were fermented in stoneware vessels. In the second stage, Galaxy cabbage was fermented simultaneously in stoneware vessels and glass jars. Cabbages were obtained from growers from the cabbage capital of Poland—the municipality of Charsznica in Małopolska. The cabbages were cut on an automatic slicer (Ma-Ga 612 p) into 2.5 mm-thick strips. Four kilograms of cabbage was weighed into Browin® (Łódź, Poland) stoneware vessels and/or glass jars, and 2.5% (*w*/*w*) NaCl (99.5%, Loba Chemie PVT Ltd., Mumbai, India) was added. A portion of weighed salt powder was added to each layer of cabbage. The cabbage was kneaded until it was covered by the juice. The surface of stoneware vessels was covered with double-layer LDPE foil (O_2 permeability 50–150 $cm^3 \times mm \times m^{-2} \times day^{-1}$ atm^{-1}) and the pressure stones were placed. The jars were closed with lids equipped with glycerol-filled fermentation tubes. Each variant was performed in triplicate. Fermentation was carried out for 14 days at 20 °C. On days 0, 1, 2, 3, 7, 10, and 14 of fermentation, 5 g of sauerkraut and 5 mL of brine were collected from each type of vessel under sterile conditions in an anaerobic chamber filled with nitrogen for chemical (determination of pH, sugar content by the DNS method, FAN concentration, selected organic acids, and sugars and polyols by HPLC) and microbial analyses (determination of the number and identification of yeasts and lactic acid bacteria).

2.2. Determination the Number of Yeasts and Their Isolation

During fermentation, the collected samples of fermenting sauerkraut were inoculated on WL agar with chloramphenicol and 2.5% (*w*/*w*) NaCl (Biocorp®, Warsaw, Poland), after appropriate dilutions, in order to analyze the number of yeasts, and the results were expressed as cfu/g sauerkraut. After incubation at 25 °C for 5 days, morphologically different colonies were isolated, and monocultures were obtained using reduction inoculation. Purified yeast cultures were maintained on slants with Sabouraud agar with chloramphenicol (Biocorp®) and kept under refrigerated conditions or frozen in 70% glycerol at −86 °C for later analyses. Cultures were differentiated using the PCR-RAPD method and identified by sequencing the ITS region and PCR-RFLP.

2.3. Determining the Number of Lactic Acid Bacteria

During fermentation, inoculations were performed under MRS agar with 2.5% (*w*/*w*) NaCl and 0.1% actidione for quantitative analysis of the developing lactic acid bacteria. Inoculations (volume: 1 mL) were performed using serial dilutions. The dilutions used for inoculation depended on the day of fermentation and the number of cfu/g expected based on preliminary tests. After incubation at 32 °C for 48 h in an incubator (17% CO_2, Esco CCL-170B-8), only plates containing 30–300 colonies were analyzed.

2.4. DNA Isolation

The analyses were performed in accordance with the manufacturer's instructions (A&A Biotechnology, Gdansk, Poland). The 48 h yeast culture was centrifuged, the supernatant was poured off, and the remaining microorganism pellet was suspended in 1 mL of sterile, nuclease-free water (NFW). The procedure was repeated, and then the pellet was suspended in 100 µL of SP solution. Then, 30 µL of chitinase and 5 µL of 1 M dithiothreitol (DTT) were added, incubated for 30 min at 50 °C, and 600 µL of LS lysing suspension and 20 µL of proteinase K solution were added. Everything was mixed and incubated for 15 min at 50 °C, and the samples were vortexed every 5 min at 1400 RPM. After incubation, the samples were vortexed for 2 min at 1400 RPM and then centrifuged for 5 min at 10,000 RPM. Then, 0.6 mL of the supernatant was collected and placed on a Mini AX Spin column in a 2 mL tube and centrifuged at 5000 RPM for 1 min. The used tube was removed, and the Mini AX Spin column was placed in a new tube. Next, 600 µL of first washing buffer (W1) was applied and centrifuged at 5000 RPM for 1 min, then the procedure was repeated with 500 µL of second washing buffer (W2). During repeated centrifugation, elution tubes were prepared, and 5 µL of neutralization buffer N was added to them. After centrifugation, the tube was carefully removed and the Mini AX Spin column was placed in the prepared elution tubes, then 150 µL of elution buffer E was added and incubated at room temperature for 5 min. Finally, the tubes with columns were centrifuged at 5000 RPM for 1 min, and the obtained purified DNA was stored under refrigerated conditions for the analyses.

2.5. RAPD-PCR

Eppendorf tubes with a capacity of 0.5 mL were placed in a water bath, and 34.75 µL of nuclease-free water (NFW) was added to them. A reaction mixture consisting of 10 µL of buffer, 0.25 µL of polymerase, 1 µL of dNTP, and 1 µL of M13 primer (5′-GAGGGTGGCGGTTCT-3′; Oligo.pl, Warsaw, Poland) was prepared in a volume appropriate to the number of samples analyzed. The thermal cycler was heated to a denaturation temperature of 95 °C. The 12.25 µL reaction mixture was pipetted into Eppendorf tubes, and 3 µL of template DNA was added. The resulting mixture was spun at a speed of 10,000 RPM at 4 °C, and then the samples were inserted into a thermal cycler and the program was run with the following parameters: initial denaturation at 95 °C for 5 min, 35 cycles (95 °C for 1 min, 36 °C for 1 min, and 68 °C for 2 min), and a final polymerization step at 68 °C for 7 min.

After the thermal cycler was finished, the tubes were removed and the samples were placed on a 2% agarose gel prepared in 1×TAE buffer with ethidium bromide, immersed in a tank filled with 5×TAE buffer. Then, 8 µL of marker and 1.6 µL of dye were placed in the two outermost wells, and run for 70 min at 100 V. After electrophoresis, the gel was taken out of the well, placed in the transilluminator, and visualized.

2.6. PCR-RFLP

ITS1 (5′ TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primers were used for amplification of the 5.8S-ITS rRNA gene region. The PCR reaction mixture (50 µL) contained 1×OneTaq Standard Reaction Buffer, 200 µM of each dNTP, 1.25 U of OneTaq DNA Polymerase (New England Biolabs, Ipswich, MA, USA), 0.2 µM of each primer (oligo.pl, Poland), and 2 µL of genomic DNA. The temperature program

consisted of initial denaturation at 94 °C for 1 min, 30 cycles (94 °C for 30 s, 55.5 °C for 1 min, and 68 °C for 3 min), and a final polymerization step at 68 °C for 5 min.

The amplified rRNA gene region (5 µL) was digested without further purification with 1 U of restriction endonucleases HhaI, HinfI, and HaeIII (Promega, Madison, WI, USA) in a 12.5 µL reaction volume using the manufacturer's instructions and conditions. The resulting region of the 5.8S-ITS rRNA gene and digested fragments were separated on 2% (*w*/*v*) agarose gels in 1×TAE buffer with ethidium bromide at 100 V for 60 min. The DNA molecular-weight-marker 100–1000 Ladder (A&A Biotechnology, Poland) was used as a length standard.

2.7. 5.8S-ITS rRNA Gene Region Sequencing

Obtained amplified products of the 5.8S-ITS rRNA gene region were purified using Clean Up AX (A&A Biotechnology, Gdansk, Poland), according to the manufacturer's instructions. The concentration of purified rRNA samples was measured at 260 nm absorbance using a NanoDrop-1000 spectrophotometer (NanoDrop Technology, Rockland, DE, USA), and then submitted for sequencing to Macrogen Inc. (Amsterdam, The Netherlands). The DNA sequences were edited, and consensus sequences were estimated manually using Mega X software. Species were identified by comparing processed sequences with those available in the GenBank NCBI database using the basic local alignment search tool (BLAST 2.15.0) at: http://www.ncbi.nlm.nih.gov/BLAST/ (accessed on 24 December 2023), considering an identity threshold of at least 98%.

2.8. Determination of Total and Reducing Sugars Using 3,5-Dinitrosalicylic Acid (DNS)

Total and reducing sugars were determined using the DNS method [6]. The neutralized samples were deproteinized using Carrez I and Carrez II solutions, then mixed, topped up with distilled water, and filtered through paper.

Part of the obtained solution was subjected to the inversion process using concentrated HCl. The liquid in the flasks was then heated in a water bath to a temperature of 68–70 °C inside the flask, and then the temperature was maintained for 5 min. After this time, the liquid was quickly cooled to a temperature of about 20 °C, three drops of methyl orange were added, and it was neutralized with 0.1 M NaOH until the liquid changed color. The solution was then poured into a 100 mL volumetric flask, brought back to the temperature of 20 °C, and topped up to the mark with distilled water.

Next, 2 mL of DNS solution and 2 mL of samples without or after inversion were measured into test tubes. The mixture was boiled for about 5–10 min, then 11 mL of distilled water was added. The contents of reducing and total sugars were determined spectrophotometrically at a wavelength of λ = 520 nm on the basis of a standard curve for various concentrations of glucose solution (0–2 g/L).

2.9. Determination of Sugars and Acids by HPLC

The determination of sugars and acids in samples taken during fermentation was performed using HPLC. Organic acids were analyzed on a Perkin Elmer Flexar FX-20 chromatograph and a Rezex ROA-Organic Acid H+ (8%) column. The sauerkraut homogenate was centrifuged, and the supernatant was filtered with syringe filters (ø = 0.45 µm). In order to obtain reliable measurements, the samples were diluted five times with distilled water. Organic acids were analyzed with a UV-Vis detector at a wavelength of 210 nm, and sugars with a refractometer. In order to calculate the concentration of individual compounds, standard curves (lactic, acetic, succinic, and oxalic acid (Sigma-Aldrich, St. Louis, MI, USA)) were previously prepared for the analyzed compounds.

Sugars and polyols were analyzed with the Shimadzu (Kyoto, Japan) NEXERA XR apparatus with a RF-20A refractometric detector, and an Asahipak NH2P-50 4.6 × 250 mm Shodex column (Showa Denko Europe, Munich, Germany), thermostated at 30 °C. The mobile phase consisted of an acetonitrile aqueous solution (70%), and the isocratic elution program (0.8 mL/min) lasted 16 min. Quantitative determinations were performed with

the use of standard curves prepared for appropriate standards: glucose, fructose, glycerol, and mannitol (Sigma-Aldrich, St. Louis, MI, USA).

2.10. Free Amine Nitrogen (FAN)

The ninhydrin method was used to determine free amino nitrogen. This method is based on the spectrometric determination of the color intensity depending on the concentration of the resulting color complex of the added reagent (ninhydrin), with NH3, as a result of boiling the mixture for 10 min. Then, the absorbance of the samples was measured at a wavelength of λ = 575 nm against distilled water with ninhydrin as a blank. A similar procedure was followed with the standard sample with glycine [7].

2.11. Sensory Analysis (QDA)

Sensory assessment of sauerkraut samples was performed using quantitative descriptive analysis (QDA) [8]. The sensory evaluation was performed by 10 trained testers, employees of the Department of Fermentation Technology and Microbiology (5 men and 5 women), aged 30 to 50 years. Fourteen sensory qualities were evaluated, which had been selected in the initial panel discussion, on a ten-point scale—overall impression, aroma (typical, pungent, off-odor), appearance (color, gloss), texture (hardness, crispiness), and taste (sour, salty, sweet, spicy, bitter, off-flavor).

2.12. Statistical Analysis

All experiments were conducted in triplicate, as was the analysis. SPSS 13.0 software was applied for analysis of statistical results. The results were presented as arithmetic means with standard error of the means (SEM) on the charts. Statistically significant differences between results ($p < 0.05$) were evaluated using multifactor variance analysis (ANOVA) with the post hoc Duncan test.

3. Results and Discussion

3.1. The Yeast Microbiota during Fermentation in Stoneware Vessels of Cabbages of Different Varieties—Preliminary Studies

The appropriate vessel used during fermentation plays an important role, as it allows the anaerobic conditions necessary for the process to be maintained, affecting the microbiota. In Poland, traditionally, stoneware vessels with pressure stones are used for sauerkraut fermentation, to prevent shredded cabbage from rising above the brine level, which may result in the development of undesirable microorganisms, such as molds or yeasts [3].

Stoneware is made from clay, kaolin, feldspar, and silica, and is characterized by relatively high porosity with a pore size of around 0.04 µm and oxygen permeability of 5 mg/liter/year [9]. Considering that the vessel is usually covered with plastic foil, it creates microaerobic conditions inside, enabling the growth of spoilage microorganisms with aerobic metabolism.

In the first (preliminary) stage of the described research, we qualitatively analyzed the yeast microbiota present during the fermentation of five different varieties of cabbage (Galaxy, Kilaton, Kronos, Zielonor, and Furious) in stoneware vessels. Due to the lack of thematically similar research articles, the results were compared with those from our earlier studies that used similar experimental conditions but different fermentation vessels (glass jars).

During the experiments, 170 yeast isolates were obtained from fermenting sauerkraut of various varieties, showing the morphological diversity of the colonies formed. Analysis of isolates by RAPD-PCR using the M13 primer showed similarity of some electrophoretic patterns, despite obtaining isolates from different cabbage varieties. This proves that the composition of the yeast microbiota on the surface of cabbage leaves is qualitatively similar and at different times during the process, typical of the plant species rather than the variety. Thirty-two cultures (biotypes) with different patterns of bands on the resulting gels were

selected for identification by PCR-RFLP reaction and Sanger sequencing of the ITS region (Table 1).

The presence of 11 strains classified into 5 yeast species was found. Three of them—*Debaryomyces hansenii*, *Rhodotorula mucilaginosa*, and *Pichia fermentans*—were identified in our previous studies, in which glass jars were used for fermentation and the entire process took place in anaerobic conditions [5]. It should, therefore, be assumed that these are microorganisms that develop in sauerkraut, regardless of the conditions during fermentation. The four identified *D. hansenii* strains developed during the initial fermentation period (up to day 4), while the other two species occurred during the first two days of fermentation and then at its end. The remaining two yeast species found—*Wickerhamomyces anomalus* and *Torulaspora delbrueckii*—were not detected during our previous studies. Their presence during cabbage fermentation, regardless of the variety used, proves that the conditions, i.e., the availability of oxygen, contributed to their development in an amount that enabled their detection.

The experiments showed the presence of three different strains of yeast of the species *W. anomalus* (Table 1). *W. anomalus* is able to grow abundantly in different fermented foods due to its fully aerobic or weakly fermentative metabolism [10]. This may explain why these yeasts were found during the fermentation of sauerkraut in stoneware vessels and were not present during its production in anaerobic conditions.

According to Passoth et al. [11], it can grow under extreme environmental stress conditions, such as low and high pH, low water activity, and high osmotic pressure. Due to these characteristics, this yeast can be a spoilage organism, for instance, in silage. Moreover, together with other species (including *S. cerevisiae*, *R. rubra*, and *D. hansenii*), *W. anomalus* was found as a natural microbiota of traditional Chinese sauerkraut [12].

W. anomalus was also isolated from fermented Pakistani sourdough [13] and from fermented bread sourdough [14], and in both cases the growth of other fungi was inhibited during fermentation. A similar phenomenon was also found during our analyses, between the third and seventh day of fermentation (Figure 1), when less diversity of yeast cultures was found, even though, according to the results of the quantitative analysis (Figure 2), the highest levels of yeast occurred at that time in these settings. This could be related to the ability of *W. anomalus* and/or *T. delbrueckii* strains to produce killer toxins, and we had already detected these types of strains in our earlier analyses during sauerkraut fermentation [5]. There is a considerable amount of published information on the widely intergeneric killing spectrum of *Wickerhamomyces* toxins against different cultures of fungi and yeast [15].

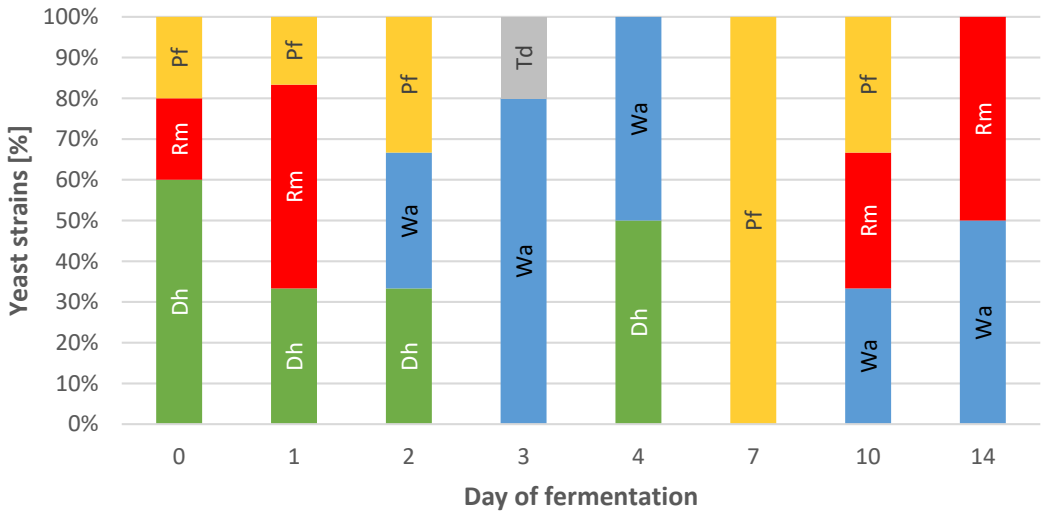

Figure 1. Distribution of yeast strains during fermentation of different cabbage varieties in stoneware vessels (Dh—*Debaryomyces hansenii*, Pf—*Pichia fermentans*, Rm—*Rhodotorula mucilaginosa*, Td—*Torulaspora delbrueckii*, and Wa—*Wickerhamomyces anomalus*).

Table 1. Identified yeast species on the basis of their lengths of restriction fragments of the 5.8S-ITS rRNA gene region and the highest 5.8S-ITS rRNA similarity score.

Strain	5.8S-ITS rRNA Gene (bp)	Restriction Fragments (bp)			Species Identification by 5.8S-ITS rRNA Gene Sequencing		
		***Cfo*I**	***Hinf*I**	***Hae*III**	**Closest Species**	**Homology %**	
Pf9	450	170 + 100 + 100 + 80	250 + 200	340 + 80 + 30	*Pichia fermentans*	100.0	MT645416
Wa1	650	575	310 + 310	600 + 50	*Wickerhamomyces anomalus*	99.0	KY657575
Wa7	650	575	310 + 310	600 + 50	*Wickerhamomyces anomalus*	99.6	KY105860
Wa22	650	575	310 + 310	600 + 50	*Wickerhamomyces anomalus*	98.4	KT580795
Rm8	640	320 + 240 + 80	340 + 225 + 75	425 + 215	*Rhodotorula mucilaginosa*	100.0	OQ692821
Rm26	640	320 + 240 + 80	340 + 225 + 75	425 + 215	*Rhodotorula mucilaginosa*	99.8	JQ293997
Td5	800	330 + 220 + 150 + 100	800	410 + 390	*Torulaspora delbrueckii*	98.2	KY105612
Dh17	650	300 + 300 + 50	325 + 325	420 + 150 + 90	*Debaryomyces hansenii*	99.0	MT192508
Dh19	650	300 + 300 + 50	325 + 325	420 + 150 + 90	*Debaryomyces hansenii*	99.2	MK275230
Dh23	650	300 + 300 + 50	325 + 325	420 + 150 + 90	*Debaryomyces hansenii*	98.1	MH429890
Dh31	650	300 + 300 + 50	325 + 325	420 + 150 + 90	*Debaryomyces hansenii*	98.3	KM816678
Cl6	370	210 + 180	180 + 160	370	*Clavispora lusitaniae*	99.0	MK312615

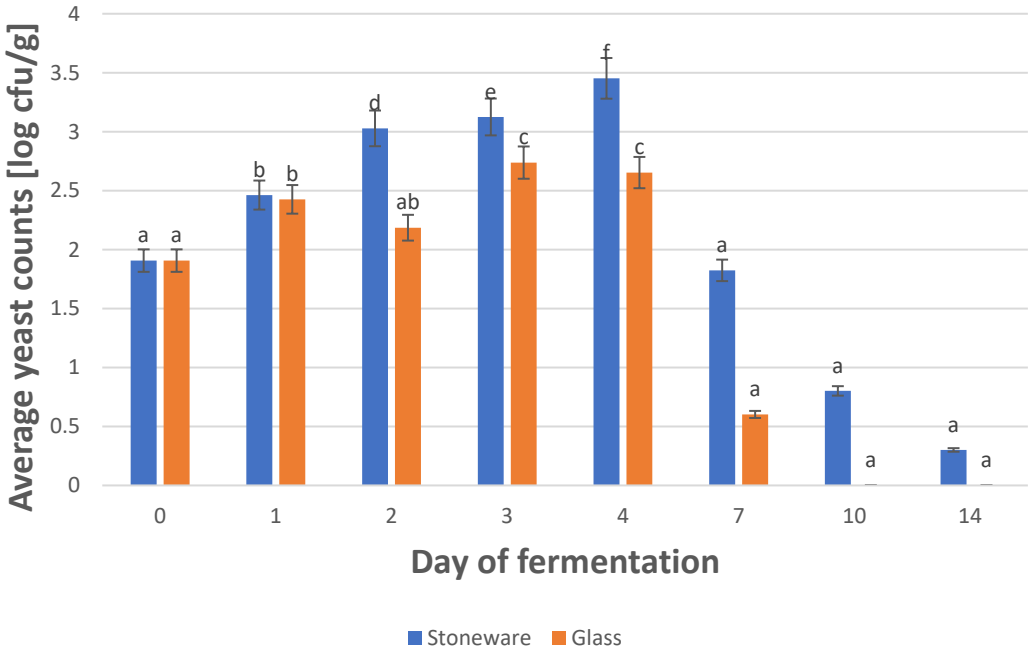

Figure 2. Changes in the amount of yeast during the fermentation of Galaxy cabbage in stoneware vessels and glass jars. Values with different roman letters (a–f) indicate statistically significant differences at $p < 0.05$.

The yeast *Torulaspora delbrueckii* was present only in the samples from the third day of fermentation of Galaxy cabbage. *T. delbrueckii* is mainly associated with wine [16] and beer fermentation [17], where it can also be used as a starter culture, enriching the produced wine with substances with a pleasant fruity and floral aroma, mainly esters. The presence of this microorganism was also found in products with high salinity, e.g., during the ripening of Feta-type cheese, where the salt concentration was approximately 8% [18].

3.2. The Influence of the Vessel Used for Fermentation on the Yeast Microbiota of Sauerkraut

Due to the large differences in the composition of the yeast microbiota during the fermentation of sauerkraut in stoneware vessels, observed in the first stage of the research in relation to previous experiments [5], in the second stage of the research, the influence of the vessel used for fermentation on the qualitative and quantitative composition of yeast and other selected parameters important for the quality of sauerkraut was analyzed. Glass jars were used, which prevent air from entering and in which anaerobic conditions are quickly created, and stoneware vessels in which the surface was covered with a plastic foil pressed with a pressure stone. The most popular variety of cabbage used for pickling in Poland—Galaxy—was used for fermentation.

The initial yeast content in the batch was 1.9 log cfu/g (Figure 2), the average amount found in previous analyses [5]. Alan and Yildiz detected much higher amounts of yeast (3.5 log cfu/mL) in Turkish cabbage used for fermentation [19]; however, according to Geeson [20], in subsequent growing seasons, there may be very large differences in the level of various microorganisms (including yeast) on the surface of fresh cabbage. Statistically significant differences between the samples were observed on the second day of fermentation (Figure 2), when inhibition of yeast cell growth at the level of 2.2–2.4 log cfu/g was observed in glass jars, while in stoneware vessels the abundance continued to increase, exceeding 3 log cfu/g. In the case of stoneware vessels, the maximum of yeast growth took place on the fourth day of fermentation, while in jars the highest number of yeast cells was recorded on the third and fourth days, and the number of yeast cells detected in stoneware vessels was over six times higher than in the jars. In the following days, there was a significant decrease in the number of yeasts; from the tenth day, yeast was no longer detected in the jars, while a small amount was found in the stoneware vessels until the

end of fermentation. A similar phenomenon was observed by Yang et al. [21], who found the maximum level of yeast on the fifth day of sauerkraut fermentation, and its content decreased in the following days. However, they obtained a significantly higher yeast count during and at the end of fermentation, which could be related to the high initial amount of yeast in fresh cabbage. Differences in the amount of yeast depending on the vessel used for fermentation may indicate different conditions, which may also have a significant impact on the qualitative composition of the microorganisms present (Figure 3).

An important observation during fermentation was the presence of film-forming yeast on the surface of the stoneware samples, while they were not observed in the jar. Characteristic colonies of microorganisms morphologically resembling mold are formed on the surface of the fermenting vegetables and perfectly tolerate high salinity, and their development may contribute to the deterioration of the quality of the product obtained. Over-proliferation of yeast can also create an undesirable yeast/fungal taste. The species most often associated with this phenomenon are the yeast *Candida mycoderma* and the mold *Galactomyces geotrichum* [4]. The presence of undesirable microorganisms (such as film-forming yeast, *Rhodotorula*, and filamentous fungi) that may negatively affect the product is one of the reasons for producers to abandon the traditional method of obtaining fermented vegetables [22], and the use of anaerobic conditions and starter cultures is increasingly common, as in other industrial fermentation processes, which increases microbiological stability, reduces losses, and improves the quality of the product created [23].

For qualitative analysis of yeast, 36 cultures were isolated, and the obtained isolates were subjected to RAPD-PCR analysis, which allowed the selection of 10 different biotypes. Sequencing of the ITS region and PCR-RFLP analysis led to the classification of isolates into five species (Figure 3; Table 1), with the yeast *D. hansenii* represented by three different strains, and *Rh. mucilaginosa* and *W. anomalus* by two strains each. They were identical to those obtained in the first (preliminary) stage of the study. The isolates also included a representative of one new species of yeast (only in jars), identified as *Clavispora lusitaniae* (Figure 3; Table 1). During the fermentation of sauerkraut, both in glass jars and stoneware vessels, a large variability in the presence of different strains was found on subsequent days of fermentation. This is a phenomenon typical of spontaneous fermentation, during which successive quantitative and qualitative changes of microorganisms occur, which is related to the presence or absence of oxygen, inhibition by formed products (such as lactic acid, ethyl alcohol, and others), depletion of nutrients, or interactions between different groups of microorganisms [4,12].

The greatest differences in the qualitative composition of yeast concerned the occurrence of representatives of the species *W. anomalus* only in the stoneware vessels, and the cultures of *Cl. lusitaniae* only in the jars. Strains that could grow anaerobically grew better in jars, and cultures that preferred a higher oxygen concentration were present in stoneware vessels. Live *W. anomalus* yeast cells were found in stoneware vessels after the first day of fermentation, and their maximum level was found on the third day, when they constituted 100% of the isolates. In the following days, their number decreased rapidly, and on the seventh day they were no longer detected. The *W. anomalus* Wa1 strain occurred between the first and third days of fermentation, while the *W. anomalus* Wa7 strain—between the second and fourth days. The differences in growth were probably related to the changing conditions in the environment between the third and fourth days of fermentation. At this time, the greatest decrease in environmental pH occurred during fermentation in stoneware vessels and an increase in the concentration of lactic and acetic acid (Table 2), as well as a sharp increase in the number of LAB (Figure 4), which may have an antagonistic effect on some yeast cultures [24]. In the first stage of experiments, *W. anomalus* cultures were also found in the final stage of fermentation (Figure 1), and this was related to the presence of the *W. anomalus* Wa22 strain, which, as it should be assumed, was not present at the discussed stage of the study.

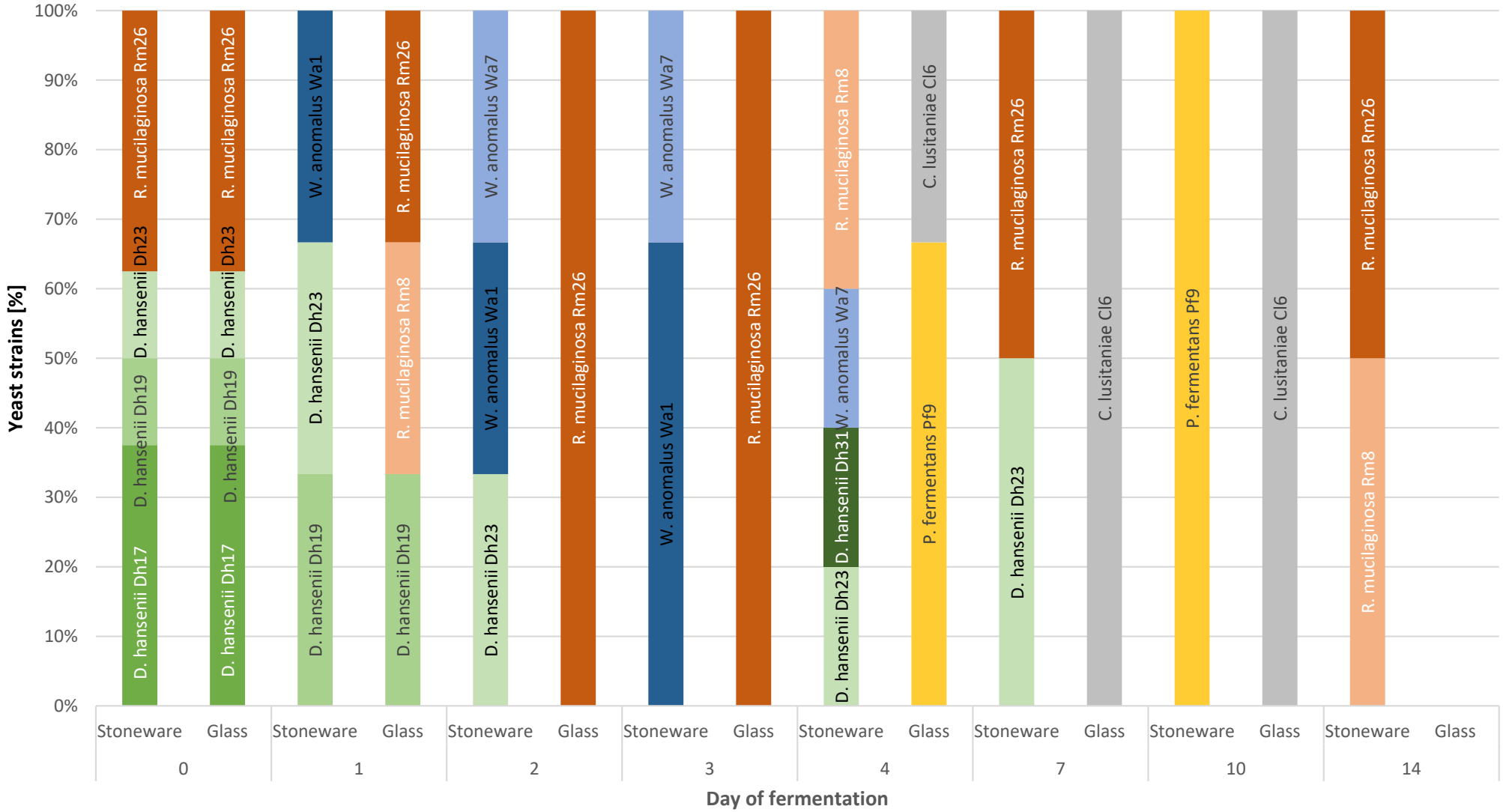

Figure 3. Distribution of yeast strains (%) during Galaxy cabbage fermentation depends on the fermentation vessel.

Table 2. A heatmap of the pH and content of selected organic acids on subsequent days of fermentation of sauerkraut using various vessels.

	Vessel/Day	0	1	2	3	4	7	10	14	SEM [1]	Sig. [2]
pH	Glass	6.04 f	5.78 ef	5.73 ef	4.64 c	4.10 b	3.82 ab	3.81 ab	3.73 a	0.17	***
	Stoneware	6.04 f	5.70 e	5.57 e	5.13 d	4.53 c	4.06 b	3.91 ab	3.86 ab	0.19	
Lactic acid	Glass	0.00 a	0.87 ab	1.05 ab	3.74 abc	4.42 bc	12.42 efg	15.78 g	15.37 fg	1.40	***
	Stoneware	0.00 a	2.01 ab	1.16 ab	2.57 ab	2.75 ab	6.95 cd	11.65 ef	10.12 de	0.86	
Oxalic acid	Glass	0.31 abc	0.36 abc	0.54 c	0.30 abc	0.50 bc	1.25 e	1.00 d	0.36 abc	0.07	***
	Stoneware	0.31 abc	0.53 c	0.27 ab	0.21 a	0.39 abc	0.82 d	0.96 d	0.15 a	0.06	
Acetic acid	Glass	0.00 a	0.98 e	1.60 g	0.47 b	1.52 fg	3.75 k	2.84 j	0.89 de	0.24	***
	Stoneware	0.00 a	0.64 bc	0.73 cd	0.84 de	0.99 e	2.38 i	1.39 f	2.07 h	0.15	
Succinic acid	Glass	6.22 f	5.85 f	8.48 g	3.53 cd	3.74 cde	2.86 abcd	1.64 ab	1.39 a	0.51	***
	Stoneware	6.22 f	5.92 f	5.64 f	4.68 def	5.42 ef	3.68 cde	1.92 abc	3.30 bcd	0.34	

[1] SEM—standard error of the mean. [2] Sig.—significance. Values with different roman letters (a–k) of the same parameter (in rows) indicate statistically significant differences at $p < 0.05$; $n = 5$; 0.001 ***. Color determination from the lowest (0%) to highest (100%) concentration of parameters. The lowest concentration of a specific parameter in a row is in the darkest red, and the highest content is in the darkest green (−1 −0.5 0 0.5 1).

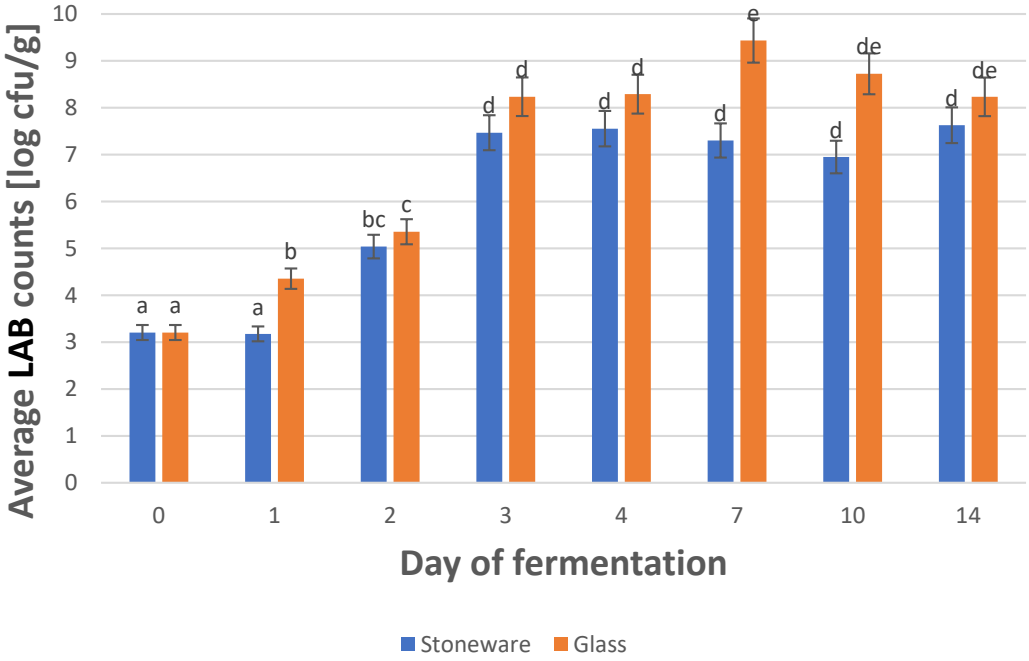

Figure 4. Changes in the number of lactic acid bacteria (LAB) during the fermentation of Galaxy cabbage in stoneware vessels and glass jars. Values with different roman letters (a–e) indicate statistically significant differences at $p < 0.05$.

As already mentioned, the yeast *W. anomalus* is characterized by fully aerobic or weak fermentative metabolism [10]. Therefore, although they are generally associated with various fermentation processes, their development is most often related to the availability of oxygen. *W. anomalus* is often among the "film-forming" yeasts associated with spoilage of beer, wine, and fermented vegetables [25], as well as bakery products [26]. According to Hung and Kyung [27], these cultures are relatively resistant to the presence of lactic acid, but sensitive to acetic acid.

The yeast *Clavispora lusitaniae* was first associated with the production of sauerkraut in our previous research [5], where they were the second most abundant yeast species, after *D. hansenii*. In the current research, they appeared on the fourth day of fermentation in glass jars and were the only ones detected on the following days (Figure 3). Our previous research has shown very good fermentation properties of these cultures, the ability to

secrete killer toxins, and resistance to relatively high salinity, high concentrations of lactic acid, and low pH [5].

Representatives of the remaining three yeast species—*D. hansenii*, *Rh. Mucilaginosa*, and *P. fermentans*—occurred during the entire fermentation of sauerkraut produced in stoneware vessels, but only in the initial stage (up to the third day) of fermentation in glass jars (Figure 3). The presence of *Rhodotorula* yeasts during vegetable fermentation is related to the natural reservoir of these microorganisms on the surface of raw vegetables [28]. These are strict aerobes whose development can be significantly limited by the creation of anaerobic conditions [29]. The presence of oxygen in stoneware vessels, also on the final days of fermentation, may enable the proliferation of those microorganisms. This may result in a decrease in the microbiological stability of sauerkraut, causing significant economic losses for producers.

3.3. The Influence of the Vessel Used for Fermentation on the LAB Content, pH, and Selected Organic Acids Level of Sauerkraut

In addition to quantitative determinations of the yeast population, lactic acid bacteria were also quantitatively analyzed during sauerkraut fermentation. Before initiating fermentation, the samples contained 3.2 log cfu/g of lactic acid bacteria (Figure 4). Differences in the number of LAB between the vessels used for fermentation were observed after 24 h, and while in stoneware vessels the amount of LAB practically did not change, in glass jars it increased by one order of magnitude. In the following days, increases in the number of bacteria were observed regardless of the vessel used for fermentation. In the jars, the maximum level of LAB was detected after 7 days of fermentation (9.43 log cfu/g), which was a result similar to that in other studies [21,30]. In the following days, the number slowly decreased, but still reached a value of 8.23 log cfu/g at the end of fermentation, higher than the highest number of LAB detected in stoneware vessels. In stoneware vessels, the maximum amount of LAB occurred on the fourth day of fermentation (7.55 log cfu/g), then decreased in subsequent measurements to increase again to the value reached after four days. The presence of oxygen during vegetable fermentation may inhibit the growth of some lactic acid bacteria, especially strictly anaerobic ones. It can also modify the metabolism of others, causing an increase in the amounts of oxidized components (such as acetic acid, aldehydes, and others) [31].

The pH value of sauerkraut (Table 2) gradually decreased during the entire fermentation, from the level of 6.04 to below 4. The greatest drop occurred between the second and fourth days in jars, and the third and seventh days in stoneware vessels. In both cases, a similar level was finally achieved, safe for fermented vegetables. The pH of fermented vegetables should be at or below 4 to limit the development of putrefactive bacteria [32]. This level was achieved significantly faster (by about 3 days) in cabbage fermented in jars, which suggests that the process itself may be shorter there.

A number of factors are responsible for obtaining the appropriate level of acidity during vegetables' fermentation, including both acid-producing bacteria and the yeast responsible for their metabolism. Among the organic acids, lactic acid and acetic acid are considered the most important in terms of influencing the pH of the sauerkraut [33]. In both vessels, the concentration of lactic acid reached its maximum level on day 10, after which the level of this compound decreased (Table 2). Statistically significant differences appeared between the samples from the seventh day of fermentation, when the concentration of lactic acid in the sauerkraut in the jars was almost twice as high as in the stoneware vessels. After the tenth day of fermentation, a decrease in the level of lactic acid was observed, with a decrease of 0.41 g/kg (2.5%) in the jars and 1.53 g/kg (13.1%) in the stoneware vessels. The conditions in the jars made it possible to maintain anaerobic conditions to a greater extent, and thus a better course of lactic fermentation, allowing for obtaining a high-quality product. At the end of fermentation, the lactic acid content should be between 1.5 and 2.0% [34]. Typically, the fermentation of sauerkraut is considered complete when it has reached a pH of approximately 3.8 or 1.5% lactic acid [35]. Such parameters were obtained

only in sauerkraut in glass jars on the tenth day of fermentation. The detected changes could be related to the microbiota present during fermentation, which created or degraded lactic acid. Moreover, the anaerobic conditions in the jar also modify the composition of the yeast microbiota, which eliminates cultures that can use lactic acid as a carbon source (deacidify), such as *Rhodotorula* yeast [36].

Acetic acid in fermented cabbages in both vessels was detected from the first day of fermentation, and several times more of this compound was found during fermentation in stoneware vessels than in jars. Acetic acid is formed by acetic acid bacteria and heterofermentative LAB, especially in aerobic conditions, as well as by some yeast cultures. They produce acetic acid mainly from sugars, but some strains can also convert lactic acid [37]. The concentration of acetic acid reached a maximum on the seventh day and then decreased in the following days. Reducing the level of acetic acid in the fermented vegetable may mean limiting the growth of the microbiota synthesizing this substance, and on the other hand, degradation/conversion of this compound to other components. In the study by Saeki et al. [38], the analyzed microorganisms were able to assimilate acetic acid after a prolonged lag phase. The analysis results showed the conversion of acetic acid to acetyl-CoA by acetyl-CoA synthetase using the TCA cycle and the glyoxylate cycle, enabling bacteria to grow on acetic acid after the depletion of other carbon sources.

Similar concentrations of oxalic acid were detected in sauerkraut regardless of the vessel used for fermentation. Drašković et al. [39] reported that the presence of this compound has a good effect on the storage of cabbage before fermentation due to its antioxidant properties and inhibitory effect on the spoilage of the raw vegetable. The above-mentioned publication proves that the concentration of this acid decreases as the storage temperature increases. It should be assumed that during fermentation, this compound is released into the environment, and the lactic acid bacteria present in it use this compound. The reduction in oxalic acid content was possibly due to the oxalate-degrading activity of lactic acid bacteria achieved by the transportation of oxalate by permease into the cells [40].

Succinic acid is one of the natural acids found in foods, such as broccoli, rhubarb, sugar beets, fresh meat extracts, various cheeses, and sauerkraut. Succinic acid is also one of the most important acids found in cabbage juice. During fermentation, it is partially metabolized by microorganisms, and sauerkraut juice contains about 50% of its initial content [41]. During sauerkraut fermentation, regardless of the vessel in which the process was carried out, a gradual decrease in the amount of succinic acid was observed (Table 2). However, the intensity of its level reduction was different—significantly higher in jars than in stoneware vessels. An inverse correlation was found between the number of lactic acid bacteria and the concentration of succinic acid. Sauerkraut obtained in glass jars contained more than two times less succinic acid than that produced in stoneware vessels.

3.4. The Influence of the Vessel Used for Fermentation on the Selected Sugars, Polyols, FAN Level, and Sensory Profile of Sauerkraut

The DNS method was used to analyze changes in the concentration of total and reducing sugars during fermentation [6]. Most of the sugars during fermentation were reducing sugars, such as glucose and fructose (Table 3). Chromatographic analysis (HPLC) of the content of these sugars during sauerkraut fermentation showed that the glucose level, regardless of the fermentation vessel used, gradually increased, while fructose decreased to zero in the case of stoneware vessels. White cabbage contains approximately 25 g/100 g DW of polysaccharides, constituting dietary fiber. They are mostly made of glucose [42]. During fermentation, the enzymatic activity of the microorganisms present causes the gradual release of simple sugars, which are used for growth and cellular metabolism [43]. Larger amounts of available sugars in the jars could have influenced the production of higher concentrations of lactic acid (Table 2). Fructose is present mainly In free, soluble form and is quickly metabolized by microorganisms. One of the products of its metabolism is mannitol [44].

Table 3. A heatmap of selected sugars, polyols, and FAN on subsequent days of fermentation of sauerkraut using various vessels.

	Vessel/Day	0	1	2	3	4	7	10	14	SEM [1]	Sig. [2]
FAN	Glass	69.6 ab	59.4 a	66.7 a	58.5 a	61.4 a	111.2 bcd	124.6 cd	137.0 d	8.4	***
	Stoneware	69.6 ab	61.3 a	61.6 a	79.6 ab	85.1 abc	136.4 d	138.6 d	139.4 d	7.0	
Total sugars	Glass	2.98 ab	4.52 cde	4.20 bcd	2.77 ab	3.17 abc	5.77 e	5.65 e	5.15 de	0.18	***
	Stoneware	2.98 ab	2.88 ab	3.01 ab	2.53 a	2.89 ab	3.23 abc	2.98 ab	3.31 abc	0.24	
Reductive sugars	Glass	1.91 cd	3.57 h	3.78 h	1.26 a	2.63 g	5.18 j	5.60 k	4.27 i	0.10	***
	Stoneware	1.91 cd	1.02 a	1.60 b	1.78 bc	2.45 fg	2.65 g	2.14 de	2.21 ef	0.30	
Glucose	Glass	1.36 b	0.84 a	1.18 ab	0.91 a	0.92 a	2.19 c	2.06 c	2.23 c	0.12	***
	Stoneware	1.36 b	1.39 b	1.41 b	0.90 a	0.86 a	6.60 e	3.34 d	0.89 a	0.39	
Fructose	Glass	1.39 e	0.37 b	0.03 a	0.71 d	0.07 a	0.71 d	0.62 cd	0.51 bcd	0.10	***
	Stoneware	1.39 e	0.31 b	0.46 bc	0.71 d	0.00 a	0.00 a	0.00 a	0.00 a	0.09	
Glycerol	Glass	0.00 a	0.00 a	0.00 a	0.10 a	0.67 c	1.78 f	1.51 e	1.03 d	0.15	***
	Stoneware	0.00 a	0.00 a	0.00 a	0.00 a	0.24 b	1.48 e	1.58 e	1.45 e	0.14	
Mannitol	Glass	1.70 b	2.04 bc	2.59 c	1.74 b	2.42 c	6.80 f	5.24 e	4.42 d	0.18	***
	Stoneware	1.70 b	2.60 c	2.52 c	2.45 c	1.70 b	0.67 a	0.52 a	0.73 a	0.37	

[1] SEM—standard error of the mean. [2] Sig.—significance. Values with different roman letters (a–k) of the same parameter (in rows) indicate statistically significant differences at $p < 0.05$; $n = 5$; 0.001 ***. Color determination from the lowest (0%) to highest (100%) concentration of parameters. The lowest concentration of a specific parameter in a row is in the darkest red, and the highest content is in the darkest green (−1 −0.5 0 0.5 1).

By the fourth day of fermentation, the mannitol level was similar in all samples (Table 3), ranging from 17.0 to 25.9 g/kg. In the following days, its amount decreased more than three-fold in stoneware vessels, while in jars it more than doubled. This phenomenon may be related to changes in the composition of the current microbiota responsible for the ongoing transformations, both yeast and bacterial. According to Johannigsmeier et al. [44], *Leuconostoc* bacteria, present during cabbage fermentation, are responsible for the production of mannitol. They carry out the hydrogenation of fructose using NADH- and NADPH-dependent enzymes [45]. *Leuconostoc* bacteria are heterofermentative lactic acid bacteria, facultatively anaerobic [46]. With a greater presence of oxygen (stoneware vessels), these bacteria may develop less strongly, and aerobic conditions may also favor the development of microbiota, which can use mannitol as a carbon source for its growth. Such microorganisms include yeast of the *Rhodotorula* genus [47].

Glycerol was not detected in samples up to the third day of fermentation (Table 3). In the following days, the amount of this polyol increased, reaching the maximum level on the seventh (jars) or tenth day of fermentation (stoneware). Then, the level of this compound decreased. Glycerol formation is usually associated with the presence of fungal microorganisms and *Saccharomyces* and non-*Saccharomyces* yeast [48]. Generally, glycerol production by prokaryotic microorganisms has not been studied as well as that by yeasts. Similar to yeasts, LAB can use enzymes of the glycerol kinase pathway for glycerol production. A process of glycerol production similar to that in yeasts may occur in LAB, also under stress conditions [49]. In the LAB group, various *Lb. plantarum* strains are most often mentioned as glycerol producers. Anoxic glycerol fermentation by various microbial species may lead to the production of a variety of other valuable compounds, such as 1,3-propanediol, 1,2-propanediol, propionic acid, succinic acid, ethanol, n-butanol, and reuterin [50].

Free amino nitrogen is very important for developing microorganisms, and it can also be a precursor to a whole range of compounds affecting the sensory properties of fermented products [51]. Changes in the FAN content during fermentation are presented in Table 3. The analysis showed no statistically significant impact of the vessel used for fermentation on its level. Maximum levels of amino nitrogen were detected in the final phase of fermentation, from the seventh day. The FAN content was influenced not only by amino acids released from the raw material, but also by those produced from dead microbial cells. High cell mortality, especially in spontaneous processes, at the end of fermentation may result in an increase in the content of the discussed group of compounds [52]. Their

too-high level in fermented products is not positively associated because it may cause turbidity and reduce the microbiological stability of the product [53].

Figure 5 presents the results of the sensory analysis of sauerkraut obtained by fermentation using two different types of vessels. The analysis showed little variation in characteristics between the obtained sauerkrauts and high acceptability of the products by the evaluators. The lack of differences in the results of the sensory analysis depending on the vessel used for fermentation could be due to the presence of small amounts of yeast cells in the cabbage used for fermentation (Figure 2), despite the detection of potentially spoilage yeast strains during fermentation in stoneware vessels (Figures 1 and 3). According to Laureiro and Querol [54], the presence of even 10^5 cells/g of yeast may not result in spoilage and does not affect the sensory characteristics. The level of microorganisms at which changes (such as discoloration, changes in taste and aroma, etc.) in food occur depend on the physicochemical properties of the product as well as the spoilage microorganism. As already stated, other authors detected much larger amounts of yeast in raw cabbage and later during its fermentation [19–21], which would result in greater changes in the sensory characteristics of sauerkraut. The statistically significant differences were found in the case of two parameters—pungent aroma and sweet taste (Figure 5). In the first case, it could be related to the different content of volatile compounds formed during fermentation. According to Li et al. [55], sulfur components that are formed from the decomposition of glycosinolates, such as allyl methyl disulfide and diallyl trisulfide, may be responsible for the pungent aroma. The greater sensation of sweet taste in sauerkraut obtained in glass jars could have been due to the almost three times higher concentration of sugars in the fermented vegetable (Table 3), mostly glucose.

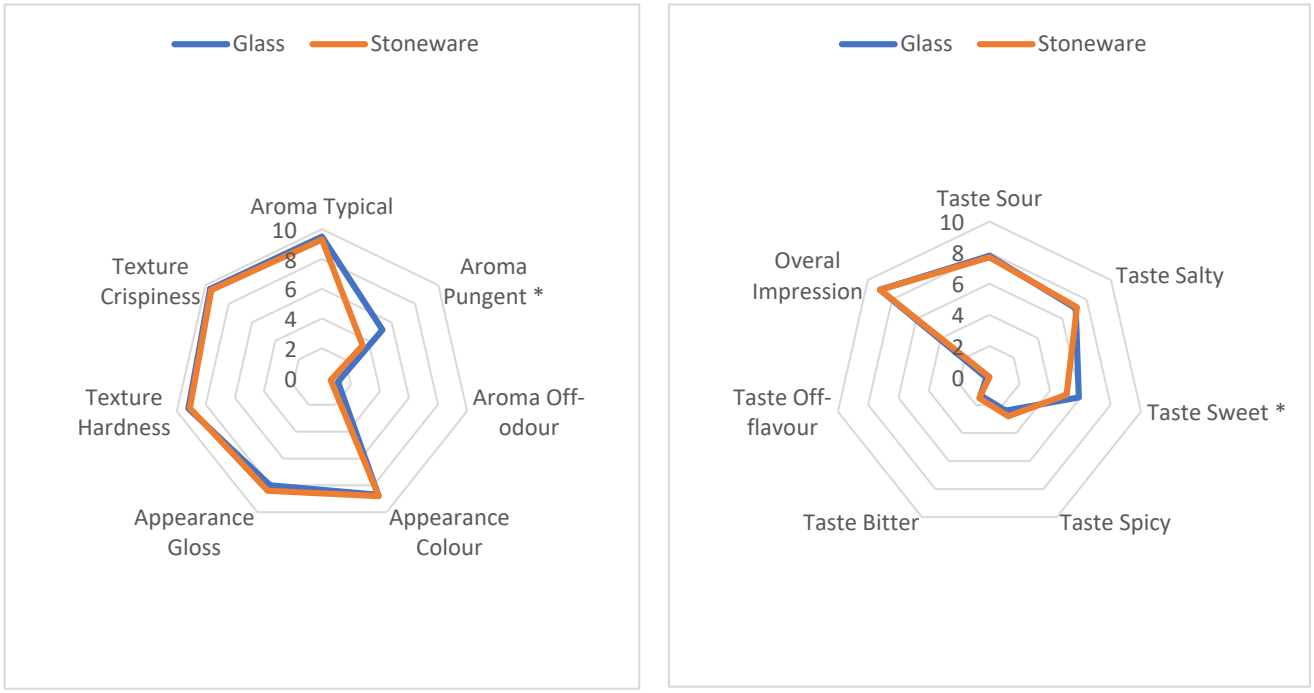

Figure 5. The spider web diagrams visualize the texture, appearance, and aroma qualities (**a**) and the taste qualities and overall impression (**b**) of sauerkraut produced using different fermentation vessels. Statistically significantly different parameters using ANOVA ($p < 0.05$) are marked with *.

4. Conclusions

In this research, we compared selected parameters important for the quality of sauerkraut during fermentation in two different vessels—glass jars and stoneware vessels. The fermentation process in glass jars had significantly more advantages: a larger number

of lactic acid bacteria, pH around 3.8, and 1.5% lactic acid, which are parameters indicating the end of fermentation of sauerkraut, were obtained on the tenth day of fermentation, and 50% more lactic acid was obtained in the finished product. The amount of yeast that may cause spoilage of sauerkraut was lower, and after the seventh day of fermentation, their presence was no longer detected. There was also no presence of film-forming yeast, such as representatives of the *Wickerhamomyces* genus.

However, further research is required in this regard, including the composition and characteristics of the bacterial microbiota (including those responsible for spoilage), the possibility of using various physical, chemical, and biological methods to stabilize products, determination of the level of individual spoilage microorganisms at which changes in sensory characteristics occur, etc. This knowledge will enable producers to obtain products of the highest quality with low economic losses.

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References

1. Steinkraus, K.H. Classification of fermented foods: Worldwide review of household fermentation techniques. *Food Control* **1997**, *8*, 311–317. [CrossRef]
2. Motarjemi, Y. Impact of small-scale fermentation technology on food safety in developing countries. *Int. J. Food Microbiol.* **2002**, *75*, 213–229. [CrossRef]
3. Satora, P.; Celej, D.; Skotniczny, M.; Trojan, N. Identyfikacja drożdży obecnych w kiszonej kapuście komercyjnej i otrzymywanej w gospodarstwach rolnych. *Żywność Nauka Technol. Jakość* **2017**, *24*, 27–36. [CrossRef]
4. Li, K.-Y. Fermentation. In *Handbook of Food and Beverage Fermentation Technology*; Hui, Y.H., Meunier-Goddik, L., Hansen, Å.S., Josephsen, J., Nip, W.K., Stanfield, P.S., Toldrá, F., Eds.; Marcel Dekker Inc.: New York, NY, USA, 2004; pp. 595–610.
5. Satora, P.; Skotniczny, M.; Strnad, S.; Ženišová, K. Yeast Microbiota during Sauerkraut Fermentation and Its Characteristics. *Int. J. Mol. Sci.* **2020**, *21*, 9699. [CrossRef]
6. Marsden, L.M.; Gray, P.P.; Nippard, G.J.; Quinlan, M.R. Evaluation of the DNS Method for Analysing Lignocellulosic Hydrolysates. *J. Chem. Technol. Biotechnol.* **1982**, *32*, 1016–1022. [CrossRef]
7. Abernathy, D.G.; Spedding, G.; Starcher, B. Analysis of protein and total usable nitrogen in beer and wine using a microwell ninhydrin assay. *J. Inst. Brew.* **2009**, *115*, 122–127. [CrossRef]
8. Gajewski, M.; Radzanowska, J. Skład chemiczny i jako sensoryczna kapusty głowiastej w zaleznosci od jej odmiany i dawki azotu stosowanej w nawozeniu mineralnym. *Żywność Nauka Technol. Jakość* **2004**, *2*, 108–120.
9. Quereda Vázquez, F.; Lorente-Ayza, M.; Ignacio, N.; Maria, D.A.; Escrig Marin, P.; Ojeda Domenech, M. Red firing clays for the manufacture of vessels for wine fermentation and maturation by means of technological processes. *Open Ceram.* **2022**, *9*, 100232. [CrossRef]
10. Padilla, B.; Gil, J.; Manzanares, P. Challenges of the Non-Conventional Yeast *Wickerhamomyces anomalus* in Winemaking. *Fermentation* **2008**, *4*, 68. [CrossRef]
11. Passoth, V.; Fredlund, E.; Druvefors, U.Ã.; Schnorer, J. Biotechnology, physiology and genetics of the yeast *Pichia anomala*. *FEMS Yeast Res.* **2006**, *6*, 3–13. [CrossRef]
12. Liu, K.-F.; Li, X.-H.; Hui, F.-L. *Yarrowia brassicae* f.a., sp. nov., a new yeast species from traditional Chinese sauerkraut. *Int. J. Sys. Evol. Microbiol.* **2018**, *68*, 2024–2027. [CrossRef] [PubMed]
13. Torres, S.S.; Verón, H.; Contreras, L.; Isla, M.I. An overview of plant-autochthonous microorganisms and fermented vegetable foods. *Food Sci. Hum. Wellness* **2020**, *9*, 112–123. [CrossRef]

14. Coda, R.; Cassone, A.; Rizzello, C.; Nionelli, l.; Cardinali, G.; Gobetti, M. Antifungal activity of *Wickerhamomyces anomalus* and *Lactobacillus plantarum* during sourdough fermentation: Identification of novel compounds and longterm effect during storage of wheat bread. *Appl. Environ. Microbiol.* **2011**, *77*, 3484–3492. [CrossRef] [PubMed]
15. Satora, P.; Tarko, T.; Sroka, P.; Blaszczyk, U. The influence of *Wickerhamomyces anomalus* killer yeast on the fermentation and chemical composition of apple wines. *FEMS Yeast Res.* **2014**, *14*, 729–740. [CrossRef] [PubMed]
16. Zhang, B.-Q.; Luan, Y.; Duan, C.-Q.; Yan, G.-L. Use of *Torulaspora delbrueckii* Co-fermentation With Two *Saccharomyces cerevisiae* Strains With Different Aromatic Characteristic to Improve the Diversity of Red Wine Aroma Profile. *Front. Microbiol.* **2018**, *9*, 606. [CrossRef] [PubMed]
17. Canonico, L.; Agarbati, A.; Comitini, F.; Ciani, M. *Torulaspora delbrueckii* in the brewing process: A new approach to enhance bioflavour and to reduce ethanol content. *Food Microbiol.* **2016**, *56*, 45–51. [CrossRef] [PubMed]
18. Rantsiou, K.; Urso, R.; Dolci, P.; Comi, G.; Cocolin, L. Microflora of Feta cheese from four Greek manufacturers. *Int. J. Food Microbiol.* **2008**, *126*, 36–42. [CrossRef]
19. Alan, Y.; Yildiz, N. Effects of *Lactobacillus* used as the starter culture on naturally fermented pickled cabbage. *Food Sci. Technol.* **2022**, *42*, e45020. [CrossRef]
20. Geeson, J.D. The Fungal and Bacterial Flora of Stored White Cabbage. *J. Appl. Bacteriol.* **1979**, *46*, 189–193. [CrossRef]
21. Yang, X.; Hu, W.; Xiu, Z.; Jiang, A.; Yang, X.; Saren, G.; Feng, K. Effect of salt concentration on microbial communities, physicochemical properties, and metabolite profile during spontaneous fermentation of Chinese northeast sauerkraut. *J. Appl. Microbiol.* **2020**, *129*, 1458–1471. [CrossRef]
22. Shih, C.T.; Hang, Y.D. Production of carotenoids by *Rhodotorula rubra* from sauerkraut brine. *LTW-Food Sci. Technol.* **1996**, *29*, 570–572.
23. Lücke, F.-K.; Zangerl, P. Food safety challenges associated with traditional foods in German-speaking regions. *Food Control* **2004**, *43*, 217–230. [CrossRef]
24. Mendoza, L.M.; de Nadra, M.C.M.; Farías, M.E. Antagonistic interaction between yeasts and lactic acid bacteria of oenological relevance. *Food Res. Int.* **2010**, *43*, 1990–1998. [CrossRef]
25. Du Toit, M.; Pretorius, I.S. Microbial spoilage and preservation of wine: Using weapons from nature's own arsenal—A review. *S. Afr. J. Enol. Vitic.* **2000**, *21*, 74–92. [CrossRef]
26. Legan, J.D.; Voysey, P.A. Yeast spoilage of bakery products and ingredients. *J. Appl. Bacteriol.* **1991**, *70*, 361–371. [CrossRef] [PubMed]
27. Hung, L.-D.; Kyung, K.H. Inhibition of yeast film formation in fermented vegetables by materials derived from garlic using cucumber pickle fermentation as model system. *Food Sci. Biotechnol.* **2006**, *15*, 469–473.
28. Shah, N.N.; Singhal, R.S. Fermented Fruits and Vegetables–Fermented Cabbage. In *Current Developments in Biotechnology and Bioengineering*; Elsevier: Amsterdam, The Netherlands, 2017; Volume 3.3.1, pp. 59–62.
29. Tkáčová, J.; Furdíková, K.; Klempová, T.; Ďurčanská, K.; Čertík, M. Screening of carotenoid-producing *Rhodotorula* strains isolated from natural sources. *Acta Chim. Slov.* **2015**, *8*, 34–38. [CrossRef]
30. Satora, P.; Skotniczny, M.; Strnad, S.; Piechowicz, W. Chemical composition and sensory quality of sauerkraut produced from different cabbage varieties. *LWT-Food Sci. Technol.* **2021**, *136*, 110325. [CrossRef]
31. Rao, Y.; Qian, Y.; Tao, Y.; She, X.; Li, Y.; Che, Z.; Li, H.; Liu, L. Influence of oxygen exposure on fermentation process and sensory qualities of Sichuan pickle (paocai). *RSC Adv.* **2019**, *9*, 38520–38530. [CrossRef]
32. Viander, B.; Maki, M.; Palva, A. Impact of low salt concentration, salt, quality on natural large-scale sauerkraut fermentation. *Food Microbiol.* **2003**, *20*, 391–395. [CrossRef]
33. Liu, Y.; Chen, X.; Li, F.; Shi, H.; He, M.; Ge, J.; Ling, H.; Cheng, K. Analysis of Microbial Diversity and Metabolites in Sauerkraut Products with and without Microorganism Addition. *Foods* **2023**, *12*, 1164. [CrossRef] [PubMed]
34. Holzapfel, W.; Schillinger, U.; Buckenhüskes, H.; Sauerkraut. *Handbook of Fermented Functional Foods*; Farnworth, E.R., Ed.; CRC Press: Boca Raton, FL, USA, 2008; pp. 395–412.
35. Andress, E.; Ingham, B.; Snyder, A.; Breidt, F. Manufacture of Traditionally Fermented Vegetable Products: Best Practice for Small Businesses and Retail Food Establishments. *Food Prot. Trends* **2021**, *40*, 251–263.
36. Daeschel, M.A.; Andersson, R.E.; Fleming, H.P. Microbial ecology of fermenting plant materials. *FEMS Microbiol. Lett.* **1987**, *46*, 357–367. [CrossRef]
37. Gomes, A.L.M.; Bueno, A.V.I.; Osmari, M.P.; Machado, J.; Nussio, L.G.; Jobim, C.C. Effects of Obligate Heterofermentative Lactic Acid Bacteria Alone or in Combination on the Conservation of Sugarcane Silage. *Front. Microbiol.* **2021**, *12*, 643879. [CrossRef] [PubMed]
38. Saeki, A.; Taniguchi, M.; Matsushita, K.; Toyama, H.; Theeragool, G.; Lotong, N.; Adachi, O. Microbiological Aspects of Acetate Oxidation by Acetic Acid Bacteria, Unfavorable Phenomena in Vinegar Fermentation. *Biosci. Biotechnol. Biochem.* **1997**, *61*, 317–323. [CrossRef]
39. Drašković, M.; Horecki, A.Y.; Šumić, Z.; Malbaša, R.; Vitas, J.; Pavlić, B.; Vakula, A. Variation of bioactive compounds content in fermented cabbage: Influence of temperature. *J. Process. Energy Agric.* **2017**, *21*, 136–141. [CrossRef]
40. Huynh, N.K.; Nguyen, D.H.M.; Nguyen, H.V.H. Effects of processing on oxalate contents in plant foods: A review. *J. Food Compos. Anal.* **2022**, *112*, 104685. [CrossRef]

41. Jeong, E.J.; Moon, D.W.; Oh, J.S.; Moon, J.S.; Seong, H.; Kim, K.Y.; Han, N.S. Development of Cabbage Juice Medium for Industrial Production of *Leuconostoc mesenteroides* Starter. *J. Microbiol. Biotechnol.* **2017**, *27*, 2112–2118. [CrossRef]
42. Wennberg, M.; Ekvall, J.; Olsson, K.; Nyman, M. Changes in carbohydrate and glucosinolate composition in white cabbage (*Brassica oleracea* var. *capitata*) during blanching and treatment with acetic acid. *Food Chem.* **2006**, *95*, 226–236. [CrossRef]
43. Kulshrestha, S.; Tyagi, P.; Sindhia, V.; Yadavilli, K.S. Invertase and its applications—A brief review. *J. Pharm. Res.* **2013**, *7*, 792–797. [CrossRef]
44. Johannigsmeier, S.D.; McFeeters, J.F.; Fleming, H.P.; Thompson, R.L. Effects of *Leuconostoc mesenteroides* Starter Culture on Fermentation of Cabbage with Reduced Salt Concentrations. *J. Food Sci.* **2007**, *72*, 166–172.
45. Bhatt, S.M.; Mohan, A.; Srivastava, S.K. Challenges in Enzymatic Route of Mannitol Production. *ISRN Biotechnol.* **2013**, *2013*, 1–13. [CrossRef] [PubMed]
46. Kostinek, M.; Specht, I.; Edward, V.A.; Pinto, C.; Egounlety, M.; Sossa, C.; Mbugua, S.; Dortu, C.; Thonart, P.; Taljaard, L.; et al. Characterisation and biochemical properties of predominant lactic acid bacteria from fermenting cassava for selection as starter cultures. *Int. J. Food Microbiol.* **2007**, *114*, 342–351. [CrossRef] [PubMed]
47. Shaigani, P.; Awad, D.; Redai, V. Oleaginous yeasts- substrate preference and lipid productivity: A view on the performance of microbial lipid producers. *Microb. Cell Fact.* **2021**, *20*, 220. [CrossRef] [PubMed]
48. Ivit, N.N.; Longo, R.; Kemp, B. The Effect of Non-*Saccharomyces* and *Saccharomyces* Non-*Cerevisiae* Yeasts on Ethanol and Glycerol Levels in Wine. *Fermentation* **2020**, *6*, 77. [CrossRef]
49. Gänzle, M.G. Lactic metabolism revisited: Metabolism of lactic acid bacteria in food fermentations and food spoilage. *Curr. Opin. Food Sci.* **2015**, *2*, 106–117. [CrossRef]
50. Kryachko, Y.; Batbayar, B.; Tanaka, T.; Nickerson, M.T.; Korber, D.R. Production of glycerol by *Lactobacillus plantarum* NRRL B-4496 and formation of hexamine during fermentation of pea protein enriched flour. *J. Biotechnol.* **2020**, *323*, 331–340. [CrossRef]
51. Lekkas, C.; Stewart, G.G.; Hill, A.; Taidi, B.; Hodgson, J. The Importance of Free Amino Nitrogen in Wort and Beer. *Tech. Q. Master Brew. Assoc. Am.* **2005**, *42*, 113–116.
52. Guadalupe-Daqui, M.; Chen, M.; Sarnoski, P.J.; Goodrich-Schneider, R.M.; MacIntosh, A.J. Impacts of Reduced (Vacuum) Pressure on Yeast Fermentation as Assessed Using Standard Methods and Automated Image Analysis. *Fermentation* **2023**, *9*, 155. [CrossRef]
53. Bell, S.-J.; Henschke, P.A. Implications of nitrogen nutrition for grapes, fermentation and wine. *Aust. J. Grape Wine Res.* **2005**, *11*, 242–295. [CrossRef]
54. Loureiro, V.; Querol, A. The prevalence and control of spoilage yeasts in foods and beverages. *Trends Food Sci. Technol.* **1999**, *10*, 356–365. [CrossRef]
55. Li, H.; Guan, H.; Zhang, X.; Xing, S.; Liu, W.; Kim, I.-C.; Gong, H. The Impact of Different Cooking Methods on the Flavor Profile of Fermented Chinese Spicy Cabbage. *Molecules* **2023**, *28*, 6539. [CrossRef] [PubMed]