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Impact of Selected Yeast Strains on Quality Parameters of Obtained Sauerkraut

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Abstract: The aim of this research was to determine the influence of yeast strains (previously isolated from the fermentation process) on selected quality parameters of sauerkraut. For this purpose, shredded and salted (2.5% *w*/*w*) cabbage of the Galaxy variety was fermented in the absence of oxygen with the addition of 2×10^6 cells of a selected yeast culture. The control sample was spontaneously fermented sauerkraut without yeast addition. The obtained sauerkraut was analysed in terms of the content of selected organic acids, sugars and polyols (HPLC), selected volatile compounds (HS-SPME-GC-TOFMS), colour (CieLAB) and aroma (QDA). Yeast *P. fermentans*, *Rh. mucilaginosa* and *W. anomalus* reduced crucial sauerkraut components such as lactic acid, glycerol, and certain volatile compounds, leading to decreased aroma intensity and acceptability. Additionally, an increase in glucosinolate decomposition products was observed. Conversely, *D. hansenii* positively influenced sauerkraut quality by enhancing lactic acid content and exhibiting similar volatile characteristics to those of the control. Two of the three samples fermented with *D. hansenii* received high sensory analysis scores akin to those of the control. Sauerkraut fermented with *Cl. lusitaniae* yeast contained elevated levels of volatile compounds—alcohols, esters and lactones—resulting in an intense floral aroma, albeit receiving lower overall ratings due to deviation from the typical profile.

Keywords: sauerkraut; yeasts; volatile components; organic acids; *Debaryomyces hansenii*

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1. Introduction

Yeasts play a minor role in sauerkraut production compared with lactic acid bacteria (LAB), but they still contribute to the fermentation process. While lactic acid bacteria are primarily responsible for converting sugars into lactic acid, which preserves the cabbage and gives sauerkraut its tangy flavour, yeasts can also be present in the fermentation environment. Yeasts can occur naturally on the surface of the cabbage or be introduced from the surrounding environment [1].

In sauerkraut production, yeasts can contribute to the fermentation process by metabolising sugars and producing alcohol and carbon dioxide. However, their contribution to the overall flavour and texture of sauerkraut is usually less significant than that of lactic acid bacteria. Furthermore, the acidic environment produced by lactic acid bacteria restricts the growth of the majority of yeasts, thereby limiting their activity during the fermentation process [2].

Although yeast is not as prominent in sauerkraut production as lactic acid bacteria, its presence underlines the complexity of the microbial interactions involved in the fermentation process. In general, the combined activity of yeast and lactic acid bacteria contributes to the unique flavour, texture and preservation of sauerkraut [3].

The quality of sauerkraut is significantly impacted by the conditions under which fermentation occurs and the microbiota involved in the process. In Poland, it is common practice to add NaCl at a concentration of 2–2.5% and to ferment the cabbage in tanks, often made of concrete. To isolate the cabbage from the external environment during

fermentation and release the juice necessary for fermentation, these tanks are often covered with a plastic bag filled with water and pressure is applied [2]. However, this system is not completely airtight and allows oxygen to enter the tank, which alters fermentation conditions and the microbiota present. The proliferation of yeasts, particularly film-forming strains, is encouraged as a result, which alters the chemical composition of the fermented vegetable. These yeasts use organic acids, especially lactic acid, as a carbon source, resulting in a decrease in acidity and an increase in pH. These changes can lead to the growth of spoilage bacteria and other spoilage microorganisms [3].

Our previous research has shown that cultures of *Debaryomyces hansenii*, *Rhodotorula mucilaginosa* and *Pichia fermentans* are consistently detected during sauerkraut fermentation, irrespective of the cabbage variety used and the fermentation conditions (anaerobic/semiaerobic). Representatives of the two species *Clavispora lusitaniae* and *Wickerhamomyces anomalus* only occur in environments with or without oxygen [4].

The aim of this research was to determine the influence of yeast strains (previously isolated from different stages of the fermentation process) on selected quality parameters of sauerkraut. The most important parameters for the quality of sauerkraut were analysed, such as the content of the main organic acids, sugars and polyols, the profile of volatile compounds and the CIE LAB colour, and a sensory analysis of the aroma of sauerkraut was carried out using the QDA (quantitative data analysis) method.

2. Materials and Methods

2.1. Raw Material and Microorganisms

Galaxy cabbage (Brassica oleracea var. capitata) was used for fermentation. The cabbages were obtained from growers in the cabbage capital of Poland (the municipality of Charsznica, Małopolska). The yeast used in the experiments was isolated in previous research [4,5]. The current research employed the most common strains found at different stages of fermentation and under anaerobic and microaerobic conditions. Table 1 presents the description of the strains used.

Table 1. Yeast strains used in experiments.

Species	Strain	Isolation		Homology [%]	
		Day of Fermentation	Vessel		
Pichia fermentans	Pf9	4	Glass	100.0	MT645416
Wickerhamomyces anomalus	Wa1	1	Stoneware	99.0	KY657575
Wickerhamomyces anomalus	Wa7	2	Stoneware	99.6	KY105860
Wickerhamomyces anomalus	Wa22	4	Stoneware	98.4	KT580795
Rhodotorula mucilaginosa	Rm8	1	Glass	100.0	OQ692821
Rhodotorula mucilaginosa	Rm26	0	Glass	99.8	JQ293997
Debaryomyces hansenii	Dh17	0	Glass	99.0	MT192508
Debaryomyces hansenii	Dh19	1	Glass	99.2	MK275230
Debaryomyces hansenii	Dh31	4	Glass	98.3	KM816678
Clavispora lusitaniae	Cl6	7	Glass	99.0	MK312615

Yeast strains were cultured in replicates on Sabouraud Dextrose LAB-AGAR medium slants at 28 °C for 24 h. Ten millilitres of sterile Sabouraud dextrose broth was inoculated with five loops of the slurry. The flasks were sealed with gauze and cotton-wool plugs, and then placed on a shaker at 20 °C for 24 h. The slurry was then transferred to 40 mL of sterile Sabouraud dextrose broth and incubated on a shaker at 20 °C for a further 24 h. At the end of yeast propagation, the number of yeast cells was determined using a Thoma

chamber. On the basis of the results obtained, the quantity of individual yeast cultures was calculated to achieve 2×10^6 cells per g of inoculated cabbage.

2.2. Fermentation of Sauerkraut

After preliminary cleaning of the cabbage from dirt and removal of the core and outer leaves until the white ones appeared, the cabbage was shredded into 2.5 mm thick strips using a sterile automatic slicer (Ma-Ga 612p). An amount of 150 g of cabbage was weighed into a 250 mL sterile glass jar. The surface was covered with powdered NaCl (99.5%, Loba Chemie PVT Ltd., Mumbai, India) to obtain a concentration of 2.5% (*w*/*w*). The cabbage was kneaded until it was covered with the juice. The samples were then inoculated with different selected yeast strains (Table 1) to obtain 2×10^6 CFU of each strain per g of cabbage. The control sample was cabbage not inoculated with yeast. The jars were sealed with caps containing fermentation tubes. Each variant was run in triplicate, and fermentation was carried out at 20 °C for 14 days. Prior to analysis, the samples were stored in zip-lock bags at −80 °C.

2.3. Determination of Sugars and Organic Acids via HPLC

HPLC was used to determine the sugars and organic acids in sauerkraut. The Shimadzu (Kyoto, Japan) NEXERA XR chromatograph equipped with a Rezex ROA-Organic Acid H+ (8%) column was used to analyse the organic acids. The sauerkraut homogenate was centrifuged, and the supernatant was filtered using syringe filters (ø = 0.45 µm). To ensure accurate measurements, the samples were diluted five times with distilled water. Organic acids were detected using a UV-Vis detector (λ = 210 nm). To calculate the concentration of each compound, we prepared standard curves for lactic acid, acetic acid, succinic acid, citric acid and oxalic acid (Sigma-Aldrich (St. Louis, MO, USA)) beforehand.

The analysis of sugars and polyols was conducted using a Shimadzu (Japan) NEXERA XR instrument with an RF-20A refractometric detector and an Asahipak NH2P-50 4.6 × 250 mm Shodex column (Showa Denko Europe, Munich, Germany) maintained at 30 °C. The mobile phase consisted of an aqueous solution of 70% acetonitrile, and the isocratic elution programme lasted for 16 min at a flow rate of 0.8 mL/min. Standard curves were used to carry out quantitative determinations for glucose, fructose, glycerol, ethanol and mannitol (Sigma-Aldrich).

2.4. Determination of Volatile Compounds (HS-SPME-GC-TOFMS)

Each homogenised sample (2 mL) was transferred to a 15 mL vial, followed by the addition of 2 mL of supersaturated NaCl solution and 0.1 mL of the internal standard solution (consisting of 50 mg/L 4-methyl-2-pentanol, 5 mg/L ethyl nonanoate and 5 mg/L anethole). Analyses were performed using an MPS autosampler (Gerstel, Mülheim an der Ruhr, Germany) equipped with automatic solid-phase microextraction (SPME) functionality. The equilibration time was set to 5 min at 40 °C. Volatile headspace compounds were extracted and concentrated using a polydimethylsiloxane-coated phase microextraction fibre (100 µm PDMS, Supelco Inc., Bellefonte, PA, USA). The fibre was positioned in the headspace (HS) above the samples at 40 °C for 35 min. The volatiles that were adsorbed on the SPME fibre underwent desorption at 250 °C for 3 min in the injector port of an Agilent Technologies 7890B chromatography system (Agilent Technologies, Santa Clara, CA, USA) that was connected to a Pegasus HT TOFMS (Time-of-Flight Mass Spectrometer) (LECO Corporation, St. Joseph, MI, USA) operating in the electron ionisation mode. Chromatographic separation was performed using an Rtx-1ms capillary column (Crossbond 100% dimethylpolysiloxane, 30 m × 0.53 mm × 0.5 µm; Restek, Bellefonte, PA, USA). Injector and detector temperatures were kept constant at 250 °C. The compounds were separated by starting at 40 °C for 3 min, followed by a temperature ramp of 8 °C/min until it reached 230 °C. Samples were then held at the maximum temperature for 9 min. Helium was used as the carrier gas at a constant electronically controlled flow rate of 1 mL/min. The transfer line and ion source temperatures were set to 250 °C, and the ion source voltage

was set to 70 eV. The analyte was transferred in splitless mode. Mass spectra were recorded in SEM mode. Compound identification was performed using mass spectral libraries and linear retention indices derived from the C6 to C30 n-alkane series. The identification of volatiles (Sigma-Aldrich) was performed qualitatively and quantitatively by comparing retention times and peak areas from both sample and standard chromatograms. Any other detected components were evaluated semi-quantitatively (in μg/L). The semi-quantitative analysis was determined by calculating the ratio between the relative peak area of each identified component and the relative peak area of the corresponding internal standard (ethyl nonanoate for esters, anethole for terpenoids and 4-methyl-2-pentanol for other components). The obtained results were analysed using the NIST database.

2.5. Colour of Sauerkraut

A colour assessment was carried out using the CIE (Lab*) system through an instrumental method. The reflectance method was used to take measurements with a Konica Minolta CM-3500d spectrophotometer (Konica Minolta, Inc., Tokyo, Japan) with reference to illuminant D65 and a visual angle of 10°. The parameters measured were L* (lightness) (L* = 0 blackness, L* = 100 whiteness), a* (amount of green) (a* < 0) or red (a* > 0), b* (amount of blue) (b* < 0) or yellow (b* > 0), C* (chroma) and h° (hue angle). Delta E (ΔE) is a measure of the difference between the colour of the test sample and that of the control. It is calculated as the square root of the sum of the differences between each of the L, a and b values.

2.6. Aroma Analysis (QDA)

The sauerkraut samples underwent aroma assessment through quantitative descriptive analysis (QDA). The evaluation was conducted by a panel of 10 trained individuals, comprising 5 men and 5 women, aged between 30 and 50 years, who are employees of the Department of Fermentation Technology and Microbiology. The assessment took place in a room designed in accordance with the ISO 8589 standard [6]. Nine aroma qualities were evaluated on a ten-point scale, including overall impression and aroma intensity, aroma typicality, acetic, sulphur, green, metallic, animal, floral and pungent. The panellists were trained to recognise specific aroma characteristics using Flavouractiv standards from Waltington, UK.

2.7. Statistical Analysis

The experiments were conducted and analysed in triplicate. However, the figures and tables show only the average values. Statistical analysis was carried out using SPSS 13.0 software. The charts present the results as arithmetic means with the standard error of the mean (SEM). Multi-factor analysis of variance (ANOVA) with the post hoc Duncan test was used to evaluate statistically significant differences between results ($p < 0.05$). PCA was performed using SPSS version 23 software (Chicago, IL, USA).

3. Results and Discussion

3.1. Organic Acids and Sugars

Yeast is consistently present during sauerkraut fermentation. The conditions prevailing during this process may increase or limit their proliferation. They also influence the qualitative composition of the yeast microbiota [4]. Previous studies have demonstrated that representatives of the species *Debaryomyces hansenii*, *Rhodotorula mucilaginosa* and *Pichia fermentans* are present during sauerkraut fermentation regardless of the prevailing conditions. Aerobic conditions facilitate the growth of the yeast *Wickerhamomyces anomalus*, while anaerobic conditions promote the development of *Clavispora lusitaniae*.

In the current research, our aim is to demonstrate how isolated yeast strains influence the quality of sauerkraut produced. To achieve this, we introduced cells of different yeast isolates into salted cabbage in quantities sufficient for them to propagate and colonise the

environment. We selected the Galaxy variety for fermentation, which is commonly utilised by sauerkraut producers in Poland.

Lactic acid is widely acknowledged as the most crucial component of sauerkraut, as it not only contributes to its characteristic sensory attributes but also enhances its microbiological stability. Fully fermented sauerkraut usually contains 1.8–2.3% acid, which is calculated as lactic acid, resulting in a pH of 3.5 or lower [7]. Throughout the fermentation process, the content of lactic acid, the main product of sauerkraut, gradually increases, ranging from 6.99 to 9.78 g/kg on day 30 [8]. Sauerkraut produced without the addition of yeast contained 9.3 g/kg of lactic acid. The remaining samples can be categorised into two groups: those exhibiting a higher content compared with that of the control and those with a lower lactic acid concentration compared with that of the control (Table 2). The former group includes sauerkraut produced with the yeast *D. hansenii*, while the latter comprises the rest of the samples. There have been no reports so far regarding the ability of the yeast *D. hansenii* to produce lactic acid. However, as indicated by previous research, certain yeast cultures have been shown to facilitate the growth of lactic acid bacteria [9]. Furthermore, *D. hansenii* has been suggested to provide essential growth factors for bacteria, including vitamins (such as lactoflavone, thiamine, pantothenic acid, nicotinic acid, folic acid and biotin), amino acids, and aroma components, as well as lipolytic and proteolytic enzymes, all of which contribute to the ripening process of certain fermented foods [10]. Yeast can potentially pose a risk as a food spoilage microorganism by consuming lactic acid, raising the pH and creating favourable conditions for the proliferation of other spoilage microorganisms [11]. The most significant reduction in lactic acid, by 2.8 g/kg compared with that of the control, was observed in sauerkraut obtained with the yeast strains *W. anomalus* Wa1 and *P. fermentans* Pf9 (Table 2). Representatives of the former *Pichia* genus are known for their ability to assimilate various carbon substrates, including organic acids [12]. Their presence, particularly in the form of a film on the surface of the product, often leads to deacidification and sensory alterations in the product [13].

The second important organic acid formed during fermentation is acetic acid [14]. Once again, the highest levels of acetic acid were observed in sauerkraut produced with the yeast *D. hansenii*. In contrast, the lowest amount of this compound, nearly half of that in the control, was generated in samples inoculated with *R. mucilaginosa* Rm26 and *P. fermentans* Pf9 (Table 2). Yeast species can produce varying amounts of acetic acid, influenced by their specific characteristics and the prevailing fermentation conditions. Some yeast strains have the ability to metabolise acetic acid into biomass, as well as a wide variety of aroma compounds, such as acetates [15]. Decreasing the concentration of acetic acid may be advantageous for sauerkraut, as this component imparts a sharp vinegary aroma to the product even in small amounts. A small amount of acetic acid is acceptable; in fact, high-quality sauerkraut typically maintains an acetic acid to lactic acid ratio of 1:4 and lower [16].

The quantity of other analysed organic acids varied slightly among the samples (Table 2). These acids originate primarily from the raw material and cabbage, and their levels typically decrease during fermentation, as they are used as a carbon source by yeast [17].

White cabbage contains 110–220 mg per g of the DW (dry weight) of fermentable sugars, mainly glucose and fructose [18]. During fermentation, microorganisms gradually use sugars for their growth and cellular metabolism [19]. The increased availability of sugars in the fermentation environment may have contributed to the production of higher concentrations of lactic acid. Fructose and glucose were the main sugars that remained in the fermented material (Table 2). The samples contained from 0.84 to 6.70 g/kg of glucose and from 2.22 to 4.67 g/kg of fructose.

Table 2. A heatmap of the content of selected organic acids, sugars and polyols in sauerkraut produced with the addition of different yeasts.

(g/kg)	Control	*D. hansenii Dh17*	*D. hansenii Dh19*	*D. hansenii Dh31*	*W. anomalus Wa1*	*W. anomalus Wa7*	*W. anomalus Wa22*	*R. mucilaginosa Rm8*	*R. mucilaginosa Rm26*	*P. fermentans Pf9*	*C. lusitaniae Cl6*	SEM [1]	Sig. [2]
Oxalic acid	1.25	1.53	1.89	1.27	1.28	1.08	1.34	1.67	1.31	1.68	1.49	0.06	ns
Citric acid	0.91 bc	0.90 bc	1.05 c	0.74 ab	0.80 bc	1.04 bc	0.78 bc	1.06 c	0.49 a	0.76 a–c	0.98 bc	0.04	**
Succinic acid	0.90	0.82	1.03	0.79	0.71	0.87	0.76	0.94	0.74	0.64	1.01	0.03	ns
Lactic acid	9.3 ab	13.4 c	10.6 bc	10.4 a–c	6.5 a	7.9 ab	7.6 ab	8.5 ab	8.2 ab	6.5 a	7.7 ab	0.5	*
Acetic acid	1.05 a–c	1.40 cd	1.69 d	1.44 cd	1.24 b–d	1.52 cd	1.02 a–c	1.46 cd	0.54 a	0.75 ab	1.45 cd	0.07	***
Glucose	4.15 cd	5.40 de	6.70 e	4.47 cd	2.02 ab	6.42 e	0.84 a	4.64 cd	3.48 bc	2.53 b	4.76 cd	0.34	***
Fructose	2.46 ab	2.62 a–c	4.17 de	3.23 a–d	4.17 de	4.67 e	3.54 cd	3.43 b–d	2.65 a–c	2.22 a	3.60 cd	0.16	***
Mannitol	5.67 ab	7.63 cd	8.80 d	6.88 b–d	6.22 a–c	8.13 cd	6.31 a–c	7.14 b–d	5.65 ab	4.35 a	7.47 b–d	0.27	***
Glycerol	0.84 a–d	0.99 d	0.88 b–d	0.77 a–d	0.65 ab	0.67 ab	0.71 a–c	0.75 a–d	0.95 cd	0.62 a	0.69 a–c	0.03	*
Ethanol	4.31 c–e	5.35 e–g	5.78 fg	5.67 fg	4.14 b–d	5.71 fg	3.30 bc	6.13 g	2.99 ab	1.96 a	4.78 d–f	0.25	***
									−1	−0.5	0	0.5	1

[1] SEM—standard error of means; [2] Sig.—significance; *, **, ***—significance at 0.05, 0.01 and 0.005 according to the least significant difference; ns—not significant. Values with different Roman letters (a–g) of the same parameter (in raw) indicate statistically significant differences at $p < 0.05$; n = 5. The concentration of a specific parameter is represented by a colour gradient ranging from dark red (lowest concentration) to dark green (highest concentration).

Fructose, mainly present in a free, soluble form, is rapidly metabolised by microorganisms. One of the metabolic byproducts of fructose metabolism is mannitol [20]. Mannitol is primarily produced by lactic acid bacteria, mainly of the *Leuconostoc* genus [21]. Yeast can utilise this component as a carbon source [22]. This phenomenon is particularly noticeable in samples fermented with yeasts of the *P. fermentans* Pf9 and *Rh. mucilaginosa* Rm26 strains, where the lowest amounts of mannitol were found. Both microorganisms have previously been implicated in the assimilation of acetic and lactic acid.

Glycerol formation is typically associated with the presence of fungal microorganisms, such as *Saccharomyces* and non-*Saccharomyces* yeast [23]. However, no significantly higher concentrations of glycerol were observed in the samples containing yeast compared with the control sample. This observation may support previous suggestions regarding the production of glycerol by prokaryotes [24]. The lowest concentration of glycerol was found in sauerkraut fermented with *Pichia fermentans* Pf9, suggesting the possible utilisation of this component as a carbon source.

The obtained sauerkraut contained between 2 and 6 g/kg of ethanol. Similar amounts of this compound have been reported in a study by Wolkers-Rooijackers et al. [25]. In most samples, we observed higher concentrations of ethanol after the addition of yeast to the fermentation than in that in the control sample. This underlines the important role of these microorganisms in introducing ethanol into the food matrix. However, two samples fermented with *Rh. mucilaginosa* Rm26 and *P. fermentans* Pf9 showed a reduction in ethanol content compared with the control. Yeast utilise the ethanol degradation pathway to redirect carbon flow towards the synthesis of acetyl-CoA. As a result, different types of ethyl esters of various acids are generated as by-products of this transformation process [26].

3.2. Volatile Compounds

Chromatographic analysis (HS-SPME-GC-MS) showed the presence of 48 different volatile compounds, mainly alcohols and esters, 13 compounds each.

The alcohol content was mainly influenced by the yeast strain added during fermentation. In general, the highest concentrations of total alcohols were observed in sauerkraut fermented with the addition of *Cl. lusitaniae* Cl6, while the lowest concentrations were associated with *P. fermentans* Pf9 (Table 3). Two groups of alcohols were identified on the basis of their formation mechanism: the first group originated from precursors present in the raw material, while the second group was microbiologically produced. Compounds with 'green' odour, such as 1-penten-3-ol, (Z)-3-hexenol, hexanol and 1-nonanol, are formed during the enzymatic oxidation of linoleic and linolenic acids. These compounds are commonly found in various fruits and vegetables, especially when they are cut or crushed during processing [27]. Our research shows that some microorganisms have the ability to enhance the release of these compounds by breaking down the structure of plant tissues through the action of various types of lytic enzymes.

Table 3. A heatmap of the content of selected alcohols and carbonyl compounds in sauerkraut produced with the addition of different yeast.

(mg/kg)			Control	*D. hansenii* Dh17	*D. hansenii* Dh19	*D. hansenii* Dh31	*W. anomalus* Wa1	*W. anomalus* Wa7	*R. mucilaginosa* Rm8	*W. anomalus* Wa22	*R. mucilaginosa* Rm26	*P. fermentans* Pf9	*C. lusitaniae* Cl6	SEM [1]	Sig. [2]	OT [mg/kg]
Alcohols																
1-Propanol, 2-methyl-	629	74	0.0 a	2.3 a	1.8 a	4.7 a	2.5 a	0.0 a	4.3 a	0.0 a	0.0 a	0.0 a	11.20 b	0.68	***	16
1-Penten-3-ol	673	57	24.4 c	0.6 a	7.9 ab	3.6 ab	0.0 a	0.0 a	13.5 a–c	0.0 a	0.0 a	18.3 bc	41.8 d	2.58	***	0.4
1-Butanol, 3-methyl-	723	55	2.4 a	36.3 b–e	37.7 c–e	64.2 e–g	72.7 g	40.5 c–f	47.8 d–g	19.9 a–d	15.8 a–c	7.7 ab	68.3 fg	4.6	***	0.41
1-Butanol, 2-methyl-	732	56	6.1 a	31.1 a–c	41.2 bc	41.2 bc	52.6 c	47.9 c	80.6 d	6.7 a	6.9 a	15.8 ab	118.0 e	6.3	***	20
2,3-Butanediol	789	45	156 g	89 def	93 ef	55 b–d	83 c–e	20 ab	50 bc	38 ab	41 ab	10 a	122 fg	8	***	1
3-Hexen-1-ol, (Z)-	831	67	4.4 a	1.8 a	3.7 a	2.4 a	4.2 a	3.6 a	3.8 a	4.3 a	2.6 a	5.3 a	9.2 b	0.4	*	0.1
1-Hexanol	865	56	2.44 ab	0.68 ab	2.61 ab	2.50 ab	0.00 a	0.00 a	3.42 bc	1.13 ab	0.61 ab	1.68 ab	6.23 c	0.40	**	2.5
1-Hexanol, 2-ethyl-	1020	57	2.6 ab	7.7 d–f	9.9 f	7.5 d–f	7.0 c–f	6.3 c–e	5.4 b–d	9.5 ef	4.2 a–d	3.5 a–c	1.7 a	0.5	***	0.024
Nonanol	1186	41	1.44 cd	3.01 f	2.45 ef	0.58 ab	0.70 a–c	0.72 a–c	2.24 ef	2.39 ef	1.24 bc	0.32 a	2.08 de	0.17	***	1
1-Decanol	1262	55	24.4 d	9.5 ab	13.2 bc	3.9 a	7.8 ab	5.4 a	17.1 c	25.3 d	13.4 bc	6.0 a	38.5 e	1.7	***	0.775
Undecanol	1364	55	60.3 c	22.7 ab	27.8 ab	8.4 a	22.3 ab	12.4 a	44.6 bc	57.6 c	42.2 bc	22.3 ab	137.2 d	6.6	***	0.5
1-Dodecanol	1472	41	9.8 c	25.6 d	23.4 d	8.6 a–c	9.8 c	3.0 a	7.7 a–c	9.1 bc	6.4 a–c	3.8 ab	26.0 d	1.6	***	0.158
1-Tetradecanol	1664	55	0.07 a	0.99 d	0.19 a	1.44 e	0.57 bc	0.22 ab	0.20 ab	0.72 cd	0.20 ab	0.21 ab	1.50 e	0.09	***	na
Carbonyl compounds																
Nonanal	1083	57	0.00 a	1.92 c–e	2.06 de	2.87 e	2.80 de	0.87 ab	0.99 a–c	2.77 de	0.00 a	1.74 b–d	1.96 c–e	0.19	***	0.001
Decanal	1209	57	1.01 a–c	2.07 de	1.59 cd	2.55 e	0.92 a–c	0.43 a	1.27 bc	0.82 a–c	0.73 ab	0.38 a	2.46 e	0.15	***	0.0003
2-Dodecanone	1383	58	0.00 a	1.09 d	0.61 c	0.91 d	0.30 b	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.07	***	460
Dodecanal	1409	41	0.13 a	1.84 c	2.01 cd	2.53 d	1.69 c	0.34 a	0.97 b	0.27 a	0.46 ab	0.16 a	1.70 c	0.16	***	0.055
Benzophenone	1585	105	2.2 ab	4.9 c	3.1 b	3.1 b	2.1 ab	0.9 a	2.3 ab	2.1 ab	1.4 a	1.0 a	4.6 c	0.3	***	na
												−1	−0.5	0	0.5	1

[1] SEM—standard error of means; [2] Sig.—significance; *, ** and ***—the significance at 0.05, 0.01 and 0.005 according to the least significant difference. Values with different Roman letters (a–g) of the same parameter (in raws) indicate statistically significant differences at $p < 0.05$; n = 5. The concentration of a specific parameter is represented by a colour gradient ranging from dark red (lowest concentration) to dark green (highest concentration). na—not available.

Alcohols are also formed microbiologically through the degradation of various compounds present in cabbage, such as amino acids (2-methyl-1-propanol, 3-methyl- and 2-methyl-1-butanol), fatty acids (1-decanol, 1-dodecanol and 1-tetradecanol) and some other compounds (2-ethyl-1-hexanol) [28]. In most cases, the introduction of yeast into the fermentation of sauerkraut did not increase the amount of these compounds compared with that in the control sample. The only exception was sauerkraut obtained with *Cl. lusitaniae* Cl6, which generally contained the highest amounts of volatile compounds, including alcohols (Table 3). This strain was characterised by the production of the highest amounts of volatile compounds on model media among representatives of this species. It was also characterised by weak killer activity and developed to contain a lactic acid content of 8 g/L [5]. 2,3-Butanediol (2,3-BD) is a bacterial metabolite produced via several intermediates, including α-acetolactate, acetoin (AC) and diacetyl, from pyruvate. The ability to produce 2,3-BD is widespread in bacteria. The Voges–Proskauer reaction, which is related to the intermediates of 2,3-BD production, is a fundamental test for bacterial classification [29]. Analyses of sauerkraut showed that the highest concentrations of 2,3-butanediol were found in the control sample. The addition of *P. fermentans* Pf9 yeast reduced the amount of the compound in question by almost 16 times. Yeast can grow on 2,3-butanediol as the sole carbon and energy source, indicating that they have the ability to degrade these metabolites. The resulting product is acetoin, which can be further degraded into simpler substances [30].

The most abundant group of carbonyl compounds in sauerkraut was of aldehydes. Most aldehydes originate from the raw material. They can be formed microbiologically during fermentation as a result of oxidation from alcohols, and the reverse process can occur simultaneously [31]. These compounds play an important role in the flavour formation of food products due to their very low aroma thresholds [32]. Of the five carbonyl compounds detected, nonanal, decanal and dodecanal had the greatest impact on the aroma of sauerkraut. Their levels were, in most cases, higher in sauerkraut to which yeasts had been added, especially *D. hansenii* and *Cl. lusitaniae*.

The second largest group of volatile compounds after alcohols was of esters (Table 4). These are mostly formed via the enzymatic condensation of alcohols and organic acids [31]. The largest amounts contained ethyl acetate, dibutyl succinate and ethyl 2-methyloctanoate. The lowest number of esters was found in the control sample and in sauerkraut obtained with *P. fermentans* Pf9 and *W. anomalus* Wa7. The highest number was found in samples with *D. hansenii* and *Cl. lusitaniae*, which differed significantly in their ester profiles. For example, sauerkraut fermented with *Cl. lusitaniae* contained the most ethyl acetate, dibutyl succinate, benzyl benzoate and ethyl hexadecanoate, *D. hansenii* Dh17—phenethyl acetate, ethyl decanoate and 1-methylethyl dodecanoate, while *W. anomalus* strains contained the most 2-butoxyethyl acetate and ethyl dodecanoate. Therefore, an excessive amount of esters may indicate a yeast infection during fermentation.

Lactones are a group of volatile organic compounds (VOCs) that are classified as esters of hydroxy acids and are defined by a cyclic ring. They are produced through lipid metabolism and have low perception thresholds [33]. Previous research has indicated lactic acid bacteria as the main producers of gamma-lactones during the fermentation of sauerkraut [34]. The results of our research indicate that yeast can also influence the formation of larger amounts of compounds from this group. The highest amounts of gamma-lactones were found in sauerkraut obtained with the addition of *Cl. lusitaniae*. This could be due to the direct synthesis of these compounds by yeast or the provision of appropriate precursors to lactic acid bacteria.

Table 4. A heatmap of the content of selected volatile esters and lactones in sauerkraut produced with the addition of different yeast.

(mg/kg)			Control	*D. hansenii* Dh17	*D. hansenii* Dh19	*D. hansenii* Dh31	*W. anomalus* Wa1	*W. anomalus* Wa7	*R. mucilaginosa* Rm8	*W. anomalus* Wa22	*R. mucilaginosa* Rm26	*P. fermentans* Pf9	*C. lusitaniae* Cl6	SEM [1]	Sig. [2]	OT [mg/kg]
Esters																
Ethyl acetate	614	43	143 a	610 ab	440 ab	1503 bc	640 ab	331 a	1008 a–c	226 a	208 a	162 a	1795 c	129	*	5
2-Butoxyethyl acetate	1061	43	1.13	1.52	3.86	1.10	5.34	1.85	3.50	5.90	3.13	2.68	0.71	0.42	ns	0.1
Ethyl octanoate	1204	88	0.23 ab	2.25 e	0.92 d	7.59 f	0.51 c	0.14 a	0.97 d	0.23 ab	0.49 bc	0.19 a	1.10 d	0.38	***	0.07
Ethyl 2-methyloctanoate	1225	102	12.0 a	84.5 c	92.8 c	45.6 b	22.0 a	12.4 a	12.1 a	12.9 a	11.7 a	13.4 a	17.0 a	5.4	***	na
Phenethyl acetate	1245	104	0.19 b–d	0.97 f	0.47 e	0.27 cd	0.17 bc	0.00 a	0.29 d	0.00 a	0.27 cd	0.11 ab	0.96 f	0.06	***	0.02
Propyl noanoate	1377	61	0.1 a	6.0 c	3.5 b	6.3 c	1.7 ab	0.2 a	0.7 a	0.1 a	0.1 a	0.1 a	0.0 a	0.5	***	na
Ethyl decanoate	1391	88	2.9 a–c	26.0 f	8.3 a–d	16.6 e	6.0 a–c	1.4 a	9.7 b–e	3.6 ab	10.6 c–e	1.9 ab	15.0 de	1.43	***	6.3
Dibutyl succinate	1560	101	104 bc	204 d	132 c	69 ab	53 ab	32 a	75 ab	93 bc	62 ab	41 a	224 d	12	***	na
Ethyl dodecanoate	1596	88	1.7 a	6.0 ab	4.2 ab	10.7 bc	21.7 d	3.5 ab	18.4 cd	6.3 ab	11.0 bc	2.2 a	3.9 ab	1.3	***	0.4
1-Methylethyl dodecanoate	1627	60	3.5 ab	8.4 d	4.9 bc	7.1 cd	2.2 ab	1.4 a	2.9 ab	2.9 ab	2.3 ab	1.2 a	6.8 cd	0.5	***	na
Benzyl benzoate	1749	105	24.0 b	45.9 c	22.3 b	38.3 c	16.9 ab	9.0 a	19.8 ab	23.9 b	18.2 ab	10.1 a	58.3 d	2.8	***	0.341
Ethyl tetradecanoate	1787	88	0.71 a	1.06 a	1.60 ab	3.48 ab	4.46 b	0.86 a	3.00 ab	4.57 b	7.76 c	1.24 ab	2.73 ab	0.44	***	4
Ethyl hexadecanoate	1979	88	1.7 a	1.6 a	1.0 a	1.9 a	1.4 a	1.1 a	4.1 c	5.4 c	3.9 bc	2.4 ab	7.2 d	0.4	***	2
Lactones																
γ-Nonalactone	1312	85	3.3 d	2.2 a–d	2.0 a–d	1.5 ab	1.8 a–c	0.8 a	2.4 b–d	3.1 cd	1.7 a–c	1.0 ab	5.8 e	0.3	***	0.007
γ-Undecalactone	1573	85	0.9 ab	2.4 c	1.8 bc	0.6 a	0.8 ab	0.4 a	0.8 ab	1.0 ab	0.9 ab	0.5 a	2.6 c	0.2	***	0.06
												−1	−0.5	0	0.5	1

[1] SEM—standard error of means; [2] Sig.—significance; *, ***—significance at 0.05 and 0.005 according to the least significant difference; ns—not significant. Values with different Roman letters (a–f) of the same parameter (in rows) indicate statistically significant differences at $p < 0.05$; n = 5. The concentration of a specific parameter is represented by a colour gradient ranging from dark red (lowest concentration) to dark green (highest concentration). na—not available.

Glucosinolates present in plants of the genus *Brassica*, are products of secondary metabolism. During fermentation, the substance can undergo hydrolysis, leading to the creation of volatile compounds like isothiocyanates, thiocyanates, nitriles and epithionitriles [35]. *Brassica* vegetables owe their 'cabbage' flavour to the presence of sulphides, disulphides, trisulphides, tetrasulphides, and other compounds formed via the hydrolysis of isothiocyanates, which can occur during heat treatment [36]. Our research revealed the presence of eight compounds from this group (Table 5). Most of them were present at levels above the threshold and thus significantly influenced the aroma of the sauerkraut obtained. In total, the highest number of these compounds was produced in samples with yeasts of all species except *D. hansenii*. This was mainly due to the low levels of carbon disulphide, methyl thiocyanate and dimethyl disulphide in these samples. Previous research suggests that halophilic bacterial species are the most likely microorganisms to use thiocyanates and related compounds as a source of sulphur, carbon and nitrogen [37]. Therefore, the formation of higher levels of these compounds may be due to increased competition for nutrients when large numbers of yeast cells are introduced into the mixture. This can also lead to a reduction in the amount of glucosinolates in the product, which may be undesirable due to their health-promoting properties [38]. Another undesirable consequence of increased levels of thiocyanate derivatives may be a change in flavour, with intense sulphuric, pungent, green and mustardy notes characterising these components [39].

Terpenes are a group of compounds that occur naturally in plants, mostly in a bound form. During fermentation, they are released or microbiologically biotransformed into others. There are more than 20 different terpenes in cabbage leaves [40]. In our research, we detected only six of them (Table 5), of which only one was found in the raw vegetable—α-terpineol—and its content was several times lower. The most abundant were β-damascenone and geranyl acetone. The introduction of yeast into the fermentation of sauerkraut affected the levels of these compounds in different ways. As in the case of the previously discussed compounds, the presence of the yeasts *D. hansenii* and *Cl. lusitaniae* increased the amount of terpenes in the final product compared with that in the control; especially, the amounts of *Rh. mucilaginosa* Rm26 and *P. fermentans* Pf9 decreased.

3.3. Colour and Aroma Profile

Food colour is often associated with product quality and serves as a key feature by which consumers evaluate and select products [41]. As consumers typically evaluate products visually before tasting them, colour plays a crucial role in consumer perception. Treatments applied to cabbage significantly affect its colour [42]. This is attributed to changes in the composition of antioxidant compounds, pH increase, the formation of browning pigments and other factors [43]. The CieLAB method was employed to assess the colour of sauerkraut and to determine the influence of yeast addition during fermentation (Table 6). Significant differences were found between the products in the a*, b*, c* and h^0 colour parameters. All sauerkraut samples analysed showed similar lightness values ranging from 62.6 to 66.7. The control sample displayed the highest values for the a*, b*, C* and h^0 coefficients, indicating a smaller share of green (a*), a greater share of yellow (b*) and a higher colour intensity (C*) compared with those in sauerkraut produced without yeast addition. The h^0 parameter values were in the yellow range (85.8–88.6). The Δe coefficient indicates the colour deviation from the control sample, with the highest differences observed in sauerkraut fermented with *W. anomalus* Wa22 and *D. hansenii* Dh17. Δe exceeding 6 indicates significant differences between two samples [44].

The aroma of sauerkraut was assessed in terms of overall aroma acceptability and intensity, and seven aroma attributes: typical, acetic, sulphur, green, metallic, animal and pungent.

The highest scores were given to sauerkraut aroma profiles obtained without the addition of yeast and with the addition of *D. hansenii* strains (Figure 1). These samples received the highest scores for the typical sauerkraut aroma, exceeding 6.5 points (Figure 2). Conversely, sauerkraut fermented with the *W. anomalus* and *Rh. mucilaginosa* strains

performed less favourably. Low scores were attributed to a low aroma intensity rather than the presence of specific off-odours. Sauerkraut produced with the *Cl. lusitaniae* Cl6 culture had the highest aroma intensity (Figure 1). This observation is consistent with the results of HS-SPME-GC-MS analysis (Tables 3–5), which showed that these samples also had the highest concentration of volatile compounds. However, despite the increased odour intensity, the aroma acceptability of these samples deteriorated significantly.

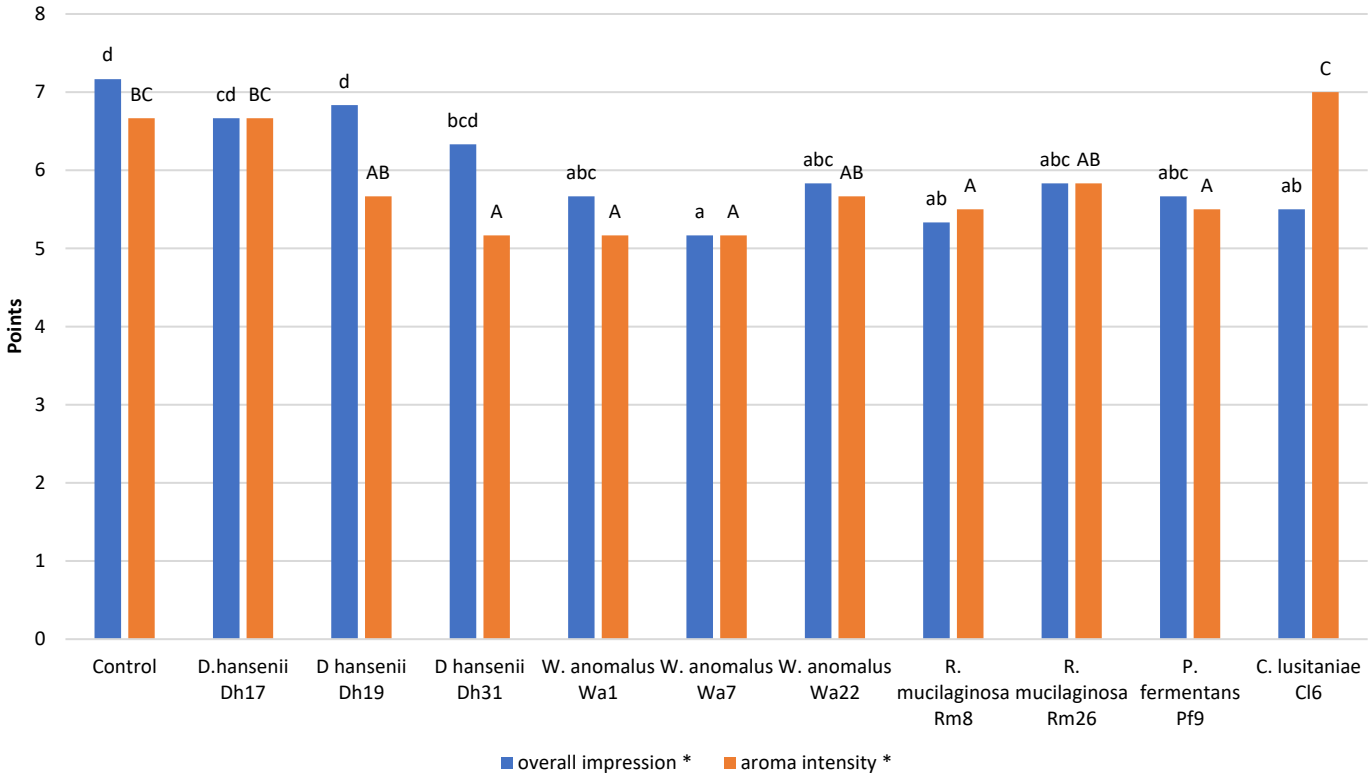


Figure 1. Overall aroma impression and aroma intensity of sauerkraut produced with the addition of different yeast cultures based on a 10-point scale. Values with different Roman letters (A–C, a–d) of the same parameter indicate statistically significant differences at $p < 0.05$; Statistically significantly different parameters determined using ANOVA ($p < 0.05$) are marked with *.

The typical aroma of sauerkraut received the highest scores, ranging from 5.8 to 7.3. The flavour of fermented cabbage is mainly influenced by components derived from the raw material, which undergo transformation during the fermentation process and thus represent a fundamental aspect of sauerkraut quality [45]. These components can be divided into two groups: the first group includes isothiocyanates and other sulphur-containing volatile organic compounds (such as sulphides and thiols), while the second group encompasses a wide range of chemicals, including alcohols, aldehydes, acids, ketones, terpenes, hydrocarbons, esters, sulphur-containing compounds and others [31].

Another highly rated aroma characteristic is the floral aroma. Lactic acid and acetic acid can both act as precursors for the formation of esters, specifically ethyl lactate and ethyl acetate, respectively, which contribute to the fruity aroma. The strains of lactic acid bacteria (LAB) present largely influence the production of these esters [34]. The highest scores for this attribute were assigned to sauerkraut fermented with *Cl. lusitaniae* Cl6 and *D. hansenii* Dh31 yeasts. In both samples, elevated levels of esters, mainly ethyl acetate, were detected. However, these samples received relatively low overall acceptability scores, suggesting that higher ester concentrations may negatively affect aroma quality.

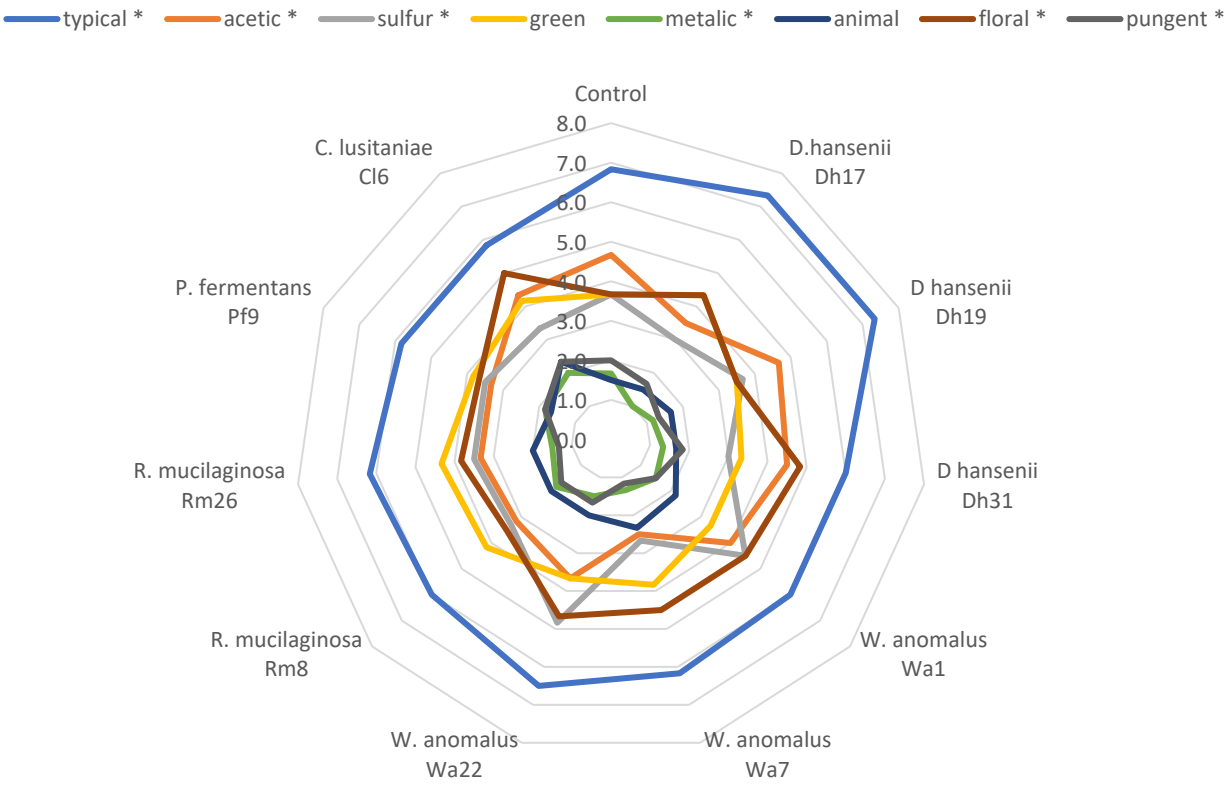


Figure 2. A spider web diagram visualising the aroma qualities of sauerkraut produced with the addition of different yeasts. Statistically significantly different parameters determined using ANOVA ($p < 0.05$) are marked with *.

Aroma attributes such as green, acetic and sulphur were rated slightly lower. The green attribute is associated with the presence of carbonyl compounds C6 and C9, which impart aromas reminiscent of green plant parts and grassiness [27]. The acetic attribute is linked to the presence of acetic acid and other volatile acids, while the sulphur attribute is associated with the presence of glucosinolate degradation products. Excessive concentrations of any of the three attributes can negatively affect the aroma of sauerkraut. In general, the sensory analysis results were consistent with the chromatography results (see Tables 3–5). However, it is important to note that the final aroma impression is the result of various interactions between the present aroma components. These interactions can create new aromas through overlapping aroma notes. Aroma compounds with similar properties can exhibit additive interactions, which lower the threshold of individual compounds. Additionally, some aroma compounds can have stronger-than-expected effects by interacting synergistically or antagonistically, resulting in a masking effect [46]. The lowest rated aromas were pungent, animal and metallic, which should be considered positive features, as these are not typical of sauerkraut and their intense presence can negatively affect the aroma experience.

Table 5. A heatmap of the content of selected sulphur compounds and terpenes in sauerkraut produced with the addition of different yeast.

(mg/kg)			Control	*D. hansenii* Dh17	*D. hansenii* Dh19	*D. hansenii* Dh31	*W. anomalus* Wa1	*W. anomalus* Wa7	*R. mucilaginosa* Rm8	*W. anomalus* Wa22	*R. mucilaginosa* Rm26	*P. fermentans* Pf9	*C. lusitaniae* Cl6	SEM [1]	Sig. [2]	OT [mg/kg]
Sulphur compounds																
Carbon disulfide	526	76	6.4 a	6.2 a	6.3 a	6.7 a	11.9 a	21.4 b	6.4 a	20.2 b	24.5 b	9.1 a	7.7 a	1.4	***	0.2
3-Butenenitrile	658	41	28.2 b–e	25.5 a–d	28.5 c–e	19.8 a	32.5 de	20.6 a–c	20.4 ab	45.8 f	34.8 e	19.9 a	23.2 a–c	1.5	***	na
Methyl thiocyanate	676	73	5.3 a–d	2.9 a	4.0 ab	2.8 a	7.0 b–d	5.4 a–d	5.1 a–c	8.5 de	7.0 bd	7.9 c–e	10.6 e	0.5	***	na
Dimethyl disulfide	729	94	10.3 a	16.3 b	9.6 a	9.9 a	23.3 de	26.5 e	17.8 bc	20.0 b–d	22.6 c–e	19.0 b–d	18.6 b–d	1.0	***	0.016
Allyl thiocyanate	812	39	0.00	0.79	0.57	0.46	0.00	0.00	1.60	0.54	0.00	0.00	0.72	0.17	ns	na
Allyl isothiocyanate	847	99	44.9 de	24.4 a	30.3 ab	34.6 bc	49.1 e	39.7 cd	38.6 cd	63.4 f	40.7 cd	45.1 de	60.1 f	2.1	***	0.04
Dimethyl trisulfide	949	126	0.95 b	1.77 d	1.19 b–d	1.19 b–d	1.59 cd	3.74 e	0.58 ab	0.91 b	0.87 b	1.15 bc	0.25 a	0.17	***	0.05
3-Butenyl isothiocyanate	958	72	0.00 a	2.27 cd	1.78 bc	3.27 def	3.88 ef	4.44 f	0.72 ab	2.53 cde	0.63 ab	0.00 a	0.71 ab	0.29	***	0.01
3-Methylthiopropyl isothiocyanate	1287	101	8.4 cd	12.2 e	4.9 ab	2.2 a	4.2 ab	1.8 a	9.0 cd	6.0 bc	4.0 ab	3.0 ab	9.9 de	0.6	***	0.006
Terpenes																
p-Cymene	1011	119	5.13	0.60	2.72	3.41	4.49	4.87	4.43	4.61	3.07	5.18	8.04	0.47	ns	0.057
Dihydromyrcenol	1053	59	2.24	3.87	4.87	3.53	4.09	4.04	2.81	4.41	2.50	2.11	2.80	0.26	ns	na
α-Terpineol	1196	93	0.00	0.37	0.47	0.31	0.32	0.15	0.19	0.21	0.05	0.00	0.00	0.04	ns	330
β-Damascenone	1371	69	10.2 d	2.7 ab	2.7 ab	1.8 a	2.3 ab	1.7 a	6.6 b–d	8.1 cd	5.2 a–c	3.6 a–c	19.0 e	1.0	***	0.002
Geranyl acetone	1451	43	5.8 ab	30.8 f	17.2 d	26.0 e	13.0 c	3.3 a	9.6 bc	5.7 a	3.9 a	2.2 a	9.9 bc	1.7	***	60
β-Ionone	1488	177	0.00 a	0.44 d	0.19 bc	0.22 c	0.00 a	0.03 a	0.00 a	0.00 a	0.12 b	0.00 a	0.00 a	0.03	***	0.007
												−1	−0.5	0	0.5	1

[1] SEM—standard error of means; [2] Sig.—significance; ***—significance at 0.005 according to the least significant difference; ns—not significant. Values with different Roman letters (a–f) of the same parameter (in rows) indicate statistically significant differences at $p < 0.05$; n = 5. The concentration of a specific parameter is represented by a colour gradient ranging from dark red (lowest concentration) to dark green (highest concentration). na—not available.

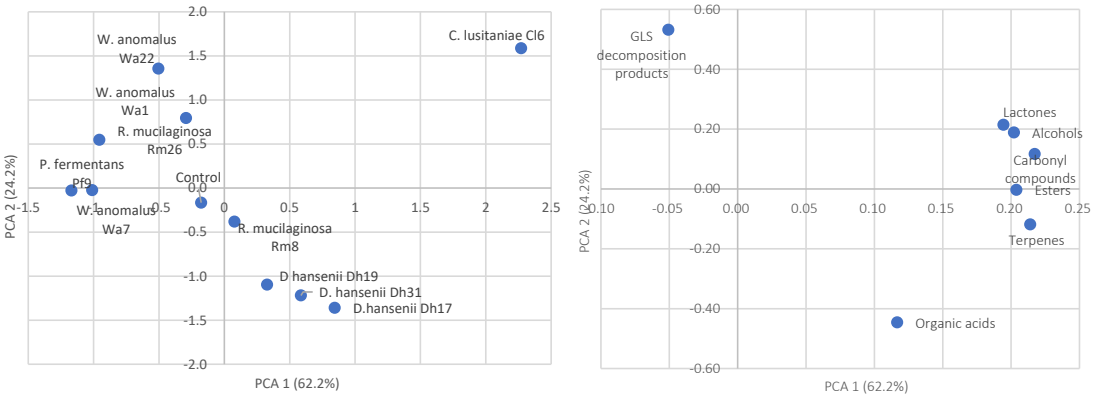
Table 6. A heatmap of CIELab values of sauerkraut produced with the addition of different yeast.

	Control	*D. hansenii* Dh17	*D. hansenii* Dh19	*D. hansenii* Dh31	*W. anomalus* Wa1	*W. anomalus* Wa7	*W. anomalus* Wa22	*R. mucilaginosa* Rm8	*R. mucilaginosa* Rm26	*P. fermentans* Pf9	*C. lusitaniae* Cl6	SEM [1]	Sig. [2]
CIE L	65.5	66.7	65.3	65.1	64.6	65.0	63.6	62.6	65.2	63.5	66.4	0.4	ns
CIE a*	0.2 b	−1.3 a	−0.8 a	−1.0 a	−0.9 a	−1.4 a	−1.5 a	−1.0 a	−0.9 a	−0.9 a	−1.4 a	0.2	*
CIE b*	29.9 c	22.6 ab	26.4 a–c	25.0 a–c	25.0 a–c	25.0 a–c	21.6 a	24.7 ab	25.9 a–c	27.0 c	26.9 bc	0.8	*
C*	29.9 c	22.7 ab	26.5 a–c	25.0 a–c	25.0 a–c	25.0 a–c	21.6 a	24.7 a–c	25.9 a–c	27.0 c	26.9 bc	0.8	*
h^0	88.6 b	86.2 ab	87.3 ab	87.7 ab	87.9 ab	86.8 ab	85.8 a	87.7 ab	87.7 ab	88.2 ab	86.9 ab	0.4	*
Δe	0.0	7.5	3.6	5.1	5.1	5.3	8.8	6.1	4.2	3.7	3.6	0.8	
									−1	−0.5	0	0.5	1

[1] SEM—standard error of means; [2] Sig.—significance; *—significance at 0.05 according to the least significant difference; ns: not significant. Values with different Roman letters (a–c) of the same parameter (in rows) indicate statistically significant differences at $p < 0.05$; n = 5. The concentration of a specific parameter is represented by a colour gradient ranging from dark red (lowest concentration) to dark green (highest concentration).

3.4. Principal Component Analysis (PCA)

Figure 3 shows the results of the principal component analysis (PCA) conducted to measure the main groups of chemical components detected in sauerkraut (expressed as the sum of the concentrations of compounds from a given group), obtained using different yeast strains. The first component (PC1) described 62.2% of the total variance, while the second one, PC2, described 24.4% of it. Positive PC1 charges were associated with the content of most of the analysed volatiles and organic acids, while negative charges were associated with glucosinolate degradation products (GLS). For PC2, negative charges were related to the content of organic acids and terpenes, while positive charges were associated with the levels of other components tested.



(**a**) (**b**)

Figure 3. Principal component analysis plot for the chemical composition of sauerkraut produced with different yeast strains (**a**) and for the corresponding loadings of selected chemical composition parameters of the samples (**b**).

The control sample, obtained without the addition of yeast, was located almost in the centre of the coordinate system, which indicates the average content of all the components analysed. To its left are the sauerkraut samples obtained with the yeasts *P. fermentans* Pf9, *W. anomalus* and *Rh. mucilaginosa* Rm26. They contained lower amounts of the components analysed than the control sample did. Samples inoculated with *P.fermentans* Pf9 stood out particularly in this respect. *Pichia fermentans* was usually detected at the end of the

fermentation of sauerkraut under anaerobic conditions [4]. In our previous research, *P. fermentans* was responsible for the production of the largest amount of volatile compounds on model substrates [5].

The introduction of cells of this microorganism into the fermentation process of sauerkraut resulted in a significant reduction in the amount of all components present in the product, both non-volatile, such as lactic acid, and volatile. *Pichia fermentans* is a typical beverage spoilage yeast found in apple juice, beer, soft drinks, must and wine [47]. It can assimilate a wide range of different carbon sources, including hexoses such as glucose and pentoses such as xylose [48]. Similarly, the yeast species *W. anomalus* is considered a yeast spoilage species [49]. Due to its aerobic metabolism, it develops slowly in the absence of oxygen [50]. This type of phenomenon was observed in the sauerkraut samples analysed. This strain neither caused excessive deacidification, which is its characteristic feature, nor did it produce large amounts of metabolites that negatively affect the quality. However, this does not change the fact that when microaerobic conditions are created, there is strong proliferation and a significant impact on the parameters of sauerkraut [4]. The discussed group of yeasts also included cultures that increased the decomposition of glucosinolates (probably, these compounds were partially metabolised as a carbon source), which resulted in an increase in the number of components produced by such decomposition [35,36]. The negative effects of this phenomenon are a reduction in the number of components with health-promoting activity and, on the other hand, the formation of a large number of compounds with an unpleasant, sulphurous aroma.

On the right side, close to the control sample, there were cabbages fermented with *Rh. mucilginosa* Rm8 yeast, which means that the qualitative compositions of these samples were similar. *Rhodotorula* yeasts are strictly aerobic organisms; therefore, their growth during sauerkraut fermentation is limited to the initial stages or to specific conditions when oxygen is available [51]. The two *Rh. mucilaginosa* strains analysed behaved differently, with the Rm26 culture significantly reducing the levels of the compounds analysed in sauerkraut, while Rm8 had no such negative effect (Tables 2–5). We observed similar large differences between these strains when analysing their presence during the fermentation of sauerkraut [4]. The Rm26 culture was present throughout the fermentation, regardless of the presence or absence of oxygen. The Rm8 strain developed more slowly, and its growth depended on aerobic conditions.

On the right, below the control sample, there are samples of sauerkraut fermented with the yeast *D. hansenii*. They formed a homogeneous group, demonstrating the high similarity of the influence of these cultures on the fermentation process and the components produced. The *D. hansenii* yeast often accompanies lactic acid bacteria (LAB) during the spontaneous fermentation of various foods [10]. In previous studies, we detected it throughout the fermentation of sauerkraut, regardless of the variety or conditions used during the process [4,5]. Current research confirms that this yeast is an important microorganism for obtaining good-quality sauerkraut. The products obtained with its participation were characterised by an increased level of lactic acid, but also by a positive aroma, which resulted in high scores in the sensory analysis.

The cabbage obtained with *Cl. lusitaniae* Cl6 is located furthest on the right. These tests were characterised by a very large number of analysed volatile compounds such as alcohols, esters and lactones. We first detected the yeast *Clavispora lusitaniae* in the fermentation process of sauerkraut [5], and at the same time we found that its growth was promoted by anaerobic conditions [4]. In the research described, we confirmed that this microorganism develops well during fermentation and does not disturb the course of lactic acid fermentation. However, it produces very large amounts of volatile compounds that modify the aroma of sauerkraut by introducing a large amount of floral notes. Unfortunately, the aroma obtained with it received a lower score in the overall evaluation due to its deviation from the typical aroma.

4. Conclusions

In our study, we investigated the effect of specific yeast cultures isolated from the fermentation process on the quality characteristics of sauerkraut. Our results confirm previous hypotheses from our published work. We identified two distinct subgroups within the microorganisms studied. One subgroup, represented by *D. hansenii*, showed a positive influence on sauerkraut quality. The second subgroup, including *W. anomalus*, *P. fermentans*, *Cl. lusitaniae* and selected *Rh. mucilaginosa* strains, had a negative effect on sensory properties and microbiological stability, particularly with regard to deacidification.

However, further research is warranted to investigate different fermentation conditions such as the presence of oxygen, salt concentration and the addition of herbs and spices. This comprehensive approach will help to achieve a stable product while minimising the risk of spoilage. In addition, research into potential starter cultures for sauerkraut fermentation should include not only lactic acid bacteria but also yeast strains such as *D. hansenii* to optimise fermentation results.

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