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Comparison of the Chemical and Aroma Composition of Low-Alcohol Beers Produced by *Saccharomyces cerevisiae* var. *chevalieri* and Different Mashing Profiles

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Abstract: Changing consumer preferences and increasing demands require adjustments in brewery operations and beer production methods. Recent trends indicate a marked decline in interest in high-alcohol beers and an increasing demand for low- and no-alcohol alternatives. The aim of this study was to evaluate and compare the volatile compound profiles produced by *Saccharomyces cerevisiae* var. *chevalieri*, a yeast strain specifically developed for non-alcoholic beer production, with a reference sample fermented with a standard *Saccharomyces cerevisiae* US-05 strain. Two mashing profiles were compared (with and without saccharification pause). The wort obtained was fermented with and without hops. The chemical composition and aroma compounds of the resulting beers were analysed using different chromatographic techniques (HPLC, GC-FID, GC-MS and CG-O). The modification of the mashing profile helped to obtain wort with about 50% lower maltose content. A lower FAN (free amino nitrogen) content was also observed, but this did not affect the fermentation process. Beers fermented with the *Saccharomyces cerevisiae* var. *chevalieri* strain had an average alcohol content of 0.5–0.8% *v*/*v*. This strain consumed about 25% of the available maltose. The resulting beers were dominated by fruity, floral and herbal aromas. In addition, beers fermented with a non-alcoholic beer strain scored highest in the sensory analysis.

Keywords: low-alcohol beers; volatile compound; aroma-active compound; special yeast strain

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1. Introduction

There is currently a dynamic increase in interest in no-alcohol and low-alcohol (NoLo) beers around the world. This trend is particularly evident in Europe and is driven by changing beer consumer preferences [1]. More and more people are interested in a healthy and active lifestyle, which leads to them looking for high-value functional products and limiting alcohol consumption [2]. Alcohol-free and low-alcohol beers are not clearly defined in EU regulations. According to Regulation 1169/2011, drinks containing less than 1.2% alcohol by volume do not need to be labelled [3]. NoLo beers are an excellent alternative to other beverages, such as sweet carbonated soft drinks, because they retain the taste and refreshing character of beer without adding significant calories [4]. In addition, these beers can help hydrate the body, making them an attractive option for people who want to be refreshed but avoid high-proof drinks [5]. The non-alcoholic beer industry in Poland has stayed at a high level for a number of years. This trend increased the most in 2019 and continues to this day [6].

There are two methods of producing low- and no-alcohol beers: biological methods (involving limiting fermentation processes) and physical methods (involving dealcoholisation processes) [7]. Physical methods involve the removal of alcohol from the beer in the final stage of production. Additional financial resources are required investment to purchase alcohol removal equipment [8]. These methods are typically divided into two groups: thermal and membrane processes [9].

Thermal processes expose the beer to a temperature of at least 60 °C for a prolonged period. This can lead to undesirable changes in the beer, such as darkening, the caramelisation of remaining sugars and deterioration of the aroma composite [10]. Conversely, membrane procedures use semi-transparent membranes by which solutes or solvents selectively migrate (permeate). As the solution passes through the membrane module, the composition of the solution changes [6].

The main aim of biological methods in the production of NoLo beers is to limit the fermentation process, which is carried out with traditional brewing yeast [11]. Already at the mashing stage, the fermentation potential of the wort is reduced by limiting the breakdown of starch into fermentable sugars (maltose, glucose) [12]. Another way to produce low-alcohol beers is to stop fermentation when the alcohol concentration reaches 0.5% v/v. However, beers produced in this way may have a sulphur aftertaste, which is removed during maturation [13]. Yeast strains that ferment the sugars present in the wort poorly or not at all are becoming increasingly popular in the production of NoLo beers [14]. The use of yeast strains with poor maltose fermentation capabilities is currently one of the best methods for producing NoLo beers. The resulting beers are characterised by an appropriate taste without a wort aftertaste [11]. In order to obtain NoLo beers with an appropriate sensory profile, many studies have been carried out on *Saccharomyces strains* with different phenotypic and genotypic profiles [15]. The *Saccharomyces ludwigii* strain, which does not have the ability to ferment maltose, is very popular. Beers produced with this strain have a low alcohol content and a fruiter, ester-like flavour [16]. This is also confirmed by research conducted by Johansson et al. [17], in which beers produced with this strain were characterised by a fruity–ester aroma with notes of apple. The *Saccharomyces cerevisiae* var. *chevalieri* strain is very popular. This yeast is currently the most widely used strain for the production of non-alcoholic beers throughout the brewing industry. Beers produced with this strain are highly accepted by consumers. The taste of the beer is mainly due to the presence of maltose, which is not used by this strain. Maltose is a sugar that adds sweetness to beer. This sugar does not occur in classically fermented beers because it is used up almost entirely by the yeast [18]. There is currently not much research related to the characteristics of this yeast strain in the brewing industry. The manufacturer declares that this strain contains an enzyme that converts phenolic acids, such as ferulic acid and cumaric acid, present in the wort, thus producing compounds affecting the taste [18]. This is very beneficial when producing low- and non-alcoholic beers using biological methods.

The main aim of the study was to investigate the influence of the yeast strain *Saccharomyces cerevisiae* var. *chevalieri*, used in the production of NoLo beers, on the flavour profile of the resulting beers. How modification of the mashing profile (one of the biological NoLo production methods) would affect the aromatic profile of the finished beers using this strain was also investigated. Low-alcohol beers produced using biological methods are often characterised by a weak aroma, so whether the changed mashing profile contributes to increasing the full flavour of the resulting beers was also investigated. To date, no results have been published that would show the aroma profile of beers produced with this yeast strain. Beers produced with the commonly used brewing yeast *Saccharomyces cerevisiae* were used as control samples. The obtained beers were analysed for physicochemical parameters, namely, turbidity, color, free amino nitrogen (FAN), alcohol, pH and sugar content, by HPLC analysis. In addition, odour compounds were analysed using GC-MS and GC-O gas chromatography.

2. Materials and Methods

2.1. Materials

Commercial pilsner malt (Viking Malt, Strzegom, Poland) and Oktawia (5.7% alpha acids) hops (PolishHops, Karczmiska, Poland) were used for the production of hopped wort. *Saccharomyces cerevisiae* (SafAle US-05, Fermentis, Warsaw, Poland) and *Saccharomyces cerevisiae* var. *chevalieri* (SafBrew LA-01, Fermentis, Warsaw, Poland) were used for fermentation.

2.2. Beer Production

2.2.1. Wort Preparation

All obtained worts were prepared using a Mash Batch R12 (1-CUBE, Havlíčkův Brod, Czech Republic). Two mashing profiles were selected. The first mashing profile is the production of Congress wort according to the EBC method [19]. The modified mashing profile was used to produce worts with a lower maltose content. For this purpose, the saccharification test (maltose production) was omitted. The mash was prepared in the same way as the Congress mash and held at 76 °C for 30 min. The containers were then cooled to 20 °C, filled with distilled water to reach a total mass of 450.0 g, and filtered through a paper filter (MN614, Oensingen, Switzerland).

2.2.2. Boiling

After the filtration process, the wort was boiled. Variant I consisted of boiling the wort without hops. Variant II involved boiling the wort with the addition of Oktawia hops (5.7% alpha acids). Then, the wort was boiled for 60 min. After completion of the boil, the hot tub was removed from the wort using Whatman class 802 filter paper. The wort was then cooled to 20 °C and brought to a common extract (9 °P) by diluting the obtained wort.

2.2.3. Fermentation Trails

The inoculation procedure was identical for both mashing profiles. After boiling, the cooled wort was inoculated with appropriate yeast strains. All variants were inoculated with 5×10^6 CFU/mL (*Saccharomyces cerevisiae* var. *chevalieri* or *Saccharomyces cerevisiae*) based on the wort extract. The concentration of yeast cells in 1 mL of suspension was determined using a Thoma chamber. Each sample (250 mL) was then placed in a 500 mL Erlenmeyer flask and fermented under anaerobic conditions. Caps with fermentation tubes filled with glycerol ensured anaerobic conditions. The fermentation was conducted at 20 °C for 8 days using a Q-CELL 240 thermostatic chamber (Alchem, Wilkowice, Poland). The kinetics of the fermentation process were monitored by measuring the mass loss, which corresponded to the release of carbon dioxide (g/L), throughout the duration of the fermentation.

2.3. Analytical Determinations

2.3.1. Physicochemical Parameters of Obtained Wort and Beer

Analysis of pH, color, turbidity, alcohol and real extract followed the methodology outlined by Pater et al. [20].

2.3.2. FAN

The free amino nitrogen content was determined using the ninhydrin method. This method involves spectrometric measurement of the color intensity, which is proportional to the concentration of the color complex formed by the reaction of ninhydrin reagent with NH3. The mixture was boiled for 10 min after adding the ninhydrin reagent. The sample's absorbance was then measured at a wavelength of 575 nm, using distilled water with ninhydrin as the baseline. A standard glycine sample was used to perform a parallel procedure [21].

2.3.3. Sugar Analysis Using a High-Performance Liquid Chromatograph (HPLC)

Sugar analysis was conducted using the method described by Satora and Pater [22], utilizing a Shimadzu NEXERA XR system (Kyoto, Japan) with an RF-20A refractometric detector. The separation was achieved on a Shodex Asahipak NH2P-50 column (4.6 × 250 mm) from Showa Denko Europe (Munich, Germany), maintained at a temperature of 30 °C. The mobile phase was a 70% aqueous acetonitrile solution, operated under isocratic conditions with a flow rate of 0.8 mL/min for a duration of 16 min. Quantitative analysis was performed using standard curves generated from glucose, fructose, maltose, maltotriose and saccharose standards obtained from Sigma-Aldrich (Poznań, Poland).

2.3.4. Odour-Active Volatile Components (HS-SPME-GC-O)

The odour-active volatile compounds of wort and beers identified by olfactometry were conducted using the method described by Pater et al. [20].

2.3.5. Analysis of Volatile Compounds Using HS-SPME-GC-MS

The analysis of volatile compounds of wort and beers was conducted using the method described by Pater et al. [20].

Volatile compounds were identified using the National Institute of Standards and Technology (NIST) database and LRIs (linear retention indices) calculated from a series of C6 to C30 n-alkanes. Quantitative identification of volatiles (Sigma-Aldrich) consisted of a comparison of the sample peak area with standard chromatograms and also with the internal standard.

2.3.6. Sensory Assessments

The sensory evaluation of the worts and the purchased beers focused on their aromas, using six sensory descriptors (fruity, floral, roasted, herbal, woody and chemical), rated on a 5-point hedonic scale in quantitative descriptive analysis (QDA). The panelists were scientific staff from the Faculty of Food Technology and Human Nutrition at the University of Agriculture in Krakow. These panelists had previously graduated from the faculty and had completed a comprehensive course on sensory analysis as part of their curriculum. The panelists were first presented with standards of different flavours to assess their recognition. They were then given the same standards at different concentrations. Only those who successfully identified the aromas at both stages were selected as panelists. The sensory evaluation of the beers was carried out by a panel of 10 selected panelists. The samples were coded and distributed to the panelists in a randomised order.

2.3.7. Statistical Analysis

The experiments were conducted and analysed in triplicate. However, the figures and tables show only the average values. The data were analysed using a one-way analysis of variance (ANOVA). The significance in the difference for each parameter was analysed separately using Tukey's post hoc test (Statistica v.10, StatSoft Inc., Krakow, Poland) and heat map test (MS Excel, Version 16.78.3).

3. Results and Discussion

3.1. Physico-Chemical Parameters of the Worts Produced by Different Mashing Profiles

One of the biological methods of producing low- and no-alcohol beers is an appropriately modified mashing profile [23]. During mashing, the starch present in the malt is broken down into fermentable sugars by the enzymes it contains (mainly α-amylase and β-amylase). In the present article, wort was produced using the Congress method and by modifying the mashing profile (mashing at a temperature of 75 °C). The purpose of modifying the mashing profile was to reduce the fermentation potential of the wort through the limitation of the breakdown from starch into fermentable sugars (mainly maltose). This effect was achieved by inactivating the enzyme α-amylase at a temperature above 65 °C [8]. Table 1 shows the physico-chemical parameters of the analysed worts. Both variants of the obtained worts were characterised by the same extract yield (9 °P), but in the case of the modified mashing, a significantly lower content of fermentable sugars was present in this extract. This was confirmed by analysing the content of individual sugars present in the wort using the HPLC method (Table 1). The applied mash profile modification helped to achieve a significantly lower content of the most desirable sugars in brewing, i.e., maltose, compared to the Congress mashing (about 25% less) and maltotriose (about 40% less). The remaining sugars, i.e., glucose, fructose and saccharose, were not statistically different between the variants analysed. Similar relationships were obtained in studies by Ivanov et al. [24], where mashing at a temperature of 77 °C resulted in a much lower content of fermentable sugars, including maltose, compared to the control mashing. These results

confirm that an appropriate modification of the mashing profile can help to achieve a lower content of fermentable sugars, which is necessary to produce the appropriate amount of alcohol by a given yeast strain.

Table 1. Physico-chemical parameters of the hopped worts produced by different mashing profiles.

Parameters	Wort (Modified Mashing)	Wort (Congress Mashing)	Sig [1]
Saccharification time [min]	>5	>5	ns
Extract [°P]	9.0 (±0.1)	9.0 (±0.1)	ns
pH	5.8 (±0.1)	6.1 (±0.1)	ns
Color [EBC]	6.2 (±0.1)	6.2 (±0.3)	ns
Turbidity [EBC]	11.4 (±0.3)	13.8 (±0.9)	ns
FAN [mg/L]	91.2 [a] (±2.3)	120.6 [b] (±3.9)	**
Maltose [g/L]	15.4 [a] (±7.1)	31.2 [b] (±1.7)	*
Maltotriose [g/L]	6.5 [a] (±0.3)	10.8 [b] (±0.9)	**
Saccharose [g/L]	3.4 (±2.2)	2.3 (±0.4)	ns
Glucose [g/L]	4.9 (±2.8)	2.6 (±0.7)	ns
Fructose [g/L]	3.8 (±3.4)	0.5 (±0.2)	ns

[1] Significance; ns—not statistically different; * and **, indicate significance at a level of 0.01–0.005 respectively, by the least significant difference. Values with different superscript Roman letters (a and b) in the same row indicate statistically significant differences according to the Duncan test ($p < 0.05$).

Another important parameter is the content of free amino nitrogen compounds (FAN), which are necessary for the growth and development of yeast and, therefore, for a proper fermentation process [24]. According to the literature, the content of free amino nitrogen compounds should be between 100 and 300 mg/L (at 12 °P) [25], converted into 9 °P, these figures would be 75 and 225 mg/L. Modification of the mashing profile resulted in bypassing not only the saccharification break (which produces maltose) but also the protein break (45–55 °C), during which the greatest amount of nitrogen compounds is produced [23]. During the mashing at a temperature of 75 °C from the malt to the wort, an adequate amount of FAN was released based on the extract of 12 °P, and, therefore, sufficient nitrogen sources were available for the initial adaptation of the yeast and subsequently through fermentation. The results obtained are, therefore, similar to those described in the article by Enders et al. [26], where the mashing profile was also modified to produce beer with reduced alcohol content. The other physico-chemical parameters of the wort obtained are not statistically different (Table 1).

3.2. Profile of Volatile Compounds in the Produced Hopped and Unhopped Worts Produced by Different Mashing Profiles

When using biological methods to produce NoLo beers, brewers/technologists focus not only on reducing the amount of alcohol synthesized by limiting fermentable sugars but also on maintaining the classic organoleptic profile of the beer [27]. Table 2 shows the content of volatile compounds (GC-MS) and odour-active compounds (GC-O) in the

worts analysed. Three main groups of compounds were present in the highest amounts (alcohols, terpenes, aldehydes). These compounds originate from the raw materials used to produce the wort (malt, hops) or are formed during the mashing and boiling stages [28]. The alcohols analysed include isobutyl alcohol, 3-methyl-1-butanol, 2-methyl-1-butanol and 2,3-butanediol (Table 2). Only the concentration of 2-methyl-1-butanol was above the detection threshold. No statistically significant differences were observed between the analysed worts. Most terpenes were present in the worts in concentrations exceeding their aroma threshold, which was also confirmed by the olfactometric analysis. The main source of terpenes in beer is the hops. This plays an important role in determining the aroma of the final product, as hop oil contains a large number of aromatically active ingredients [29]. In the worts analysed, the dominant terpene was linalool with a detection threshold of 5 µg/L. This compound is characterised by floral and lavender aromas. According to the literature, linalool is a good indicator of hop flavour [30]. The other dominant aromas transferred from the hops to the worts during boiling are pine, woody, rose and floral, derived from compounds such as β-pinene, trans-linalool oxide, citronellol and geraniol. Research by other scientists also shows that after boiling, worts are dominated by aromas derived from hops, i.e., terpenes [29]. Statistically significant differences were observed between citronellol and geraniol. The worts produced by the Congress method were characterised by a significantly higher concentration of these compounds, which is also confirmed by the results of the intensity of individual aromas presented in Table 3. Hop isomerisation quantifies the efficiency with which alpha acids introduced during boiling are converted to iso-alpha acids in the wort [31]. The efficiency of isomerisation is affected by temperature, intensity and duration of boiling, hopping rate, pH of the wort and absorption of bitter compounds on the protein break [32]. The higher levels of citronellol and geraniol in the wort after Congress mashing may be due to the use of a protein break, which partially degrades the protein present in the malt [33]. In a study by Ganz et al. [31], it was found that increased coagulation of proteins during the mashing stage resulted in increased isomerisation of alpha acids.

The next group of compounds analysed was the aldehydes (Table 2). There are several methods for creating aldehydes during the production of brewing wort. The most important of these are oxidation of unsaturated fatty acids, Maillard reactions and Strecker amino acid degradation [32]. Four aldehydes were selected in the produced wort (pentanal, furfural, heptanal and methional) based on the results obtained after olfactometric analysis. These compounds were characterised by fruity, bready and cooked vegetable odours. The concentration of furfural was significantly higher in the worts after modified mashing than after conventional mashing (Table 2). Furfural is formed in the wort during boiling as a result of Maillard reactions [34]. The increased content of this compound in the wort after modifying the mashing profile could be caused by mashing at high temperatures (75 °C). It can be concluded that at this stage, Maillard reactions began to occur, during which larger amounts of dextrins were also produced [35]. However, the higher concentration of this compound did not have a negative effect on the sensory perception of the wort analysed, which is confirmed by the intensity concentrations shown in Table 3. Conversely, methional was characterised by a concentration above the detection threshold in both worts. Ditrych et al. [36] identified malt as the primary source of aldehydes like methional. These compounds are introduced into the wort during boiling, and their concentrations tend to increase with storage. Methional, which has a boiled potato aroma, can negatively impact beer aroma at higher concentrations. However, in the olfactometric analysis conducted, methional did not adversely affect the aroma of the wort (Table 3).

Table 2. Content of volatile and odour-active compounds of hopped worts analysed.

[µg/L]	*m/z*	LRI [4]	Threshold [3]	Wort (Modified Mashing)	Wort (Congress Mashing)	SEM [1]	Sig [2]	GC-O Descriptors [5]
Alcohols								
Isobutyl alcohol [s]	43, 41, 33	609	3600	5.8	3.2	0.7	ns	X
3-methyl-1-butanol	42, 55, 70	716	1000	496.9	688.6	56.4	ns	X
2-methyl-1-butanol	41, 57, 70	724	15.9	221.2	189.1	14.7	ns	X
2,3-butanediol [s]	45, 57, 75	1492	4500	2.7	2.4	0.9	ns	X
Terpenes								
β-pinene	41, 69, 93	969	4	14.5	18.3	2.7	ns	Pine [H]
β-myrcene	41, 69, 93	981	13	136.3	299.1	49.9	ns	Slightly floral with spicy notes [F]
Limonene	68, 79, 93	1025	65	7.06	6.5	0.5	ns	X
Trans-linalool oxide	43, 59, 94	1077	5	30.4	20.9	2.8	ns	Woody [H]
Linalool	55, 71, 93	1089	6	237.3	335.1	27.1	ns	Flower and lavender [FL]
Citronellol	41, 67, 69	1205	8	16.7 [b]	29.1 [a]	3.1	*	Rose [FL]
Geraniol	41, 69, 93	1232	4	142.5 [b]	243.9 [a]	23.6	*	Floral [FL]
4-Terpineol [s]	71, 93, 111	1179	1.5	5.5	6.3	0.3	ns	Pine [H]
Aldehydes								
Pentanal	44, 58, 41	688	280	4.9	2.8	0.6	ns	Fruity, nutty [FR, R]
Furfural	96, 39, 67	825	250	3.5	1.9	0.4	*	Bready [R]
Heptanal	70, 44, 55	889	15	2.8	3.2	0.4	ns	Fruity [FR]
Methional [s]	48, 104, 76	909	0.3	1.9	1.6	0.2	ns	Boiled potatoes [V]
Others								
Acetophenone	51, 77, 105	1036	65	1.59	1.4	0.1	ns	X
Benzothiazole	69, 108, 135	1196	80	12.1	6.9	1.3	ns	X
Verbenol [s]	43, 59, 119	1146	1800	116.9	21.1	22.6	**	Herbal [H]

[1] SEM—standard error of the mean. [2] Significance; ns—not statistically different; * and ** indicate significance at a level of 0.05–0.01 and 0.01–0.005 respectively, by the least significant difference. Values with different superscript Roman letters (a and b) in the same row indicate statistical differences according to the Duncan test ($p < 0.05$). [4] LRI—linear retention index; the amount of components was determined. [3] Threshold in beer [37]. OAV > 1. [5] Aroma descriptor perceived at the sniffing port of the GC-O. X—not detected in the GC-O analysis. Aroma group of detected aroma descriptors is signified by letters in brackets—roasted (R), fruity (FR), floral (FL), herbaceous (H), chemical (C) and Vegetable (V). [s]—concentration of given compounds calculated relative to the internal standard SD < 5%.

Table 3. Heatmap of odour-active compound intensities detected by GC-O in the hopped worts obtained (Congress and modified mashing).

Compounds	LRI [1]	Wort (Modified Mashing)	Wort (Congress Mashing)
β-pinene	969	1.0	1.0
β-myrcene	981	1.0	1.0
Trans-linalool oxide	1077	1.0	1.0
Linalool	1089	1.0	1.0
Citronellol	1205	0.8	1.0
Geraniol	1232	0.8	1.0
4-Terpineol	1179	0.8	1.0
Pentanal	280	0.5	0.5
Furfural	825	0.5	0.5
Heptanal	889	0.5	0.5
Methional	909	1.0	1.0
Verbenol	1146	0.5	0.5

[1] LRI—linear retention index. The lowest intensity of aromas in these columns is in the darkest red, the average concentration in orange and the highest intensity is in the darkest green. SD < 5%.

3.3. Fermentation Kinetics of Beers Produced from Worts after Different Mashing Profiles with Saccharomyces cerevisiae var. chevalieri or S. cerevisiae US-05 Strains

The wort obtained from the Congress and the modified mashing were inoculated with the yeast used for the production of non-alcoholic beers (*Saccharomyces cerevisiae* var. *chevalieri*) and, as a control, with the yeast commonly used in brewing for the production of ale beers, *Saccharomyces cerevisiae* US-05. The kinetics of the fermentation process were measured from the day the wort was inoculated with a specific amount of each yeast strain (Figure 1). The analysis was carried out until no changes in the amount of CO_2 released (g/L) were observed in the following days of the process. As expected, the *Saccharomyces cerevisiae* US-05 yeast strain started fermentation from the first day of the process and the intensity of fermentation was significantly higher compared to the yeast strain intended for the production of NoLo beers (*Saccharomyces cerevisiae* var. *chevalieri*). As a result of the reduced maltose content, the yeast releases less carbon dioxide during the fermentation of the wort resulting from the modified mash compared to the Congress mash (Figure 1). This is also confirmed by the alcohol content results shown in Table 4. However, this did not correspond to the production of the amount of alcohol shown in Table 4. In the case of *Saccharomyces cerevisiae* var. *chevalieri*, fermentation started slowly and this trend was maintained throughout the fermentation period. From a sensory point of view, incomplete fermentation of maltose, as is the case with the *Saccharomyces cerevisiae* var. *chevalieri* strain, can contribute to the production of sweet beers [38]. The use of alternative yeasts that do not ferment maltose offers a compelling approach to producing low-alcohol beers with aromatic complexity. These yeasts also help to reduce aldehydes in the wort, thereby eliminating the "wort" taste commonly found in low-alcohol beers [39]. Interesting results were observed in the case of fermentation with and without the addition of hops. The samples with hop addition fermented better than the others. Adamenko and Kawa-Rygielska [40] focused their research on the influence of hops on the production of NoLo beers. They found that the hop variety and quantity had a direct effect on the fermentation process of low-alcohol and non-alcoholic beers. Due to the low alcohol and high carbohydrate content, the production of NoLo beers carries the risk of the growth of undesirable organisms [41]. It is, therefore, planned to investigate in detail how the anti-bacterial properties of hops protect NoLo beers against infections during fermentation.

Table 4. Physico-chemical parameters of the low-alcohol beers produced using different mashing methods, with or without the addition of hops and with different yeast strains for fermentation.

Parameters	Beer (Modified Mashing)				Beer (Congress Mashing)				Sig [1]
	***Saccharomyces cerevisiae* var. *chevalieri* without Hops**	***Saccharomyces cerevisiae* var. *chevalieri* with Hops**	***Saccharomyces cerevisiae* without Hops**	***Saccharomyces cerevisiae* with Hops**	***Saccharomyces cerevisiae* var.** chevalieri without Hops	***Saccharomyces cerevisiae* var. *chevalieri* with Hops**	***Saccharomyces cerevisiae* without Hops**	***Saccharomyces cerevisiae* with Hops**	
Colour [EBC units]	4.8 ^{cb} (±0.6)	5.3 ^{b} (±0.5)	4.6 ^{cb} (±0.4)	5.8 ^{a} (±0.1)	4.3 ^{c} (±0.1)	5.1 ^{cb} (±0.2)	4.3 ^{c} (±0.1)	5.4 ^{b} (±0.2)	*
Turbidity [EBC units]	4.9 ^{b} (±1.1)	7.3 ^{b} (±2.1)	6.4 ^{b} (±2.3)	12.7 ^{a} (±2.9)	6.4 ^{b} (±0.2)	5.6 ^{b} (±1.5)	12.9 ^{a} (±0.1)	8.2 ^{b} (±1.1)	**
pH	4.5 (±0.1)	4.4 (±0.1)	4.4 (±0.1)	4.5 (±0.1)	4.1 (±0.0)	4.5 (±0.0)	4.1 (±0.0)	4.5 (±0.0)	ns
Ethanol [% *v*/*v*]	0.5 ^{f} (±0.1)	0.8 ^{e} (±0.1)	2.4 ^{c} (±0.1)	2.7 ^{d} (±0.0)	0.5 ^{f} (±0.1)	0.5 ^{a} (±0.0)	3.5 ^{b} (±0.0)	3.8 ^{a} (±0.0)	***
Real extract [% *w*/*w*]	6.8 ^{a} (±0.3)	5.9 ^{b} (±0.2)	2.8 ^{c} (±0.4)	3.1 ^{c} (±0.1)	7.1 ^{a} (±0.2)	7.2 ^{a} (±0.1)	3.0 ^{c} (±0.1)	3.1 ^{c} (±0.0)	***
FAN [mg/L]	61.5 ^{ab} (±9.9)	44.5 ^{b} (±2.8)	44.4 ^{b} (±3.8)	58.9 ^{b} (±17.0)	73.7 ^{a} (±3.2)	49.4 ^{b} (±5.8)	44.3 ^{b} (±3.9)	54.8 ^{b} (±5.6)	*
Maltose [g/L]	11.9 ^{b} (±2.1)	7.5 ^{b} (±0.8)	0.7 ^{c} (±0.2)	0.0 ^{d} (±0.0)	29.5 ^{a} (±2.8)	28.5 ^{a} (±1.4)	0.0 ^{d} (±0.0)	0.0 ^{d} (±0.0)	***
Maltotriose [g/L]	6.1 ^{b} (±0.9)	3.1 ^{c} (±0.3)	0.4 ^{d} (±0.3)	0.1 ^{d} (±0.0)	7.2 ^{b} (±0.4)	10.0 ^{a} (±0.6)	0.1 ^{d} (±0.0)	0.1 ^{d} (±0.0)	***
Saccharose [g/L]	0.5 (±0.6)	0.0 (±0.0)	0.1 (±0.1)	0.1 (±0.0)	0.2 (±0.2)	0.1 (±0.0)	0.1 (±0.1)	0.1 (±0.1)	ns
Glucose [g/L]	0.9 (±2.9)	0.2 (±0.2)	0.5 (±0.4)	0.4 (±0.3)	0.2 (±0.4)	0.5 (±0.0)	0.3 (±0.3)	0.8 (±0.6)	ns
Fructose [g/L]	3.8 (±3.4)	0.6 (±0.5)	1.6 (±0.9)	1.5 (±0.8)	1.4 (±0.9)	2.8 (±0.2)	2.3 (±0.3)	2.3 (±1.7)	ns

[1] Significance; ns—not statistically different, *, **, and *** indicate significance at a level of 0.05–0.01, 0.01–0.005 and <0.005, respectively, by the least significant difference. Values with different superscript Roman letters (a–f) in the same row indicate statistically significant differences according to the Duncan test ($p < 0.05$).

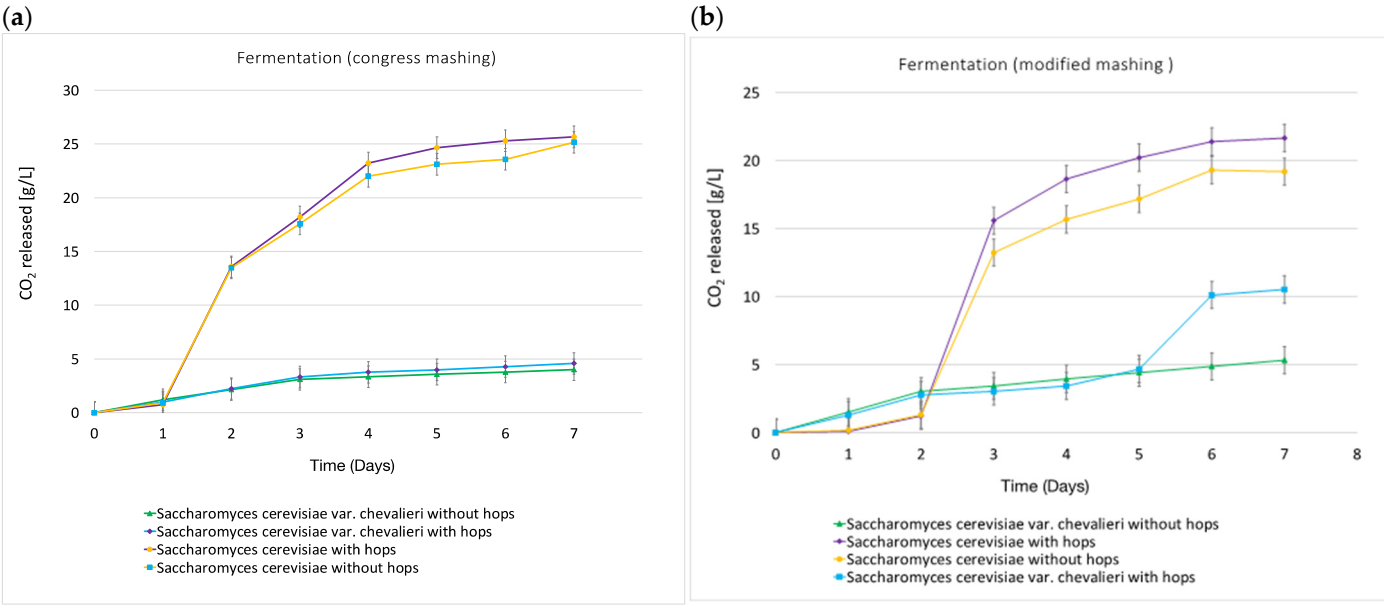

Figure 1. Fermentation kinetics of beers obtained from wort after Congress (**a**) and modified mashing (**b**) with *Saccharomyces cerevisiae* var. *chevalieri* and *Saccharomyces cerevisiae*, n = 3; STD < 5%.

3.4. Physicochemical Parameters of Beers Produced from Worts after Different Mashing Profiles with Saccharomyces cerevisiae var. chevalieri or S. cerevisiae US-05 Strains

Table 4 shows the physico-chemical parameters of the beers obtained. The colour of a beer is an important sensory attribute because it must correspond to the style of the beer and this is the first characteristic that the consumer notices. The appearance of the product, including the colour, is an important quality factor [42]. The beers obtained had a colour in the range of 4.3–5.8 EBC units. These values are consistent with pale beers produced from 100% Pilsner malt [43]. Modification of the mashing profile and different yeast strains did not affect the colour of the resulting beers. Differences were observed in beers with and without hops. This is also confirmed by the research of Adamenko and Kawa-Rygielska [40]. In their research, the authors found that the colour of non-alcoholic beers is determined by the hop variety as well as its form and quantity. Another important parameter that gives the consumer the first visual impression of the quality of the beer is its turbidity. The formation of haze has a negative effect on the organoleptic properties and clarity of the beer [44]. The beers obtained are characterised by an appropriate level of turbidity, which is also confirmed by research carried out by other scientists [45].

pH is also a key parameter in beer production. During fermentation, various metabolites produced by the yeast—including organic acids—can decrease this quality control parameter [46]. All beers analysed had similar pH values (4.1–4.5), appropriate for a given beer style [47]. The results obtained in terms of ethanol concentration indicate that the yeast used, *Saccharomyces cerevisiae* var. *chevalieri*, makes it possible to obtain beer with an ethanol content of 0.5% *v*/*v* in the case of the Congress mashing. During the fermentation process, these yeasts used almost none of the available maltose and also assimilated less free nitrogen compounds FAN (Table 4). In turn, after modifying the mashing profile, these yeasts contributed to obtaining beers with an average alcohol content of 0.5–0.8% *v*/*v*, using 25% of the available maltose (Table 4). Therefore, in the case of this yeast strain, the mashing process must be modified in the first stage to minimise the production of glucose and produce maltose and dextrins instead. This yeast does not use maltose for fermentation and the resulting beers are rich in dextrins, which is very desirable in the case of NoLo beers [14,48]. As for beers inoculated with *Saccharomyces cerevisiae* yeast (US-05), they produced 3.0–3.1% *v*/*v* ethanol in the case of Congress mashing, where there was a higher content of fermentable sugars (maltose). In this case, the yeast used 100% of the available maltose. After modifying the mashing profile (lower amount of fermentable

sugars), the yeast produced an average alcohol content of 2.4–2.7% v/v. This proves that the applied modification of the mashing profile is suitable for traditional yeast strains used in brewing because by reducing the amount of maltose, a significantly lower alcohol content is produced. In the case of maltotriose, it is not surprising that the yeast *Saccharomyces cerevisiae* used almost the all available sugar in both mash profiles. *Saccharomyces cerevisiae* var. *chevalieri* did not use the sugars at all after the Congress mashing. As for the remaining sugars (glucose, fructose, saccharose), both strains had similar sugar content in all variants, and the obtained concentrations of individual sugars did not differ statistically (Table 4).

3.5. Content of Odour-Active Compounds in Low-Alcohol Beers Produced Using Different Mashing Methods, with or without the Addition of Hops, and with Different Yeast Strains for Fermentation

The quality of fermented beverages depends largely on the type of yeast strain used in their production, the alcohol concentration and the extract, as well as aroma compounds [49]. Therefore, appropriate modification of the mashing profile or the use of specific yeast strains in the fermentative production of non-alcoholic beers may help preserve the metabolites responsible for their sensory profile [50]. Table 5 shows the content of odour-active compounds found in the beers analysed. During the production of the beers, variants without and with the addition of hops were considered in order to study how the yeast strain used influences the aromatic profile of the obtained beers (*Saccharomyces cerevisiae* var. *chevalieri* and *Saccharomyces cerevisiae* US-05). In the beers analysed, 28 odour-active aroma compounds were detected, including 8 alcohols, 9 esters, 8 terpenes and 3 compounds classified as other. The detected aromas were divided into six odour groups: roasted (R), fruity (FR), floral (FL), herbaceous (H), chemical (C) and animal (A).

The first group analysed was the higher alcohols, which affect the taste of beer by increasing the perception of alcohol and giving a warmer sensation in the mouth. The process of biosynthesis of these compounds requires the involvement of several genes and is directly related to the metabolism of amino acids via the Ehrlich pathway [51,52]. Among the alcohols analysed, the highest concentrations were found in compounds such as 2-methyl-1-propanol, which gives the beer a mild and sweet aroma [53]. The highest amounts of this compound were produced during fermentation with the yeast *Saccharomyces cerevisiae* US-05, regardless of the mashing profile used. In the case of 2-methyl-1-butanol (malt and sweet aroma), beers fermented with *Saccharomyces cerevisiae* also contained higher concentrations of this compound after modification of the mashing profile. With respect to the Congress mashing, beers without the addition of hops after fermentation with *Saccharomyces cerevisiae* var. *chevalieri* were characterised by a higher value of 2-methyl-1-propanol. The concentration of this compound in all the samples analysed was above the detection limit, which is also confirmed by the analyses of the intensity of the individual aromas presented in Table 6. Higher alcohols such as 1-hexanol also impart herbal and green aromas to the beer. This compound was present above the detection limit in the beers analysed and the highest concentration was observed in beers fermented with the yeast strain *Saccharomyces cerevisiae*. 2-Phenylethanol was also found above the detection limit in all analysed samples. A significantly higher concentration of this compound was observed in beers mashed according to the Congress method without the addition of hops, both after fermentation with *Saccharomyces cerevisiae* var. *chevalieri* and *Saccharomyces cerevisiae*. The aromatic alcohol 2-phenylethanol has a sweet rose aroma and has a positive effect on the aroma of beer. This compound is also thought to mask the perception of dimethyl sulphide (DMS) [54]. The higher alcohols analysed in this paper contributed significantly to the final aroma of the beers obtained, as confirmed by the results of the olfactometric analysis. Both the strain used for the production of non-alcoholic beers (*Saccharomyces cerevisiae* var. *chevalieri* as well as *Saccharomyces cerevisiae* strain) after modification of the mashing profile, contributed to the production of a significantly higher content of these compounds compared to low-alcoholic and non-alcoholic beers produced by other methods [55,56].

Of the nine esters analysed, seven had a concentration above the detection limit (Table 5). The dominant aromas belonged to the group of fruity and floral compounds.

Esters are formed intracellularly as a result of the fermentation of yeast cells [57]. One of the most important esters with flavour and aroma effects in beer is ethyl acetate, which gives beer a floral and solvent aroma [58], which was also confirmed by olfactometric analysis. The highest concentration of ethyl acetate was found in beer without the addition of hops fermented with the yeast *Saccharomyces cerevisiae* var. *chevalieri*, compared to other variants (Table 5). The values obtained are higher than in the studies carried out by Ramsey et al. [56]. Ethyl propionate also had a significant effect on the flavour of the beers obtained. This compound is characterised by a pineapple aroma. The highest concentration of this compound was found in beers after modified mashing fermented with the yeast strain *Saccharomyces cerevisiae*. In the case of ethyl hexanoate, the highest concentration of this compound occurred in beers with hops in both mashing profiles (Table 5). This compound is characterised by a fruity and red apple aroma. These values (ethyl propionate and ethyl hexanoate) are similar to the concentrations of compounds found in beers according to the table in the article by Romero-Rodriguez et. al. [59]. Another ester that contributed significantly to the sensory profile of the beers obtained is ethyl octanoate with an apple, banana and pineapple aroma [60]. The highest concentration of this compound was found in hopped beers mashed by the Congress method and fermented with both yeast strains. In the current research, the beers obtained were characterised by a higher content of esters compared to those analysed by Riu-Aumatell et al. [61].

In addition to active secondary flavour metabolites, brewer's yeast influences the taste of beer through the biotransformation of hop-derived flavour compounds [62,63]. As hops are the main source of terpenes in beer [30], these compounds were only detected in the hopped beers. Compounds derived from hops mainly contributed herbal, fruity and floral aromas to the beer (Table 5). During fermentation, the concentration of linalool, which is characterised by a floral and lavender aroma, increased in beers with a modified mashing profile. Kaltner's research [64] also demonstrated such relationships, suggesting that fermentation releases glycoside-related flavour compounds that enhance aroma. In addition, Belgian researchers have shown that beta-glucosidase activity is strain-independent and appears to be beneficial during beer fermentation [65]. In the present study, a significant increase in the concentration of this compound was observed in both strains after the end of fermentation. The decrease in geraniol concentration during fermentation is also very interesting; again, the greatest decrease was observed in beers after Congress mashing (Table 5). Yeast can biotransform some monoterpene alcohols and hydrogenate geraniol to citronellol [61], a trend observed in the present study. The concentration of citronellol increased in all beers analysed (regardless of the mashing performed).

In addition to alcohols, terpenes and esters, the beers analysed also contained other key compounds such as acetophenone, decanone and benzothiazoles. These compounds were characterised by floral and chemical aromas. Decanal and benzothiazoles had concentrations above the detection limit, which is also confirmed by the results of the intensity of individual compounds during olfactometric analysis (Table 6).

All the beers obtained were subjected to a sensory analysis (QDA). The beers with hop addition after modified mashing received the highest score (total score) for both yeast strains (almost 4.5/5 points). They also received the highest scores in the floral, fruit and hop aroma categories (Figure 2). Beers after modified mashing fermented with the *Saccharomyces cerevisiae* var. *chevalieri* strain without the addition of hops were characterised by a woody and roasted aroma. In the case of samples prepared using the Congress mash, *Saccharomyces cerevisiae* var. *chevalieri* fermented without hops was characterised by a woody and chemical aroma.

Table 5. Odour-active compounds of the low-alcohol beers produced using different mashing methods, with or without the addition of hops and with different yeast strains for fermentation.

				Beer (Modified Mashing)				Beer (Congress Mashing)						
[µg/L]	*m/z*	LRI [5]	Threshold [3]	*Saccharomyces cerevisiae* var. *chevalieri* without Hops	*Saccharomyces cerevisiae* var. *chevalieri* with Hops	*Saccharomyces cerevisiae* without Hops	*Saccharomyces cerevisiae* with Hops	*Saccharomyces cerevisiae* var. *chevalieri* without Hops	*Saccharomyces cerevisiae* var. *chevalieri* with Hops	*Saccharomyces cerevisiae* without Hops	*Saccharomyces cerevisiae* with Hops	SEM [1]	Sig [2]	GC-O descriptors [4]
Alcohols														
1-propanol-2-methyl	43, 41, 33	609	3600	662.2 ^{b}	802.6 ^{b}	3118.1 ^{a}	2709.3 ^{a}	1180.5 ^{b}	2151.7 ^{a}	3428.2 ^{a}	3719.4 ^{a}	281.6	***	Mild and sweet [FR]
1-butanol [s]	56, 41, 31	650	20,000	0 ^{c}	0 ^{c}	8.1 ^{a}	6.8 ^{a}	4.2 ^{b}	9.8 ^{a}	15.7 ^{a}	13.4 ^{a}	0.9	**	Sweet, alcoholic [R]
3-methyl-1-butanol	42, 55, 70	716	1000	73,156 ^{c}	104,556 ^{b}	123,488 ^{b}	131,358 ^{b}	124,822 ^{b}	201,993 ^{a}	124,777 ^{b}	119,681 ^{b}	11011	**	Bready, alcoholic, fruity [R, FR]
2-methyl-1-butanol	41, 57, 70	724	15.9	18,891 ^{d}	26,966 ^{dc}	42,574 ^{b}	41,586 ^{b}	32,137 ^{c}	57,030 ^{a}	36,747 ^{c}	38,329 ^{c}	3276	***	Malt and sweet [FR]
1-pentanol, 4-methyl- [s]	56, 41, 69	852	1800	1.1 ^{a}	6.4 ^{a}	2.3 ^{a}	2.9 ^{a}	1.2 ^{a}	0.8 ^{b}	3.9 ^{a}	1.5 ^{a}	0.4	*	Pungent [C]
1-hexanol [s]	56, 43, 69	889	10	76.5 ^{b}	88.6 ^{b}	78.1 ^{b}	99.5 ^{a}	33.1 ^{c}	69.2 ^{b}	27.8 ^{c}	28.2 ^{c}	5.3	**	Herbal, green [H]
2-phenylethanol	91, 65, 122	1091	1000	2441 ^{b}	2539 ^{b}	2714 ^{b}	2805 ^{b}	2260 ^{b}	3531 ^{a}	2961 ^{b}	3041 ^{ab}	215.6	*	Rose [FL]
2,3-butanediol [s]	45, 57, 75	1492	4500	2.5	7.3	188.1	139.5	42.4	0	31.9	6.5	21.8	ns	Buttery, creamy [A]
Esters														
Ethyl acetate	43, 61, 70	598	5000	7381 ^{c}	15,169 ^{c}	13,224 ^{c}	16,205 ^{c}	13,400 ^{c}	42,323 ^{a}	20,463 ^{b}	16,331 ^{c}	2164	**	Floral and solvent [FL]
Ethyl propionate [s]	57, 29, 102	695	7	2.4 ^{b}	6.7 ^{b}	25.2 ^{a}	34.5 ^{a}	2.5 ^{b}	21.3 ^{a}	8.03 ^{b}	24.3 ^{a}	2.5	**	Pineapple [F]
Isobutyl acetate	43, 56, 73	758	1100	173.3 ^{b}	655.2 ^{b}	459.6 ^{b}	600.3 ^{b}	245 ^{b}	1724.9 ^{a}	282.8 ^{b}	306.4 ^{b}	106.7	*	Fruit, apple and banana [FR]
Ethyl butyrate	43, 71, 88	784	150	16 ^{c}	30.8 ^{c}	130.8 ^{b}	164.5 ^{a}	14 ^{c}	74.9 ^{b}	30.1 ^{c}	41.8 ^{c}	11.8	**	Pineapple, sweet and fruity [FR]
1-Butanol 3-methyl-, acetate	43, 55, 70	860	220	124.7 ^{c}	719.8 ^{b}	244.1 ^{c}	351.4 ^{c}	253 ^{c}	1743.3 ^{a}	121.8 ^{c}	174.3 ^{c}	114.9	*	Fruity and apple [FR]
Ethyl valerate	57, 85, 88	883	1	0.2	0.5	1.6	2.9	0.7	1.5	0.7	1.1	0.2	ns	Yeast and fruit [FR]
Ethyl hexanoate	43, 88, 99	980	200	66.4 ^{b}	307.0 ^{a}	242.6 ^{a}	257.8 ^{a}	129.1 ^{b}	432.6 ^{a}	122.3 ^{b}	218.6 ^{a}	27.7	*	Fruity and red apple [FR]
Ethyl octanoate	57, 88, 101	1179	70	252.7 ^{c}	816.2 ^{b}	1058.8 ^{b}	792.9 ^{b}	707.9 ^{b}	1204.2 ^{a}	902.2 ^{b}	1245.6 ^{a}	71.4	***	Apple, banana, pineapple [FR]
Ethyl laurate	88, 101, 55	1577	5000	54.6 ^{c}	533.9 ^{b}	160.4 ^{c}	98.5 ^{c}	156.9 ^{c}	1062.7 ^{a}	97.8 ^{c}	199.1 ^{c}	63.3	*	Fruity [FR]

Table 5. *Cont.*

[µg/L]	*m/z*	LRI [5]	Threshold [3]	Beer (Modified Mashing)				Beer (Congress Mashing)				SEM [1]	Sig [2]	GC-O descriptors [4]
				Saccharomyces cerevisiae var. *chevalieri* without Hops	*Saccharomyces cerevisiae* var. *chevalieri* with Hops	*Saccharomyces cerevisiae* without Hops	*Saccharomyces cerevisiae* with Hops	*Saccharomyces cerevisiae* var. *chevalieri* without Hops	*Saccharomyces cerevisiae* var. *chevalieri* with Hops	*Saccharomyces cerevisiae* without Hops	*Saccharomyces cerevisiae* with Hops			
Terpenes														
β-pinene	41, 69, 93	969	4	0 ^{b}	4.6 ^{a}	0 ^{b}	2.3 ^{a}	0 ^{b}	0.7 ^{b}	0 ^{b}	0.4 ^{b}	1.5	*	Pine [H]
β-myrcene	41, 69, 93	981	13	0 ^{b}	144.6 ^{a}	0 ^{b}	95.4 ^{a}	0 ^{b}	6.4 ^{b}	0 ^{b}	1.4 ^{b}	18.9	*	Slightly floral with spicy notes [F]
Limonene	68, 79, 93	1025	65	0 ^{e}	3.2 ^{a}	0 ^{e}	2.3 ^{b}	0 ^{e}	1.3 ^{c}	0 ^{e}	0.7 ^{d}	0.4	***	Lemon and citrus [FR]
Trans-linalool oxide	43, 59, 94	1077	5	0 ^{c}	22.2 ^{a}	0 ^{c}	24.8 ^{a}	0 ^{c}	11.8 ^{b}	0 ^{c}	17.2 ^{b}	2.0	***	Woody [H]
Linalool	55, 71, 93	1089	6	0 ^{c}	679.6 ^{a}	0 ^{c}	536.1 ^{a}	0 ^{c}	393.5 ^{ab}	0 ^{c}	221.5 ^{b}	34.8	**	Flower and lavender [FL]
Citronellol	41, 67, 69	1205	8	0 ^{e}	97.4 ^{b}	0 ^{e}	118.3 ^{a}	0 ^{e}	57.8 ^{c}	0 ^{e}	37.5 ^{d}	5.1	***	Rose [FL]
Geraniol	41, 69, 93	1232	4	0 ^{d}	72.6 ^{b}	0 ^{d}	199.6 ^{a}	0 ^{d}	52.9 ^{c}	0 ^{d}	55.1 ^{c}	13.5	***	Floral [FL]
4-Terpineol [s]	71, 93, 111	1179	1.5	0 ^{c}	6.1 ^{a}	0 ^{c}	6.2 ^{a}	0 ^{c}	4.2 ^{b}	0 ^{c}	1.2 ^{c}	0.5	***	Pine [H]
Others														
Acetophenone	51, 77, 105	1036	65	1.5	8.3	2.7	5.5	4.3	1.7	2.2	7.7	0.7	ns	Sweet, pungent and chemical [C]
Decanal	41, 43, 57	1182	0.1	283.8 ^{a}	1223.1 ^{b}	590.5 ^{a}	426.8 ^{a}	620.5 ^{a}	1663.3 ^{b}	402.3 ^{a}	749.6 ^{a}	96.3	**	Aldehydic, citrus and floral [FL]
Benzothiazole	69, 108, 135	1196	80	103.9 ^{a}	137.7 ^{a}	107.2 ^{a}	125.3 ^{a}	684.6 ^{b}	70.3 ^{a}	299.7 ^{a}	376.3 ^{a}	39.7	**	Gasoline and rubber [C]

[1] SEM—standard error of the mean. [2] Significance; ns—not statistically different; *, **, and *** indicate significance at a level of 0.05–0.01, 0.01–0.005, and <0.005, respectively, by the least significant difference. Values with different superscript Roman letters (a–e) in the same row indicate statistical differences according to the Duncan test ($p < 0.05$). [5] LRI—linear retention index; the amount of components was determined. [3] Threshold in beer [37]. OAV > 1. [4] Aroma descriptor perceived at the sniffing port of the GC-O. X—not detected in the GC-O analysis. Aroma group of detected aroma descriptors indicated by letters in brackets—roasted (R), fruity (FR), floral (FL), herbaceous (H), chemical (C) and animal (A). [s]—concentration of given compounds calculated relative to the internal standard, SD < 5.

Table 6. Heatmap of odour-active compound intensities detected by GC-O in the low-alcohol beers produced using different mashing methods, with or without the addition of hops and with different yeast strains for fermentation.

		Beer (Modified Mashing)				Beer (Congress Mashing)			
Compound	**[1] LRI**	***Saccharomyces cerevisiae* var. *chevalieri* without Hops**	***Saccharomyces cerevisiae* var. *chevalieri* with Hops**	***Saccharomyces cerevisiae* without Hops**	***Saccharomyces cerevisiae* with Hops**	***Saccharomyces cerevisiae* var. *chevalieri* without Hops**	***Saccharomyces cerevisiae* var. *chevalieri* with Hops**	***Saccharomyces cerevisiae* without Hops**	***Saccharomyces cerevisiae* with Hops**
Isobutyl alcohol	609	0.5	0.5	0.8	0.5	0.5	0.5	0.8	1.0
1-butanol	650	0.0	0.0	0.5	0.5	0.5	0.5	0.5	0.5
3-methyl-1-butanol	716	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2-methyl-1-butanol	724	0.5	0.5	1.0	1.0	0.8	1.0	0.8	0.8
1-pentanol, 4-methyl-	852	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
1-hexanol	889	0.8	0.8	0.8	1.0	0.5	0.8	0.5	0.5
2-phenylethanol	1091	0.8	0.8	0.8	0.8	0.8	1.0	0.8	1.0
2,3-butanediol	1492	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Ethyl acetate	598	0.5	0.5	0.5	0.5	0.5	1.0	0.8	0.5
Ethyl propionate	695	0.5	0.5	1.0	1.0	0.5	1.0	1.0	1.0
Isobutyl acetate	758	0.5	0.5	0.5	0.5	0.5	0.8	0.5	0.5
Ethyl butyrate	784	0.5	0.5	0.8	1.0	0.5	0.8	0.8	0.8
1-Butanol 3-methyl-, acetate	860	0.5	1.0	1.0	1.0	1.0	1.0	0.5	0.5
Ethyl valerate	883	0.5	0.5	0.8	0.8	0.5	0.8	0.5	0.8
Ethyl hexanoate	980	0.5	1.0	1.0	1.0	0.5	1.0	0.5	1.0
Ethyl octanoate	1179	0.5	0.8	1.0	0.8	0.8	1.0	0.8	1.0
Ethyl laurate	1577	0.5	1.0	0.5	0.5	0.5	1.0	0.5	0.5

Table 6. *Cont.*

Compound	[1] LRI	Beer (Modified Mashing)				Beer (Congress Mashing)			
		Saccharomyces cerevisiae var. *chevalieri* without Hops	*Saccharomyces cerevisiae* var. *chevalieri* with Hops	*Saccharomyces cerevisiae* without Hops	*Saccharomyces cerevisiae* with Hops	*Saccharomyces cerevisiae* var. *chevalieri* without Hops	*Saccharomyces cerevisiae* var. *chevalieri* with Hops	*Saccharomyces cerevisiae* without Hops	*Saccharomyces cerevisiae* with Hops
β-pinene	969	0.0	1.0	0.0	0.5	0.0	0.5	0.0	0.5
β-myrcene	981	0.0	1.0	0.0	1.0	0.0	0.5	0.0	0.5
Limonene	1025	0.0	0.5	0.0	0.5	0.0	0.5	0.0	0.5
Trans-linalool oxide	1077	0.0	1.0	0.0	1.0	0.0	0.8	0.0	0.8
Linalool	1089	0.0	0.8	0.0	1.0	0.0	0.5	0.0	0.5
Citronellol	1205	0.0	0.8	0.0	1.0	0.0	0.5	0.0	0.5
Geraniol	1232	0.0	0.8	0.0	1.0	0.0	0.5	0.0	0.5
4-Terpineol	1179	0.0	0.5	0.0	0.5	0.0	0.5	0.0	0.5
Acetophenone	1036	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Decanal	1182	0.5	1.0	0.5	0.5	0.5	1.0	0.5	0.8
Benzothiazole	1196	0.8	0.8	0.8	0.8	1.0	0.5	0.8	0.8

[1] LRI—linear retention index. The lowest intensity of aromas in these columns is in the darkest red, and the highest intensity is in the darkest green. SD < 5%.

Figure 2. Sensory analysis (QDA) of the low-alcohol beers produced using modified mashing (**a**) or Congress mashing (**b**), with or without the addition of hops and with different yeast strains for fermentation. n = 5; STD < 5%.

4. Conclusions

In conclusion, the study demonstrates the effectiveness of modifying the mashing profile to produce low- and no-alcohol beers with reduced fermentable sugars, particularly maltose. This is confirmed by the results obtained for, among other things, the content of individual sugars. The modification of the mashing profile (omission of the saccharification pause) contributed to the production of approximately 25% less of the most important sugar in brewing, i.e., maltose. Thanks to this, the commonly used yeast (*Saccharomyces cerevisiae* US-05) produced beers with an average content of 2.4–2.7% v/v during fermentation, which was much lower compared to the Congress mashing (3.5–3.8% v/v). This approach not only addresses fermentation potential but also ensures sufficient free amino nitrogen compounds for yeast growth and fermentation. The beers obtained with this method were also characterised by a rich sensory profile with high consumer acceptance. In the case of the yeast strain for the production of alcohol-free beer (*Saccharomyces cerevisiae* var. *chevalieri*), with a standard mashing profile (with a saccharification pause), it contributed to obtaining beers with an alcohol content of 0.5% v/v. For this strain, it is, therefore, important to choose a mashing profile that minimises glucose production by producing a higher amount of maltose. In addition, the production of low-alcohol beers using *Saccharomyces cerevisiae* var. *chevalieri* contributed to the production of beers with the desired sensory profile. These beers were characterised by a very rich aromatic profile, including higher alcohols, esters and terpenes. Higher levels of compounds such as β myrcene and 4-terpineol were observed, which also had a significant impact on the sensory profile of the beers obtained. In addition, beers with hops after modification of the mashing profile obtained the highest score (almost 4.5/5 points) in the sensory analysis. The sensory analysis underlines the importance of hop addition and yeast selection and highlights the potential to produce flavourful non-alcoholic beverages while maintaining traditional beer characteristics. Overall, the results emphasise the importance of tailored brewing techniques and yeast strains in achieving desired alcohol levels and sensory experiences in low-alcohol and non-alcoholic beers.

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