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Versatility of *Saccharomyces cerevisiae* 41CM in the Brewery Sector: Use as a Starter for "Ale" and "Lager" Craft Beer Production

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Abstract: Craft breweries tend to use special raw materials and also special ingredients (spices, herbs, fruits) to typify beers, but the metabolic activities of yeasts play a primary role in defining the sensory characteristics of this beverage. *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* are yeast species usually used for ale and lager beer production. The selection and use of new yeast starters with peculiar technological and enzymatic characteristics could represent the key point for the production of beers with good and distinctive organoleptic properties. In this study, the fermentative performance of *S. cerevisiae* 41CM yeast isolated from the vineyard environment for ale and lager craft beer production on a laboratory scale was evaluated. The commercial yeast *S. cerevisiae* Fermentis S-04 and *S. pastorianus* Weihenstephan 34/70 were used as reference strains. *S. cerevisiae* 41CM showed fermentative kinetics similar to commercial starters, both in lager (12 °C) and ale (20 °C) brewing. In all beers brewed, the largest percentage of volatile compounds synthesized during the fermentation were alcohols, followed by esters, terpenes, and aldehydes. In particular, *S. cerevisiae* 41CM starter contributed a higher relative percentage of esters in the ale beer than that detected in the lager beer, without ever synthesizing unwanted volatile compounds.

Keywords: top and bottom fermenting yeast; craft beers; microbrewing

Citation: Testa, B.; Coppola, F.; Letizia, F.; Albanese, G.; Karaulli, J.; Ruci, M.; Pistillo, M.; Germinara, G.S.; Messia, M.C.; Succi, M.; et al. Versatility of *Saccharomyces cerevisiae* 41CM in the Brewery Sector: Use as a Starter for "Ale" and "Lager" Craft Beer Production. *Processes* **2022**, *10*, 2495. https://doi.org/10.3390/pr10122495

Academic Editor: Antonio D. Moreno

Received: 26 October 2022
Accepted: 21 November 2022
Published: 24 November 2022

1. Introduction

Beer is a fermented beverage made from these primary ingredients: water, malted cereal grain (such as barley), hops, and yeast. The choice of a yeast strain to be used for brewing is crucial for achieving beers with desirable, unique, and distinctive sensory properties [1]. The two main brewer's yeasts are *Saccharomyces cerevisiae* and *Saccharomyces pastorianus*, a natural hybrid between *S. cerevisiae* and the newly discovered *Saccharomyces eubayanus* [2,3].

S. cerevisiae, traditionally used for "ale" beer, is characterized by specific properties, such as flocculation and ethanol tolerance, and requires temperatures ranging between 15 °C and 25 °C for optimal fermentation [4]. On the other hand, *S. pastorianus*, used for "lager" beer [5], ferments well at temperatures between 8 and 12 °C [6] and differs in its ability to metabolize maltotriose and its high flocculation [7,8].

In the last decade, an interest in craft beer has emerged in several countries, where sales have grown faster than the common industrial beer [9]. Craft brewers tend to use special ingredients such as spices, herbs, and fruits to enhance the beer flavor, but yeasts

and their metabolic behavior have the main role in defining the organoleptic characteristics of the beer [10]. Therefore, the selection and use of new yeast strains with peculiar metabolic properties could represent the key point in differentiating products in the brewery sector, especially for local producers [11,12]. Recent metagenomics studies indicate that the natural fungal biodiversity is immense and largely unexplored, and the commercial yeast strains available represent a small fraction of the natural biodiversity [13,14]. This evidence suggests that the environment hosts yeast strains that could prove biotechnologically viable in industrial fermentation, including beer production. [15]. In recent years, researchers have been working to create and expand the pool of yeasts for the brewery sector, including also the creation in the laboratory of new strains generated by interspecific hybridization [3] or isolated from non-brewing environments [16]. In order to evaluate some fermentative performances, in this study we tested the suitability of *S. cerevisiae* 41CM isolated from the vineyard environment in "ale" and "lager" beer production. This strain was selected for its good technological properties and, in particular, cryotolerance, low hydrogen sulfide production, and flocculation ability [17].

2. Materials and Methods

2.1. Yeasts Cultures and Microbial Media

S. cerevisiae 41CM (accession number to GenBank OM037660) belonging to the Di.A.A.A (Department of Agricultural, Environmental and Food Sciences; Campobasso, Italy) collection was used as a new brewing starter. As a reference, the following two commercial yeast strains were used: "Bottom-fermenting yeast" *S. pastorianus* Weihenstephan 34/70 (Fachhochschule Weihenstephan, Freising, Germany) and "Top-fermenting yeast" *S. cerevisiae* S-04 (Fermentis, Lesaffre, Maisons-Alfort Cedex, France). The yeasts were cultured aerobically at 20 °C in 250 mL of YEPD broth (Thermo Fisher Scientific, Waltham, MA, USA) using 500 mL Erlenmeyer flasks maintained under stirring using a digital orbital shaker (Heathrow Scientific, Vernon Hills, IL, USA) set at 150 rpm. After 24 h, the broth cultures were centrifuged at 5000 rpm for 10 min at 4 °C, and the cell pellets, after washing with saline solution (0.9% NaCl), were obtained. Finally, cell pellets were resuspended into 10 mL of beer wort and used as inoculum. Cell density of inoculum was assessed by Thoma Hemocytometer Counting Chamber (Thermo Fisher Scientific).

The yeast viable cell counts at the beginning and during the alcoholic fermentation were evaluated using WL nutrient agar medium (Thermo Fisher Scientific) and by plate incubation for 48 h at 28 °C in aerobic conditions.

2.2. Wort Production

Beer wort was brewed in a local craft microbrewery (Birra del Contado, Cercemaggiore CB, Molise, Italy). For the production of blonde ale wort, Pilsen and Munich malts were used (Château Pale ale, Castle Malting, Lambermont, Belgium) and Saaz and East Kent Golding hops (Barth-Hass, Nürnberg, Germany) were added during the boiling phase. Instead, Pilsen and Vienna malts (Château Pale ale, Castle Malting, Lambermont, Belgium), and Saaz and Chinook hops (Barth-Hass, Nürnberg, Germany) for international pale lager wort production were used.

The main analytical characteristics of the worts, meeting the requirements established by BJCP [18], were as follows: pH 5.72, °Plato 12.3, density (original gravity) 1.049 g/cm^3, 20 IBU (International Bitterness Unit) and FAN (free amino nitrogen) 268 mg/L for the blonde ale style; pH = 5.70, °Plato 12.0, density (original gravity) 1.048 g/cm^3, 18 IBU and FAN 258 mg/L for international pale lager style.

2.3. Fermentation Trials

Four fermentative tests were carried out using the following yeast starters: *S. cerevisiae* 41CM (41CM ale); *S. cerevisiae* S-04 (S-04 ale); *S. cerevisiae* 41CM (41CM lager), and *S. pastorianus* W-34/70 (W-34/70 lager). The primary fermentation was conducted at 20 °C for tests with 41CM and S-04 starters, and at 12 °C for tests with 41CM and W-34/70 starter,

respectively. For each test, 25 L of wort was inoculated with approximately 10^6 yeast cells/mL. The fermentative tests were conducted in 30 L stainless-steel fermenters. All tests were performed in triplicate. Fermentations were monitored by measuring the pH decrease, ethanol production, and yeast cell viability count and were stopped when ethanol concentration remained stable for three consecutive days. At the end of the primary fermentation (15 days), 3.5 g/L of sucrose (priming) was added to the fermented products. The secondary fermentation and maturation were carried out at 20 °C for ale beer (41CM ale and S-04 ale) and 12 °C for lager beer (41CM lager and W-34/70 lager) using glass bottles with a 0.33 L capacity, amber color, corona stopper, standard model. After 30 days, the beers were subjected to physicochemical and volatile compound analysis.

2.4. Physicochemical Analysis

The density and ethanol were determined according to methods designated by the European Brewery Convention (EBC) [19]. Glycerol, acetaldehyde, acetic acid, and DL-lactic acid were determined using enzymatic kits (Steroglass, Perugia, Italy) according to the manufacturer's instructions. Diacetyl (2,3-butanedione) was determined according to the method of Alvarez et al. [20]. FAN and IBU were determined, respectively, according to the method described by Dukes et al. [21] and Kishimoto et al. [22]. All these determinations were performed spectrophotometrically using a BioSpectrometer basic (Eppendorf, Hamburg, Germany). The pH measurement was conducted using a pHmeter (Crison basic 20, Barcelona, Spain). All the analyses were carried out in triplicate.

2.5. Aroma Volatile Compounds Analysis

Volatile compounds were determined by static headspace solid-phase micro-extraction (HS-SPME) technique according to Alves et al. [23]. Briefly, 2 mL of sample was placed into a 4 mL headspace vial (Supelco Co., Bellefonte, PA, USA), sealed with a PTFE/silicon septum (Supelco Co., Bellefonte, PA, USA) for analysis. The vial was heated at 40 °C for 30 min in a water bath prior to SPME headspace sampling. Extraction was performed using SPME fibers (Supelco Co., Bellefonte, PA, USA) coated with either 50/30 µm of divinylbenzene–carboxen–polydimethylsiloxane (DVB–CAR–PDMS). The fibers were conditioned before use by heating them in the injection port of the GC system at 270 °C for 1 h. After the extraction time, the fibers were recovered and transferred to the injection port of the GC, where the compounds were thermally desorbed at 250 °C for 4 min. A fiber cleaning step of 10 min at the conditioning temperature with the split valve opened was performed in the GC injector after every chromatographic run to remove any absorbed residue. Before the acquisitions, a blank test was performed under the same experimental conditions to check for possible impurities.

GC–MS analyses were performed using an Agilent 7890B series gas chromatograph (Agilent Technologies, Milan, Italy) coupled with an Agilent 5977A mass selective detector (MSD) equipped with an HP-5MS capillary column (30 m × 0.25 mm ID, 0.5 µm film thickness, J&W Scientific Inc., Folsom, CA, USA). The desorption step was carried out in the splitless mode (5 min) with a programmed temperature from 60 °C to 250 °C at 5 °C/min, with a final holding time of 10 min. The carrier gas was helium at a flow rate of 1.25 mL/min. Spectra were recorded in the electron impact mode (ionization energy, 70 eV) in a range of 15–550 amu at 2.9 scans/s. The identification of volatile compounds was achieved by comparing mass spectra with those of the data system library (NIST11, $p > 90\%$) and, wherever possible, by comparing retention times (R.T.) and mass spectra with those of commercially available standards. A mixture of a continuous series of straight-chain hydrocarbons, C5–C40 (Alkane Standard Solution C5–C40, Sigma Aldrich, Milan, Italy), was injected into an HP-5MS column under the same conditions previously described for the beer sample to obtain the linear retention indices (RIs) [24]. The relative abundance of each compound was calculated using the integrated peak area data from the GC-MS trace.

2.6. Statistical Analysis

All experiments were carried out in triplicate. The results were expressed as the mean ± SD (n = 3). Yeast cell counts were analyzed by ANOVA (IBM SPSS Statistics 21). Physicochemical parameters were analyzed using a t test with Welch's correction.

3. Results and Discussion

3.1. pH Evolution and Alcohol Production during Fermentation

Fermentations were monitored by measuring the pH decrease and the ethanol production. The evolution of these parameters is shown graphically in Figure 1 and the relative numerical data are shown in Tables S1 and S2 (Supplementary Materials). The primary fermentations had a regular course and were completed in all the tests conducted. The pH from an initial value of about 5.70 gradually decreased, until it reaches final values between 4.43 and 4.50 without significant differences, both in the lager and ale beers obtained with the use of *S. cerevisiae* 41CM, *S. pastorianus* W-34/70, and *S. cerevisiae* S-04 starters. The ethanol kinetic in the ale and lager beers, obtained with the use of the *S. cerevisiae* 41 CM strain, showed similar evolution compared to commercial strains. As regards the yeast viable cell count, both for lager and ale beers, an increase of one logarithmic cycle was found after 4 days of fermentation with values that stabilized at about 7.0 log CFU/mL until the end of primary fermentation. The numeric data for yeast viable cell count are reported in Table S3 (Supplementary Materials). Overall, the fermentative kinetics of *S. cerevisiae* 41CM are comparable to those of the two commercial starters; the performance of this new starter has shown that its versatility would allow it to be used in the production of both lager beer at 12 °C and ale beer at 20 °C.

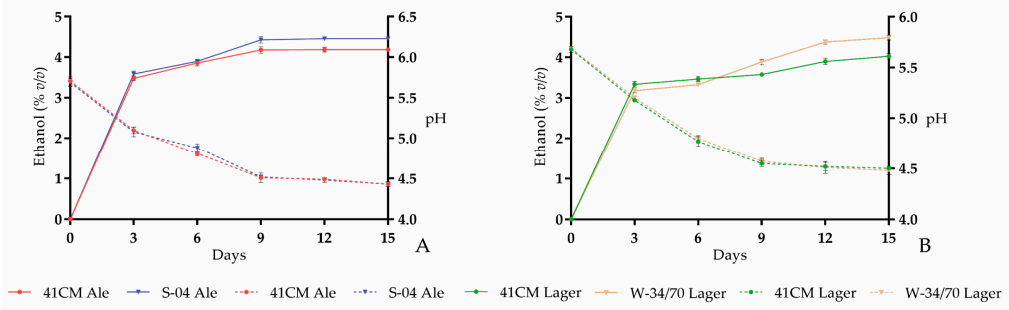

Figure 1. Evolution of pH (dashed lines) and ethanol (continuous lines) during the primary fermentation of ale (**A**) and lager (**B**) beers. Error bars indicate ± SD (n = 3).

3.2. Main Physicochemical Characteristic of the Beers

The data of the physicochemical analysis of the beers are reported in Tables 1 and 2. Significant differences between ale and lager beers obtained with the use of *S. cerevisiae* 41CM, *S. pastorianus* W-34/70, and *S. cerevisiae* S-04 were found, except for pH and final density.

Table 1. Physicochemical parameters of the ale beers obtained using *S. cerevisiae* 41CM and *S. cerevisiae* S-04 starters. Different lowercase letters in each row represent significant differences ($p < 0.05$). Results are shown as mean ± SD (n = 3).

	41CM Ale	**S-04 Ale**
pH	4.43 ± 0.03 ^{a}	4.43 ± 0.02 ^{a}
Acetic acid (mg/L)	60.86 ± 0.50 ^{a}	80.89 ± 0.40 ^{b}
Ethanol (% *v/v*)	4.20 ± 0.02 ^{a}	4.47 ± 0.04 ^{b}
Glycerol (mg/L)	1556.66 ± 68.24 ^{a}	1685.33 ± 26.02 ^{b}
D,L-lactic acid (mg/L)	47.90 ± 2.72 ^{a}	57.80 ± 3.30 ^{b}
Diacetyl (mg/L)	0.10 ± 0.01 ^{a}	0.12 ± 0.01 ^{b}
Acetaldehyde (mg/L)	2.10 ± 0.06 ^{a}	2.40 ± 0.03 ^{b}
Density (g/cm^3)	1.010± 0.01 ^{a}	1.011± 0.01 ^{a}

Table 2. Physicochemical parameters of the lager beers obtained using *S. cerevisiae* 41CM and *S. pastorianus* W-34/70 starters. Different lowercase letters in each row represent significant differences ($p < 0.05$). Results are shown as mean ± SD ($n = 3$).

	41CM Lager	W-34/70 Lager
pH	4.50 ± 0.01 [a]	4.48 ± 0.04 [a]
Acetic acid (mg/L)	70.37 ± 0.70 [a]	90.88 ± 0.90 [b]
Ethanol (% *v/v*)	4.05 ± 0.07 [a]	4.51 ± 0.06 [b]
Glycerol (mg/L)	1289.33 ± 13.05 [a]	1196.01 ± 13.52 [b]
D,L-lactic acid (mg/L)	20.73 ± 0.73 [a]	28.23 ± 0.40 [b]
Diacetyl (mg/L)	0.11 ± 0.01 [a]	0.29 ± 0.02 [b]
Acetaldehyde (mg/L)	1.33 ± 0.06 [a]	1.40 ± 0.04 [a]
Density (g/cm^3)	1.013 ± 0.01 [a]	1.012 ± 0.01 [a]

At the end of fermentations and maturation, the ethanol content in the beers obtained with the use of *S. cerevisiae* 41CM strain was, respectively, 4.20 % *v/v* in ale and 4.05 % *v/v* in lager beers, significantly different with respect to beers obtained using *S. cerevisiae* S-04 (4.47 % *v/v*) and *S. pastorianus* W-34/70 (4.51% *v/v*) strains.

Acetic acid is the main component of volatile acids in beer and an excess of it can give an unwanted hint of vinegar. The concentration of this acid found in ale and lager beers, fermented using *S. cerevisiae* 41CM strain were, respectively, 60.86 mg/L and 70.37 mg/L, significantly lower with respect to beers obtained with the use of *S. cerevisiae* S-04 (80.89 mg/L) and *S. pastorianus* W-34/70 (90.88 mg/L) strains.

This compound is formed during yeast metabolism through the oxidation of acetaldehyde or the reabsorption and metabolization of pyruvic acid [25]. Regardless of the starter used, in the beers produced in our tests, the quantities of acetic acid remained below the threshold value of 100 mg/L [26].

Glycerol is a desirable alcohol and positively influences beer flavor intensity, fullness, and body [27]. In ale and lager beers where the *S. cerevisiae* 41CM strain was used, the glycerol content detected was 1556.66 mg/L and 1289.33 mg/L, respectively. Significantly different were the amounts of glycerol found in beers obtained by the use of *S. cerevisiae* S-04 (1685.33 mg/L) and *S. pastorianus* W-34/70 (1196.01 mg/L) strains.

As regards the concentration of DL-lactic acid, the *S. cerevisiae* 41CM strain produced amounts of this acid of 20.73 mg/L (lager beer) and 47.90 mg/L (ale beer), significantly lower compared to the beers obtained with the use of *S. pastorianus* W-34/70 (28.23 mg/L) and *S. cerevisiae* S-04 (57.80 mg/L) strains. This acid is mainly derived from yeast metabolism and was reported to have a threshold flavor of about 400 mg/L [26]. Therefore, in all the beers obtained in our tests, this acid does not exert a direct effect on the flavor, while it contributes to the pH of the beer.

Diacetyl (2,3-butanedione) is a vicinal diketone product of yeast metabolism and is more characteristic of ale than lager beers; its concentration is usually used as an indicator of wort fermentation or maturation quality [20]. Diacetyl, mainly in lager beer, is considered an undesirable volatile compound above the taste threshold of about 0.10–0.15 mg/L since it could give the beer a stale milk aroma and flavor [28]. Our results showed a high variability regarding the diacetyl production; in particular, the *S. cerevisiae* 41CM strain used both for ale and lager beer produced lower amounts of this compound (0.10 and 0.11 mg/L) compared to the beers obtained by commercial *S. cerevisiae* S-04 and *S. pastorianus* W-34/70 strains (0.12 and 0.29 mg/L, respectively). Yeast cells hold the enzymes required to reduce diacetyl to acetoin and then to 2,3-butanediol as well as those required to reduce 2,3-pentanedione to 2,3-pentanediol. This elimination of diacetyl and 2,3-pentanedione occurs at the end of the conventional main fermentation period and during the maturation of beer. These reduced compounds have a much higher flavor threshold and their presence is acceptable at the concentrations usually found in beer.

The detected amounts of acetaldehyde, both in ale and lager beers, were between 1.33 and 2.40 mg/L, below the threshold perception values (5–15 mg/L). Acetaldehyde is

an "off-flavor" known to have the aroma and flavor of green apple or pumpkin peel [29]. During fermentation, the yeast produces acetaldehyde as an intermediate compound in the conversion of glucose to ethanol. However, at the end of the alcoholic fermentation and during maturation the yeast completely converts the vast majority of acetaldehyde to ethanol so that any residual amount falls below the flavor threshold. If the fermentation is less than optimal, the conversion of acetaldehyde to ethanol may remain incomplete [30].

3.3. Volatile Compound Characteristics

The complex flavor of the beers can largely be attributed to biochemical activities within the yeast cell during fermentation processes.

Flavor-active substances produced by fermenting yeast cells can be divided into five main groups: sulfur-containing molecules, organic acids, higher alcohols, carbonyl compounds, and volatile esters [29].

In Table 3 are reported the volatile compounds identified in the beers brewed using the different starter yeasts.

Table 3. Main volatile compounds identified (expressed as percentage of the peak area of each compound compared to the total area) in the ale and lager beers produced by different starters.

Peak No.	Compound	R.T.	$RI_{Lit.}$ [2]	$RI_{Exp.}$ [3]	41CM Ale Area (%) ± S.E. [1]	S04 Ale Area (%) ± S.E. [1]	41CM Lager Area (%) ± S.E. [1]	W-34/70 Lager Area (%) ± S.E. [1]
	Alcohols:							
1	Ethyl alcohol	2.63	-	<500	42.85 ± 0.55	48.73 ± 0.68	50.54 ± 1.01	37.65 ± 0.42
2	1-butanol, 3-methyl-	4.36	731	734	0.64 ± 0.03	2.70 ± 0.06	3.25 ± 0.06	1.64 ± 0.05
3	1-butanol, 2-methyl-	4.41	735	736	4.71 ± 0.06	3.35 ± 0.07	2.86 ± 0.06	2.28 ± 0.06
4	1-Hexanol	7.66	868	870	0.13 ± 0.01	0.17 ± 0.01	-	0.08 ± 0.01
7	1-Heptanol	10.97	963	966	-	0.12 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
11	1-Hexanol, 2-ethyl	12.9	1031	1026	0.14 ± 0.01	0.93 ± 0.04	0.19 ± 0.01	0.13 ± 0.01
12	1-Octanol	14.26	1060	1063	0.12 ± 0.01	0.16 ± 0.02	0.21 ± 0.03	0.11 ± 0.01
16	Phenethyl alcohol	15.7	1119	1121	9.11 ± 0.15	13.07 ± 0.21	11.67 ± 0.11	7.26 ± 0.21
22	1-Decanol	20.19	1275	1279	0.05 ± 0.01	0.11 ± 0.01	0.07 ± 0.01	0.03 ± 0.00
24	2-Undecanol	20.98	1301	1303	-	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.00
	Total alcohols				57.75 ± 0.83	69.37 ± 1.11	68.87 ± 1.30	49.27 ± 0.78
	Esters:							
5	3-methylbutyl ester	7.91	874	876	5.82 ± 0.06	2.57 ± 0.05	5.44 ± 0.07	2.31 ± 0.05
9	Hexanoic acid, ethyl ester	11.94	995	996	2.97 ± 0.06	3.16 ± 0.04	2.64 ± 0.05	3.02 ± 0.04
10	Acetic acid, hexyl ester	12.4	1015	1017	0.10 ± 0.03	0.12 ± 0.02	0.32 ± 0.03	0.13 ± 0.02
13	Heptanoic acid, ethyl ester	15.13	1092	1095	0.05 ± 0.00	0.11 ± 0.02	0.89 ± 0.04	0.10 ± 0.01
18	Octanoic acid, ethyl ester	18.11	1194	1196	18.14 ± 0.28	14.03 ± 0.28	8.19 ± 0.17	31.12 ± 0.41
21	Acetic acid, 2-phenylethyl ester	19.85	1253	1255	1.54 ± 0.04	1.55 ± 0.05	3.09 ± 0.07	1.61 ± 0.05
23	Nonanoic acid, ethyl ester	20.85	1291	1294	0.08 ± 0.01	0.09 ± 0.01	0.04 ± 0.00	0.08 ± 0.01
26	9-Decenoic acid, ethyl ester	23.24	1382	1387	5.19 ± 0.06	3.67 ± 0.04	1.94 ± 0.05	5.54 ± 0.05
27	Decanoic acid, ethyl ester	23.43	1393	1397	6.45 ± 0.06	2.67 ± 0.04	5.26 ± 0.06	4.77 ± 0.06
28	Octanoic acid, isopentyl ester	24.7	1441	1446	0.05 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00
29	Dodecanoic acid, ethyl ester	28.16	1594	1597	0.55 ± 0.04	0.15 ± 0.02	0.66 ± 0.05	0.09 ± 0.01
	Total esters				40.94 ± 0.64	28.14 ± 0.57	28.49 ± 0.59	48.80 ± 0.71
	Aldehydes:							
15	Nonanal	15.33	1102	1105	0.04 ± 0.00	0.04 ± 0.00	0.09 ± 0.01	0.08 ± 0.01
19	Decanal	18.35	1203	1201	0.05 ± 0.00	0.09 ±0.01	0.12 ± 0.01	0.17 ± 0.02
	Total aldehydes				0.09 ± 0.00	0.13 ± 0.01	0.21 ± 0.02	0.25 ± 0.03
	Terpenes:							
14	Linalool	15.2	1108	1106	0.45 ± 0.03	0.66 ± 0.05	-	0.62 ± 0.05
20	Citronellol	18.99	1236	1232	0.16 ± 0.01	0.52 ± 0.04	0.61 ± 0.04	0.47 ± 0.03
	Total terpenes				0.61 ± 0.04	1.18 ± 0.09	0.61 ± 0.04	1.09 ± 0.08
	Others:							
8	Sulcatone	11.56	971	974	0.10 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	0.06 ± 0.01
17	Octanoic acid	17.47	1193	1191	0.20 ± 0.00	0.13 ± 0.01	0.31 ± 0.02	0.12 ± 0.01
25	Gamma-Nonalactone	22.7	1358	1361	-	0.05 ± 0.01	0.10 ± 0.01	0.02 ± 0.00
	Total others				0.30 ± 0.02	0.26 ± 0.03	0.49 ± 0.04	0.20 ± 0.02

[1] N = 3 replicates; [2] RI_{Lit} = linear retention index from the literature; [3] RI_{Exp} = determined linear retention index against mixture of n-alkanes (C5–C40) on HP-5MS column.

Alcohols represented the major group in all the beers, followed by esters, terpenes, and aldehydes. The ale beer obtained with the use of *S. cerevisiae* 41CM showed the lowest percentage amounts of total alcohols (57.75%), concerning the beer obtained with the use of *S. cerevisiae* S-04 (69.37%). Conversely, in lager beers, *S. cerevisiae* 41CM contributed to obtaining a percentage of total alcohols (68.87%) higher than that obtained with the use of *S. pastorianus* W-34/70 (49.27%).

After ethyl alcohol (Peak No. 1), phenethyl alcohol (Peak No. 16) had the highest relative percentage, ranging from 7.26% (W-34/70 lager) to 13.07% (S-04 ale). *S. cerevisiae* 41CM contributed to obtaining a higher relative percentage of phenethyl alcohol in lager (11.67%) than in ale beers (9.11%). In particular, phenethyl alcohol is an important aromatic compound which gives the beer an elegant and delicate rose fragrance [31].

The other most present alcohols were 1-butanol, 2-methyl, and 1-butanol, 3-methyl which give the beer hints of whiskey, malt, and burntness [32].

The production of higher alcohols is directly associated with the metabolism of amino acids, which are the main source of nitrogen in cereal-based wort [33].

Alcohols play a direct role in taste perception or through interactions with other beer components due to the synergistic and antagonistic effects these compounds induce on taste perception [34].

Moreover, higher alcohols are precursors of esters that contribute to the impression of a fruity aroma. In fact, higher alcohols and esters are necessary for the aromatic profile of a high-quality beer [12].

The flavor profiles of beer can principally be attributed to the enzymatic activities during fermentation within the yeast cell, where sugars are converted to ethanol and volatile compounds such as higher alcohols and esters, which are simultaneously formed as by-products of yeast metabolism [35].

Esters are a class of very important volatile compounds that generally characterize beer with fruity–floral aromas [36]. These compounds are mainly produced by yeast metabolism in the intracellular space by condensation between the acetyl-CoA derivatives of aliphatic acids with alcohols and are rapidly diffused through the yeast cell membrane in the fermentation medium due to their lipid-soluble characteristics. In addition, transesterification of natural hop-derived methyl esters into ethyl esters by yeast can occur [35].

In ale beer produced with *S. cerevisiae* 41CM, there was a higher relative percentage of total esters (40.94%) than in beer obtained with the use of *S. cerevisiae* S-04 (28.14%). On the other hand, in the production of lager beer at 12 °C, *S. pastorianus* W-34/70 contributed to obtaining a higher percentage of total esters (48.80%) than with the use of *S. cerevisiae* 41CM (28.49%).

It has been demonstrated that the esters diffuse amongst the cells and fermenting medium depending on the yeast species used and the temperature, since most esters are retained at lower temperatures. The higher proportion of esters produced remains inside cells of lager yeasts, and in the fermenting medium for ale yeasts [29,37], this explains the different relative percentage of total esters in ale beer (40.94%) compared to lager beer (28.49%) obtained with the use of *S. cerevisiae* 41CM strain.

In particular, octanoic acid, an ethyl ester (ethyl octanoate; Peak No. 18), was the most abundant in lager beers, with relative percentages ranging from 8.19% (41CM lager) to 31.12% (W-34/70 lager).

Other more detected esters, with relative percentages ranging between around 1.0% and 6.0%, were: 3-methylbutyl ester (Peak No. 5); hexanoic acid, an ethyl ester (Ethyl hexanoate; Peak No. 9); 9-Decenoic acid, an ethyl ester (Ethyl 9-decenoate; Peak No. 26), and Acetic acid, a 2-phenylethyl ester (phenyl ethyl acetate; Peak No. 21).

Generally, esters have low odor thresholds. However, the presence of different esters can have a synergistic effect on the individual flavors, which means that esters can also affect beer flavors well below their individual threshold concentrations [38]. Ethyl hexanoate has a low concentration threshold at 0.005 mg/L, ethyl octanoate at 0.5 mg/L and ethyl decanoate at 1.5 mg/L [35]. In particular, ethyl hexanoate, ethyl octanoate, and ethyl

decanoate are also considered as highly positive flavor attributes of beer aroma related to flowery and fruity descriptors [39]. The aroma of 3-methylbutyl ester is described as banana and pineapple, while the phenyl ethyl acetate is described as roses and honey [39].

The different enzymatic activities, as esterase, are influenced by temperature and are strain-specific; the temperature has a major impact on beer fermentation and maturation, in fact, and as the fermentation temperature increases, there is a decrease in acetaldehyde and proximal diketones and an increase in esters and higher alcohols [40].

Among the volatile compounds, the presence of aldehydes such as nonanal and decanal with total relative percentages between 0.09% (*S. cerevisiae* 41CM ale) and 0.25% (*S. pastorianus* W-34/70 lager) was detected. Aldehydes constitute oxidized counterparts of the corresponding alcohols and play a very important role in beer flavor, being responsible for the fresh and slightly green notes of barley.

Many of the aldehydes present in wort are converted by reduction reaction due to the action of yeasts during fermentation by reduction to their saturated ethanol counterparts [41].

On the other hand, the re-oxidation of higher alcohols to carbonyls during the aging of beer may render them flavor-active again. Aldehydes are also synthesized by yeast as intermediates in the formation of ethanol through the decarboxylation of keto acids. The presence of active yeasts in the maturation phase is a prerequisite for satisfactorily low aldehyde levels in the final product.

The aliphatic aldehydes have a green, grass-like aroma, a quality that might be considered desirable at low concentrations; in particular, nonanal and decanal have been related to citrus and orange-peel descriptors. During beer storage, increases in levels of staling aldehydes coincide with the sensory perception of undesirable aging off-flavors [29].

In beers, terpenic compounds are mainly derived from the hop essential oils which are added to the wort during the boiling process. These compounds are related to pleasant aromas such as citrus, flowers and lilac [42,43]. The terpenes detected in the beers produced in our tests were linalool and citronellol, with relative total percentages ranging from 0.61% to 1.18%. Recently, King and Dickinson [44,45] reported the biotransformation of monoterpene alcohols by yeast metabolism. Geraniol can be mainly transformed into β-citronellol and adjunctively to linalool. The coexistence of these terpenes enhances the "fruity", "citrus" and "green" characteristics of the beer [46].

4. Conclusions

In a market totally fueled by standardized industrial products, craft breweries seek to meet new consumer demands by producing genuine and high-quality beers, with distinctive organoleptic characteristics. Therefore, the use of new ingredients during the brewing process and, above all, the sourcing of new starter yeasts could represent a breakthrough for new beers that satisfy even the most demanding consumers in terms of quality and sensory characteristics. The present work highlighted the versatility and good fermentative aptitudes of the *S. cerevisiae* 41CM yeast strain isolated from the vineyard, which makes its use different in beer production. In fact, its ability to ferment at 12 °C and 20 °C is a very interesting feature since, unlike commercially available *S. cerevisiae* yeast strains, it could be used both for the production of ale and lager beers. These preliminary results are important to foster further investigations conducted in order to evaluate organoleptic quality and shelf life, a very important aspect of unfiltered and unpasteurized beers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr10122495/s1, Table S1. pH changes in ale and lager beers during alcohol fermentation, using *S. cerevisiae* 41CM, *S. cerevisiae* S-04 and *S. pastorianus* W-34/70 starters. Table S2. Ethanol evolution in ale and lager beers during alcohol fermentation, using *S. cerevisiae* 41CM, *S. cerevisiae* S-04 and *S. pastorianus* W-34/70 starters. Table S3. Yeast viable cell count (log CFU/mL) in ale and lager beers during alcohol fermentation.

Author Contributions: Conceptualization, M.I. and B.T.; methodology, B.T and M.I.; software, F.L. and G.A.; validation, F.C., F.V. and M.S.; formal analysis, B.T., J.K., G.S.G. and M.P.; investigation, P.T. and B.T.; resources, S.J.L., F.C. and M.R.; data curation, B.T., M.I. and F.L.; writing—original draft preparation, M.I. and B.T.; writing—review and editing, M.I.; visualization, M.C.M.; supervision, M.I. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data presented in this study are available in the Supplementary Materials.

Conflicts of Interest: The authors declare no conflict of interest.

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