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Unmalted Cereals, Oenological Yeasts, and in-Bottle Sugar Addition as Synergic Strategies to Enhance the Quality of Craft Beers

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Abstract: Craft beer quality is the result of the complex interactions among ingredients. The purpose of this work was to assess the influence of combinations of cereal mixtures, yeast strains, and sucrose added for the refermentation in bottle on the physico-chemical and sensory characteristics of the resulting beers in order to maximize their antioxidant content and overall quality. More in depth, brewing trials were carried out with 16 combinations of 2 cereal mixtures (made of 60% malted barley/40% unmalted durum or soft wheat), 4 oenological *Saccharomyces cerevisiae* strains (17290 and 14061 isolated from Negroamaro; 9502 and 9518 from Susumaniello musts), and 2 concentrations of sucrose for refermentation (6 and 9 g/L). If maximizing the total phenolic content is the goal, the best beers were those obtained from the mixtures containing durum wheat and fermented by *S. cerevisiae* 17290 and 14061. Instead, the best sensory results were obtained from brewing the mixture containing the unmalted common wheat and fermented by *S. cerevisiae* 9518 thanks to their persistent foam; high turbidity, alcohol content, effervescence, and body; and low saltiness and sourness. The physico-chemical and sensory quality of beers were mainly affected by the cereal mixtures and secondarily by yeasts. The quantity of sucrose added for refermentation affected only CO_2, residual sugar, and foam.

Keywords: antioxidant; craft brewing; grape/wine yeasts; physico-chemical indices; sensory profile; refermentation; unmalted cereals

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1. Introduction

Craft beer production can be inspired by traditional brewing styles to build the loyalty of regular consumers, but it can be also performed according to innovative approaches in order to intercept the preferences of new consumers. The ways to differentiate the final products include the rediscovering of ancient grains or the use of novel starchy materials [1], the choice of new cross-over yeast cultures [2], and the modification of the brewing procedures [3].

Among the 103 beer styles categorized by the Brewers Association [4], the Belgian top-fermented witbier beers are arousing the interest of craft beer consumers for their persistent foam, pale yellow/light golden yellow color, cloudy appearance, slightly sour taste, low content of bitter substances, and moderate alcohol content [5]. These characteristics are due to ingredients such as pale barley malt, unmalted wheat (generally added in quantities of 40–60%), low-flocculating (ale) yeasts, and flavor enhancers such as curaçao bitter orange peel and coriander.

The effects of different unmalted wheats (*Triticum aestivum, T. durum, T. monococcum* and *dicoccum*, *T. spelta*—spelt) have been poorly investigated. Baiano et al. [3] studied the influence of unmalted cereals, hops, and yeasts on several characteristics of white craft beers. They found that the highest overall quality scores were assigned to beers made with common wheat of cv. Risciola combined with hop of cv. Columbus and to beers produced with durum wheat of cv. Dauno III combined with hop of cv. Cascade. Studies performed on the brewing of *Triticum aestivum* wheats highlighted that different varieties of the same species can differently affect volatile profile and foam stability of the resulting white craft beer [6].

The yeast strains employed to promote the alcoholic fermentation also affect the beer quality. Although most strains used in ale beer fermentation belong to the *Saccharomyces cerevisiae* species, different autochthonous strains can produce different intermediate metabolites and by-products, mainly in the form of volatile compounds [7,8]. Furthermore, yeast strains are known to affect chemical composition, sensory properties, and antioxidant capacity of beer [9]. In recent years, strains of oenological origins have been studied for their use as brewing starters. Canonico et al. [10] investigated the use of yeast selected in a winery environment for the refermentation of beers previously fermented with a classic starter strain, finding significantly higher concentrations of compounds able to impart fruity and flowery aromas.

Although there is a large amount of literature concerning the priming composition during the refermentation step [11–13], poorly or not investigated at all is the quantity of sugar added for refermentation and its effects on physico-chemical and organoleptic characteristics of beers.

In the present study, white craft beers were formulated starting from three ingredients, namely starchy materials, yeast strains, and sucrose added for the refermentation in bottle. In particular, two cereal mixtures of malted barley and unmalted grains (alternatively durum wheat or common wheat), four *Saccharomyces cerevisiae* strains isolated from fermenting Negroamaro and Susumaniello grape musts, and two concentrations of sucrose to promote the secondary fermentation were used. The purpose of the work was to evaluate the influence of such ingredient combinations on several physico-chemical indices that are known to affect antioxidant properties and sensory quality of a beer. To the best of our knowledge, the single and interactive effects of these three ingredients have been studied for the first time in this work. A further element of novelty of this work was the use of oenological yeasts both for fermentation and refermentation steps.

2. Materials and Methods

2.1. Ingredients Used in the Brewing Trials

Malted barley of cv. Fortuna was provided by Agroalimentare Sud (Melfi, Italy). The unmalted durum wheat cv. Dauno III and common wheat cv. Risciola were obtained from plants grown from selected seeds supplied by CREA-CI Research Centre (Foggia, Italy) and cultivated in the fields of Soc. Cooperativa Agricola Valleverde (Bovino, Foggia, Italy).

Dried hop cones of cv. Cascade, characterized by citrusy and floral flavors and by a 6.7% α-acid content, bitter orange peels, and coriander, were purchased at Birramia (Querceta, Italy).

For the wort fermentation trials, four *S. cerevisiae* strains collected by the ITEM Microbial Culture Collection of CNR-ISPA (http://www.ispacnr.it/collezioni-microbiche, accessed on 21 December 2023) and described in previous studies [8,14] were used: 17290 and 14061, isolated from fermenting Negroamaro grape must and characterized by high ester production and by high terpenes production, respectively; 9502 and 9518, isolated from fermenting Susumaniello grape must and characterized by high ester and alcohol production (the first) and by intermediate ester, terpene, and alcohol production (the second). The suitable cell quantity for the fermentation trials was calculated as described by Thesseling et al. [15]. Briefly, yeast strains were inoculated (1/1000 *v/v*) in 10 mL of Yeast Extract Peptone Dextrose (YEPD) from cryopreserved stock and grown overnight at 30 °C. Then, yeasts were further propagated by inoculating the previous cultures in 1 L of the same media and incubating at 30 °C for 24 h in an orbital shaker incubator at 150 rpm. After incubation, cells were centrifuged and resuspended in a sterile saline solution (NaCl 0.86%).

2.2. Brewing Procedure

The beers were elaborated with 60% of malted barley and 40% of unmalted cereal both to respect the minimum amount of malt established by the Italian law on beer [16] and to pursue the highest beer differentiation using the maximum permitted percentage of unmalted cereal. More in depth, sixteen types of white craft beers (Table 1) were obtained by combining two cereal mixtures, namely barley malt-unmalted durum wheat (DW) or barley malt-unmalted common wheat (CW); four *S. cerevisiae* strains (17290, 14061, 9502, or 9518); and two concentrations of sucrose for the refermentation in the bottle, i.e., 6 g/L (S6) or 9 g/L (S9).

Table 1. Combination of ingredients (2 types of cereal mixtures, 4 *S. cerevisiae* strains, and 2 sugar levels for refermentation) used to produce the 16 types of beers.

Beer Acronyms	Cereal Mixture	*S. cerevisiae* Strain	Sugar Levels
DW-17290-S6	60% malted barley-40% unmalted durum wheat (DW)	Isolated from Negroamaro (17290)	6 g/L
DW-17290-S9	60% malted barley-40% unmalted durum wheat (DW)	Isolated from Negroamaro (17290)	9 g/L
DW-14061-S6	60% malted barley-40% unmalted durum wheat (DW)	Isolated from Negroamaro (14061)	6 g/L
DW-14061-S9	60% malted barley-40% unmalted durum wheat (DW)	Isolated from Negroamaro (14061)	9 g/L
DW-9502-S6	60% malted barley-40% unmalted durum wheat (DW)	Isolated from Susumaniello (9502)	6 g/L
DW-9502-S9	60% malted barley-40% unmalted durum wheat (DW)	Isolated from Susumaniello (9502)	9 g/L
DW-9518-S6	60% malted barley-40% unmalted durum wheat (DW)	Isolated from Susumaniello (9518)	6 g/L
DW-9518-S9	60% malted barley-40% unmalted durum wheat (DW)	Isolated from Susumaniello (9518)	9 g/L
CW-17290-S6	60% malted barley-40% unmalted common wheat (CW)	Isolated from Negroamaro (17290)	6 g/L
CW-17290-S9	60% malted barley-40% unmalted common wheat (CW)	Isolated from Negroamaro (17290)	9 g/L
CW-14061-S6	60% malted barley-40% unmalted common wheat (CW)	Isolated from Negroamaro (14061)	6 g/L
CW-14061-S9	60% malted barley-40% unmalted common wheat (CW)	Isolated from Negroamaro (14061)	9 g/L
CW-9502-S6	60% malted barley-40% unmalted common wheat (CW)	Isolated from Susumaniello (9502)	6 g/L
CW-9502-S9	60% malted barley-40% unmalted common wheat (CW)	Isolated from Susumaniello (9502)	9 g/L
CW-9518-S6	60% malted barley-40% unmalted common wheat (CW)	Isolated from Susumaniello (9518)	6 g/L
CW-9518-S9	60% malted barley-40% unmalted common wheat (CW)	Isolated from Susumaniello (9518)	9 g/L

A total of 3 technological replicates were performed for each of the 16 beers. The amounts of ingredients per 100 L of finished beer were the following: water, 135 L (115 L for mashing and 20 for sparging); barley malt and unmalted wheat, 14.75 and 9.75 kg, repectively; 100 g of hop cones (~15 IBU bitterness); 100 g of bitter orange peels; 100 g of coriander. The brewing trials were carried out in a Braumeister system (Speidel Tank-und Behälterbau GmbH, Ofterdingen, Germany).

The malted and unmalted cereals of each mixture (DW or CW) were coarsely ground through a 2-roller mill (Albrigi Luigi, Stallavena, Verona, Italy), setting a mill gap of 0.5 ± 0.1 mm. The obtained coarse flour was added to the mashing water (preliminarily heated to 45 °C and kept under stirring until the mash-off). The mashing steps were the following: increase of the temperature from 46 to 52 °C; protein rest (54 °C, 10 min); β-amylase rest (63 °C, 50 min); α-amylase rest (70 °C, 50 min); mash-off (81 °C, 15 min). The temperature increase rate was set at 1.5 °C/min. The final wort pH was close to 5.4. The exhausted solid fraction was separated from the wort, crossed by the sparge water at 81 °C, and left to drain. The resultant wort was boiled for 55 min. Five minutes after the start of boiling, bitter orange peels, coriander, and hop cones were added. The wort (original gravity 1.053 ± 0.005) was cooled at room temperature, whirlpooled to remove solid residues, and inoculated with the yeast liquid culture to have an initial concentration of $\sim 1 \times 10^7$ cells/mL. The fermentation step was conducted at 20 ± 2 °C for 21 ± 1 days (final gravity 1.015 ± 0.001) while the subsequent maturation was carried out at 4 ± 1 °C for 4 days. Finally, beer was racked, inoculated with the same yeast strain used for the first fermentation ($\sim 1 \times 10^5$ cells/mL to guarantee a regular fermentation), added with sucrose (6 or 9 g/L), and packaged into 750 mL glass brown bottles. The bottled beer was conditioned at 20 ± 1 °C for 1 month and stored at 5 ± 1 °C until analyses.

2.3. Analyses of the Cereal Mixtures

Moisture and ash contents were quantified as % using the AACC methods 44-15.02 and 08-01.01, respectively [17]. Phenolics were extracted according to Gandolpho et al. [18] and quantified (TPC, mg of gallic acid/100 g dm) using the Folin–Ciocalteu reagent [19]. The beer phenolic profiles were analyzed according to Baiano et al. [3] using an HPLC-DAD system (Agilent 1100 Liquid Chromatograph, Santa Clara, CA, USA) equipped with a 100 mm × 4.6 mm × 3 μm RP-C18 Gemini column (Phenomenex, Aschaffenburg, Germany). The phenolic compounds were identified by comparing their retention times and spectra with those of 18 pure standards. The concentration of each phenolic compound (mg/100 g dm) was determined by comparing the peak area with those of standard curves at two wavelengths: 280 and 320 nm. The antioxidant activity (AA) of the extracts was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity [20] and quantified through a calibration curve prepared with Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as mmol of Trolox per g of dry matter.

2.4. Analyses of Beers

2.4.1. Basic Analyses

The pH values were measured through a BASIC 20 pH meter (CRISON, Modena, Italy). Soluble solids (as Brix) were measured by a Digital "Pocket" Refractometer (ATAGO Co., Ltd., Tokyo, Japan), and the values were corrected on the basis of the beer alcohol content [21]. Color was spectrophotometrically determined at 430 nm on degassed and filtered (0.45 μm) beers and quantified on the EBC scale [22]. The carbon dioxide content (as mg CO_2/L) was determined though the HI 3818 Carbon Dioxide Test Kit (Hanna Instruments, Padova, Italy). Alcohol content (%), titratable acidity (g lactic acid/L), and volatile acidity (g acetic acid/L) were determined as described in Baiano et al. [3].

2.4.2. Organic Acids, Sugars, and Glycerol

Organic acids were analyzed through an HPLC-DAD system (Agilent 1100 Liquid Chromatograph, Santa Clara, CA, USA) on an Agilent Hi-Plex H (300 × 7.7 mm) column with internal particles of 8.0 µm (Agilent Technologies, Santa Clara, CA, USA) at 210 nm, according to Coelho et al. [23].

Maltodextrin, maltotriose, maltose, glucose, fructose, and glycerol concentrations were quantified through the same type of column used for organic acid analysis. A mobile phase made of deionized water at a constant flow rate of 0.6 mL/min and a run time of 30 min were applied. The detection was carried out through a Refractive Index Detector (RID).

Quantification of individual organic acids and sugars was performed through the ChemStation software (G2170BA, Agilent Santa Clara, CA, USA) using a five-point regression curve ($r^2 \geq 0.99$) on the basis of authentic standards. The results were expressed as mg/mL.

2.4.3. Total Phenolic Content, Phenolic Profile, and Antioxidant Activity

The TPC was estimated through the Folin–Ciocalteu method [24] and expressed as mg gallic acid equivalents per liter of beer. The AA was determined as already described for the cereal mixtures, and the results were expressed as % mmol of Trolox per liter of beer. The phenolic profile was analyzed by the HPLC system described in the Section 2.3 according to Baiano et al. [3].

2.4.4. Sensory Descriptive Analysis

The trained sensory panel was formed through recruitment, selection, and training steps. The technical standards ISO 11035:1994, 5496:2006, 3972:2011, and 8586:2021 [25–28] were followed for selection and training. For the recruitment steps, 30 aspiring panellists were asked to complete a questionnaire about the availability, motivation, possible allergies, etc. During the selection step, the recruited people were subjected to preliminary sensory tests to evaluate their ability to recognize different types of aromas, individuate their physiological thresholds of fundamental tastes (sweet, salty, acidic and bitter) alone and in binary solutions (sweet and acid, sweet and bitter), evaluate their ability to use the intensity scale of the beer sensory properties, and discriminate beers with different characteristics. During training, references (beers with specific characteristics) served as illustrative stimuli for generating sensory terminologies. The panel was constituted by six judges aged between 40 and 65 years, experienced in alcoholic beverage sensory evaluation, and in possession of a sommelier or technical wine taster certificate. The panel size met the ISO technical standard 11035:1994 [25]. Panellists performed a Quantitative Descriptive Analysis (QDA) as described by Baiano et al. [3]. They were asked to evaluate five visual (for foam: color, amount, and persistence; for liquid portion: color and turbidity), four gustatory (sweetness, bitterness, saltiness, sourness/acidity), and three tactile (alcoholic, effervescence, and body) attributes. The panellists also gave a comprehensive and objective score of the sensory quality of each sample evaluated after its swallowing (overall quality) [29]. All descriptors and the overall quality were evaluated on a 5-point scale, except for those referring to foam color (1 = white, 2 = rose, 3 = cream, or 4 = capuchin) and liquid color (1 = pale straw yellow, 2 = straw yellow, 3 = golden yellow, or 4 = amber).

2.4.5. Statistical Analysis

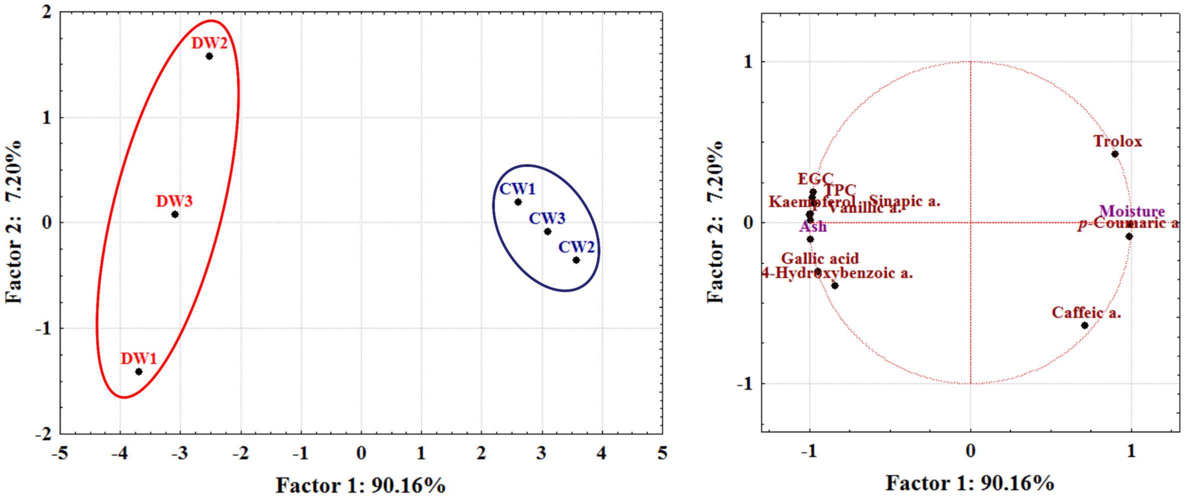
Each analysis was performed at least three times for each of the three technological replicates. Thus, the mean values and standard deviations were calculated on 3 × 3 raw data. A one-way Analysis of Variance (ANOVA, $p < 0.05$) was applied to cereal mixtures to highlight significant differences among samples. A three-way ANOVA followed by LSD test ($p < 0.05$) was applied to highlight the single and interactive effects of barley malt/unmalted cereal mixtures (DW or CW), yeast strains (17290, 14061, 9502, and 9518), and sugar levels for in-bottle refermentation (6 g/L and 9 g/L) on physico-chemical and sensory aspects of the beers. Principal Component Analysis (PCA) was first applied to the cereal mixtures in order to highlight their overall differentiation level. Then, PCA was applied to check whether the 16 types of beers could be distinguished from each other according to their physico-chemical and sensory indices. The Pearson correlation coefficients (p-value < 0.01) were determined in order to find significant correlations among beer characteristics (Table S1). The package Statistica for Windows V. 7.0. (Statsoft, Tulsa, OK, USA) was used.

3. Results and Discussion

3.1. Characteristics of the Cereal Mixtures

In order to verify the existence of differences between the starting cereal mixtures, their composition has been studied and compared. DW had significantly lower moisture (7.7 ± 0.1%) and higher ash content (3.53 ± 0.24%) than CW (8.5 ± 0.1% moisture and 2.65 ± 0.02% ash), thus being a greater source of mineral components. As durum and common wheats were grown in the same locality and then subjected to the same cultural practices, the ash difference can be ascribed to belonging to different species [30]. In order to give an insight into molecules known for their bioactive effects and their role in haze formation, TPC, phenolic profiles, and antioxidant activity of the cereal mixtures have been compared. The presence of durum wheat ensured greater TPC than common wheat (338 ± 2 and 279 ± 4 mg/100 dm, respectively). These data have a double interpretation, as low TPC is considered as an indicator of low haze formation in the future beers, but a recent trend is to increase the antioxidant content of alcoholic beverages to counterbalance the negative effects of the alcohol content by preventing alcohol-induced markers of inflammation, oxidative stress, and angiogenesis [31]. Despite the higher phenolic content of DW compared to CW, the latter exerted a greater antioxidant activity (1.85 ± 0.05 vs. 1.36 ± 0.25 mmol Trolox/g dm). Concerning the specific phenolic profiles, *p*-coumaric acid was detected in CW (2.0 ± 0.1 mg/100 g dm) but not in DW, while kaempferol and gallic acid were detected in higher concentrations in the cereal mixture containing durum wheat (17.1 ± 0.3 and 3.7 ± 0.2 vs. 11.3 ± 0.3 and 3.2 ± 0.1 mg/100 g dm). Compounds such as epicatechin, ferulic acid, epigallocatechingallate, rutin, resveratrol, rosmaric acid, and quercetin were not detected either in DW or in CW, while the acids sinapic, caffeic, and 4-hydroxybenzoic were found in concentrations around 1 mg/100 g dm in both the cereal mixtures. To complete the phenolic profiles of the cereal mixtures, epigallocatechin and vanillic acid were not detected in CW but were present in DW with concentrations of 11.6 ± 1.0 and 1.1 ± 0.1 mg/100 g dm, respectively.

PCA was applied to the two starting cereal mixtures, and the first two factors accounted for 97.4% of the variance in the whole data set. The two cereal mixtures appear clearly separated and homogeneously grouped in the factor plan (Figure 1), with DW samples located in the left quadrant and CWs placed in the right quadrant (Figure 1a). The positive loadings of actor 1 are associated with high values of antioxidant activity, moisture, and concentration of *p*-coumaric acid. In contrast, the negative loadings of factor 1 are associated with high amounts of TPC, kaempferol, epigallocatechin, gallic, and vanillic acids and ash (Figure 1b).



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

Figure 1. PCA of the cereal mixtures: projection of (**a**) mixture samples and (**b**) their physico-chemical indices on the factorial plane.

3.2. Physico-Chemical Characteristics of the Beers

3.2.1. Physico-Chemical Indices and Concentrations of Sugars, Glycerol, and Organic Acid

The interactions among cereal mixtures, yeast strains, and sugar levels significantly affected all parameters (Tables 2 and 3), with the exception of the fumaric acid concentration. The single effects of the three considered factors were statistically significant too, except for the influence of sugar level on alcohol content and volatile acidity, thus they are discussed point by point. The beer color was in the range from 6.6 to 9.2 on the EBC scale, with significant single effects of cereal mixture and yeast. More in depth, beers produced from CW showed higher EBC values, as a consequence of the higher carotenoid content of Risciola, together with beers fermented by strains isolated from Susumaniello must, probably due to the different ability of *S. cerevisiae* strains to adsorb colored compounds on their cell walls [32]. The average alcohol content shows a high variability among samples, comprising between 3.10% (DW-14061-S6) and 6.47% (CW-17290-S6). The lowest alcohol % of DW beers is explained by the lower carbohydrate contents of durum wheat (71%) with respect to that of common wheat (75%) [33], while the low fermentative performances of *S. cerevisiae* 14061 depended on its slowest maltotriose fermentation [2]. The average beer soluble solids ranged from 3.8 °Bx (CW-17290-S6) to 7.5 °Bx (DW-14061-S9) and were consistent with the results concerning the alcohol content. Since the contribution of CO_2 content to the freshness of craft beers, brewing yeasts are specifically selected to quickly produce CO_2 in moderate sugar medium. The beer CO_2 content was composed of between 4.60 and 7.53 g/L, with positive effects exerted by the use of DW mixture, *S. cerevisiae* 9502, and, as expected, by the addition of the highest sucrose amount.

Table 2. Influence of cereal mixtures, yeasts, and sugar levels on some physico-chemical parameters and on the contents of sugars and glycerol in the beers.

Beer Acronyms	Color (EBC)	Alcohol Content (%)	CO_2 (g/L)	Soluble Solids (Brix)	Sugars (mg/mL)					Glycerol (mg/L)
					Maltodextrins	Maltotriose	Maltose	Glucose	Fructose	
Interactive effects (Cereal mixtures × Yeasts × Sugar levels)										
DW-17290-S6	6.6 ± 0.1 ^{a}	4.09 ± 0.02 ^{bc}	6.75 ± 0.25 ^{ef}	6.7 ± 0.1 ^{i}	23.98 ± 0.76 ^{h}	2.83 ± 0.25 ^{fg}	3.91 ± 0.15 ^{h}	10.77 ± 0.50 ^{j}	0.99 ± 0.16 ^{ad}	2.40 ± 0.09 ^{ac}
DW-17290-S9	6.6 ± 0.0 ^{a}	6.64 ± 0.01 ^{f}	7.53 ± 0.07 ^{h}	6.0 ± 0.2 ^{f}	28.88 ± 0.15 ^{i}	4.89 ± 0.48 ^{h}	10.05 ± 1.32 ^{i}	8.38 ± 0.90 ^{i}	1.21 ± 0.06 ^{df}	2.60 ± 0.05 ^{d}
DW-14061-S6	6.7 ± 0.0 ^{a}	3.10 ± 0.10 ^{a}	6.60 ± 0.05 ^{e}	7.8 ± 0.1 ^{j}	12.60 ± 0.90 ^{b}	16.54 ± 0.6 ^{j}	3.49 ± 0.02 ^{gh}	3.84 ± 0.09 ^{ef}	4.55 ± 0.29 ^{g}	2.31 ± 0.11 ^{ab}
DW-14061-S9	6.7 ± 0.0 ^{a}	3.27 ± 0.05 ^{a}	6.63 ± 0.13 ^{e}	7.5 ± 0.0 ^{k}	15.09 ± 0.04 ^{e}	14.73 ± 0.40 ^{i}	3.42 ± 036 ^{fg}	4.72 ± 0.11 ^{h}	5.66 ± 0.45 ^{h}	2.48 ± 0.31 ^{bd}
DW-9502-S6	7.4 ± 0.1 ^{c}	4.52 ± 0.10 ^{cd}	7.00 ± 0.25 ^{fg}	6.1 ± 0.1 ^{fg}	33.09 ± 0.35 ^{j}	2.45 ± 0.17 ^{bd}	3.04 ± 0.07 ^{ef}	12.27 ± 0.42 ^{k}	1.13 ± 0.08 ^{cf}	2.20 ± 0.18 ^{a}
DW-9502-S9	7.2 ± 0.0 ^{b}	4.77 ± 0.02 ^{d}	7.10 ± 0.10 ^{g}	6.3 ± 0.1 ^{gh}	29.73 ± 0.89 i	2.61 ± 0.09 ^{df}	2.80 ± 0.05 ^{ce}	18.64 ± 0.67 ^{l}	1.30 ± 0.11 ^{f}	2.51 ± 0.09 ^{cd}
DW-9518-S6	8.4 ± 0.3 ^{d}	5.80 ± 0.09 ^{e}	4.60 ± 0.10 ^{a}	4.6 ± 0.2 ^{d}	16.28 ± 0.70 ^{f}	2.54 ± 0.15 ^{cf}	2.39 ± 0.24 ^{bd}	4.27 ± 0.39 ^{g}	0.96 ± 0.09 ^{ac}	3.35 ± 0.07 ^{ef}
DW-9518-S9	8.0 ± 0.2 ^{d}	6.06 ± 0.04 ^{ef}	4.63 ± 0.28 ^{a}	4.8 ± 0.2 ^{d}	18.14 ± 0.67 ^{g}	2.33 ± 0.30 ^{ad}	2.50 ± 0.37 ^{bd}	0.54 ± 0.16 ^{a}	0.86 ± 0.17 ^{ab}	3.38 ± 0.07 ^{ef}
CW-17290-S6	8.2 ± 0.1 ^{d}	6.47 ± 0.04 ^{f}	4.95 ± 0.05 ^{b}	3.8 ± 0.2 ^{a}	14.78 ± 1.40 ^{de}	2.08 ± 0.16 ^{a}	1.61 ± 0.09 ^{a}	3.17 ± 0.10 ^{b}	1.05 ± 0.09 ^{be}	3.37 ± 0.04 ^{ef}
CW-17290-S9	7.3 ± 0.0 ^{bc}	4.31 ± 0.02 ^{bd}	5.13 ± 0.13 ^{b}	4.7 ± 0.^{d}	14.00 ± 0.71 ^{cd}	2.59 ± 0.15 ^{df}	2.21 ± 0.04 ^{b}	4.18 ± 0.09 ^{fg}	1.22 ± 0.03 ^{ef}	3.79 ± 0.33 ^{gh}
CW-14061-S6	8.3 ± 0.1 ^{d}	6.27 ± 0.0 ^{ef}	5.90 ± 0.10 ^{d}	4.1 ± 0.1 ^{b}	13.72 ± 0.40 ^{cd}	2.19 ± 0.25 ^{ab}	2.82 ± 0.14 ^{de}	3.34 ± 0.15 ^{bc}	0.97 ± 0.06 ^{ac}	3.31 ± 0.02 ^{ef}
CW-14061-S9	7.4 ± 1.2 ^{c}	6.20 ± 0.02 ^{ef}	6.13 ± 0.03 ^{d}	4.6 ± 0.3 ^{d}	15.83 ± 0.57 ^{ef}	2.24 ± 0.20 ^{ac}	2.76 ± 0.18 ^{ce}	3.09 ± 0.04 ^{b}	1.12 ± 0.27 ^{cf}	3.42 ± 0.18 ^{ef}
CW-9502-S6	9.2 ± 0.1 ^{e}	6.28 ± 0.03 ^{ef}	5.88 ± 0.18 ^{d}	4.4 ± 0.1 ^{c}	8.90 ± 1.57 ^{a}	2.86 ± 0.18 ^{fg}	2.18 ± 0.09 ^{b}	3.76 ± 0.31 ^{de}	0.79 ± 0.12 ^{a}	3.27 ± 0.21 ^{e}
CW-9502-S9	8.1 ± 0.0 ^{d}	6.19 ± 0.04 ^{ef}	6.08 ± 0.13 ^{d}	5.3 ± 0.2 ^{e}	12.30 ± 1.81 ^{b}	2.95 ± 0.10 ^{g}	2.46 ± 0.07 b^{d}	3.30 ± 0.10 ^{bc}	0.86 ± 0.06 ^{ab}	3.50 ± 0.21 ^{f}
CW-9518-S6	8.0 ± 0.1 ^{d}	6.15 ± 0.03 ^{ef}	4.60 ± 0.10 ^{a}	5.3 ± 0.1 ^{e}	13.67 ± 0.98 ^{c}	2.48 ± 0.07 ^{b}e	2.37 ± 0.11 b^{c}	3.71 ± 0.07 ^{ce}	1.09 ± 0.22 ^{cf}	3.78 ± 0.16 ^{g}
CW-9518-S9	8.3 ± 0.1 ^{d}	3.73 ± 1.55 ^{ab}	5.45 ± 0.45 ^{c}	6.4 ± 0.1 ^{h}	17.68 ± 0.58 ^{g}	2.79 ± 0.13 ^{eg}	2.32 ± 0.01 ^{b}	3.38 ± 0.08 ^{bd}	1.06 ± 0.22 ^{be}	3.98 ± 0.15 ^{h}
Significance	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Single effect of cereal mixtures										
DW	7.2 ^{a}	4.78 ^{a}	6.35 ^{b}	6.2 ^{b}	22.22 ^{b}	6.12 ^{b}	3.95 ^{b}	7.93 ^{b}	2.08 ^{b}	2.65 ^{a}
CW	8.1 ^{b}	5.70 ^{b}	5.51 ^{a}	4.8 ^{a}	13.86 ^{a}	2.52 ^{a}	2.34 ^{a}	3.49 ^{a}	1.02 ^{a}	3.55 ^{b}
Significance	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Single effect of yeasts										
17290	7.2 ^{a}	5.38 ^{b}	6.09 ^{b}	5.3 ^{a}	20.41 ^{c}	3.09 ^{c}	4.45 ^{d}	6.62 ^{c}	1.12 ^{b}	3.04 ^{b}
14061	7.3 ^{a}	4.70 ^{a}	6.31 ^{c}	6.0 ^{c}	14.31 ^{a}	8.93 ^{d}	3.12 ^{c}	3.75 ^{b}	3.08 ^{c}	2.88 ^{a}
9502	8.0 ^{b}	5.44 ^{b}	6.51 ^{d}	5.5 ^{b}	21.00 ^{d}	2.72 ^{b}	2.62 ^{b}	9.49 ^{d}	1.02 ^{b}	2.87 ^{a}
9518	8.2 ^{b}	5.43 ^{b}	4.82 ^{a}	5.3 ^{a}	16.44 ^{b}	2.54 ^{a}	2.39 ^{a}	2.97 ^{a}	0.99 ^{a}	3.62 ^{c}
Significance	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Single effect of sugar levels										
S6	7.9 ^{a}	5.33 ^{a}	5.78 ^{a}	5.3 ^{a}	17.12 ^{a}	4.2^{5} ^{a}	2.73 ^{a}	5.64 ^{a}	1.44 ^{a}	3.00 ^{a}
S9	7.4 ^{a}	5.14 ^{a}	6.09 ^{b}	5.7 ^{b}	18.96 ^{b}	4.39 ^{b}	3.57 ^{b}	5.78 ^{b}	1.66 ^{b}	3.21 ^{b}
Significance	ns	ns	Y	Y	Y	Y	Y	Y	Y	Y

In the columns, different letters correspond to significant differences at $p < 0.05$ by LSD multiple range test; Y, significant; ns, not significant.

Table 3. Influence of cereal mixtures, yeasts, and sugar levels on pH, acidity, and the organic acid profiles of the beers.

Beer Acronyms	**pH**	**Titratable Acidity (g/L)**	**Volatile Acidity (g/L)**	**Organic Acids (mg/mL)**				
				Citric	**Malic**	**Succinic**	**Lactic**	**Acetic**
Interactive effects (cereal mixtures ×yeasts × sugar levels)								
DW-17290-S6	4.17 ± 0.02 ^{cd}	3.51 ± 0.08 ^{i}	2.19 ± 0.02 ^{c}	0.86 ± 0.03 ^{fg}	0.92 ± 0.06 ^{c}	7.15 ± 0.11 ^{h}	0.72 ± 0.02 ^{ce}	3.95 ± 0.09 ^{j}
DW-17290-S9	4.01 ± 0.01 ^{b}	1.56 ± 0.02 ^{e}	0.35 ± 0.02 ^{a}	0.63 ± 0.08 ^{c}	0.71 ± 0.05 ^{b}	4.64 ± 0.20 ^{d}	0.64 ± 0.07 ^{b}	3.84 ± 0.09 ^{j}
DW-14061-S6	3.79 ± 0.06 ^{a}	6.30 ± 0.04 ^{k}	4.32 ± 0.01 ^{d}	0.90 ± 0.07 ^{h}	1.20 ± 0.04 ^{e}	7.92 ± 0.08 ^{i}	0.77 ± 0.03 ^{fg}	1.89 ± 0.02 ^{cd}
DW-14061-S9	3.79 ± 0.03 ^{a}	6.50 ± 0.03 ^{l}	5.58 ± 0.06 ^{e}	0.65 ± 0.04 ^{c}	0.85 ± 0.02 ^{c}	5.10 ± 0.19 ^{e}	0.58 ± 0.03 ^{a}	1.40 ± 0.06 ^{a}
DW-9502-S6	3.97 ± 0.06 ^{b}	3.36 ± 0.03 ^{h}	2.17 ± 006 ^{c}	1.04 ± 0.12 ^{i}	1.39 ± 0.08 ^{f}	8.14 ± 0.07 ^{i}	0.74 ± 0.04 ^{df}	3.22 ± 0.16 ^{i}
DW-9502-S9	3.97 ± 0.06 ^{b}	3.75 ± 0.03 ^{j}	2.22 ± 0.06 ^{c}	0.86 ± 0.07 ^{fg}	0.88 ± 0.12 ^{c}	3.94 ± 0.23 ^{c}	0.84 ± 0.02 ^{hi}	2.79 ± 0.04 ^{h}
DW-9518-S6	4.40 ± 002 ^{h}	1.30 ± 0.0 ^{c}	0.32 ± 0.02 ^{a}	0.78 ± 0.06 ^{d}	0.72 ± 0.03 ^{b}	3.20 ± 0.20 ^{b}	0.75 ± 0.04 ^{df}	1.61 ± 0.08 ^{ac}
DW-9518-S9	4.39 ± 0.03 ^{h}	1.46 ± 0.02 ^{d}	0.38 ± 0.02 ^{a}	0.49 ± 0.02 ^{a}	0.64 ± 0.04 ^{ab}	2.70 ± 0.11 ^{a}	0.70 ± 0.02 ^{cd}	1.54 ± 0.01 ^{ab}
CW-17290-S6	4.29 ± 0.04 ^{fg}	1.79 ± 0.01 ^{g}	0.22 ± 0.03 ^{a}	0.83 ± 0.02 ^{df}	1.06 ± 0.08 ^{d}	5.23 ± 0.29 ^{e}	0.69 ± 0.03 ^{bc}	2.30 ± 0.35 ^{ef}
CW-17290-S9	4.25 ± 0.08 ^{ef}	3.71 ± 0.02 ^{j}	2.18 ± 0.08 ^{c}	0.90 ± 0.03 ^{gh}	1.10 ± 0.17 ^{de}	5.88 ± 0.30 ^{f}	0.68 ± 0.03 ^{bc}	2.41 ± 0.52 ^{eg}
CW-14061-S6	4.32 ± 0.0 ^{g}	1.50 ± 0.01 ^{d}	1.23 ± 0.99 ^{b}	0.85 ± 0.06 ^{eg}	0.91 ± 0.10 ^{c}	6.21 ± 0.33 ^{g}	0.82 ± 0.04 ^{gh}	2.50 ± 0.62 ^{fh}
CW-14061-S9	4.21 ± 0.02 ^{de}	1.17 ± 0.00 ^{b}	0.30 ± 0.01 ^{a}	0.79 ± 0.03 ^{de}	0.88 ± 0.10 ^{c}	7.00 ± 0.48 ^{h}	0.88 ± 0.02 ^{i}	2.50 ± 0.53 ^{fh}
CW-9502-S6	4.24 ± 0.04 ^{ef}	1.46 ± 0.03 ^{d}	0.33 ± 0.03 ^{a}	0.56 ± 0.03 ^{b}	0.69 ± 0.13 ^{ab}	4.12 ± 0.09 ^{c}	0.99 ± 0.03 ^{j}	2.12 ± 0.08 ^{de}
CW-9502-S9	4.22 ± 0.04 ^{de}	1.66 ± 0.01 ^{f}	0.29 ± 0.01 ^{a}	0.94 ± 0.03 ^{h}	0.92 ± 0.05 ^{c}	5.69 ± 0.20 ^{f}	1.28 ± 0.05 ^{l}	2.73 ± 0.25 ^{gh}
CW-9518-S6	4.12 ± 0.01 ^{c}	1.34 ± 0.01 ^{c}	0.30 ± 0.01 ^{a}	0.68 ± 0.02 $_{c}$	0.57 ± 0.14 ^{a}	3.46 ± 0.30 ^{b}	0.77 ± 0.05 ^{ef}	1.80 ± 0.00 ^{bc}
CW-9518-S9	4.11 ± 0.07 ^{c}	1.12 ± 0.0 ^{a}	0.35 ± 0.01 ^{a}	0.82 ± 0.03 ^{df}	1.12 ± 0.16 ^{de}	4.77 ± 0.17 ^{d}	1.08 ± 0.08 ^{h}	2.22 ± 0.02 ^{ef}
Significance	Y	Y	Y	Y	Y	Y	Y	Y
Single effect of cereal mixtures								
DW	4.06 ^{a}	3.47 ^{b}	2.19 ^{b}	0.77 ^{a}	0.91 ^{a}	5.35 ^{a}	0.72 ^{a}	2.53 ^{b}
CW	4.22 ^{b}	1.72 ^{a}	0.65 ^{a}	0.80 ^{a}	0.90 ^{a}	5.29 ^{a}	0.90 ^{b}	2.32 ^{a}
Significance	Y	Y	Y	ns	ns	ns	Y	Y
Single effect of yeasts								
17290	4.18 ^{c}	2.64 ^{c}	1.23 ^{b}	0.80 ^{b}	0.95 ^{b}	5.72 ^{c}	0.68 ^{a}	3.13 ^{d}
14061	4.03 ^{a}	3.87 ^{d}	2.85 c	0.80 ^{b}	0.96 ^{b}	6.56 ^{d}	0.76 ^{b}	2.07 ^{b}
9502	4.10 ^{b}	2.56 ^{b}	1.25 ^{b}	0.85 ^{c}	0.97 ^{b}	5.47 ^{b}	0.96 ^{d}	2.71 ^{c}
9518	4.25 ^{d}	1.30 ^{a}	0.33 ^{a}	0.69 ^{a}	0.76 ^{a}	3.53 ^{a}	0.82 ^{c}	1.79 ^{a}
Significance	Y	Y	Y	Y	Y	Y	Y	Y
Single effect of sugar levels								
S6	4.16 ^{b}	2.57 ^{a}	1.38 ^{a}	0.81 ^{b}	0.93 ^{b}	5.68 ^{b}	0.78 ^{a}	2.42 ^{a}
S9	4.12 ^{a}	2.62 ^{b}	1.45 ^{a}	0.76 ^{a}	0.89 ^{a}	4.96 ^{a}	0.83 ^{b}	2.43 ^{a}
Significance	Y	Y	ns	Y	Y	Y	Y	ns

In the columns, different letters correspond to significant differences at $p < 0.05$ by LSD multiple range test; Y, significant; ns, not significant.

The quali-quantitative composition of sugars remained in the final beers, and their glycerol contents were strongly influenced by the interaction of the three factors, showing a remarkable variability among the 16 beers (Table 2). The concentrations of the individual sugars were in the following ranges: maltodextrins, 8.9–33.09 mg/mL; maltotriose, 2.08–16.54 mg/mL; maltose, 1.61–10.05 mg/mL; glucose, 0.54–18.64 mg/mL; fructose, 0.79–5.66 mg/mL. Instead, the production of glycerol comprised between 2.20 mg/L and 3.98 mg/L. DW beers had the highest concentrations of all the residual sugars as a consequence of the higher starch degradation occurring during brewing, which was in turn related to the higher endo-β-glucanase and endo-1,4-β-D-xylanase activities detected in durum wheat [34]. Furthermore, the beers produced from CW showed the highest glycerol content. *S. cerevisiae* 9518 showed the fastest fermentation of mono- (Pearson correlation coefficient between fructose and alcohol was −0.64), di-, and trisaccharides (Pearson correlation coefficient between maltotriose and alcohol was −0.6)—consistently with its high ability to produce alcohols—and a good ability to metabolize maltodextrins. The utilization of *S. cerevisiae* 17290 resulted in beers with the highest maltose content and high maltodextrin concentrations; as well, *S. cerevisiae* 9502 had the slowest maltodextrin and glucose fermentation capacity. However, both the strains produced suitable ethanol content. The fermentation performed by *S. cerevisiae* 14061 produced beers with the highest maltotriose and fructose contents consistently with their lowest alcohol and maltodextrin contents. *S. cerevisiae* 14061 and 9502 also produced the lowest glycerol amounts. As expected, the beers obtained with the addition of 9 g sucrose/L showed the highest concentrations of all residual sugars and glycerol, and the latter was a response to the highest osmotic stress. In fact, yeast cells produce and accumulate glycerol to adapt the intracellular osmolarity to that of the environment, thus preventing dehydration [35]. This ability is technologically exploited to confer body and fullness to the beer, so researchers generally select highly producing glycerol strains [36]. However, when yeast cells activate the so-called High Osmolarity Glycerol pathway, this diversion of carbon flux towards glycerol synthesis may also limit the production of CO_2 (correlation coefficient between CO_2 and glycerol was −0.77).

The concentration of organic acids has been investigated because of its contribution to pH, titratable acidity (freshness), and volatile acidity (off-flavors). Table 3 highlights the significant interactive effects of cereal mixtures, yeasts, and sugar levels on organic acids and correlated variables. The average pH was in the range 3.79 (DW-14061-S6 and DW-14061-S9)–4.40 (DW-9518-S6), and these values are consistent with those detected in commercial Belgian white beers [37]. DW-14061-S9 also had the highest titratable acidity, which is a trait typical of beers produced with unmalted cereals. Regardless of the high volatile acidity of DW-14061 beers, they had only moderate contents of acetic acid, which is undesired for its vinegary flavor. This behavior is probably the result of the specific intracellular carbon flows of *S. cerevisiae* 14061: the flow is higher in the tricarboxylic acid cycle and lower towards ethanol and acetate, as highlighted by the low alcohol and acetic acid content of the beers fermented by this strain. The lowest pH and the highest acidity values found in CW beers were due to the strongest buffer potential of their hydrolysed protein, in particular when fermented by *S. cerevisiae* 14061. An opposite behavior was observed in beers fermented by *S. cerevisiae* 9518, which also had the lowest content of acetic acid. The refermentation following the highest sucrose addition resulted in lower pH values and higher titratable acidity without changes of the alcohol content (greater carbon flow in the tricarboxylic acid cycle and lower towards ethanol).

Except for fumaric acid, whose amount was always below 0.01 g/L, the average concentrations of the other organic acids varied widely among the beers and were in the following decreasing order: succinic (2.70–8.14 g/L), acetic (1.40–3.95 g/L), malic (0.57–1.39 g/L), lactic (0.58–1.28 g/L), and citric (0.49–1.04 g/L). As can be seen, succinic acid is the primary organic acid, whose interest is related to its ability to impart salty and bitter flavors to the beer [38]. The single effect of cereal mixtures was statistically significant only for lactic and acetic acids, whose concentrations were higher in CW and DW, respectively.

The effects of yeast were of greater interest since organic acids are mainly produced during alcoholic fermentation. Citric acid is an exception, since it is generally already present in wort, although it can be produced in the Krebs cycle. Consequently, the alcohol content was inversely correlated with titratable acidity, volatile acidity, and malic acid (−0.78 and −0.77, −0.54, respectively), while citric acid was positively correlated with malic and succinic acids (+0.75 and +0.69, respectively). The beers fermented by *S. cerevisiae* 14061 showed the highest content of succinic acid, while those fermented by *S. cerevisiae* 9502 had the highest citric, malic, and lactic acid concentrations. Finally, the lowest lactic acid and the highest acetic acid contents were detected in beers inoculated with *S. cerevisiae* 17290, while *S. cerevisiae* 9518 gave rise to beers with the lowest concentrations of citric, malic, and succinic acids.

3.2.2. Phenolic Component and Antioxidant Activity

As discussed in Section 3.1, the phenolic component of the cereal mixture is only partially released during mashing, and this explains the generally moderate phenolic content of beer with respect to wine [39]. The average TPC of the beers ranged between 345 mg/L of CW-9518-S6 and 429–449 mg/L of the DW beers, fermented by the strains isolated from Negroamaro, independently on the amount of sugar added for refermentation (Table 4). DW beers showed higher TPC than CW consistently with the differences in total phenolics detected between the starting cereal mixtures. The differences among TPCs of beers produced according to the same brewing procedure starting from the same ingredients but fermented by different strains can be related to the following two phenomena: the ability of some *S. cerevisiae* strains to produce phenolics [40]; the different ability of the yeast cell wall to release or adsorb phenolic molecules. These concentrations are considerably greater than those (221–364 mg/L) detected by Baiano et al. [3] in beers obtained through brewing with the same cereal mixtures as a result of the higher cereal-to-water ratio, the change in the mashing steps, and the increased hop addition. The antioxidant activity varied from 0.52–0.55 mmol Trolox/L (CW-14061-S6 and DW-14061-S6) to 0.98 mmol Trolox/L (DW-9502-S6) to highlight the highest positive effect of durum wheat in the cereal mixture and the fermentation performed by *S. cerevisiae* 9502.

Eighteen phenolic compounds were retrieved in all the samples, although in different concentrations: nine phenolic acids (gallic, 4-hydroxybenzoic, vanillic, caffeic, syringic, chlorogenic, ferulic, *p*-coumaric, and sinapic); four flavanols (catechin, epicatechin, epigallocatechin, and epicatechingallate); three flavonols (rutin, quercetin, and kaempferol); one hydroxystilbene (resveratrol) (Table 4). The phenolics detected in the highest concentrations were chlorogenic acid (4.8–14.0 mg/L), epigallocatechin (3.8–10.9 mg/L), epicatechin (6.9–10.0 mg/L), rosmarinic acid (4.6–9.1 mg/L), kaempferol (2.0–8.9 mg/L), gallic acid (3.5–7.3 mg/L), epicatechingallate (2.6–6.0 mg/L), vanillic acid (0.3–6.0 mg/L), and sinapic acid (2.8–4.8 mg/L). The other compounds were detected in concentrations lower than 2.1 mg/L. The significant single effects exerted by cereal mixture and yeast strain on all the phenolics indicate that phenolics were released from the cereal matrix during mashing and were synthetized, released, and absorbed in different amount by yeasts. DW beers had the highest concentrations of gallic, 4-hydroxybenzoic, caffeic, ferulic, *p*-coumaric, sinapic, and rosmarinic acids as well as of catechin, resveratrol, and querceti,. The other compounds occurred in greater concentrations in CW beers. The lowest concentrations of most of the phenolic compounds were detected in beers fermented by *S. cerevisiae* 9518 (gallic, 4-hydroxybenzoic, vanillic, chlorogenic, ferulic, *p*-coumaric, sinapic, and rosmarinic acids; catechin, epigallocatechin, epicatechingallate, rutin, and resveratrol) and in those fermented by *S. cerevisiae* 9502 (caffeic, ferulic, *p*-coumaric, rosmarinic acids; epicatechin, epigallocatechin, resveratrol, quercetin, and kaempferol). The highest contents of the individual phenolics were detected in beers fermented by *S. cerevisiae* 17290 (gallic, 4-hydroxybenzoic, caffeic, ferulic, *p*-coumaric, sinapic, and rosmarinic acids; catechin, resveratrol) and *S. cerevisiae* 14061 strains (vanillic, syringic, and chlorogenic acids; epicatechin, epicatechingallate, epigallocatechin, rutin, kaempferol). No differences were highlighted between the two sugar levels for most individual phenolics.

Table 4. Influence of cereal mixtures, yeasts, and sugar levels on the total phenolic content, antioxidant activity, and phenolic profile of the beers.

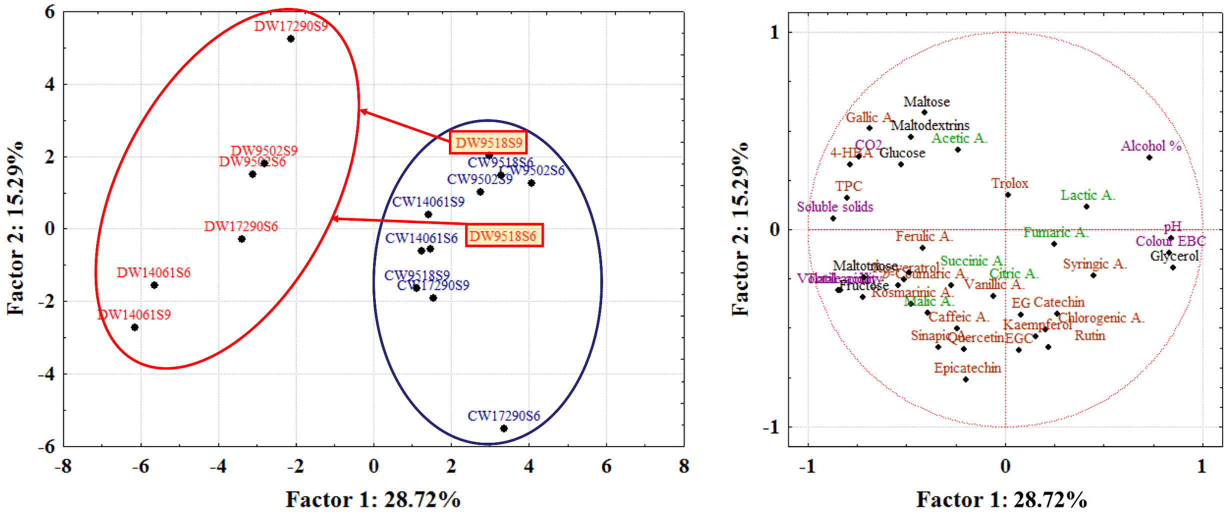
Beer Acronyms	TPC (mg/L)	AA (mmol Trolox/L)	Phenolics (mg/L)																	
			Gallic Acid	**4-HBA**	**Catechin**	**Vanillic Acid**	**Caffeic Acid**	**Syringic Acid**	**Epicatechin**	**Chlorogenic Acid**	**EGC**	**Ferulic Acid**	***p*-Coumaric Acid**	**Sinapic Acid**	**EG**	**Rutin**	**Resveratrol**	**Rosmarinic Acid**	**Quercetin**	**Kaempferol**
Interactive effects (cereal mixtures × yeasts × sugar levels)																				
DW-17290-S6	439 ± 12 ^{e}	0.57 ± 0.01 ^{ab}	7.3 ± 0.3 ^{i}	1.9 ± 0.1 ^{h}	0.5 ± 0.1 ^{bd}	4.8 ± 0.0 ^{g}	0.9 ± 0.0 ^{c}	0.6 ± 0.0 ^{e}	7.8 ± 0.0 ^{d}	10.3 ± 0.1 ^{g}	9.4 ± 0.5 ^{e}	1.8 ± 0.0 ^{b}	1.2 ± 0.0 ^{b}	4.8 ± 0.3 ^{d}	6.0 ± 0.2 ^{g}	0.9 ± 0.1 ^{a}	1.6 ± 0.0 ^{ab}	8.5 ± 0.8 ^{e}	1.6 ± 0.0 ^{e}	6.5 ± 0.3 ^{g}
DW-17290-S9	449 ± 23 ^{e}	0.88 ± 0.04 ^{e}	7.1 ± 0.1 ^{hi}	2.1 ± 0.0 ^{i}	1.0 ± 0.0 ^{f}	2.0 ± 0.1 ^{b}	1.2 ± 0.0 ^{e}	0.2 ± 0. ^{c}	6.9 ± 0.0 ^{a}	6.4 ± 0.2 ^{d}	3.9 ± 0.2 ^{a}	1.9 ± 0.0 ^{c}	1.2 ± 0.0 ^{b}	2.9 ± 0.1 ^{a}	2.7 ± 0.2 ^{a}	0.9 ± 0.0 ^{a}	1.4 ± 0.0 ^{a}	4.8 ± 0.2 ^{a}	1.3 ± 0.0 ^{b}	6.3 ± 0.4 ^{g}
DW-14061-S6	433 ± 12 ^{e}	0.55 ± 0.02 ^{a}	6.8 ± 0.0 ^{g}	1.9 ± 0.1 ^{h}	0.2 ± 0.1 ^{a}	3.6 ± 0.0 ^{d}	1.0 ± 0.0 d	0.1 ± 0.0 ^{b}	9.5 ± 0.1 ^{f}	12.4 ± 0.6 ^{h}	3.8 ± 0.3 ^{a}	1.8 ± 0.0 ^{b}	1.2 ± 0.0 ^{b}	4.8 ± 0.2 ^{d}	2.6 ± 0.1 ^{a}	1.1 ± 0.1 ^{ab}	1.5 ± 0.0 ^{ab}	8.9 ± 0.1 ^{ef}	1.4 ± 0.0 ^{c}	5.9 ± 0.5 ^{fg}
DW-14061-S9	429 ± 8 ^{e}	0.87 ± 0.06 ^{de}	6.8 ± 0.0 ^{g}	1.9 ± 0.1 ^{h}	1.0 ± 0.1 ^{f}	3.0 ± 0.4 ^{c}	1.3 ± 0.0 ^{e}	0.2 ± 0.1 ^{c}	9.0 ± 0.3 ^{e}	6.8 ± 0.0 ^{de}	10.6 ± 0.8 ^{f}	2.0 ± 0.0 ^{c}	1.4 ± 0.0 ^{c}	4.9 ± 0.2 ^{d}	5.3 ± 0.0 ^{f}	1.3 ± 0.0 ^{b}	1.7 ± 0.0 ^{b}	6.8 ± 0.2 ^{c}	1.6 ± 0.1 ^{e}	4.9 ± 0.0 ^{e}
DW-9502-S6	375 ± 50 ^{dc}	0.98 ± 0.11 ^{f}	6.7 ± 0.1 ^{fg}	1.9 ± 0.0 ^{h}	0.5 ± 0.1 ^{bc}	3.9 ± 0.1 ^{e}	1.0 ± 0.0 ^{d}	0.4 ± 0.0 ^{d}	7.0 ± 0.1 ^{ab}	4.8 ± 0.2 ^{a}	4.2 ± 0.1 ^{a}	1.8 ± 0.0 ^{b}	1.1 ± 0.0 ^{ab}	4.2 ± 0.0 ^{c}	4.8 ± 0.2 ^{de}	1.2 ± 0.0 ^{b}	1.6 ± 0.0 ^{ab}	8.5 ± 0.8 ^{e}	1.4 ± 0.0 ^{c}	2.5 ± 0.0 ^{b}
DW-9502-S9	422 ± 19 ^{de}	0.78 ± 0.04 ^{bd}	7.0 ± 0.0 ^{h}	1.7 ± 0.1 ^{f}	0.5 ± 0.0 ^{bd}	3.6 ± 0.2 ^{d}	1.1 ± 0.0 ^{d}	0.2 ± 0.1 ^{c}	7.3 ± 0.2 ^{bc}	5.9 ± 0.1 ^{bc}	6.8 ± 0.1 ^{c}	1.9 ± 0.0 ^{c}	1.2 ± 0.0 ^{b}	3.7 ± 0.2 ^{b}	5.3 ± 0.0 ^{f}	1.4 ± 0.0 ^{b}	1.6 ± 0.0 ^{ab}	9.0 ± 0.3 ^{f}	1.3 ± 0.0 ^{b}	2.5 ± 0.1 ^{b}
DW-9518-S6	394 ± 13 ^{cd}	0.76 ± 0.02 ^{bc}	6.0 ± 0.1 ^{d}	1.5 ± 0.0 ^{e}	0.6 ± 0.1 ^{cd}	2.2 ± 0.1 ^{b}	1.2 ± 0.1 ^{e}	nd ^{a}	7.2 ± 0.1 ^{b}	7.7 ± 0.4 ^{f}	5.0 ± 0.1 ^{b}	2.0 ± 0.0 ^{c}	1.0 ± 0.0 ^{ab}	4.8 ± 0.2 ^{d}	4.6 ± 0.0 ^{d}	1.3 ± 0.2 ^{b}	1.6 ± 0.0 ^{ab}	9.1 ± 0.1 ^{f}	1.6 ± 0.0 ^{e}	8.3 ± 0.0 ^{h}
DW-9518-S9	382 ± 3 ^{bc}	0.82 ± 0.06 ^{ce}	6.2 ± 0.1 ^{e}	0.9 ± 0.1 ^{b}	0.7 ± 0.0 ^{e}	2.2 ± 0.2 ^{b}	0.9 ± 0.0 ^{c}	0.1 ± 0.0 ^{b}	7.5 ± 0.3 ^{c}	7.0 ± 0.1 ^{e}	4.0 ± 0.0 ^{a}	1.9 ± 0.0 ^{c}	0.9 ± 0.0 ^{a}	2.9 ± 0.2 ^{a}	4.3 ± 0.1 ^{c}	1.1 ± 0.0 ^{ab}	1.5 ± 0.0 ^{ab}	5.8 ± 0.2 ^{b}	1.5 ± 0.0 ^{d}	2.0 ± 0.1 ^{a}
CW-17290-S6	375 ± 22 ^{ac}	0.84 ± 0.03 ^{ce}	3.5 ± 0.0 ^{a}	0.8 ± 0.1 ^{a}	2.1 ± 0.1 ^{h}	6.0 ± 0.1 ^{h}	1.5 ± 0.0 ^{g}	0.7 ± 0.0 ^{ef}	10.0 ± 0.1 ^{g}	13.9 ± 0.2 ^{i}	10.9 ± 0.7 ^{f}	1.9 ± 0.0 ^{c}	1.2 ± 0.0 ^{b}	4.5 ± 0.2 ^{cd}	5.9 ± 0.0 ^{g}	2.5 ± 0.1 ^{c}	1.4 ± 0.0 ^{a}	8.3 ± 0.6 ^{e}	1.6 ± 0.0 ^{e}	8.9 ± 0.2 ^{i}
CW-17290-S9	362 ± 17 ^{ab}	0.79 ± 00.02 ^{be}	6.2 ± 0.1 ^{e}	1.1 ± 0.0 ^{c}	0.7 ± 0.0 ^{e}	0.3 ± 0.0 ^{a}	1.0 ± 0.0 ^{d}	0.5 ± 0.0 ^{de}	7.2 ± 0.1 ^{b}	14.0 ± 0.1 ^{i}	6.6 ± 0.0 ^{c}	1.8 ± 0.0 ^{b}	1.0 ± 0.0 ^{ab}	4.6 ± 0.1 ^{cd}	5.0 ± 0.2 ^{e}	2.8 ± 0.1 ^{d}	1.6 ± 0.0 ^{ab}	4.6 ± 0.0 ^{a}	1.4 ± 0.0 ^{c}	9.2 ± 0.2 ^{i}
CW-14061-S6	378 ± 3 ^{bc}	0.52 ± 0.05 ^{a}	6.3 ± 0.0 ^{e}	1.7 ± 0.0 ^{fg}	1.5 ± 0.0 ^{g}	3.5 ± 0.2 ^{d}	0.9 ± 0.0 ^{c}	0.4 ± 0.0 ^{d}	8.0 ± 0.1 ^{d}	6.1 ± 0.6 ^{cd}	7.9 ± 0.1 ^{d}	1.8 ± 0.0 ^{b}	1.5 ± 0.0 ^{c}	4.6 ± 0.4 ^{cd}	4.9 ± 0.7 ^{e}	1.2 ± 0.0 ^{b}	1.5 ± 0.0 ^{ab}	6.3 ± 0.7 ^{bc}	1.4 ± 0.0 ^{c}	3.5 ± 0.1 ^{c}
CW-14061-S9	372 ± 15 ^{ac}	0.69 ± 0.10 ^{b}	6.2 ± 0.0 ^{e}	1.5 ± 0.0 ^{e}	0.6 ± 0.0 ^{d}	2.3 ± 0.1 ^{b}	1.0 ± 0.0 ^{d}	0.3 ± 0.0 ^{c}	7.3 ± 0.1 ^{bc}	7.6 ± 0.0 ^{f}	10.7 ± 0.8 ^{f}	1.9 ± 0.0 ^{c}	1.0 ± 0.0 ^{ab}	3.7 ± 0.2 ^{b}	5.0 ± 0.1 ^{e}	1.2 ± 0.0 ^{b}	1.5 ± 0.0 ^{ab}	7.5 ± 0.0 ^{d}	1.4 ± 0.0 ^{c}	5.1 ± 0.5 ^{e}
CW-9502-S6	360 ± 10 ^{ab}	0.78 ± 0.08 ^{bd}	5.7 ± 0.1 ^{bc}	1.1 ± 0.0 ^{c}	0.8 ± 0.0 ^{e}	4.1 ± 0.1 ^{f}	0.7 ± 0.0 ^{a}	0.4 ± 0.0 ^{d}	7.0 ± 0.0 ^{ab}	12.3 ± 0.0 ^{h}	6.8 ± 0.3 ^{c}	1.7 ± 0.0 ^{a}	1.0 ± 0.0 ^{ab}	3.9 ± 0.4 ^{b}	3.9 ± 0.2 ^{b}	1.4 ± 0.0 ^{b}	1.5 ± 0.0 ^{ab}	4.60 ± 0.0 ^{a}	1.2 ± 0.0 ^{a}	4.8 ± 0.1 ^{e}
CW-9502-S9	360 ± 12 ^{ab}	0.75 ± 0.10 ^{bc}	5.9 ± 0.0 ^{cd}	1.8 ± 0.0 ^{g}	0.8 ± 0.0 ^{e}	4.2 ± 0.2 ^{f}	0.7 ± 0.0 ^{a}	0.8 ± 0.0 ^{g}	7.1 ± 0.0 ^{ab}	10.9 ± 0.1 ^{g}	6.5 ± 0.3 ^{c}	1.8 ± 0.0 ^{b}	1.0 ± 0.0 ^{ab}	3.7 ± 0.0 ^{b}	5.3 ± 0.0 ^{f}	0.9 ± 0.0 ^{a}	1.5 ± 0.0 ^{ab}	4.5 ± 0.3 ^{a}	1.2 ± 0.0 ^{a}	5.2 ± 0.5 ^{e}
CW-9518-S6	345 ± 6 ^{a}	0.70 ± 0.02 ^{b}	5.5 ± 0.1 ^{b}	1.2 ± 0.1 ^{d}	0.3 ± 0.0 ^{a}	2.2 ± 0.0 ^{b}	0.8 ± 0.0 ^{b}	0.7 ± 0.0 ^{f}	7.5 ± 0.1 ^{c}	5.5 ± 0.2 ^{b}	6.3 ± 0.1 ^{c}	1.7 ± 0.0 ^{a}	1.1 ± 0.1 ^{ab}	3.9 ± 0.3 ^{b}	3.8 ± 0.1 ^{b}	0.9 ± 0.0 ^{a}	1.5 ± 0.0 ^{ab}	4.4 ± 0.1 ^{a}	1.4 ± 0.0 ^{c}	4.2 ± 0.30 ^{d}
CW-9518-S9	355 ± 9 ^{ab}	0.74 ± 0.04 ^{bc}	6.6 ± 0.1 ^{f}	1.4 ± 0.0 ^{e}	0.5 ± 0.0 ^{b}	3.7 ± 0.2 ^{d}	1.4 ± 0.0 ^{f}	0.7 ± 0.1 ^{fg}	9.9 ± 0.2 ^{g}	6.2 ± 0.6 ^{d}	9.2 ± 0.5 ^{e}	1.7 ± 0.0 ^{a}	1.2 ± 0.0 ^{b}	2.8 ± 0.2 ^{a}	3.7 ± 0.3 ^{b}	1.0 ± 0.0 ^{a}	1.6 ± 0.0 ^{ab}	7.9 ± 0.0 ^{e}	1.6 ± 0.0 ^{e}	8.6 ± 0.0 ^{h}
Signif.	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Single effect of cereal mixtures																				

DW	415 ^{b}	0.78 ^{b}	6.7 ^{b}	1.7 ^{b}	0.9 ^{b}	3.2 ^{a}	1.1 ^{b}	0.2 ^{a}	7.8 ^{a}	7.7 ^{a}	6.0 ^{a}	1.9 ^{b}	1.2 ^{b}	4.1 ^{b}	4.5 ^{a}	1.2 ^{a}	1.6 ^{b}	7.7 ^{b}	1.5 ^{b}	4.7 ^{a}
CW	343 ^{a}	0.70 ^{a}	5.7 ^{a}	1.3 ^{a}	0.6 ^{a}	3.3 ^{b}	1.0 ^{a}	0.6 ^{b}	8.0 ^{b}	9.6 ^{b}	8.1 ^{b}	1.8 ^{a}	1.1 ^{a}	4.0 ^{a}	4.7 ^{b}	1.5 ^{b}	1.5 ^{a}	6.0 ^{a}	1.4 ^{a}	6.2 ^{b}
Signif.	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Single effect of yeasts																				
17290	406 ^{b}	0.77 ^{b}	6.0 ^{a}	1.5 ^{b}	1.1 ^{d}	3.3 ^{c}	1.2 ^{c}	0.5 ^{d}	8.0 ^{b}	11.1 ^{c}	7.7 ^{b}	1.8 ^{a}	1.1 ^{a}	4.2 ^{c}	4.9 ^{c}	1.8 ^{c}	1.5 ^{a}	6.6 ^{a}	1.4 ^{b}	7.7 ^{d}
14061	403 ^{b}	0.66 ^{a}	6.5 ^{c}	1.7 ^{d}	0.8 ^{c}	3.1 ^{b}	1.1 ^{b}	0.2 ^{a}	8.5 ^{c}	8.2 ^{b}	8.3 ^{c}	1.9 ^{b}	1.3 ^{b}	4.5 ^{d}	4.5 ^{b}	1.2 ^{b}	1.6 ^{b}	7.4 ^{b}	1.4 ^{b}	4.8 ^{b}
9502	379 ^{a}	0.81 ^{c}	6.3 ^{b}	1.6 ^{c}	0.6 ^{b}	4.0 ^{d}	0.9 ^{a}	0.5 ^{cd}	7.1 ^{a}	8.5 ^{b}	6.1 ^{a}	1.8 ^{a}	1.1 ^{a}	3.9 ^{b}	4.8 ^{c}	1.2 ^{b}	1.5 ^{a}	6.6 ^{a}	1.3 ^{a}	3.7 ^{a}
9518	369 ^{a}	0.71 ^{b}	6.1 ^{a}	1.3 ^{a}	0.5 ^{a}	2.6 ^{a}	1.1 ^{b}	0.4 ^{b}	8.0 ^{b}	6.6 ^{a}	6.1 ^{a}	1.8 ^{a}	1.1 ^{a}	3.6 ^{a}	4.1 ^{a}	1.1 ^{a}	1.5 ^{a}	6.8 ^{a}	1.5 ^{b}	5.8 ^{c}
Signif.	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Single effect of sugar levels																				
S6	387 ^{a}	0.7 ^{a}	6.0 ^{a}	1.5 ^{a}	0.8 ^{a}	3.8 ^{b}	1.0 ^{a}	0.4 ^{a}	8.0 ^{b}	9.1 ^{b}	6.8 ^{a}	1.8 ^{a}	1.2 ^{a}	4.4 ^{b}	4.6 ^{a}	1.3 ^{a}	1.5 ^{a}	7.3 ^{b}	1.4 ^{a}	5.6 ^{a}
S9	391 ^{a}	0.8 ^{a}	6.5 ^{b}	1.6 ^{a}	0.7 ^{a}	2.7 ^{a}	1.1 ^{a}	0.3 ^{a}	7.8 ^{a}	8.1 ^{a}	7.3 ^{b}	1.9 ^{a}	1.1 ^{a}	3.7 ^{a}	4.6 ^{a}	1.3 ^{a}	1.5 ^{a}	6.4 ^{a}	1.4 ^{a}	5.5 ^{a}
Signif.	ns	ns	Y	ns	ns	Y	ns	ns	Y	Y	Y	ns	ns	Y	ns	ns	ns	Y	ns	ns

In the columns, different letters correspond to significant differences at $p < 0.05$ by LSD multiple range test; Y, significant; ns, not significant. TPC, total phenolic content. AA, antioxidant activity. 4-HBA, 4-hydroxybenzoic acid. EGC, epigallocatechin. EG, epicatechingallate.

3.2.3. PCA Applied to the Beer Physical and Chemical Characteristics

PCA applied to the data set consisting of physico-chemical indices, sugars, glycerol, organic acids, and phenolic concentrations (Figure 2) highlighted that the beers were clearly grouped according to the type of cereal mixture used (DW and CW). More in depth, DW beers are placed in the portion of the Cartesian plane identified by negative values of factor 1 (Figure 2a) and characterized by a high level of acidity (both titratable and volatile), antioxidant activity, carbon dioxide, soluble solids, sugars, acetic acid, and TPC (Figure 2b). Instead, CW beers are placed in the quadrants identified by positive values of factor 1 (Figure 2a) and distinguished by high pH and high alcohol, glycerol, and lactic acid contents (Figure 2b). However, the variance explained by the first two factors was low (~44%) as a consequence of the levelling effects exerted by the application of the same brewing procedure. Furthermore, there are two outliers, represented by the DW beers fermented by *S. cerevisiae* 9518, which showed physico-chemical characteristics similar to those of CW beers. The graphic representation also highlights that only 14061 and 9502 strains were able to impart common physico-chemical characteristics to the beers and that, instead, the possibility of diversifying the physical and chemical quality of the beers by varying the quantity of sugar added during secondary fermentation is limited to the variables related to acidity, carbon dioxide, and residual sugars.



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

Figure 2. PCA of the physical and chemical characteristics of beers: projection of (**a**) beers and (**b**) data concerning basic analyses (in violet), organic acids (in green), sugars and glycerol (in black), and antioxidants (in brown).

3.2.4. Sensory Characteristics

According to the sensory analyses (Table 5), all the beers had in common a white foam and intermediate scores for the overall flavor intensity and the yeast flavor, without significant single and interactive effects of the considered factors. Furthermore, the sugar level did not exert significant effects on many other variables, namely turbidity, all gustatory and tactile characteristics, as well as the overall quality.

Table 5. Influence of cereal mixtures, yeasts, and sugar levels on sensory characteristics of beers.

Beer Acronyms	**Color**		**Foam**		**Turbidity**	**Gustatory Characteristics**				**Tactile Characteristics**			**Overall Quality**
	Foam	**Liquid**	**Amount**	**Persist.**		**Sweetn.**	**Bittern.**	**Saltiness**	**Acidity/Sourness**	**Alcohol.**	**Effervesc.**	**Body**	
Interactive effects (cereal mixtures × yeasts × sugar levels)													
DW-17290-S6	1.0 ± 0.0 ^{a}	2.0 ± 0.6 ^{ab}	1.8 ± 0.4 ^{b}	1.2 ± 0.4 ^{a}	2.0 ± 0.0 ^{a}	2.2 ± 0.4 ^{ab}	2.0 ± 0.0 ^{a}	2.8 ± 0.4 ^{b}	3.2 ± 0.4 ^{c}	2.7 ± 0.5 ^{ab}	2.5 ± 0.5 ^{a}	2.3 ± 0.5 ^{bc}	2.3 ± 1.0 ^{abc}
DW-17290-S9	1.0 ± 0.0 ^{a}	1.7 ± 0.8 ^{a}	2.3 ± 0.5 ^{bc}	1.5 ± 0.5 ^{a}	2.0 ± 0.0 ^{a}	2.2 ± 0.4 ^{ab}	2.3 ± 0.5 ^{ab}	2.7 ± 0.5 ^{b}	3.3 ± 0.5 ^{cd}	2.7 ± 0.5 ^{ab}	2.8 ± 0.4 ^{ab}	2. 3 ± 0.5 ^{ab}	3.0 ± 0.9 ^{cd}
DW-14061-S6	1.0 ± 0.0 ^{a}	1.8 ± 0.8 ^{ab}	1.0 ± 0.0 ^{a}	1.0 ± 0.0 ^{a}	2.2 ± 0.4 ^{ab}	2.0 ± 0.0 ^{a}	2.3 ± 0.8 ^{ac}	2.7 ± 0.5 ^{b}	4.0 ± 0.0 ^{d}	2.5 ± 0.5 ^{ab}	2.3 ± 0.5 ^{a}	2.2 ± 0.4 ^{ab}	1.7 ± 0.8 ^{a}
DW-14061-S9	1.0 ± 0.0 ^{a}	1.7 ± 0.8 ^{a}	1.0 ± 0.0 ^{a}	1.0 ± 0.0 ^{a}	2.2 ± 0.4 ^{ab}	2.2 ± 0.4 ^{ab}	2.2 ± 0.4 ^{ab}	2.8 ± 0.8 ^{b}	3.8 ± 0.4 ^{cd}	2.3 ± 0.5 ^{a}	2.3 ± 0.5 ^{a}	2.0 ± 0.0 ^{a}	1.8 ± 0.8 ^{ab}
DW-9502-S6	1.0 ± 0.0 ^{a}	2.0 ± 0.0 ^{ab}	3.0 ± 0.0 ^{cd}	2.3 ± 0.5 ^{b}	2.3 ± 0.5 ^{ac}	2.0 ± 0.0 ^{a}	2.8 ± 0.4 ^{b}	2.3 ± 0.5 ^{ab}	3.0 ± 0.9 ^{cd}	2.3 ± 0.5 ^{a}	2.7 ± 0.5 ^{ab}	2.3 ± 0.5 ^{ab}	2.7 ± 0.8 ^{bc}
DW-9502-S9	1.0 ± 0.0 ^{a}	2.0 ± 0.6 ^{ab}	3.2 ± 0.8 ^{de}	2.7 ± 0.8 ^{bc}	2.2 ± 0.4 ^{ab}	2.3 ± 0.5 ^{ab}	2.5 ± 0.5 ^{ab}	2.5 ± 0.8 ^{ab}	3.0 ± 0.6 ^{c}	2.5 ± 0.5 ^{ab}	2.8 ± 0.8 ^{ab}	2.3 ± 0.5 ^{ab}	3.0 ± 0.6 ^{cd}
DW-9518-S6	1.0 ± 0.0 ^{a}	2.2 ± 0.8 ^{ab}	4.7 ± 0.5 ^{gh}	4.2 ± 0.8 ^{fg}	3.7 ± 0.8 ^{e}	2.5 ± 0.5 ^{ab}	3.0 ± 0.9 ^{c}	2.3 ± 0.5 ^{ab}	2.3 ± 0.5 ^{a}	2.8 ± 0.4 ^{ab}	3.5 ± 0.5 ^{c}	3.2 ± 0.8 ^{b}	4.2 ± 0.8 ^{e}
DW-9518-S9	1.0 ± 0.0 ^{a}	1.8 ± 0.8 ^{ab}	4.8 ± 0.4 ^{h}	4.3 ± 0.5 ^{fg}	2.8 ± 1.0 ^{bd}	2.8 ± 0.8 ^{c}	2.5 ± 0.5 ^{ac}	2.3 ± 0.5 ^{ab}	2.3 ± 0.5 ^{a}	2.7 ± 0.5 ^{ab}	2.8 ± 0.8 ^{ab}	2.8 ± 0.8 ^{ab}	3.8 ± 0.8 ^{de}
CW-17290-S6	1.0 ± 0.0 ^{a}	2.3 ± 0.5 ^{ab}	3.8 ± 1.0 ^{ef}	3.3 ± 0.5 ^{de}	2.0 ± 0.0 ^{a}	2.2 ± 0.4 ^{ab}	2.7 ± 0.8 ^{ac}	2.7 ± 0.5 ^{b}	2.8 ± 0.4 ^{b}	2.8 ± 0.4 ^{ab}	3.7 ± 0.8 ^{c}	3.3 ± 0.8 ^{b}	3.8 ± 0.8 ^{de}
CW-17290-S9	1.0 ± 0.0 ^{a}	2.3 ± 0.8 ^{ab}	4.5 ± 0.5 ^{fh}	4.0 ± 0.6 ^{fg}	2.0 ± 0.0 ^{a}	2.0 ± 0.0 ^{a}	2.8 ± 0.4 ^{bc}	2.3 ± 0.5 ^{ab}	2.3 ± 0.5 ^{a}	3.0 ± 0.6 ^{b}	3.5 ± 0.8 ^{c}	3.2 ± 0.8 ^{b}	3.8 ± 0.4 ^{de}
CW-14061-S6	1.0 ± 0.0 ^{a}	2.3 ± 0.5 ^{ab}	4.7 ± 0.8 ^{gh}	4.5 ± 0.8 ^{g}	2.0 ± 0.0 ^{a}	2.5 ± 0.5 ^{ab}	2.5 ± 0.5 ^{ac}	2.5 ± 0.5 ^{ab}	2.3 ± 0.5 ^{a}	2.8 ± 0.4 ^{ab}	3.8 ± 0.4 ^{c}	3.3 ± 0.5 ^{b}	4.2 ± 0.8 ^{e}
CW-14061-S9	1.0 ± 0.0 ^{a}	2.2 ± 0.8 ^{ab}	4.3 ± 1.0 ^{fh}	4.3 ± 0.5 ^{fg}	2.0 ± 0.0 ^{a}	2.3 ± 0.05 ^{ab}	2.5 ± 0.5 ^{ac}	2.5 ± 0.5 ^{ab}	2.3 ± 0.5 ^{a}	3.0 ± 0.6 ^{b}	3.5 ± 0.8 ^{ab}	3.2 ± 0.8 ^{b}	4.2 ± 1.0 ^{e}
CW-9502-S6	1.0 ± ±0.5 ^{a}	2.0 ± ±0.9 ^{ab}	4.8 ± ±0.4 ^{h}	4.5 ± ±0.5 ^{g}	2.7 ± 1.0 ^{ad}	2.2 ± ±0.4 ^{ab}	2.7 ± ±0.5 ^{bc}	2.4 ± ±0.5 ^{ab}	2.2 ± ±0.4 ^{a}	2.7 ± ±0.5 ^{ab}	3.3 ± ±0.5 ^{ab}	3.2 ± 0.4 ^{b}	3.8 ± ±0.8 ^{de}
CW-9502-S9	1.0 ± 0.0 ^{a}	2.2 ± 0.8 ^{ab}	4.8 ± 0.4 ^{h}	4.5 ± 0.5 ^{g}	2.8 ± 0.8 ^{bd}	2.7 ± 0.5 ^{b}	2.5 ± 0.5 ^{ac}	2.3 ± 0.5 ^{ab}	2.2 ± 0.4 ^{a}	2.8 ± 0.4 ^{ab}	2.8 ± 1.0 ^{ab}	2.8 ± 1.0 ^{ab}	3.8 ± 1.0 ^{de}
CW-9518-S6	1.0 ± 0.0 ^{a}	2.5 ± 0.8 ^{b}	4.2 ± 0.8 ^{fh}	3.2 ± 0.4 ^{cd}	3.3 ± 0.8 ^{de}	2.5 ± 0.5 ^{ab}	2.5 ± 0.5 ^{ac}	2.3 ± 0.5 ^{ab}	2.3 ± 0.5 ^{ab}	2.8 ± 0.4 ^{ab}	2.8 ± 0.8 ^{ab}	2.8 ± 0.8 ^{ab}	4.2 ± 1.0 ^{e}
CW-9518-S9	1.0 ± 0.0 ^{a}	2.5 ± 0.5 ^{b}	4.0 ± 0.6 ^{fg}	3.8 ± 0.8 ^{ef}	3.0 ± 1.1 ^{ce}	2.7 ± 0.5 ^{b}	2.5 ± 0.5 ^{ac}	2.2 ± 0.4 ^{a}	2.5 ± 0.5 ^{ab}	2.8 ± 0.4 ^{ab}	3.0 ± 0.9 ^{b}	3.0 ± 0.6 ^{ab}	4.0 ± 0.6 ^{e}
Significance	ns	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Single effect of cereal mixtures													
DW	1.0 ^{a}	1.9 ^{a}	2.7 ^{a}	2.3 ^{a}	2.4 ^{a}	2.3 ^{a}	2.5 ^{a}	2.6 ^{a}	3.1 ^{b}	2.6 ^{a}	2.7 ^{a}	2.4 ^{a}	2.8 ^{a}
CW	1.0 ^{a}	2.3 ^{b}	4.4 ^{b}	4.0 ^{b}	2.5 ^{a}	2.4 ^{a}	2.6 ^{a}	2.4 ^{a}	2.4 ^{a}	2.8 ^{b}	3.3 ^{b}	3.1 ^{b}	4.0 ^{b}
Significance	ns	Y	Y	Y	ns	ns	ns	ns	Y	Y	Y	Y	Y
Single effect of yeasts													
17290	1.0 ^{a}	2.1 ^{a}	3.1 ^{b}	2.5 ^{a}	2.0 ^{a}	2.1 ^{a}	2.5 ^{ab}	2.6 ^{b}	2.9 ^{b}	2.8 ^{a}	3.1 ^{a}	2.8 ^{a}	3.2 ^{a}
14061	1.0 ^{a}	2.0 ^{a}	2.7 ^{a}	2.7 ^{a}	2.1 ^{a}	2.2 ^{a}	2.4 ^{a}	2.6 ^{b}	3.1 ^{b}	2.7 ^{a}	3.0 ^{a}	2.7 ^{a}	3.0 ^{a}
9502	1.0 ^{a}	2.1 ^{a}	3.9 ^{c}	3.5 ^{b}	2.5 ^{b}	2.3 ^{a}	2.6 ^{b}	2.4 ^{ab}	2.6 ^{a}	2.6 ^{a}	2.9 ^{a}	2.7 ^{a}	3.3 ^{a}
9518	1.0 ^{a}	2.2 ^{a}	4.4 ^{d}	3.9 ^{c}	3.2 ^{c}	2.6 ^{b}	2.5 ^{ba}	2.3 ^{a}	2.4 ^{a}	2.8 ^{a}	3.0 ^{a}	3.0 ^{b}	4.0 ^{b}
Significance	ns	ns	Y	Y	Y	Y	Y	Y	Y	ns	ns	Y	Y

Single effect of sugar levels													
S6	1.0 ^{a}	2.1 ^{a}	3.5 ^{a}	3.0 ^{a}	2.5 ^{a}	2.2 ^{a}	2.6 ^{a}	2.5 ^{a}	2.8 ^{a}	2.7 ^{a}	3.1 ^{a}	2.8 ^{a}	3.3 ^{a}
S9	1.0 ^{a}	2.0 ^{a}	3.6 ^{b}	3.3 ^{b}	2.4 ^{a}	2.4 ^{a}	2.5 ^{a}	2.5 ^{a}	2.7 ^{a}	2.7 ^{a}	2.9 ^{a}	2.7 ^{a}	3.4 ^{a}
Significance	ns	ns	Y	Y	ns	ns	ns	ns	ns	ns	ns	ns	ns

In the columns, different letters correspond to significant differences at $p < 0.05$ by LSD multiple range test; Y, significant; ns, not significant.

The sensory evaluation of beer color showed a low variability, with the mean scores ranging from 1.7 to 2.5 and with a significant single effect of the cereal mixture only. Consistently, with the instrumental evaluation of color, the highest sensory scores were attributed to the beers produced from the mixture containing the common wheat. The Pearson correlation coefficient between the color sensorially and instrumentally evaluated was low (0.27 at p-value < 0.01). The reason is that the EBC color of the samples was included in a narrow range corresponding to a straw-pale gold color, i.e., colors whose differences are not correctly detectable by the human eye. In addition, the color perceived by the human eye is also influenced by the effervescence of the beer and the ability of CO2 bubbles to reflect light.

The single effects of yeast and sucrose level were statistically not significant. The scores concerning quantity and persistence of foam were in the ranges 1.0–4.8 and 1.0–4.5, respectively. CW-9518-S9 beers obtained the highest scores for both variables. According to the literature [41], the interactions between cereal proteins (especially the non-modified proteins of the unmalted wheat) and the hop acids are the main effects responsible for foaming, while yeast proteins predominantly affect foam persistence. Despite having the same amount of protein (11.1% soft wheat and 11.3% durum wheat), the improving effect of wheat on foam stability depends on wheat variety [42]. The positive effects of the increasing levels of sucrose on the foam characteristics were due to the highest quantity of carbon dioxide produced. Turbidity, whose score ranged between 2.0 and 3.7, was affected only by yeast. Beers fermented by *S. cerevisiae* 9518 showed the highest turbidity, while beers fermented by the strains isolated from Negroamaro showed the lowest values of this parameter. These findings, obtained under standardized conditions, confirm the influence of the choice of strain on haze formation as a consequence of their aptitude to release variable quantities of macromolecular material to the medium as a function of the glycosylation pattern of cell wall mannoproteins [43]. If this does not represent a problem in the case of craft beers, the capability of a strain to generate considerable haze can be an obstacle in the production of bright beers.

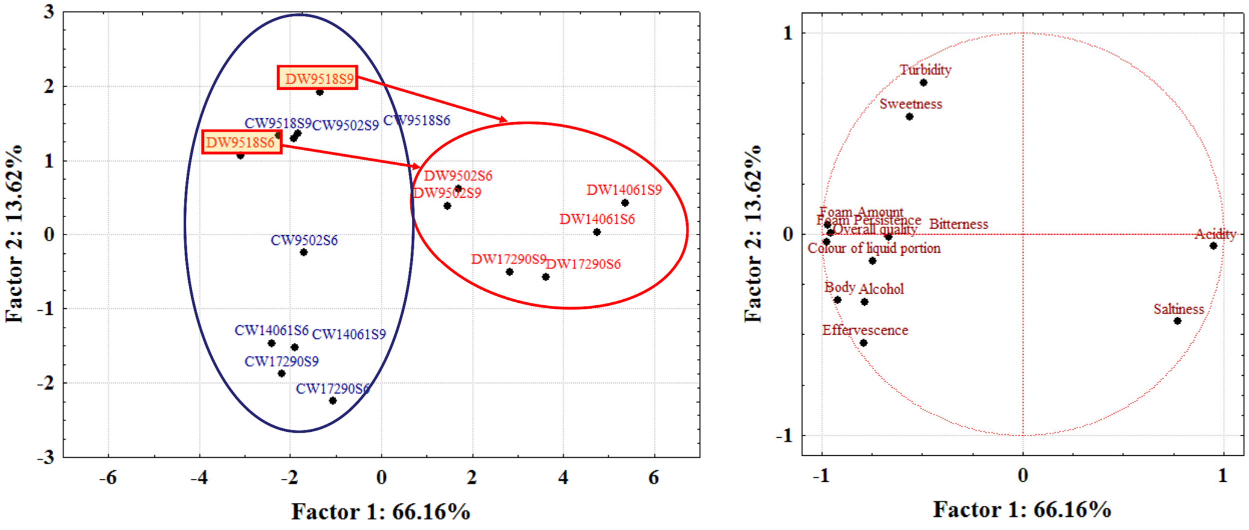
Sweetness (2.0–2.8), bitterness (2.0–3.0), and saltiness (2.2–2.8) scores were affected in different ways only by yeast: the greatest sweetness was perceived in beers fermented by *S. cerevisiae* 9518, which also produced a high quantity of glycerol, a compound having a sweet taste; consistently with the evaluation of the hoppy flavor, the bitter taste was perceived with greater and lesser intensity in the beers fermented by 9502 and 14061, respectively; finally, the salty taste was perceived with greater and lesser intensity in the beers fermented by the strains isolated from Negroamaro must and by *S. cerevisiae* 9518, respectively. Remarkable differences were found in sourness (scores from 2.2 to 4.0), with the highest intensity evaluated in beers produced from the unmalted durum wheat (which also showed the lowest pH values and the highest acidity) and fermented by *S. cerevisiae* 14061 strain. Beers fermented by the strains isolated from Susumaniello must had the lowest sourness scores (together with the highest pH and the lowest acidity).

Regarding the tactile characteristics, the beers were evaluated as moderately alcoholic (2.3–3.0). The beers perceived as the most alcoholic were those produced from malted barley-unmalted common wheat, consistently showing the highest alcohol content. A remarkable variability among beers was observed for effervescence (2.3–3.8): the beers perceived as the most sparkling were those produced from CW mixture, consistently showing the highest foam quantity and persistence. The body ranged from 2.0 to 3.2, with the highest scores obtained by the beers produced from CW mixture and fermented by *S. cerevisiae* 9518, which were also the products having the greater glycerol contents.

The overall rating scores showed a greater variability (1.7–4.2), with the highest scores assigned to the beers produced from CW mixture and fermented by *S. cerevisiae* 9518. These beers had the following characteristics: persistent foam; high turbidity; high perception of the alcoholic content; high effervescence; great body; low saltiness and sourness. These findings complied with the Pearson correlation coefficients between overall quality and foam quantity and persistence (+0.72), effervescence (+0.57), body (+0.75), and sourness (−0.54). According to the lower perceived quality of the sour beer, the overall quality was positively correlated with pH. The overall quality of the experimental beers was inversely correlated with their residual sugars (correlation coefficients: −0.68 with soluble solids; −0.64 with maltotriose; −0.62 with fructose), their volatile acidity (−0.67), and their TPC (−0.58), while it was positively correlated with color (+0.62) and glycerol content (+0.63).

3.3. Principal Component Analysis Applied to the Beer Sensory Characteristics

Similarly to what was observed for the chemical-physical characteristics, the PCA of the sensory data allowed us to group the beers into homogeneous clusters by type of cereal mixture, and the two outliers already highlighted in Figure 2a are easily observable in Figure 3a. However, the variance of the first two factors was significantly greater (about 80%), highlighting the close dependence of both the sensory characteristics and the overall sensory quality of beers on the starting cereal materials and, in the second instance, on the inoculated yeasts. DW beers are located in the portion of the Cartesian plane identified by positive values of factor 1, while CW beers are in the part of the plane characterized by negative values of factor 1 (Figure 3a). The first group is characterized by a high level of acidity/sourness, while CW beers are distinguished by a high amount and persistence of foam, high effervescence, high body and alcohol, and high overall quality (Figure 3b).



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

Figure 3. PCA applied to the beer sensory characteristics: (**a**) samples and (**b**) analytical data.

4. Conclusions

The statistical analysis of the experimental data highlighted remarkable differences among the cereal mixtures, mainly consisting of the highest antioxidant activity of CW and the highest TPC and ash content of DW. However, these differences were partially mitigated by the brewing procedures. Furthermore, the first most influent factor was represented by the cereal mixture, followed by yeast strain. The sucrose added for refermentation exerted its influence only on acidity, residual sugars, carbon dioxide, and amount and persistence of the foam and not, as expected, on gustatory-tactile characteristics and on the overall sensory quality. As a result, the PCA separately applied on physico-chemical and sensory data allowed us to distinguish the beers mainly by cereal mixtures and in a second instance by yeasts.

Regarding physico-chemical quality, the beers fermented by the two strains isolated from Negroamaro must showed the highest phenolic contents. From a sensory point of view, the beers obtained from CW wort, especially if fermented by *S. cerevisiae* 9518, were considered the best mainly thanks to their higher overall quality, effervescence, amount and persistence of foam, alcohol, and body and their lower sourness and saltiness.

Regarding coherence of the beer characteristics with the brewing style, the high turbidity (low flocculation) given by *S. cerevisiae* 9518 make it suitable for the production of Belgian-style beers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/beverages10010008/s1, Table S1: Pearson correlation coefficient (p-value < 0.01) between couples of beer characteristics.

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