

Autochthonous Ingredients for Craft Beer Production

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Abstract: Innovation in the beer market focuses on research into the different ingredients that make up this popular and sought-after beverage. Some of these innovations have focused on the use of autochthonous ingredients, which bring added value to products, reduce production costs and environmental impact, and provide beers with special organoleptic characteristics. This study focuses on the use of autochthonous ingredients from the Community of Madrid and the design of four recipes that are suited to these ingredients and consumer demand. In terms of the ingredients used, the micromalting of barley grown in the CM was carried out, while hops (Cascade variety for bittering hops and wild hops Rayo verde Tajuña and Torreta Tajuña for aroma) and yeast strains (*Saccharomyces cerevisiae* G 520 and G 354) were selected from previous studies, which were also sourced from the CM. For the barley malt, parameters such as protein content were determined, which was at the maximum limit but did not affect the final quality of the beers. The content of α - and β -acids in the hops was also determined, being higher in the Cascade variety. Different physicochemical analyses (GC, aromatic compounds, bitterness, colour, SO₂, polyphenols, and antioxidant capacity) were carried out to determine the quality of the beers obtained from the four recipes designed, as well as tastings by a panel of experts and consumers. Finally, two recipes were rated the best by a panel of experts and consumers, where the cereal, hop, and fruit flavours stood out for their aromatic profile.

Keywords: craft beer; autochthonous ingredient; aroma compounds; barley malt; hop; yeasts



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1. Introduction

Beer is a globally appreciated and consumed beverage. In recent years, the beer sector has seen its market increase as consumers increasingly demand more craft beer [1]. This is why brewers have sought innovation in the revival of beer styles, the use of new ingredients, and new methods for brewing low-ethanol beer [2]. However, the growth in the industry has been influenced by the COVID-19 pandemic. Many craft breweries were affected, but the rest sought adaptive solutions, such as the use of alternative resources [3–5]. As part of the search for new ingredients, there is a commitment to using ingredients that are specific to a region in order to promote local agriculture, as well as the use of resources (hops and wild yeasts) that have not yet been exploited in the field of beer. With this in mind, there is a great deal of interest in the ingredients within the brewing process, namely water, barley malt, hops, and yeast. Indeed, the use of raw materials other than the usual ones or from other cultivars could bring different flavours, aromas, and sensory attributes to beer [6]. Additionally, the use of indigenous resources could reduce production and supply costs, as well as create added value to the final products.

Water is a key ingredient in brewing, and the quality of the beer depends on it, as it influences several parameters such as wort pH, yeast flocculation, α -amylase activity, hop utilisation, colour, mouthfeel, and palatability of the final product [6–9]. In order to obtain

a particular style of beer that resembles those produced in their cities of origin, a number of adjustments are made to the mineral composition of the water (mainly carbonate, sulphate, iron, and manganese) to avoid undesirable sensory attributes, colours, or flavours.

One of the main ingredients in beer production is cereals, which mainly provide nutrients in the form of sugars for fermentation to take place. The sugar found in cereals is starch, but this must be hydrolysed by the action of enzymes to transform it into sugars that can be fermented by yeasts. This process is called malting and consists mainly of germinating the cereals, which are then dried and kilned. The different types of malts are obtained according to temperatures and kilning times, such as base malts (pale), special malts (caramel), and roasted malts (chocolate, coffee), in order to be able to produce different styles of beer [10]. Barley (*Hordeum vulgare* L.) and wheat (*Triticum durum* Desf. and *Triticum aestivum* L.) are the most widely used cereals in the malting industry, with barley malt accounting for around 90% of the world's production [10]. Moreover, the use of regional cereal cultivars means the regionalisation of beer and the addition of value to beer.

The next indispensable ingredient in brewing beer is hops (*Humulus lupulus* L.), the importance of which lies in their bitterness, which contributes to the characteristic aroma and flavour of the beer. These sensory characteristics depend on the hop variety used, as well as its format (flower, pellet, or extracts) [6]. Brewers traditionally divide hops into two categories: those that are used for their bittering contribution, where the best known are Target, Admiral, Nugget, Pride of Ringwood, and Super Pride, and those that provide pleasant hop aromas, such as Fuggles, Goldings, Saaz, Willamette, Cascade, and Cluster. Hops are also geographically related, so they are divided according to their origin (American, German, British, and European) [6].

Another important ingredient in beer brewing is yeast since, during fermentation, in addition to producing CO₂ and ethanol, it produces aromatic compounds that contribute to the beer organoleptic profile, as well as the production of other byproducts that can contribute antioxidant capacity to the beer, among other characteristics. Therefore, because of these characteristics that can contribute to the organoleptic profile of beers, they are considered as indigenous ingredients of certain regional beers, such as the Münchener Bier [11]. The yeasts used to brew beer are mainly divided into two groups: ale and lager. Ale beers are made mainly with yeasts of the genus *Saccharomyces cerevisiae* and are capable of fermenting at warmer temperatures (16–22 °C), in addition to fermenting at the top of the fermenter. In the lager group, yeasts that are the natural hybrid between the species of *S. cerevisiae* and *Saccharomyces bayanus*, known as *Saccharomyces pastorianus* (syn. *Saccharomyces carlsbergensis*), are used. These yeasts ferment at lower temperatures (6–16 °C) and carry out the process at the bottom of the fermenter [12]. Since they are a fundamental ingredient, cultivar starters are increasingly being demanded in brewing, and scientists and brewers have focused on being able to bring added value to beer.

This project aims to offer a first approach to the production of beer through the use of autochthonous raw materials from the Community of Madrid (water, barley malt, hops, and yeasts), as well as the design of recipes that enhance these ingredients and meet the demand of the regional craft beer consumer.

2. Materials and Methods

2.1. Beer Ingredients

The ingredients used in brewing for the different recipes were water, malted barley, hops, and yeast, all of which were obtained in the Community of Madrid. Each of these ingredients contributes to the final taste and aroma of the beer.

2.1.1. Water

Water makes up 90% of the beer in terms of quantity. Thus, it must meet chemical and microbiological requirements, as well as certain parameters that will affect clarity, flavour, odour, the absence of colour, and, thus, the quality of the beer. Factors that can contribute

to water quality, therefore, are pH, alkalinity, ion concentration, and microbial counts, as well as the absence of disinfection byproducts.

The water used for the brewing process showed the following characteristics (Table 1), as measured with CDR FoodLab kits and equipment (CDR s.r.l, Florence, Italy).

Table 1. Analysed parameters in water using CDR FoodLab.

Bicarbonates	Calcium	Zinc	Magnesium	Potassium	Sulphate	Chlorides	pH
49.50 ± 0.50	24.50 ± 2.40	0.06 ± 0.01	4.70 ± 0.00	<50	<10	<50	8.21 ± 0.38

Data are expressed in mg L⁻¹.

In order to obtain a pH suitable for fermentation, a total CaSO₄ (Brewferm, Beverlo, Belgium) addition of 59 g was carried out during the mashing and washing of the grain.

2.1.2. Barley Malt

The cereal used for the recipe design was the barley of the two-row Beka variety. This cereal was sown in an experimental field belonging to the IMIDRA Institute in Aranjuez, which belongs to the Community of Madrid. Once collected, the basic parameters, such as germination capacity (100%), germination energy (97.5%), and total protein (14.95%), were analysed. The malting process of the barley was then carried out to promote the synthesis of enzymes necessary for the subsequent hydrolysis of starch into fermentable sugars. A micromalting process was performed, according to the following phases:

- Steeping phase: Grains were soaked with tap water at 18 °C for 7 h, followed by a water-free rest period of 17 h at 18 °C. This phase was carried out over 48 h with a final rest period of 48 h, by which time the first rootlets had appeared.
- The germination phase was carried out for 48–72 h at 18 °C, maintaining humidification and oxygenation during the entire process. This phase was finished once the roots and plumule had reached the same length, with the final water content of the barley grains being 40%.
- The kilning phase was conducted in forced-air ovens at a temperature of 52 °C for 24 h, followed by 2 h at 80 °C. In addition, part of the malt obtained was also kilned at 150 °C for 1 h. The final water content was less than 5%.

After the malting process, the malt was stored in hermetically sealed bags at 14 °C. The barley malt obtained was analysed by the AETCM Laboratories (Spanish Association of Beer and Malt Technicians, Coslada, Madrid, Spain), certified according to the UNE-EN-ISO 9001:2015 standard [13] for the following parameters: congress mash (EBC Methods 4.5.1 and 4.5.2 [14,15]), colour (EBC Method 4.7.1 [16]), β-glucans (EBC Method 4.16.3 [17]), soluble nitrogen (EBC Method 4.9.1 [18]), total nitrogen (EBC Method 4.3.1 [19]), Kolbach index (MEBACK Method R-205.12.999 [20]), and diastatic power (EBC Method 4.12.1 [21]).

2.1.3. Hops

Hops are responsible for providing bitterness to the beer, as well as aromas; thus, for the design of the recipes, the Cascade variety was used as bittering hops and two wild varieties isolated from the Tajuña area (Madrid, Spain) were used as aromatic hops. Both the commercial and wild varieties were grown on an experimental field as well as the barley, in Aranjuez. These varieties were selected from previous trials to produce the best yields for cultivation in the region. After harvesting, the hops were analysed by AETCM Laboratories for the following characteristics: essential oils (EBC Method 7.10 [22]), polyphenols (EBC Method 7.14 [23]), and α and β- acids (EBC Method 7.7 [24]).

2.1.4. Yeast Strains

Two *S. cerevisiae* yeast strains, previously selected for their adequate organoleptic profile and fermentation characteristics for brewing production [25], were used in this project:

- *S. cerevisiae* GENL 520: The beers brewed with this yeast were mainly characterised by a fruity profile, with a banana aroma and flavour, as well as being phenolic. The beers showed medium bitterness, high body, and persistence. The balance between aroma and flavour was high.
- *S. cerevisiae* GENL 354: The beers brewed with this strain were noted for their sweet, fruity taste and accentuated bitterness. They showed a medium body, as well as low persistence, which makes the beer more drinkable. The beers were balanced in aroma and flavour.

2.2. Recipes, Wort Obtention, and Fermentation 16 L

The recipes were designed to produce a pale ale beer style, which could be accepted by the general public. Four recipes were brewed, where the variations were carried out in the yeast strains and hops added for aroma (Figure 1).

Recipe A	Recipe B	Recipe C	Recipe D
Pale Barley malt: 4 kg	Pale Barley malt: 4 kg	Pale Barley malt: 4 kg	Pale Barley malt: 4 kg
Kilned malt: 200 g	Kilned malt: 200 g	Kilned malt: 200 g	Kilned malt: 200 g
Hops for bitterness: 20 g Cascade	Hops for bitterness: 20 g Cascade	Hops for bitterness: 20 g Cascade	Hops for bitterness: 20 g Cascade
Hops for aroma: 20 g Rayo Verde Tajuña	Hops for aroma: 20 g Rayo Verde Tajuña	Hops for aroma: 20 g Torreta Tajuña	Hops for aroma: 20 g Torreta Tajuña
Yeast strain: G 520	Yeast strain: G 354	Yeast strain: G 520	Yeast strain: G 354

Figure 1. Recipes designed for the preparation of the different beers using the autochthonous ingredients from the Community of Madrid.

The mashing conditions for obtaining the beer wort were an initial volume of 11 L at 63 °C for 1 h, and then 15 L wash water at 65 °C. The bittering hops were added from minute zero during the wort boil, whereas the aroma hops were added at minute 40. The final volume of beer wort obtained was 15–16 L, with a density of 10.34 ± 0.54 °P and a pH of 5.45 ± 0.10 . After the beer wort was obtained, it was cooled and transferred to 20 L atmospheric fermenters locked with a Müller valve to allow the CO₂ to escape from the system, where fermentation was performed at 18 °C with an inoculum concentration of 10^6 cells mL⁻¹. The fermentations were carried out in duplicate using inoculums grown sequentially in YPD broth medium (1% bacteriological peptone, 2% yeast extract, and 2% glucose; all *w/v*) (Condalab, Madrid, Spain) at 28 °C for 48 h. The fermentation evolution was controlled daily by sugar density and temperature measurements until constant density values (two days in a row with the same measurement).

After fermentation was completed, 7 g L⁻¹ of glucose (Condalab, Madrid, Spain) was added, bottled, and left at around 20 °C for carbonation for 1 week. It was then stored at 4–5 °C for at least one month for maturation.

2.3. Fermentation Control and Determination of Yeast Concentration during the Process

Triplicate samples were taken daily for every fermenter during beer elaboration trials. The samples (1 mL) for the qPCR study were centrifuged, and the pellets were immediately cryopreserved until Real-Time quantitative PCR analysis. For total cultivable yeast counts, samples correctly prediluted were spread on YPD plates using an automatic plater of Petri dishes (easySpiral[®], Interscience, St. Nom, France). For colony counting, the manufacturer's instructions were followed.

DNA Extraction and Real-Time qPCR Assays

The DNA extraction from yeast pellet samples was carried out using the DNeasy Plant mini kit (QIAGEN, Valencia, CA, USA), as described previously [26,27]. Yeast cell pellets were washed twice with sterile water. Previous to DNA extraction, the cells were subjected to physical break-down with 700 µL of AP1 buffer into 2 mL microcentrifuge tubes containing 0.5 mm diameter glass beads. The tubes were shaken at the maximum rate in a mixer mill (Retsch GmbH, Haan, Germany) and centrifuged for 1 min. Subsequently, the supernatant was transferred to a sterile tube and purified according to the manufacturer's manual.

The qPCR analysis was carried out on QuantStudio™ 5 (Applied Biosystems, Carlsbad, CA, USA) equipment. The PCR reactions were performed in optical-grade 96-well plates (Applied Biosystems), with 25 µL final volumes containing 5 µL of DNA template, 0.7 µM of primers (SC1 and SC2; [28]), and 12.5 µL of SYBR Green Master Mix (Roche Diagnostics GmbH, Mannheim, Germany). Triplicate samples were used. The amplification conditions were as follows: 95 °C for 10 min, 40 cycles of 95 °C for 15 s, 60 °C for 1 min, and 72 °C for 30 s.

Previously, standard curves for each *S. cerevisiae* strain were created by plotting the cycle threshold (C_T) values of the qPCR performed on a dilution series of yeast cells in YPD media (10^8 to 10^3 cells mL⁻¹) against the log input cells mL⁻¹. From the slope of standard curves, the amplification efficiency (E) was calculated using the formula $E = (10^{-1/\text{slope}}) - 1$ [29].

2.4. Beer Analyses

After the maturation phase, the beers were analysed for different parameters. All beers were previously degassed to carry out the analyses using a grade 2 V (Whatman, Maidstone, UK) filter.

2.4.1. CDR FoodLab

The CDR FoodLab (BeerLab 1.6.2 software, www.cdrfoodlab.com (accessed on 15 January 2024), verified by the international reference analysis laboratory Campden BRI (www.campdenbri.co.uk, accessed on 15 January 2024), was used to determine the following parameters in beer: colour (range 1–100 EBC—European Brewing Convention), bitterness (range 5–100 IBU—International Bitterness Unit), lactic acid (D + L) (range 150–3500 mg L⁻¹), vicinal diketones (VDKs) (range 0.05–2 mg L⁻¹), polyphenols (range 5–550 mg L⁻¹), and SO₂ (range 1–30 mg L⁻¹). The equipment is based on enzymatic reactions and spectrometric measurements for the determination of the different parameters.

2.4.2. Volatile Compounds

A gas chromatograph 6850 (GC-FID, Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a flame ionisation detector was used for the identification of the volatile compounds. A total of 33 major aromatic compounds were determined and grouped into families: higher alcohols, esters, acids, aldehyde-ketones, lactones, and phenols. A DB-WAX (60 m × 0.32 mm i.d. and 0.5 µm film) column was used (Agilent Technologies, Inc., Santa Clara, CA, USA). The carrier gas used was helium with a flow of 2 mL min⁻¹. The oven temperature started at 40 °C for 5 min and was increased to 200 °C at a rate of 3 °C min⁻¹. The splitless injection mode was used, and the temperatures of the injector and detector were set to 200 °C. The extraction of volatile compounds was carried out

according to the method from Ortega et al. [30] based on liquid-phase microextraction with dichloromethane (DCM, Panreac, Barcelona, Spain). Briefly, the beer samples (2.7 mL) were shaken for 90 min at 250 rpm together with bi-distilled water (6.3 mL), ammonium sulphate (3.9 g) (Panreac-AppliChem, Barcelona, Spain), DCM (250 μ L), and the internal standards (20 μ L of each). Subsequently, the vials were centrifuged, and the aliquot was collected and deposited at the bottom, where the aromas were extracted. The identification and quantification of compounds were performed using four internal standards (2-butanol, 4-methyl-2-pentanol, 4-hydroxy-4-methyl-2-pentanone, and 2-octanol). The levels of the different compounds were determined by calibration lines for each compound ($R^2 = 0.9830\text{--}0.9999$). Each sample was analysed in triplicate.

2.4.3. Residual Sugars, Glycerol, and Ethanol Determination by HPLC

Analyses (fermentable sugars: maltotriose, maltose, glucose and fructose, glycerol and ethanol) were carried out on a Thermo HPLC system consisting of a Dionex Ultimate 3000 (Thermo Scientific, Waltham, MA, USA) equipped with a quaternary pump and an autosampler, a column compartment provided with a temperature controller, and a 520 refractive index detector (ERC). Chromatographic separations were performed on an ion-exclusion column, Rezex ROA-Organic Acid H+ (8%), 150×7.8 mm (Phenomenex, Torrance, CA, USA) using water with hydrogen sulphide (0.005 N, HPLC grade, Panreac, Barcelona, Spain) in the mobile phase with a flow rate of 0.6 mL min^{-1} at 60°C . The injection volume was 10 μ L. The samples were previously filtered through a 0.22 μm filter. All calibrations produced a linear response with an R^2 value > 0.9991 over the concentration range analysed.

2.4.4. Antioxidant Capacity

The antioxidant capacity of beers was measured using an e-BQC lab device (Bioquochem, Asturias, Spain, www.bioquochem.com, accessed 15 January 2024) with TEAC assay (Trolox Equivalent Antioxidant Capacity). The device measures the redox potential of the samples and expresses it in micro-Coulombs (μC) [31]. The measurements are expressed as two values: one indicating the antioxidant capacity of compounds with the highest rate of free radical scavenging (Q1) and the other one with the lower rate of free radical scavenging (Q2). For the TEAC assay, a solution of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox 8 mM L^{-1} in methanol 5% and pH 4.5) (Sigma, Aldrich, São Paulo, Brazil) was used. A Trolox calibration curve was elaborated (Q1, $R^2 = 0.9974$; Q2, $R^2 = 0.9876$; QT = Q1 + Q2), with the different concentrations versus the measurements obtained with the e-BQC equipment, where the antioxidant activity of the beers was expressed as millimoles of Trolox equivalents per litre (mmol TE L^{-1}).

2.5. Organoleptic Profile

Sensory evaluation was performed by twelve trained and experienced panellists (seven male and five female). Different descriptors were used to describe their absence or presence level on a scale from 0 to 5. Three groups of attributes were evaluated on the basis of appearance (colour and foam retention), aroma (yeast, fruity, banana, phenolic, diacetyl, and hop), and taste (alcohol, sweet, salty, acidic, bitterness, astringency, effervescence, warmth, slickness, and body). The tasting tests were conducted in a suitable room, and the beer samples were kept at room temperature for 30 min prior to testing. All beer samples were numbered and anonymous. The average of the main results was plotted in a radar chart for the different attributes analysed for each beer. This analysis allows us to carry out a formal descriptive analysis of each of the beers since, by evaluating the intensity of each attribute, we can obtain an aromatic profile of the beers. In addition, and together with the overall evaluation of the beers, we can rate the overall quality of the beers (EBC Method 13.10) [32].

2.6. Consumer Analysis

A total of 110 consumers (55 male and 55 female), regular users of beer between 18 and 75 years of age (24 between 18 and 36 years, 39 between 37 and 56 years, and 47 between 57 and 75 years old), participated in the consumer tests. The consumer test was conducted in the sensory room from IMIDRA in individual booths. Beer samples (50 mL) were served at 10 °C.

Hedonic (attribute-diagnostic questionnaire) and purchase intent ratings were obtained from the consumers during the tasting session, in which consumers tasted one set of four beers. The 9-point hedonic scale was used for bitterness, acidity, and overall impression scaling (where 1 = extremely dislike, 5 = neither like nor dislike, and 9 = extremely like) and the rest of the attributes were analysed according to check-all-that-apply (CATA) questions [33]. The questionnaire was displayed to consumers using the Sensesbit 1.6.2 software (TasteLab, Galicia, Spain, www.sensesbit.com, accessed 15 January 2024).

2.7. Statistical Analysis

The data obtained from chemical analysis, aroma compounds, and antioxidant capacity were statistically analysed, and their means and standard deviations are presented. The statistical processing of data was carried out using R Studio 4.1 software (Integrated Development for R. RStudio, PBC, Boston, MA, USA). The applied methods used PCA and a correlation matrix to visualise the samples of beer in an n-dimensional space and define the samples associated with the different parameters analysed. The one-way ANOVA test with Tukey post hoc test on a significance level of $p < 0.05$ was also used to identify the homogenous groups between samples. The identification of significant data correlation was performed with the Pearson test.

3. Results and Discussion

3.1. Ingredient Characteristics

3.1.1. Barley Malt

Barley cereal is the main ingredient used for brewing beer, and two types of barley can be used: six-row and two-row. Their main difference is that two-row barley produces a malt with a lighter extract, lighter colour, and lower enzyme content than six-row barley [34]. In order to determine the quality of the barley, different parameters such as the hot water extract (HWE) of the kernel, kernel weight, β -glucan and protein content, friability, α -amylase activity, viscosity, and soluble nitrogen ratio (SNR) were analysed [35,36]. In order to carry out the brewing process, the barley must be malted, a process which increases enzyme activity, soluble proteins, and the availability of polysaccharides (starch) [37]. Malting is the process of incompletely germinating cereal grain, where, due to a series of enzymatic degradations of the grain endosperm, starch is obtained. During the mashing process, the starch is degraded by enzymatic reactions to the fermentable sugars necessary for the alcoholic fermentation by the yeasts. Malting consists of three phases: steeping to ensure good water absorption by the grain (between 15% and 40% moisture); germination to allow partial embryo growth, enzyme synthesis, and partial endosperm breakdown; and kilning to ensure the stability of the product. Table 2 shows the results obtained from the malting process.

The total protein of the barley was 14.95%, which is within the normal range for barley (between 8% and 15%) [37]. In general, a high protein content in barley is not desirable for brewing, as it can affect the carbohydrate content of the barley, reducing it [38,39]. Likewise, a high protein content can contribute positively to foam stability and negatively to beer haze [36]. However, both parameters were positively assessed by the tasting panel of experts, as well as the consumers. This could, therefore, be one of the reasons for the low density found after mashing. Compared to other studies [40], total protein content, germination capacity, and germination energy were found to be similar.

Table 2. Characteristic properties of the malt used.

Parameters	Barley Malt
Congress Mash:	
Saccharification rate (min)	10.00 ± 0.00
Speed of filtration (h)	<1.00 ± 0.00
Odour	normal
Fine–coarse difference (%)	2.60 ± 0.28
pH of wort	5.96 ± 0.06
Colour (EBC)	4.50 ± 1.13
β-glucans (mg L ⁻¹)	217.15 ± 40.31
Soluble nitrogen content (%)	5.85 ± 1.34
Total nitrogen (%)	14.35 ± 0.92
Kolbach Index	40.50 ± 6.36
Diastatic power (° WK)	399.00 ± 24.04

Data are means ± standard deviations of two samples.

Regarding the parameters analysed for barley malt, in the congress mash, the filtration time was less than 1 h (considered a normal time for the modification of β-glucans [40]). Filtration times of less than 1 h can speed up the wort brewing process and make it necessary to use less time in the brewing process. For the saccharification rate (10 min), the values were between the normal range of saccharification (10–15 min [40,41]). Saccharification is the process by which the complex sugars (starch) present in barley are hydrolysed to simpler sugars by β-amylase so that they can be used by yeast to produce beer, which is why, in this case, barley malt presented a good saccharification time. The pH of the wort obtained with this malt was 5.96, which would be within the specific range of the European Beer Convention. This pH would be similar to that obtained by this variety in other locations [42]. It has been shown that pH values between 5 and 6.6 can affect the photolytic activity of malt [43]. Additionally, the pH value can affect yeast growth during fermentation, which is the reason pH adjustments were subsequently made with calcium sulphate, and as in the preliminary trials, the onset of fermentation was delayed.

The colour of the malt obtained was 4.5 EBC, despite not being a malt variety specified by the EBC. The variations in wort colour may be due to the malt kilning process but can also be influenced by non-enzymatic browning reactions; the Maillard reaction, which takes place during kilning in the malting process; and the boiling of the wort in the brewing process. Here, sugars interact with amino acids, producing a variety of odours and flavours. This reaction is the basis of the aroma industry, and the type of amino acid involved determines the resulting flavour and colour [44].

β-glucans are an important factor in determining their water solubility, viscosity, and gelatinisation [45]. A high β-glucan content can lead to an increase in viscosity, which causes a reduction in the filtration rate and high turbidity of the beer. This is why brewers demand a β-glucan content of less than 178 mg L⁻¹ [46]. The value obtained in this case in the micromalting was 217.15 mg L⁻¹ of β-glucans; however, as mentioned above, the results in terms of filtration were optimal, as well as the appearance of the beer, as will be seen later in the tasting.

The soluble nitrogen content was 5.85%, which is an adequate amount of amino acids that is essential for the subsequent fermentation of proteins [47].

The Kolbach index indicates the proteolytic enzyme content of the malt as well as its disaggregation. Therefore, its importance is also related to the total nitrogen content, as it expresses the ratio between the soluble nitrogenous components determined in the wort and the total content of the nitrogenous components in the malt. The usual range of this index is from 36 to 44% [48]. The obtained value was 40.50, similar to the results shown by Bravi et al. [49]. Another study compares the Kolbach index in barley malt and buckwheat malt [50], with the value for barley malt (38%) being similar to that obtained in the present study (40.50%). In contrast, these authors concluded that in the case of the buckwheat

malt samples (Kolbach index, 24–26%), there was insufficient disaggregation, and, thus, we recommend the use of exogenous proteolytic enzymes during the mashing process.

The diastatic power determines the combined activity of the enzymes β -amylase, α -amylase, dextrinase, and α -glucosidase, which hydrolyse starch to obtain fermentable sugars (maltose, maltotriose, glucose, fructose, and sucrose) [51]. The diastatic power of the malt was 399.00 °WK, which is within the optimum range of 152–500 °WK [21]. These values were also higher than those obtained in other studies where the commercial varieties were analysed with an average diastatic power of 273 °WK [52].

3.1.2. Hops

Hops are another important ingredient of beer. The unfertilised female inflorescences of this plant, which belongs to the Cannabaceae family, were used. The importance of this ingredient lies in the fact that it provides bitterness as well as contributes to the characteristic aroma and flavour of the beer. Hops also contribute other characteristics to the quality of the beer, as they improve the formation and stability of the foam and have antibacterial properties, thus protecting it from the deterioration caused by certain micro-organisms in the beer [53]. The key compounds in hops that give these characteristics to beers are resins and essential oils. Table 3 shows the values obtained for the essential oils, α -, and β -acids, as well as the polyphenols of the hops used in the recipe design. The variety that showed significant differences was Cascade hops.

Table 3. Characteristic properties of the hops used.

Parameters	Cascade	Rayo Verde Tajuña	Torreta Tajuña
Essential oils (mL)	0.51 ± 0.03 ^a	0.05 ± 0.00 ^b	0.05 ± 0.00 ^b
Polyphenols (%)	1.00 ± 0.10 ^a	0.59 ± 0.09 ^b	0.90 ± 0.10 ^a
α-acids:			
Cohumulone (%)	2.06 ± 0.16 ^a	0.25 ± 0.05 ^b	0.21 ± 0.02 ^b
Adhumulone (%)	5.83 ± 0.13 ^a	1.03 ± 0.05 ^b	0.80 ± 0.10 ^b
β-acids:			
Colupulone (%)	2.18 ± 0.18 ^a	1.29 ± 0.09 ^b	1.03 ± 0.03 ^b
Adlupulone (%)	2.48 ± 0.18 ^a	2.02 ± 0.02 ^b	1.63 ± 0.13 ^c

Data with different superscript letters within each row are significantly different (Tukey tests: $p < 0.05$).

Resins are classified into two main subfractions: hard and soft resins. Hard resins come from the oxidation and polymerisation of soft resinous substances, while soft resins correspond to humulones (α -acids) and lupulones (β -acids). During the wort brewing process, hops are added at the wort boiling stage, where the α -acids undergo oxidative isomerisation into iso- α -acids (iso-humulones). This isomerisation is responsible for about 70% of the bitterness of the beer [54]. The resin values that can be found in the hops ranged from 2.2% to 11.5% for α -acids and 2.2% to 11.2% for β -acids [55]. In this case, there was a noticeable difference between Cascade hops, with a total of 7.89% α -acids and 4.66% β -acids, and wild hops, with a content of 1.01–1.28% α -acids and 2.66–3.31% β -acids, which is the reason that the Cascade variety was used as a bittering hop in the recipes. On the other hand, essential oils, mainly terpenoids, are extracted during late and dry hopping and are responsible for the aroma of the beer [56]. The essential oil content of hops usually ranges between 0.5% and 3% [57], and, in particular, the oil content of the Cascade hops is between 0.7% and 1.4% [58]. In this case, the Cascade variety cultivated in the Madrid region has an oil content inferior to the minimum varietal oil content. Despite the low content of wild hops, sensory differences were observed between the beers brewed with each of these hops. The variety of hops used contributes to different sensory attributes perceived in the beer [59].

The polyphenols from hops play an important role in beer. They modify certain characteristics of beer, such as bitterness, astringency, body, and fullness, as well as imparting antioxidant capacity to the beer. During wort boiling, about 70% of the total polyphenols

in the beer are extracted from the hops, although their final concentration depends on their polarity as well as their tendency to form complexes with the proteins present in the wort [60,61]. The content of polyphenols that can be found in the dry matter of hops can vary between 4.5% and 16% [55], so in this case, where the three hops were used, the concentrations found were lower (between 0.59% and 1%). In the study carried out by Rodolfi et al. [58], clear differences were observed in the production of xanthohumol (the major polyphenol found in hops with antioxidant properties), depending on the area of cultivation, with the highest concentrations found in those from German areas.

3.2. Study of Yeast Inoculated Population during Beer Fermentation

Most investigations using the qPCR application in beer had been directed at the detection of spoilage microorganisms during beer elaboration [62–64]. In the current work, this qPCR technique was used to monitor inoculated *S. cerevisiae* strains in beer fermentation in comparison with a culture-dependent method. The *S. cerevisiae* strain populations were monitored using YPD plates for the total cultivable cells and by qPCR for total yeast populations (Figure 2). In order to determine the sensitivity and detection limits of the qPCR, standard curves of each *S. cerevisiae* strain (G 520 and G 354) were performed with the same pair of primers, and Table 4 shows the slope, intersection, correlation coefficient (R^2), and efficiency of the standard curves. The assays were linear over six orders of magnitude, and the detection limits for both strains were at concentrations as low as 10^3 cells mL^{-1} .

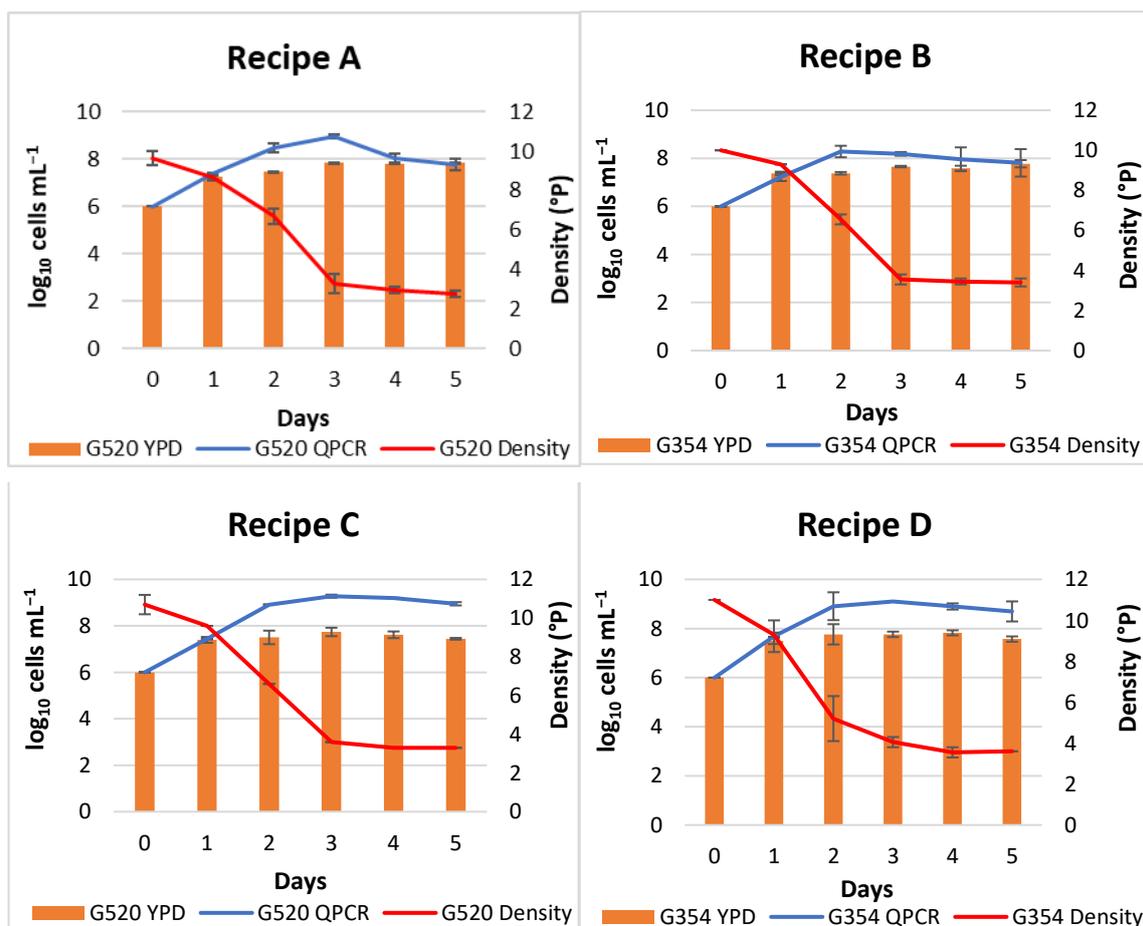


Figure 2. Yeast population dynamics during the four different fermentation recipes. Results, expressed as \log_{10} cells mL^{-1} , were obtained using YPD culture media (columns) and qPCR analysis (blue lines) for *S. cerevisiae* G 520 and G 354 strains; sugar density values (grey lines) are expressed as Plato degrees ($^{\circ}\text{P}$). All fermenters were inoculated with an initial concentration of 10^6 cell mL^{-1} (day 0).

Table 4. Standard curves performed for each yeast strain.

Yeast Strain	Slope	Intersection	R ²	Efficiency
<i>S. cerevisiae</i> G 520	-3.17 ± 0.05	38.39 ± 0.32	0.986 ± 0.02	106.83 ± 2.31
<i>S. cerevisiae</i> G 354	-3.36 ± 0.08	40.03 ± 0.47	0.997 ± 0.00	98.37 ± 3.15

The influence of the beer wort matrix on qPCR efficiency in comparison with YPD media was studied in previous works [27]. It was reported that the differences observed were not statistically significant ($p < 0.05$); thus, the construction of standard curves in different matrices does not substantially change the results, and YPD can be used to quantify the yeast population in beer fermentation.

The *S. cerevisiae* yeast strains used in the present work were isolated from the final phase of fermentation in Tempranillo (G 520) and in Garnacha (G 354) wine elaboration from wineries belonging to D.O.P. “Vinos de Madrid”. Currently, numerous studies focus on craft beer production with *Saccharomyces* strains from different sources, but these focus on potential capacity in brewing [25,65–67]. In a previous work [25], G520 and G354 strains were not able to consume all maltotriose and maltose of wort. However, both showed a good performance, with an apparent attenuation of 72% in comparison with that of the commercial strain S-04 (82.2%).

It is worth noting that the density values (Figure 2) decreased until the third day for both trials and both *S. cerevisiae* strains when the slow decrease in density coincided (in time) with the stabilisation of *S. cerevisiae* populations. During the fermentation of recipes A and B, with the difference being the aroma hops used (Rayo Verde Tajuña), the cell concentration measured by plating and qPCR was similar. However, cell growth in G 520 beer elaboration was higher than in G 354 beer, with a maximum cultivable population of 6.79×10^7 cells mL⁻¹ (day 5) with respect to 5.94×10^7 cells mL⁻¹ (day 5). In addition, its fermentative capacity also seemed to be greater, ending with a final sugar density of 2.75 °P in G 520 and 3.40 °P in G 354. In recipes C and D (aroma hop Torreta Tajuña), this could be seen, as there were greater differences in cell enumeration between plating and qPCR. In this case, it was the G 354 beer that reached the highest cultivable cell concentration, with a value of 6.69×10^7 cell mL⁻¹ (day 4) and 5.47×10^7 cell mL⁻¹ (day 3) for the G 520 beer. As for the sugar density values, there were no major differences between the final levels (3.30 °P in the G 520 beer and 3.60 °P in the G 354 beer).

In all cases (Figure 2), it was found that cell quantification was very similar for plating and for qPCR during the first day of fermentation. The exponential phase occurs until 2–3 days of fermentation, the stationary phase occurs around day 3, and the cell death phase occurs until the end of fermentation on day 5. In contrast, the qPCR analysis reported the highest values of total yeast in all subsequent days. This might be because of the viability of yeasts, and, therefore, its cultivable state is more favourable in the first 24 h. Then, the differences observed in enumeration between plating and qPCR would be due to the presence of dead cells in the sample since the qPCR technique detects both viable and dead cells. These results are in accordance with other works [68,69]. However, this behaviour is most commonly noted for recipes C and D in comparison with recipes A and B, where the cell concentration is very similar for both analysis methods. These differences may be due to a large presence of viable but non-cultivable (VBNC) populations in beers or due to the over-estimation of yeasts by the amplification of DNA from dead yeast cells, as was also reported by Hierro et al. [68] in wine elaboration. The wort has extraneous impurities and technologically important constituents that adversely influence the yeast cells. On the one hand, it was described that plant polyphenols increase the biological stability in a final beer; on the other hand, this can have a harmful effect on the physiological state of yeasts, culture growth, and fermentation evolution [70]. Other authors have studied the influence of α -acids from hops in connection with the growth inhibition of the brewer yeast (*S. cerevisiae*), in particular, the transition to hop-extract-induced VBNC *S. cerevisiae* [71]. The range of isomerised α -acids contents in hoppy beers is normally found between 20 and

80 mg L⁻¹ [72,73]. Despite *S. cerevisiae* tolerance to certain concentrations of isomerised α-acids, such quantities affect the cellular metabolism of this yeast. Xiao et al. [71] observed that the treatment of the *S. cerevisiae* YS86 strain with isomerised hop extracts (300 mg L⁻¹) induced the complete entry of the yeast into the VBNC state. Thus, yeast fermentation efficiency decreased, with lower alcohol production and higher residual sugars, as well as lower aromatics and esters in the final beer. In this study, the main difference between recipes A and B and recipes C and D is the hops used for the aroma. Contrary to previous observations by other authors [64], the highest population differences between the plating and qPCR analyses were observed for recipes C and D, which presented the lowest α-acids content (Table 3). In this case, they cannot be directly related to the differences in cell enumeration.

3.3. Physicochemical Characteristics of the Obtained Beers

3.3.1. CDR Beer Analysis

After the maturation of the beers obtained from the four different recipes, the different parameters were analysed, as shown in Table 5.

Table 5. Main analytical characteristics of the beers obtained using four different recipes, analysed with CDR FoodLab.

Recipes	Lactic Acid mg L ⁻¹	Colour EBC	Bitterness IBU	VDKs mg L ⁻¹	SO ₂ mg L ⁻¹	Polyphenols mg L ⁻¹
A	273.50 ± 10.50 ^a	17.5 ± 0.50 ^a	11.80 ± 1.10 ^a	0.29 ± 0.05 ^a	3.80 ± 0.20 ^c	269.5 ± 6.50 ^a
B	294.00 ± 29.00 ^a	16.00 ± 0.00 ^{ab}	11.15 ± 0.55 ^a	0.17 ± 0.01 ^{bc}	12.45 ± 0.95 ^a	299.5 ± 12.50 ^a
C	209.5 ± 21.50 ^b	15.5 ± 0.50 ^{bc}	10.80 ± 0.80 ^a	0.23 ± 0.03 ^{ab}	4.60 ± 0.90 ^{bc}	267.5 ± 41.50 ^a
D	282.5 ± 18.50 ^a	14.0 ± 1.00 ^c	10.70 ± 0.70 ^a	0.10 ± 0.02 ^c	6.70 ± 1.60 ^b	327.5 ± 59.50 ^a

Data are means ± standard deviations of two beer samples. Data with different superscript letters within each column are significantly different (Tukey tests: *p* < 0.05). Recipes A and C: strain G 520; recipes B and D: strain G 354. Recipes A and B: Rayo Verde Tajuña hops; recipes C and D: Torreta Tajuña hops.

Lactic acid is formed from pyruvate during the fermentation process carried out by yeasts, with the main substrate being glucose. However, the physiological role of lactic acid production and the molecular mechanisms remain unknown [74]. The lactic acid concentration obtained in each of the recipes did not vary greatly between the recipes and yeast strains, with results ranging from 209.5 to 294 mg L⁻¹. Likewise, none of the values exceeded the perception threshold for lactic acid (400 mg L⁻¹) [75].

As seen above for malt, the colour of the beer is one of its main characteristics in terms of appearance. It depends mainly on the type of malt used and the melanoidins produced during the malting process. The colour values obtained were between 14.00 and 17.50 EBC, corresponding to the colour range of a pale ale beer. Some studies have related the colour of beers to the polyphenols produced during the brewing process [76,77]; however, in this case, there is no direct correlation between both parameters.

The hop used to impart bitterness to the beer in all four recipes was the Cascade variety, with the same quantity used in all of them; thus, there were no notable differences between the recipes. The bitterness of the beer can also be influenced by the yeast strains used during fermentation, as it has been shown that α-acid molecules can adhere to the yeast cell walls and settle at the bottom of the fermenter when the fermentation process is completed, thus reducing the bitterness of the beer [78–80]. In this case, the ranges obtained were 10.7 to 11.8 IBU, with no relationship between the yeast strains used.

The most important vicinal diketones (VDKs) to consider in beer quality are diacetyl and 2,3-pentanedione. They impart a strong toffee and buttery aroma and are, therefore, considered undesirable aromas for most beer styles. VDKs are formed by yeasts during the fermentation process as they excrete an intermediate of valine synthesis, α-acetolactate, which spontaneously decarboxylates to diacetyl. The total VDK value obtained in the different beers was statistically significant and ranged between 0.10 and 0.29 mg L⁻¹, with

the perception threshold for diacetyl and 2,3-pentanedione being 0.15 and 0.9 mg L⁻¹, respectively [81]. However, none of these undesirable aromas were detected in both the expert panel tastings and the consumer tastings. When comparing these results with those previously observed using these yeasts [25], strain G 520 produces higher concentrations of VDKs. Additionally, its increased production may be due to an increase in the amount of proteins available in the beer wort, as they are the main source for yeast metabolism [82].

Yeasts also produce SO₂ from the amino acids cysteine and methionine during fermentation. The importance of this lies in the fact that it is a compound that acts on the stability of beer flavour, as well as having antioxidant capacity [83]. The taste threshold for SO₂ is 25 mg L⁻¹; thus, in none of the beers was this exceeded (from 3.80 to 12.45 mg L⁻¹). Therefore, even if it was added to the beer at a later stage to provide stability, it would still be below the threshold [84,85]. SO₂ can be found either free or in the form of bisulphite when bound to carbonyl compounds, masking its bad taste and thus slowing down the oxidation process of the beer [86]. Several studies have related SO₂ concentration to parameters such as fermentation temperature, wort pH, yeast composition, oxygenation composition, wort oxygenation, and the enzymes active in the sulphur metabolism pathway [86,87]. According to studies carried out by Pickerell et al. [83], lower glycogen content in the wort could lead to poor yeast growth and, thus, increase SO₂ production. Further studies would be needed to determine this; however, according to the results obtained from the yeast concentration throughout the fermentation process, the yeast population was not affected. In contrast, the studies performed by Duan et al. [87] also did not give similar results to those from this project, where a high protein concentration led to an increase in SO₂ production.

Polyphenols are compounds with an antioxidant character, as they are involved in the elimination of free radicals and the chelation of metals. The major contribution of polyphenols in beer comes from malt and hops. These compounds can also influence bitterness and beer astringency [57]. These compounds are also modified during beer storage, as they react with proteins and form high molecular weight species and haze. The values detected in the beers obtained (ranging from 267.5 to 327.5 mg L⁻¹) were found to be within the average for other commercial beers (between 270 and 600 mg L⁻¹) [88]. Likewise, according to the tasting results, bitterness and astringency were not influenced by these polyphenol values. Similarly, there was no clear correlation between polyphenols and antioxidant capacity in this study. These results are not in agreement with those reported in other works [89,90]. There were also no significant differences between the different recipes.

3.3.2. Residual Sugars, Glycerol, and Ethanol Determination Using HPLC

Table 6 shows the results obtained for the four recipes after fermentation. The data were statistically significant, with differences between the recipes. As can be seen, the apparent attenuation was similar for both yeast strains (ranging from 67.5% to 69.5%), with the expected values being the same as those in previous trials (72% for both) [25].

Table 6. Fermentation parameters analysed using HPLC and the apparent attenuation of the beers obtained.

Recipes	Residual Fermentable Sugars (g L ⁻¹)		Apparent Attenuation (%)	Ethanol % (v/v)	Glycerol (g L ⁻¹)
	Maltotriose	Maltose			
A	8.40 ± 0.44 ^a	2.39 ± 0.02 ^b	69.5 ± 0.50 ^a	3.25 ± 0.06 ^a	1.72 ± 0.05 ^a
B	8.83 ± 0.07 ^a	2.50 ± 0.03 ^a	67.5 ± 0.50 ^{bc}	3.21 ± 0.03 ^a	1.68 ± 0.00 ^{ab}
C	9.24 ± 0.66 ^a	2.15 ± 0.03 ^c	69.0 ± 1.00 ^{ab}	3.33 ± 0.04 ^a	1.59 ± 0.02 ^b
D	8.90 ± 0.01 ^a	2.04 ± 0.06 ^d	67.0 ± 0.00 ^c	3.05 ± 0.07 ^b	1.47 ± 0.07 ^c

Data are means ± standard deviations of two beer samples. Data with different superscript letters within each column are significantly different (Tukey tests: *p* < 0.05). Recipes A and C: strain G 520; recipes B and D: strain G 354. Recipes A and B: Rayo Verde Tajuña hops; recipes C and D: Torreta Tajuña hops. Initial sugar composition of the wort: glucose, 14.47 mg L⁻¹; fructose, 3.50 mg L⁻¹; maltose, 44.33 mg L⁻¹; and maltotriose, 12.26 mg L⁻¹.

As for residual sugars, both strains fermented glucose and fructose completely, leaving only residual maltose and maltotriose with similar values. Similar maltose and maltotriose fermentation results were obtained when these strains were pre-selected from previous trials [25]. The presence of residual sugars can influence the physical properties of the beer, such as viscosity, thus contributing to a fuller-bodied beer, as well as a stronger mouthfeel [91,92]. Similarly, the beers obtained using the different recipes had similar ethanol values between 3.05% and 3.33% (*v/v*). Another parameter resulting from fermentation that was assessed was glycerol, as it also contributes to the body and mouthfeel of the beer [93,94]. The concentration of this compound in the beer is related to cell growth rate, ethanol production, and sugar utilisation. When compared to previous trials [25], where ethanol production was higher (5.07% (*v/v*) for both strains), the glycerol content was also higher (3.19 and 3.63 mg L⁻¹). This fact can be contrasted with this study, as the volume of alcohol was lower, and, therefore, the glycerol values were between 1.47 and 1.72 mg L⁻¹.

3.4. Main Volatile Compound Production

The final quality of beer is due to many factors, including the aroma compounds produced during brewing. There is a large number of aroma substances in beer, which can be categorised into different groups, such as esters, higher alcohols, fatty acids, aldehydes, ketones, hydrocarbons, ethers, sulphur compounds, alicyclic compounds, aromatic compounds, and heterocyclic compounds, among others. These compounds can individually, synergistically, or antagonistically influence both the aroma and flavour of beer [95–100]. These compounds can also come from the different ingredients used in the brewing process, either because they are part of these ingredients or because they are formed during the brewing process [54].

A total of 33 aromatic compounds were analysed, of which the main ones detected are listed in Table 7. The beers showed significant differences for all the aroma compounds analysed. Furthermore, the concentrations of the different aroma compounds were significantly higher in recipes A and C, where yeast strain G 520 was used.

Table 7. Main volatile compounds found in the four beers obtained.

Recipes	A	B	C	D
Higher alcohols				
Isobutanol	33.72 ± 0.94 ^a	13.17 ± 1.76 ^b	35.24 ± 3.43 ^a	12.17 ± 0.65 ^b
1-butanol	0.14 ± 0.00 ^a	0.13 ± 0.00 ^b	nd	nd
Isoamyl alcohol	107.80 ± 0.36^b	82.07 ± 2.90^c	116.85 ± 4.91^a	76.43 ± 3.32^c
Methionol	0.47 ± 0.13 ^a	0.32 ± 0.00 ^a	0.35 ± 0.04 ^a	0.33 ± 0.01 ^a
Benzyl alcohol	0.05 ± 0.00 ^b	0.07 ± 0.01 ^a	0.04 ± 0.00 ^b	0.04 ± 0.00 ^b
β-phenylethanol	26.69 ± 1.44 ^a	11.04 ± 0.07 ^b	32.92 ± 1.41 ^a	15.23 ± 4.40 ^b
Esters				
Isoamyl acetate	2.92 ± 0.27^a	1.32 ± 0.02^b	2.38 ± 0.11^a	0.78 ± 0.36 ^b
Ethyl butyrate	0.03 ± 0.03 ^b	0.18 ± 0.00 ^a	0.11 ± 0.00 ^{ab}	0.09 ± 0.09 ^{ab}
Ethyl isovalerate	0.15 ± 0.00 ^a	0.14 ± 0.01 ^a	0.14 ± 0.00 ^a	0.14 ± 0.01 ^a
Ethyl hexanoate	0.32 ± 0.00^a	0.33 ± 0.01^a	0.29 ± 0.00^{ab}	0.20 ± 0.08 ^b
Ethyl octanoate	0.08 ± 0.01 ^a	0.10 ± 0.01 ^a	0.11 ± 0.00 ^a	0.09 ± 0.03 ^a
2-phenylethyl acetate	0.11 ± 0.00 ^a	0.05 ± 0.00 ^c	0.10 ± 0.00 ^b	0.05 ± 0.00 ^c
Fatty acids				
Butyric acid	0.64 ± 0.01 ^a	0.58 ± 0.02 ^b	0.56 ± 0.01 ^{bc}	0.54 ± 0.00 ^c
Hexanoic acid	3.12 ± 0.21 ^a	4.43 ± 0.00 ^a	3.34 ± 0.12 ^a	3.26 ± 0.09 ^a
Octanoic acid	7.70 ± 1.64 ^b	13.14 ± 0.02 ^a	10.44 ± 0.55 ^{ab}	9.68 ± 3.65 ^{ab}
Decanoic acid	2.74 ± 0.13 ^b	8.83 ± 1.24 ^a	4.10 ± 0.05 ^b	4.16 ± 2.67 ^b

Data, expressed as mg L⁻¹, are means ± standard deviations of two independent experiments. nd = not detected. Compounds above their threshold levels are marked in bold. Data with different superscript letters within each row are significantly different (Tukey tests: *p* < 0.05).

Higher alcohols are the most abundant compounds in beer. There are about 80 higher alcohols in beer, the total concentration of which can be between 100 and 300 mg L⁻¹,

with their main resource being malt and yeast [54]. The same amount and type of barley malt was used for the four recipes, so the variations observed in their concentration are due to the yeast strains used. When the concentration is below 300 mg L^{-1} , they will bring freshness and floral and pleasant notes, adding complexity to the beers [101]. The total higher alcohols produced in the beers obtained from the different recipes ranged between 104.19 and 185.40 mg L^{-1} , with the highest concentrations produced in those recipes where the G 520 yeast strain was used (recipes A and C). The concentrations of the higher alcohols detected were similar for the four recipes, except for isobutanol, isoamyl alcohol, and β -phenylethanol, for which higher concentrations were obtained in the recipes where yeast strain G 520 was used. Likewise, only values above the perception threshold (70 mg L^{-1}) were obtained for isoamyl alcohol [102]. This compound contributes sweet, alcoholic, and banana flavours. In general, strains selected for ale fermentations have been found to produce higher concentrations of higher alcohols than lagers [103].

The second most important group of aromatic compounds found in beer are esters because, although they are found in low concentrations, their perception thresholds are low. These compounds can come from malt, hops, or yeast and can be formed at any time during the brewing process [54]. As with the higher alcohols, the highest concentrations occurred for recipes A and C (between 3.13 and 3.60 mg L^{-1}). As for the concentrations of the different compounds detected, isoamyl acetate and ethyl hexanoate were the ones for which values above the threshold were obtained (1.4 mg L^{-1} and 0.21 mg L^{-1} , respectively) [104,105]. Isoamyl acetate contributes banana aromas, while ethyl hexanoate contributes green apple aromas. In spite of this, these aromas did not stand out in the tasting, as in the overall evaluation, other aromas stood out above these.

On the other hand, different fatty acids were also detected, the origin of which can be malt, hops, or yeast [54]. The total concentration of fatty acids that can be found in beer is around $15\text{--}30 \text{ mg L}^{-1}$, with the most abundant being the short–medium-chain fatty acids (C4–C10) [106]. Among them are those that contribute the commonly called caprylic aroma (hexanoic, octanoic, and decanoic acid). It is an undesirable aroma in brewing, but it is usually found in lager beers and in 20% of ale beers [76]. However, in the beers analysed, the threshold was not exceeded for any of the three compounds (8 mg L^{-1} for hexanoic, 15 mg L^{-1} for octanoic, and 100 mg L^{-1} for decanoic) [107]. In addition, other short-chain fatty acids, such as butyric acid, which can increase during fermentation due to the action of yeasts, were also studied [77]. This compound contributes to “cheesy” or “sickly” off-flavours; however, it was not detected above the threshold in any of the beers (2 mg L^{-1}) [75].

3.5. Antioxidant Activity

Beer is a globally consumed beverage that is a source of natural antioxidants, such as phenolic compounds from barley and hops [108–111]. However, yeasts can also influence the composition of phenolic compounds and, thus, the antioxidant capacity of beer [112]. Table 8 shows the results of the antioxidant activity of the beers obtained using the four different recipes. Significant differences in the data were mainly observed for slow-acting antioxidants.

Table 8. Antioxidant activity of the beers obtained using different recipes.

Recipes	Q1	Q2	Qt
A	3.39 ± 0.00^a	9.41 ± 0.13^{ab}	12.81 ± 0.13^b
B	3.58 ± 0.16^a	9.75 ± 0.09^a	13.34 ± 0.07^a
C	3.36 ± 0.09^a	9.15 ± 0.21^b	12.51 ± 0.30^{bc}
D	3.53 ± 0.06^a	8.73 ± 0.18^c	12.26 ± 0.12^c

Data are means \pm standard deviations of two independent experiments, expressed as millimoles of Trolox equivalents per litre (mmol TE L^{-1}). Q1, fast-acting antioxidants; Q2, slow-acting antioxidants; Qt, total antioxidants. Data with different superscript letters within each column are significantly different (Tukey tests: $p < 0.05$). Recipes A and C: strain G 520; recipes B and D: strain G 354. Recipes A and B, Rayo Verde Tajuña hops; recipes C and D: Torreta Tajuña hops.

The levels of total antioxidant capacity (Qt) found in the four beers were similar, ranging between 12.26 and 13.34 (mmol TE L⁻¹), which is also within the average of those obtained with the previously tested strains (13.03 and 13.43 mmol TE L⁻¹) [25]. In this case, as there were no major differences between the beers obtained using the different recipes, no direct relationship was found between the hops and the yeast strains used. However, it should be noted that the values obtained are higher than those observed in other studies on antioxidant capacity analysis in beer (from 424.77 to 10,508.47 µmol TE L⁻¹) [113].

3.6. Principal Component Analysis (PCA)

Principal component analysis (PCA) was used to assess the trends of the analysed data and the different recipes used. Two principal components were analysed, where PC1 (Dim1) explained up to 59.70% of the total variance, and PC2 (Dim2) explained another 29.30%. Figure 3 shows a scatter plot, where both components are plotted against the most prominent parameters of the beers.

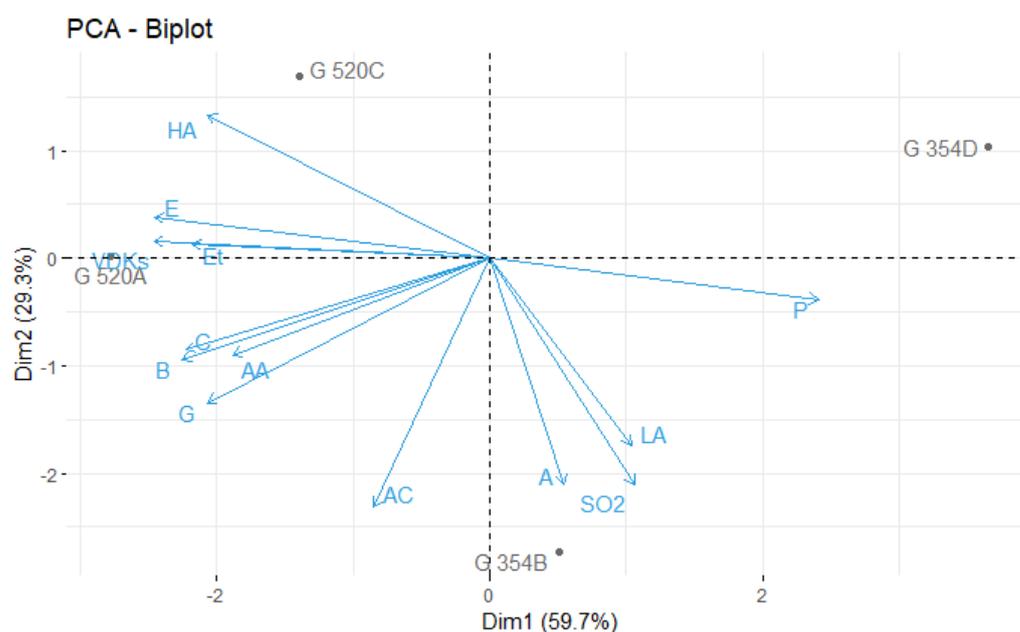


Figure 3. Projection of the beers on the axes formed by PC1 (Dim1) and PC2 (Dim2). Each object is the average of the two corresponding experimental beers. B, bitterness; G, glycerol; AC, antioxidant capacity; Et, ethanol; A, total fatty acids; HA, total higher alcohols; E, total esters; LA, lactic acid; C, colour; VDKs, vicinal diketones; AA, apparent attenuation; P, polyphenols. G 520A: recipe A; G 354B: recipe B; G 520C: recipe C; G 354D: recipe D.

The beers obtained using recipes A and C were both situated in the positive region of PC2, which is associated with total higher alcohols, total esters, ethanol, and vicinal diketones; therefore, they can be considered parameters associated with the yeast strain (G 520), as they are the main producers of higher alcohols and esters. Conversely, recipes B and D, which used the same yeast strain (G 354), were in the positive zone of PC1; however, only recipe B showed a trend towards the production of fatty acids, lactic acid, and SO₂. Therefore, the PCA showed a clustering of recipes that is more dependent on the yeast strains used than the hops for aroma.

3.7. Sensory Analysis

Beer is composed of numerous aromatic components, both volatile (higher alcohols, esters, sulphur compounds, and hop aroma compounds) and non-volatile (hop bittering components), as well as compounds that contribute to mouthfeel characteristics (such as

alcohol, carbonation, and polyphenols). It is the interaction of all these compounds that will define the organoleptic profile of a beer.

The beers obtained from the four designed recipes were subjected to sensory analysis, the data of which are illustrated in Figure 4.

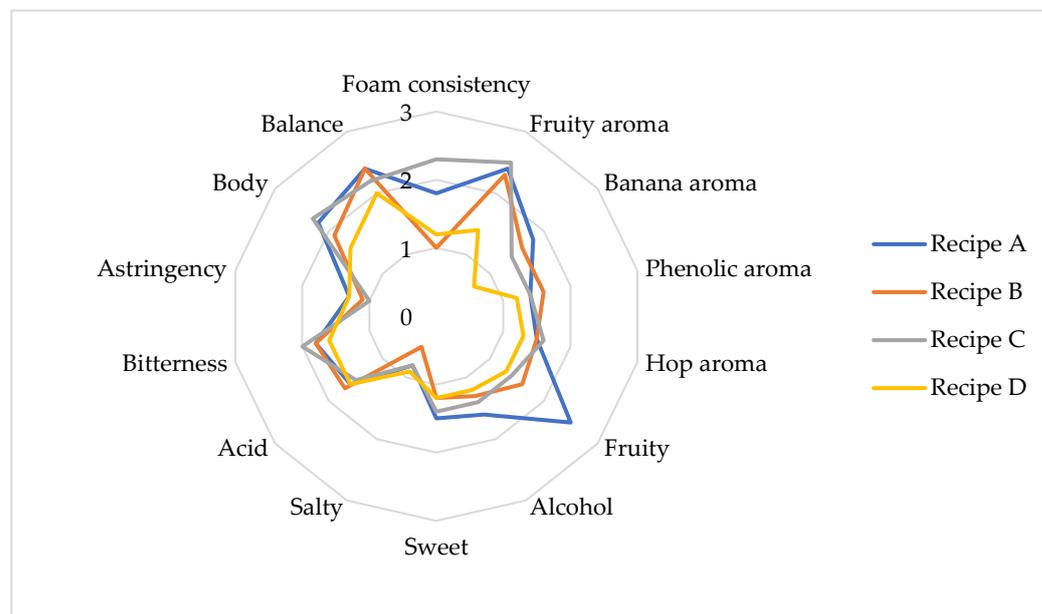


Figure 4. Sensory analysis of the four different beers obtained (from 0 to 3). Visual attributes: foam consistency. Aroma attributes: fruity aroma, phenolic aroma, banana aroma, and hop aroma; flavour attributes: fruity, alcohol, sweet, bitter, salty, acid, and astringent; overall attributes: body and balance.

As can be seen in the graph, recipe A stands out from the rest because it has a much fruitier taste than the rest of the recipes, where it was not as noticeable. The fruity aroma is also one of the outstanding attributes in all the recipes with average values, except in recipe D. This is related to the results of the aromatic compounds analysed, as recipes A, B, and C presented higher total concentrations of esters than recipe D. Despite not exceeding the detection thresholds for the compounds analysed, the synergism between the compounds means that these are accentuated in tasting.

The sensory perception of hops can be related to the IBU values obtained; however, some beer components, such as sweetness or polyphenol content, may contribute to increasing or masking this sensation [114]. In this case, the IBU values obtained were not high, which was subsequently reflected in the tasting, as the beers were not excessively bitter in their character.

In terms of general attributes, the beers were rated as balanced and full-bodied, with the best body ratings for recipes A and C, for which the G 520 yeast was used.

The overall assessment showed that the four different recipes produced pleasant and aromatic sensory profiles with specific aromatic characteristics; however, recipes A and C (yeast strain G 520) stood out against B and C (yeast strain G 354) for their fruity aroma and flavour. It was found that, in general, consumers have a greater preference for beers with fruity profiles and bitterness that is not too pronounced [115].

3.8. Consumer Analysis

The design of a brewing recipe involves a final consumer analysis to determine consumer acceptability [116].

In order to evaluate the four beers obtained using the different recipes, 110 volunteer consumers were recruited. A prerequisite for participation in the study was to be a regular beer drinker, as the beers to be evaluated were craft beers. The panel was composed of 50% women and 50% men, aged between 18 and 75 years. In terms of frequency of beer

consumption, 97.27% of the panel reported frequent beer consumption several times a week or at the weekend. Furthermore, 97.27% consumed both commercial and craft beer, while 32.73% only consumed commercial lager.

A frequency analysis of the data was conducted using CATA questions, as shown in Table 9.

Table 9. Analysis of the data frequency obtained by CATA questions.

Sensory Attributes	Response (n = 110)			
	A	B	C	D
Appearance				
Visual impression				
Very hazy	31	44	35	29
Hazy	17	14	35	29
Dull	38	35	26	30
Clear	15	13	9	15
Bright	9	4	5	7
Aroma				
Aroma intensity				
Low	49	34	41	37
Low-medium	24	27	30	26
Medium	32	35	29	38
Medium-high	4	11	10	8
High	1	3	0	1
Taste				
Acidity				
Low	35	40	48	46
Low-medium	43	49	40	44
Medium	7	10	6	11
Medium-high	21	8	12	9
High	4	3	4	0
Bitterness				
Low	29	27	42	38
Low-medium	40	45	38	44
Medium	26	26	20	16
Medium-high	12	7	8	10
High	3	5	2	2
Aftertaste intensity				
Short	54	49	42	45
Short-medium	9	9	19	24
Medium	19	17	19	8
Medium-long	27	34	27	30
Long	1	1	3	3

In general, the beers were visually identified as having some hazy appearance, a medium-low aroma intensity, and low taste in acidity and bitterness, as well as a short aftertaste, which makes the beers more easily drinkable. The results concurred with the expert tasting panel.

The aromatic attributes (Figure 5) most detected by consumers were hops and cereal (ranging from 37.27% to 57.27%). The beers were then highlighted for their yeasty and fruity aroma. In terms of flavour attributes, hops, cereal, yeast, and fruity continued to be the most predominant, along with a roasted flavour. The hops in recipes A (Rayo Verde Tajuña aromatic) and C (Torreta Tajuña aromatic) should be highlighted, as their detection at the flavour level stood out over aroma.

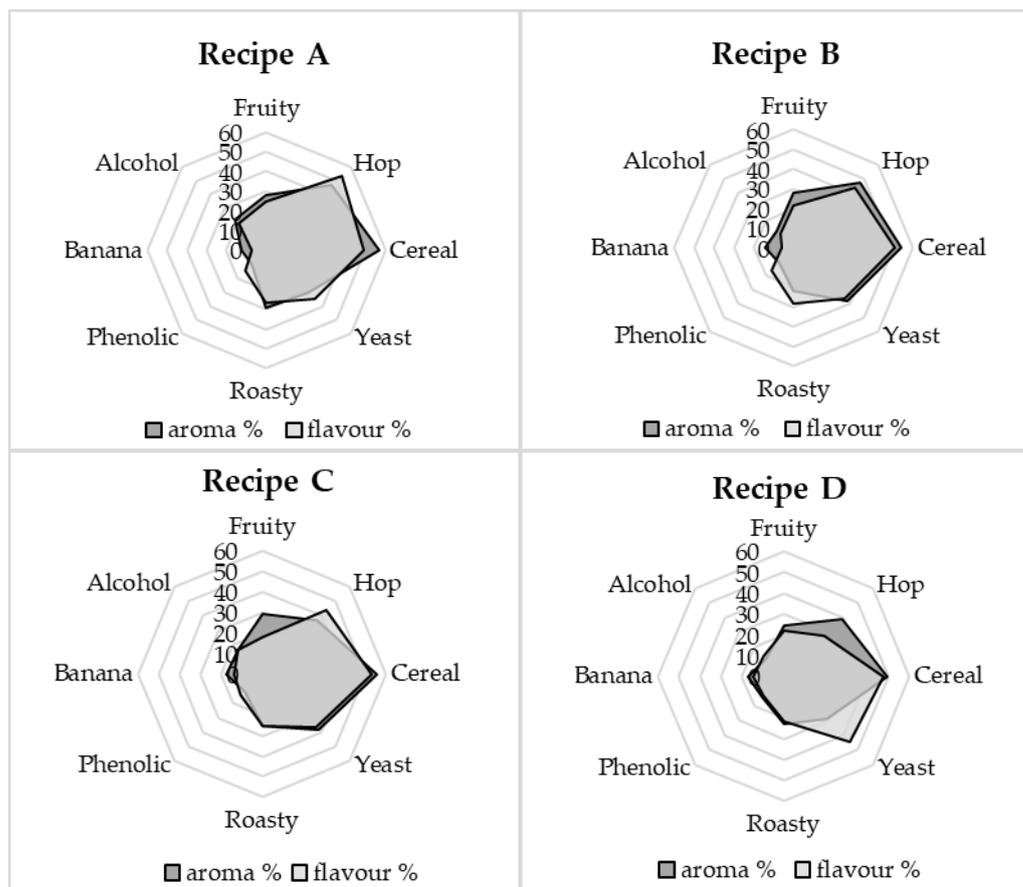


Figure 5. Percentage of detection by consumers for each of the analysed attributes in the aroma and flavour for the four beers.

When contrasting these results with those obtained by the panel of trained experts, as well as the different analyses performed, these are beers that have an aromatic profile of predominantly fruit, cereal, and hop flavours and aromas. Additionally, some studies suggest that consumers have a preference for beers with conventional flavours, such as cereal, hops, and fruit [117].

The overall rating of the four beers was very similar, with the best-rated being those from recipes A and C. The consumers were also asked about their purchase intention, which, for these recipes, ranged from 62.73% to 66.36%.

4. Conclusions

This paper evaluated the design of four pale ale beer recipes using autochthonous ingredients from the Community of Madrid. The use of indigenous ingredients (water, malt, hops, and yeast) is an important factor that helps to create important connections with the territory, attracting more consumers, as well as representing an added value to the products and favouring the agricultural sector of the region.

As regards the composition of the hops used, it was seen that the commercial variety (Cascade) could be used as a bittering hop, while the wild varieties (Rayo Verde Tajuña and Torreta Tajuña) could be used as aroma hops, as the content of α - and β -acids was much lower than in the Cascade variety. On the other hand, for the variety of barley malt used (Beka), the values obtained were similar to those of other malting barleys on the market in terms of quality and characteristics. Although the protein values found were high, it was not a negatively assessed parameter in the final beers. As far as fermentation is concerned, the yeasts used to brew the beers showed good fermentation capacities, as well as an outstanding organoleptic profile.

In general, the beers obtained were well rated by both the expert panel and the consumers, with the best rated being recipes A and C, which used the same yeast strain (G 520) but different aromatic hops. These were considered to be beers with an organoleptic profile; prominent hop, cereal, and fruit aromas and flavours; and a medium balance and body, which made the beers more drinkable. Future studies may focus on testing these recipes on an industrial scale and adjusting them if necessary, so interested brewers may then develop the recipes and launch the beers to market.

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