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Preliminary Study of the Effect of Short Maceration with Cherry and Oak Wood Chips on the Volatile Composition of Different Craft Beers

Ana C. Correia [1], Maria L. González-SanJosé [2], Miriam Ortega-Heras [2] and António M. Jordão [1,3,*]

[1] Agrarian Higher School, Food Industries Department, Polytechnic Institute of Viseu, 3500-606 Viseu, Portugal; anacorreia@esav.ipv.pt
[2] Department of Food Science and Technology, University of Burgos, 09001 Burgos, Spain; marglez@ubu.es (M.L.G.-S.); miriorte@ubu.es (M.O.-H.)
[3] Chemistry Research Centre (CQ-VR)—Food and Wine Chemistry Lab., 5001-801 Vila Real, Portugal
* Correspondence: antoniojordao@esav.ipv.pt

Abstract: In the last few years, the production and consumption of craft beers has increased notably. However, there is restricted knowledge about the potential impact of chips from different wood species on beer quality. Thus, this work aimed to evaluate the effect of the addition of wood chips from cherry and oak species—after the fermentation was completed and during a brief maceration time—on the volatile composition of three different beer groups (Ale, Lager, and Porter) through a headspace solid-phase dynamic extraction (HS-SPDE) and GC-MS analysis. Fifty-six volatile compounds from different chemical families (esters, alcohols, terpenes, acids, aldehydes, ketones, and pyrazines) were detected, identified, and considered in this study. In general, the volatile composition of the beers macerated with wood chips was very similar to that of the control beers. However, the control beers showed higher volatile compound levels. The results suggest potential interactions between beer volatile compounds and the wood chips during maceration. The outcomes of this research could be of practical interest to brewers since they could improve the knowledge of the impact of short-time contact and low wood chip concentration on the volatile composition of different craft beers.

Keywords: cherry; craft beers; oak; volatile compounds; wood chips

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1. Introduction

Beer is one of the most popular alcoholic beverages worldwide, being the third most popular drink overall after water and tea. In 2022, global beer production amounted to about 1.89 billion hectoliters (data available at statistica.com accessed on 23 July 2023). In the last decade, the brewing sector has observed the emergence of many craft beer breweries and microbreweries that have opened worldwide. According to the Brewers of Europe Report 2020, the number of European microbreweries has seen an unprecedented increase from 1992 in 2008 to more than 9500 in 2020 and the number continues to grow. Similarly, a constant increase in craft breweries and craft beer sales has also been observed in European countries [1]. The Europe Craft Beer Market is projected to register a compound annual growth production rate of 8.62% during the forecast period of 2022–2027.

In 2022, the Beer Style Guidelines published by the Brewers Association (www.brewersassociation.org accessed on 24 July 2023) describe three main groups of beer styles: Ale, Lager, and Hybrid/mixed Lager or Ales. However, inside each beer style group, there are a lot of different substyles (87 for the Ale style group, 33 for Lager style, and 34 for Hybrid/mixed Lager or Ales).

Beer is a very complex alcoholic beverage in terms of chemical composition, ingredients, and sensory styles. In fact, the wide range of combinations of malts, yeasts, hops, and in some beers, other products such as different cereals, spices, or fruits, along with the different brewing methods, have a strong influence on the developments of the beer's characteristics, namely, in chemical and aroma profiles [2]. Regardless, the demand for special and different beers with new sensory properties seems to be one of the reasons for the high increase in the craft beers market [3].

Volatile compounds are substances that contribute significantly to the organoleptic properties of beverages, including beers. According to Palamand and Aldenhoff [4], the volatile fraction of beer can be composed of over 800 different compounds. However, only several tens of these can be flavor-active, being the main compounds responsible for the flavor sensation of the beers. These compounds are diverse chemicals, including esters, fatty acids, alcohols, sulfur, furanic compounds, carbonyl compounds, monoterpenes, C_{13}-norisoprenoids, and volatile phenols [5,6]. In addition, the volatile composition of beers depends on different factors, namely, raw material [2] and the brewing process, specifically the technological steps and yeast metabolism [7,8]. Furthermore, several volatile compounds are also formed during the beer aging process [9,10].

For several beverages, such as wine, whiskey, and vinegar, one of the most common ways of aging is using wooden barrels, particularly from oak, that allow beverages to have specific characteristics, namely, in terms of their aroma. During the beverage wood contact, there is mainly an extraction process, together with other chemical phenomena, such as oxidations, polymerization, and polycondensation reactions [11,12]. In addition, the high number of wood extractable compounds can play a significant role in the flavor of alcoholic beverages since they change their volatile composition [13]. In beers, the maturation in wood, particularly in oak, is not new in breweries. However, there is a new interest in this process to obtain the flavor characteristics that this practice can provide for beers [10,14]. In general, only a few works published on this subject describe the use of oak barrels, usually with previous use in wine aging, for the beer's conservation and aging [15]. According to Sanna and Pretti [16], the use of wine oak barrels has great interest among craft brewers to allow some of the vinous character of the barrel to permeate the beer. Guimarães et al. [17] described the use of wood barrels and cubes from different Brazilian woods (chestnut, balsam, oak, and amburana), and pointed out the effect of each wood on the phenolic and sensory profile of Lager beer styles. Previously, other authors described the impact of oak fragments (chips and cubes) on beer volatile composition using a single beer style [15,18]. The referenced works demonstrated the value of woods for beers conservation or aging, but they are focused mainly on the oak wood species and only studied the effects on a single beer style.

To deepen the knowledge of the volatile composition of diverse types of craft beers and to contribute to the state-of-the-art of the effect of wood chips on these beers, the volatile composition of different craft beers with and without maceration with oak wood chips was studied. The study includes three different beer types, and two different wood chips (oak and cherry species), being, as far as we know, the first report about the effect of cherry chips on craft beers. It should be highlighted that the aim of this work was not to compare types of beers but to study the effect of different wood chips on the volatile composition of the beer types considered.

2. Materials and Methods

2.1. Craft Beers and Wood Chip Species

The craft beer samples were kindly provided by the brewery 8ª Colina company (Lisbon, Portugal). Thus, three different craft beers, each one belonging to the groups of Ale, Lager, and Porter were produced. For the Ale and Porter beers, worts were fermented with *Saccharomyces cerevisiae* yeast strain (Safale S-04^{TM}), while for Lager beer, worts were fermented with *Saccharomyces pastorianus* (Safale S-23^{TM}). Both yeasts were provided by Fermentis Division of S.I. Lesaffre (Lille, France) and were used according to the instructions of the supplier. The fermentation process was completed in 2 weeks keeping the temperature around 18 °C for Ale and Porter beers, while for lager beer the fermentation was completed in 2 months at 10 °C of temperature. In addition, a commercial hop blend Trident™ was used and provided by Hopteiner (Mainburg, Germany). Finally, the malts used to produce each beer type were supplied by the company Castel Malting (Lambermont, Belgium). For the Porter beer a Château CafeLight™ malt was used, while Château Pilser 2RS™ and Château Ale Nature™ malts were used for Ale and Lager beers production, respectively.

Each beer style showed the following characteristics: Ale beer, 5.2% (v/v) ethanol content, 34 International Bitterness Units (IBUs), and a pH value 4.0; Lager beer, 5.0% (v/v) ethanol content, 25 IBUs of bitterness, and a pH value 4.2; and Porter beer, 5.5% (v/v) ethanol content, 38 IBUs of bitterness, and a pH value 4.5.

Chips from two different wood species were used: cherry (*Prunus avium*) from Italy forests and oak (*Quercus petraea*) from France forests, supplied by AEB Bioquímica Portuguesa SA (Viseu, Portugal). All wood chips used presented a medium toasting level (20 min at 160–170 °C) previously submitted to a natural drying process and with a particle dimension of around 8 mm (average size).

2.2. Experimental Conditions

After the fermentation process, the craft beers studied were macerated in contact with the different wood chips (concentration of 1.0 g/L) during 30 aging days at a temperature of 18 °C (except for Lager beer which was maintained at a temperature of 13 °C) and slightly stirred twice a week. Ale and Lager beers were macerated with cherry wood chips, and Porter beer was macerated with oak wood chips. This selection was made based on previous assays (unpublished data) to identify the chips with the best sensorial results. Each assay was prepared in stainless steel containers of 30 L capacity and under isobaric conditions. Beer carbonation was carried out by pumping 1.8 bar of carbon dioxide (CO_2) using an automatic gas pressure regulator. For each beer, a control beer (without wood chip contact) stored in a 30 L stainless steel container under the same temperature and pressure was also made. After the maceration period, the beers were manually bottled in 330 mL glass bottles previously flushed with CO_2 to remove oxygen. Manually bottled beers were stored at ambient temperature until analysis. Two bottles of each type of beer were analyzed, each in duplicate.

2.3. Beer Volatile Compound Profile

The isolation and identification of volatile compounds were carried out following the method proposed by Castro et al. [5]. The isolation of the volatile compounds was carried out using a headspace solid-phase dynamic extraction (HS-SPDE) coupled with a gas chromatograph (GLC, Agilent Technologies HP 6890N) with a mass spectrometer (Agilent Technologies 5973) fully controlled by a CTC-CombiPAL autosampler (Bender and Hobein, Zurich, Switzerland). A PDMS/AC fiber (90% of polydimethylsiloxane and 10% of active charcoal, Chromotech, Germany) was used after its conditioning (heating at 200 °C for 1 h).

An amount of 5 mL of each beer sample and a stir bar were introduced into 10 mL glass vials. The vials were encapsulated with a metallic cap of chlorobutyl/polytetrafluoroethylene seal (Chromacol Ltd., Welwyn Garden City, UK). Each vial was equilibrated for 15 min at 60 °C and then a dynamic extraction was carried out under the following conditions: agitation speed 250 rpm, extraction temperature 60 °C, extraction strokes 55, extraction volume 1 mL, fiber temperature 55 °C, and fill/eject speed 50 µL/s. For analysis, the following conditions were used: desorption volume, 1 mL; injection port temperature, 250 °C; Carbowax 20M column (60 × 0.32 mm, 0.25 µm film thickness, Quadrex Corporation, Symta, Madrid, Spain); helium with 1 mL/min flow rate as the carrier; oven conditions of 35 °C initial temperature, increasing 3 °C/min until 230 °C and held for 5 m; and mass detection in electronic impact mode (70 eV). The isolated beer volatile components were identified by comparison of obtained mass spectra with mass spectral data from the NIST library. The volatile analysis was carried out in duplicate from each sampled bottle and the results were expressed as peak area values.

2.4. Statistical Analysis

The data are shown as mean ($n = 4$, two bottles $\times$ 2 analysis) $\pm$ standard deviation. Results obtained were statistically tested via analysis of variance (ANOVA, one-way). Tukey's test ($p < 0.05$) was applied to the data to determine significant differences between all craft beers elaborated for each volatile compound. In addition, Student's *t*-test ($p < 0.05$; $p < 0.01$; ($p < 0.001$)) was also applied between each craft beer style (with and without wood chip maceration). Finally, a study using a principal component analysis (PCA) allowed us to evaluate the global effect of wood chip contact on the volatile composition of beers. All analyses were performed using SPSS software version 25.0 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

The use of different wood species, particularly oak [15,18], but also, chestnut, balsam, and amburana woods [17], have been studied for beer aging in recent years. However, the maceration of different craft beers with cherry and oak wood chips during a short time is not usual and, consequently, it is not possible to have a real perception of the potential impact on beer volatiles.

This work shows the volatile fingerprint of craft beers of three different types (Ale, Lager, and Porter) macerated with wood chips (oak and cherry species) for 30 days. Fifty-six different volatiles were identified in the studied craft beers. They were: three acetates, five acids, seven alcohols (not including ethanol), three aldehydes, twelve ethyl esters, three furfuryl derivates, two ketones, two pyrazines, fourteen terpenes, and five other non-grouped compounds.

It is important to note that the results obtained in this work are based on average peak areas and, consequently, give a semiquantitative estimation of the volatile compound profiles of the tested beers. As a result, it was not possible to have precise information about their actual participation in the aroma profile of the studied beers. However, sensorial odor descriptors for each volatile compound identified, described in previous works, are shown in Table 1.

Table 1. Sensorial odor descriptors for the volatile compounds determined in the different craft beer types studied.

Volatile Compounds	Sensorial Odor Descriptors	References
Alcohols		
2-Methyl-1-propanol	Malty	[6,19]
1-Octen-3-ol	Mushroom	[20]
1-Heptanol	Violet, herbal	[21]
2-Nonanol	Fruity, rose	[22]
1-Octanol	Nuts, coconut, oily	[10]
1-Nonanol	Fatty	[23]
Phenethyl ethanol	Flowery, honey	[19]
Ethyl esters		
Hexanoic acid ethyl ester	Rancid, fatty, fruity	[24]
Decanoic acid ethyl ester	Rancid, waxy, soap	[24]
Dodecanoic acid ethyl ester	Metallic, fatty	[25]
Heptanoic acid ethyl ester	Sweaty, fruity	[26]
Octanoic acid ethyl ester	Sweaty, fatty	[24]
Nonanoic acid ethyl ester	Sweaty, fruity	[27]
Tetradecanoic acid ethyl ester	Fatty, soapy, waxy	[28]
Trans-4-decenoic ethyl acid	Fruity	[29]
9-Decenoic acid ethyl ester	Rancid, sweaty	[7]
Undecanoic acid ethyl ester	Bitterness, dairy	[30]
Benzoic acid ethyl ester	Chemical	[31]
Diethyl succinate	Vinous, floral	[32]

Table 1. *Cont.*

Volatile Compounds	Sensorial Odor Descriptors	References
Acetates		
Ethyl acetate	Pineapple, fruity	[33]
Isoamyl acetate	Banana, apple, solvent	[24]
Phenylethyl acetate	Floral, pleasant	[34]
Acids		
Acetic acid	Sour, vinegar, pungent	[26]
Hexanoic acid	Rancid, fatty	[24]
2,5-Dimetil, 4-hexenoic acid	----	----
Octanoic acid	Sweaty, fatty	[24]
Decanoic acid	Rancid, waxy, soap	[24]
Aldehydes		
Octanal	Fatty, orange, lemon	[26]
Nonanal	Citrus-like, fatty	[26]
Decanal	Sweet, green, fruity	[35]
Ketones		
6-Methyl-5-hepten-2-one	Fruity	[8]
2-Undecanone	Fruity	[8]
Terpenes		
2-β-Pinene	Woody	[36]
Δ-3-Carene	Resin, sweet, lemon	[37]
β-Phellandrene	Mint	[37]
DL-Limonene	Lemon, citric	[38]
Eucalyptol	Mint, pepper	[39]
β-Ocimene	Herb, sweet	[37]
Linalool	Aniseed, lemon	[10]
α-Humulene	Herbal, woody	[2,40]
β-Citronellol	Flowery	[10]
Geranyl acetone	Fruity	[41]
β-Caryophyllene	Spice, citrus	[8]
Cis-Calamenene	Herb, clove	[26]
Δ-Cadinene	Wood, herbaceous	[42]
Cadalene	Spicy	[43]
Furfuryl derivates		
Furfural	Bread, almond, sweet	[7]
2-Acetylfuran	Peanut, sweet	[44]
2-Furanmeethanol (furfuryl alcohol)	Hay, moldy	[10]
Pyrazines		
2-Methyl pyrazine	Cocoa, roasted	[45]
3-Ethyl-2,6-dimethyl-pyridine	Earth, nutty	[7]
Other compounds		
p-Allyl anisole	Spicy, anise	[46]
Eugenol	Spicy, medicinal	[47]
2-Acetylpyrrole	Nutty, herbal	[44]
Methoxy phenyl oxime	Green, bitter	[48]
Acetoin	Mushroom, sweet	[45]

3.1. Volatile Fingerprint of Craft Beers

3.1.1. Alcohols

Usually, the higher alcohols are most volatiles in fermented beverages and beer is not an exception [49]. Among them, amyl alcohol (C_5) is the volatile compound present at the highest concentration followed by isobutyl alcohol, which can exert a very negative effect on beer aroma at high concentrations, while other alcohols (C_6 or higher) are present at low levels [6,9]. The levels of amyl alcohol detected in this study were very low, being in some cases so small that data gave a very high variation coefficient (more than 130%); for that reason, this compound was not considered in this study (Table 2).

Table 2. Average peak area, standard deviation, and coefficient of variation in volatile compounds determined via SPME-GC-MS from three different craft beer types macerated during 30 days in contact with cherry and oak wood chips.

Compounds (†)	Craft Beer Types					
	Ale		Lager		Porter	
	Ale	Ale + Ch	Lager	Lager + Ch	Porter	Porter + Oak
Alcohols *Higher*						
2-Methyl-1-propanol	6.45×10^{6} b * $\pm 1.13 \times 10^{6}$ (17.5%) (††)	3.80×10^{6} b $\pm 1.21 \times 10^{5}$ (3.2%)	2.37×10^{6} b * $\pm 6.35 \times 10^{5}$ (26.8%)	3.54×10^{6} b $\pm 2.57 \times 10^{5}$ (7.3%)	1.42×10^{7} a $\pm 2.41 \times 10^{6}$ (16.9%)	1.20×10^{7} a $\pm 2.74 \times 10^{6}$ (22.9%)
>C_6 Alcohols						
1-Octen-3-ol	6.74×10^{5} d $\pm 7.14 \times 10^{4}$ (10.6%)	8.48×10^{5} c $\pm 8.62 \times 10^{4}$ (10.2%)	1.44×10^{6} a *** $\pm 1.06 \times 10^{5}$ (7.3%)	9.58×10^{5} c $\pm 6.18 \times 10^{4}$ (6.5%)	1.18×10^{6} b *** $\pm 3.42 \times 10^{4}$ (2.9%)	9.05×10^{5} c $\pm 3.11 \times 10^{4}$ (3.4%)
1-Heptanol	6.27×10^{5} c $\pm 1.30 \times 10^{5}$ (20.8%)	7.63×10^{5} c $\pm 1.61 \times 10^{4}$ (2.1%)	1.05×10^{6} b ** $\pm 5.28 \times 10^{4}$ (5.%)	8.13×10^{5} c $\pm 6.23 \times 10^{4}$ (7.7%)	1.40×10^{6} a ** $\pm 1.01 \times 10^{5}$ (7.3%)	1.04×10^{6} b $\pm 4.51 \times 10^{4}$ (4.3%)
2-Nonanol	6.77×10^{5} b * $\pm 7.13 \times 10^{4}$ (10.5%)	2.74×10^{5} c $\pm 1.28 \times 10^{4}$ (4.7%)	1.39×10^{6} a ** $\pm 2.23 \times 10^{5}$ (16.0%)	8.35×10^{5} b $\pm 2.72 \times 10^{4}$ (3.3%)	3.24×10^{5} c $\pm 3.99 \times 10^{4}$ (12.3%)	3.05×10^{5} c $\pm 2.89 \times 10^{4}$ (9.5%)
1-Octanol	2.76×10^{6} a,b $\pm 3.57 \times 10^{5}$ (12.9%)	2.31×10^{6} b $\pm 5.17 \times 10^{4}$ (2.2%)	1.75×10^{6} c * $\pm 1.64 \times 10^{5}$ (9.3%)	1.37×10^{6} c $\pm 1.40 \times 10^{5}$ (10.2%)	3.14×10^{6} a * $\pm 1.33 \times 10^{5}$ (4.2%)	2.51×10^{6} b $\pm 4.16 \times 10^{5}$ (16.6%)
1-Nonanol	n.q.	2.24×10^{6} a $\pm 1.15 \times 10^{5}$ (5.1%)	n.q.	1.39×10^{6} b $\pm 2.49 \times 10^{5}$ (18.0%)	1.85×10^{6} a,b $\pm 4.28 \times 10^{5}$ (23.2%)	1.41×10^{6} b $\pm 1.61 \times 10^{5}$ (11.4%)
Total average peak area >C_6 Alcohols	*4.06×10^{6} d *** $\pm 2.90 \times 10^{5}$ (7.1%)*	*6.44×10^{6} b $\pm 5.21 \times 10^{4}$ (0.8%)*	*5.63×10^{6} c $\pm 4.47 \times 10^{5}$ (7.9%)*	*5.36×10^{6} c $\pm 3.87 \times 10^{5}$ (7.2%)*	*7.88×10^{6} a ** $\pm 4.56 \times 10^{5}$ (5.8%)*	*5.82×10^{6} c,b $\pm 6.03 \times 10^{5}$ (10.3%)*
Aromatic						
Phenethyl ethanol	1.14×10^{8} b $\pm 5.91 \times 10^{6}$ (5.2%)	1.16×10^{8} b $\pm 3.27 \times 10^{6}$ (2.8%)	1.02×10^{8} b ** $\pm 4.02 \times 10^{6}$ (3.9%)	8.64×10^{7} b $\pm 6.84 \times 10^{6}$ (7.9%)	2.39×10^{8} a $\pm 2.49 \times 10^{7}$ (10.4%)	2.34×10^{8} a $\pm 4.60 \times 10^{7}$ (19.7%)
Total average peak area	**1.25×10^{8} b**	**1.26×10^{8} b**	**1.09×10^{8} b ***	**9.53×10^{7} b**	**2.61×10^{8} a**	**2.52×10^{8} a**
Ethyl Esters *Major*						
Hexanoic acid ethyl ester	1.09×10^{8} a $\pm 2.08 \times 10^{7}$ (19.1%)	8.16×10^{7} b $\pm 2.21 \times 10^{6}$ (2.7%)	7.73×10^{7} b ** $\pm 1.03 \times 10^{7}$ (13.4%)	1.19×10^{8} a $\pm 1.15 \times 10^{7}$ (9.7%)	3.46×10^{7} c $\pm 5.79 \times 10^{6}$ (16.7%)	4.88×10^{7} c $\pm 1.70 \times 10^{7}$ (34.8%)
Decanoic acid ethyl ester	4.78×10^{8} a ** $\pm 2.41 \times 10^{7}$ (5.0%)	2.90×10^{8} b $\pm 1.98 \times 10^{7}$ (6.8%)	2.96×10^{8} b ** $\pm 6.04 \times 10^{7}$ (20.4%)	1.30×10^{8} c $\pm 8.42 \times 10^{6}$ (6.5%)	8.41×10^{7} d,c $\pm 2.04 \times 10^{7}$ (24.3%)	5.26×10^{7} d $\pm 1.91 \times 10^{7}$ (36.3%)
Dodecanoic acid ethyl ester	5.59×10^{7} b $\pm 1.71 \times 10^{7}$ (30.6%)	5.08×10^{7} b $\pm 4.13 \times 10^{6}$ (8.1%)	8.37×10^{7} a ** $\pm 1.44 \times 10^{7}$ (17.2%)	4.22×10^{7} b $\pm 6.21 \times 10^{6}$ (14.7%)	2.05×10^{7} c $\pm 2.32 \times 10^{6}$ (11.3%)	3.76×10^{7} c,b $\pm 1.72 \times 10^{7}$ (45.8%)
Total average peak area of major ethyl esters	*6.42×10^{8} a ** $\pm 2.70 \times 10^{7}$ (4.2%)*	*4.22×10^{8} b $\pm 2.18 \times 10^{7}$ (5.2%)*	*4.57×10^{8} b ** $\pm 8.20 \times 10^{7}$ (17.9%)*	*2.91×10^{8} c $\pm 1.10 \times 10^{7}$ (3.8%)*	*1.22×10^{8} d $\pm 6.08 \times 10^{6}$ (5.0%)*	*1.15×10^{8} d $\pm 6.60 \times 10^{7}$ (57.6%)*
Ethyl Esters *Minor*						
Heptanoic acid ethyl ester	3.41×10^{6} a,b * $\pm 5.85 \times 10^{5}$ (17.2%)	2.13×10^{6} c $\pm 9.69 \times 10^{4}$ (4.5%)	4.42×10^{6} a $\pm 5.25 \times 10^{5}$ (11.9%)	3.86×10^{6} a $\pm 3.13 \times 10^{5}$ (8.1%)	4.26×10^{6} a ** $\pm 5.81 \times 10^{5}$ (13.6%)	2.65×10^{6} c,b $\pm 4.35 \times 10^{5}$ (16.4%)
Octanoic acid ethyl ester	2.65×10^{6} a $\pm 5.65 \times 10^{5}$ (21.3%)	1.49×10^{6} b $\pm 1.57 \times 10^{4}$ (1.1%)	1.69×10^{6} a,b * $\pm 9.58 \times 10^{4}$ (5.7%)	1.26×10^{6} b $\pm 3.51 \times 10^{4}$ (2.8%)	2.24×10^{6} a,b $\pm 9.69 \times 10^{4}$ (4.3%)	1.30×10^{6} b $\pm 3.33 \times 10^{5}$ (25.7%)
Nonanoic acid ethyl ester	9.24×10^{6} a *** $\pm 7.25 \times 10^{5}$ (7.8%)	4.82×10^{6} b $\pm 7.33 \times 10^{4}$ (1.5%)	4.51×10^{6} b $\pm 1.12 \times 10^{6}$ (24.9%)	3.06×10^{6} c $\pm 6.57 \times 10^{5}$ (21.5%)	3.02×10^{6} c ** $\pm 3.63 \times 10^{5}$ (12.0%)	1.56×10^{6} d $\pm 5.31 \times 10^{5}$ (34.0%)
Tetradecanoic acid ethyl ester	1.03×10^{7} a * $\pm 1.73 \times 10^{6}$ (16.8%)	6.54×10^{6} b $\pm 7.04 \times 10^{5}$ (10.8%)	8.73×10^{6} a * $\pm 1.26 \times 10^{6}$ (14.4%)	6.47×10^{6} b $\pm 1.30 \times 10^{6}$ (20.1%)	5.18×10^{6} b $\pm 3.45 \times 10^{5}$ (6.7%)	4.48×10^{6} b $\pm 3.76 \times 10^{5}$ (8.4%)
Trans-4-decenoic acid ethyl ester	1.41×10^{6} a $\pm 1.40 \times 10^{5}$ (10.0%)	n.q.	1.01×10^{6} a $\pm 1.32 \times 10^{5}$ (13.1%)	n.q.	n.q.	n.q.
9-Decenoic acid ethyl ester	4.34×10^{5} a $\pm 5.92 \times 10^{4}$ (13.6%)	5.39×10^{5} a,b $\pm 3.63 \times 10^{4}$ (6.7%)	6.27×10^{5} a * $\pm 1.41 \times 10^{5}$ (22.4%)	3.50×10^{5} a,b $\pm 7.95 \times 10^{4}$ (22.7%)	3.29×10^{5} b $\pm 3.73 \times 10^{4}$ (11.3%)	4.09×10^{5} a,b $\pm 1.42 \times 10^{5}$ (34.7%)
Undecanoic acid ethyl ester	7.23×10^{5} a $\pm 6.35 \times 10^{4}$ (8.8%)	6.93×10^{5} a $\pm 7.96 \times 10^{4}$ (11.5%)	6.25×10^{5} a,b $\pm 1.54 \times 10^{5}$ (24.6%)	5.11×10^{5} c,a,b $\pm 1.12 \times 10^{5}$ (22.0%)	4.06×10^{5} c,b $\pm 7.39 \times 10^{4}$ (18.2%)	2.99×10^{5} c $\pm 1.22 \times 10^{5}$ (40.7%)
Benzoic acid ethyl ester	1.01×10^{6} b ** $\pm 4.68 \times 10^{4}$ (4.6%)	2.57×10^{6} a $\pm 1.37 \times 10^{5}$ (5.4%)	6.48×10^{5} c ** $\pm 3.50 \times 10^{4}$ (5.4%)	1.05×10^{6} b $\pm 1.14 \times 10^{5}$ (10.9%)	7.84×10^{5} c $\pm 1.05 \times 10^{5}$ (13.4%)	6.43×10^{5} c $\pm 1.40 \times 10^{5}$ (21.7%)
Diethyl succinate	4.79×10^{5} c $\pm 2.27 \times 10^{4}$ (4.7%)	4.55×10^{5} c $\pm 4.11 \times 10^{3}$ (0.9%)	n.q.	2.00×10^{5} c $\pm 1.23 \times 10^{4}$ (6.2%)	1.11×10^{6} b * $\pm 9.50 \times 10^{4}$ (8.6%)	1.75×10^{6} a $\pm 3.96 \times 10^{5}$ (22.6%)
Total average peak area of minor ethyl esters	*2.94×10^{7} a * $\pm 2.93 \times 10^{6}$ (9.9%)*	*1.92×10^{7} b $\pm 4.51 \times 10^{5}$ (2.3%)*	*2.14×10^{7} b ** $\pm 1.63 \times 10^{6}$ (7.6%)*	*1.54×10^{7} c $\pm 1.67 \times 10^{6}$ (10.9%)*	*1.60×10^{7} c *** $\pm 1.28 \times 10^{6}$ (8.0%)*	*1.08×10^{7} d $\pm 8.72 \times 10^{5}$ (8.1%)*
Total average peak area	**6.72×10^{8} a ****	**4.41×10^{8} b**	**4.79×10^{8} b ****	**3.07×10^{8} c**	**1.38×10^{8} d**	**1.25×10^{8} d**
Acetates						
Ethyl acetate	7.14×10^{7} a,b $\pm 7.60 \times 10^{6}$ (10.7%)	5.45×10^{7} c $\pm 9.28 \times 10^{6}$ (17.0%)	6.33×10^{7} c,b $\pm 1.61 \times 10^{7}$ (25.4%)	7.60×10^{7} a,b $\pm 2.91 \times 10^{6}$ (3.8%)	7.23×10^{7} a,b * $\pm 5.84 \times 10^{6}$ (8.1%)	8.47×10^{7} a $\pm 4.87 \times 10^{6}$ (5.8%)
Isoamyl acetate	4.51×10^{7} c $\pm 6.62 \times 10^{6}$ (14.7%)	4.29×10^{7} c $\pm 1.14 \times 10^{6}$ (2.7%)	8.61×10^{7} b ** $\pm 9.07 \times 10^{6}$ (10.5%)	1.05×10^{8} a $\pm 3.15 \times 10^{6}$ (3.0%)	1.03×10^{8} a * $\pm 1.20 \times 10^{7}$ (11.6%)	8.14×10^{7} b $\pm 5.66 \times 10^{6}$ (7.0%)
Phenylethyl acetate	2.94×10^{7} d $\pm 2.33 \times 10^{6}$ (7.9%)	2.64×10^{7} d $\pm 4.30 \times 10^{5}$ (1.6%)	5.64×10^{7} c * $\pm 7.49 \times 10^{6}$ (13.3%)	3.96×10^{7} d $\pm 6.78 \times 10^{6}$ (17.1%)	8.10×10^{7} b $\pm 5.57 \times 10^{6}$ (6.9%)	9.83×10^{7} a $\pm 1.50 \times 10^{7}$ (15.3%)
Total average peak area	**1.46×10^{8} c ***	**1.24×10^{8} c**	**2.06×10^{8} b**	**2.20×10^{8} b**	**2.57×10^{8} a**	**2.64×10^{8} a**
Acids						
Acetic acid	7.05×10^{6} a * $\pm 1.02 \times 10^{6}$ (14.4%)	2.92×10^{6} b $\pm 1.27 \times 10^{5}$ (4.4%)	2.98×10^{6} b *** $\pm 6.39 \times 10^{5}$ (21.4%)	7.91×10^{6} a $\pm 8.15 \times 10^{5}$ (10.3%)	1.39×10^{6} c *** $\pm 9.37 \times 10^{4}$ (6.7%)	7.16×10^{6} b $\pm 1.09 \times 10^{6}$ (15.2%)
Hexanoic acid	1.40×10^{7} b $\pm 2.07 \times 10^{6}$ (14.8%)	1.33×10^{7} b $\pm 1.74 \times 10^{6}$ (13.0%)	1.71×10^{7} a $\pm 1.25 \times 10^{6}$ (7.3%)	1.72×10^{7} a $\pm 1.76 \times 10^{6}$ (10.2%)	6.31×10^{6} c $\pm 8.47 \times 10^{5}$ (13.4%)	8.25×10^{6} c $\pm 1.76 \times 10^{6}$ (21.4%)
2,5-Dimetil, 4-hexenoic acid	1.34×10^{6} a $\pm 7.0 \times 10^{4}$ (5.2%)	1.19×10^{6} a $\pm 2.54 \times 10^{4}$ (2.1%)	1.08×10^{6} a $\pm 1.59 \times 10^{5}$ (14.7%)	1.38×10^{6} a $\pm 3.25 \times 10^{4}$ (2.4%)	n.q.	1.06×10^{6} a $\pm 2.20 \times 10^{5}$ (20.7%)
Octanoic acid	9.60×10^{7} a $\pm 1.05 \times 10^{7}$ (11.0%)	9.69×10^{7} a $\pm 4.44 \times 10^{6}$ (4.6%)	1.04×10^{8} a $\pm 1.47 \times 10^{7}$ (14.2%)	9.09×10^{7} a $\pm 7.04 \times 10^{6}$ (7.7%)	3.37×10^{7} b $\pm 3.26 \times 10^{6}$ (9.7%)	3.00×10^{7} b $\pm 8.05 \times 10^{6}$ (26.8%)
Decanoic acid	1.48×10^{7} b * $\pm 3.61 \times 10^{6}$ (24.4%)	2.24×10^{7} a $\pm 3.37 \times 10^{5}$ (1.5%)	2.25×10^{7} a * $\pm 7.65 \times 10^{6}$ (34.0%)	1.14×10^{7} c,b $\pm 1.93 \times 10^{6}$ (16.9%)	6.44×10^{6} d,c $\pm 1.50 \times 10^{6}$ (23.0%)	4.72×10^{6} d $\pm 1.61 \times 10^{6}$ (34.0%)
Total average peak area	**1.29×10^{8} a**	**1.37×10^{8} a**	**1.42×10^{8} a**	**1.28×10^{8} a**	**4.78×10^{7} b**	**5.00×10^{7} b**
Aldehydes						
Octanal	1.72×10^{6} a $\pm 4.83 \times 10^{5}$ (28.1%)	1.37×10^{6} a $\pm 1.83 \times 10^{5}$ (13.3%)	8.88×10^{5} a * $\pm 4.49 \times 10^{4}$ (5.1%)	1.31×10^{6} a $\pm 3.26 \times 10^{5}$ (24.9%)	1.27×10^{6} a $\pm 4.12 \times 10^{5}$ (32.5%)	1.25×10^{6} a $\pm 1.72 \times 10^{5}$ (13.8%)
Nonanal	1.39×10^{7} a ** $\pm 6.02 \times 10^{5}$ (4.3%)	8.65×10^{6} b $\pm 1.07 \times 10^{5}$ (1.2%)	4.18×10^{6} c $\pm 1.49 \times 10^{6}$ (35.6%)	8.02×10^{6} b $\pm 1.66 \times 10^{6}$ (20.7%)	7.81×10^{6} b $\pm 1.50 \times 10^{6}$ (19.3%)	6.77×10^{6} b $\pm 2.12 \times 10^{5}$ (3.1%)

Table 2. *Cont.*

Compounds (†)	Craft Beer Types					
	Ale		Lager		Porter	
	Ale	Ale + Ch	Lager	Lager + Ch	Porter	Porter + Oak
Decanal	6.33×10^6 a $\pm 2.22 \times 10^6$ (35.1%)	8.62×10^6 a $\pm 1.15 \times 10^6$ (13.3%)	3.37×10^6 b ** $\pm 2.33 \times 10^5$ (6.9%)	8.76×10^6 a $\pm 1.97 \times 10^6$ (22.5%)	6.63×10^6 a $\pm 1.68 \times 10^6$ (25.3%)	6.63×10^6 a $\pm 1.65 \times 10^6$ (24.9%)
Total average peak area	**1.69×10^7 a**	**1.86×10^7 a**	**6.34×10^6 a ****	**1.81×10^7 a**	**1.34×10^7 a**	**1.10×10^7 a**
Ketones						
6-Methyl-5-hepten-2-one	6.76×10^5 b $\pm 5.73 \times 10^4$ (8.5%)	5.68×10^5 b $\pm 3.79 \times 10^4$ (6.7%)	1.45×10^6 a ** $\pm 1.43 \times 10^5$ (9.9%)	8.69×10^5 b $\pm 1.93 \times 10^5$ (22.2%)	1.55×10^6 a $\pm 7.44 \times 10^5$ (48.2%)	n.q.
2-Undecanone	1.97×10^5 c ** $\pm 3.65 \times 10^4$ (18.6%)	3.86×10^5 b $\pm 2.21 \times 10^4$ (5.7%)	4.27×10^5 b $\pm 4.89 \times 10^4$ (11.4%)	n.q.	6.41×10^5 a $\pm 1.69 \times 10^5$ (26.4%)	n.q.
Total average peak area	**8.72×10^5 b ***	**9.54×10^5 b**	**1.87×10^6 a *****	**8.69×10^5 b**	**1.41×10^6 a,b**	**(--)**
Terpenes						
Monoterpenes						
2-*β*-Pinene	3.80×10^6 a * $\pm 1.25 \times 10^6$ (32.9%)	3.15×10^5 b $\pm 7.76 \times 10^2$ (0.2%)	4.11×10^6 a ** $\pm 9.70 \times 10^5$ (23.6%)	3.96×10^5 b $\pm 9.97 \times 10^4$ (25.2%)	4.37×10^5 b $\pm 1.40 \times 10^5$ (32.2%)	7.00×10^5 b $\pm 2.41 \times 10^5$ (34.4%)
Δ-3-Carene	4.16×10^6 a $\pm 4.55 \times 10^5$ (10.9%)	n.q.	2.41×10^6 a $\pm 5.38 \times 10^5$ (22.3%)	n.q.	3.85×10^6 a $\pm 2.00 \times 10^6$ (52.1%)	5.48×10^5 b $\pm 1.18 \times 10^5$ (21.5%)
β-Phellandrene	3.10×10^7 a ** $\pm 5.88 \times 10^7$ (19.0%)	6.45×10^6 b $\pm 8.81 \times 10^3$ (0.1%)	2.70×10^7 a ** $\pm 5.61 \times 10^6$ (20.8%)	6.41×10^6 b $\pm 1.19 \times 10^6$ (18.5%)	6.41×10^6 b $\pm 1.06 \times 10^6$ (16.5%)	6.92×10^6 b $\pm 3.36 \times 10^6$ (48.5%)
DL-Limonene	7.44×10^7 a * $\pm 2.59 \times 10^7$ (34.8%)	5.67×10^6 c $\pm 1.22 \times 10^6$ (21.5%)	2.43×10^7 b *** $\pm 3.84 \times 10^6$ (15.8%)	3.49×10^6 c $\pm 9.90 \times 10^5$ (28.4%)	6.97×10^6 c $\pm 1.61 \times 10^6$ (23.1%)	7.16×10^6 c $\pm 9.67 \times 10^5$ (13.5%)
Eucalyptol	4.23×10^6 b * $\pm 3.52 \times 10^5$ (8.3%)	1.36×10^6 c $\pm 3.56 \times 10^5$ (26.1%)	6.89×10^6 a ** $\pm 2.01 \times 10^6$ (29.2%)	2.53×10^6 c,b $\pm 5.44 \times 10^5$ (21.5%)	n.q.	4.18×10^6 b $\pm 1.58 \times 10^6$ (37.8%)
β-Ocimene	3.33×10^6 a $\pm 9.09 \times 10^5$ (27.3%)	2.14×10^6 a,b $\pm 1.21 \times 10^5$ (5.7%)	3.20×10^6 a ** $\pm 3.83 \times 10^5$ (12.0%)	2.15×10^6 a,b $\pm 6.13 \times 10^4$ (2.8%)	2.38×10^6 a,b * $\pm 5.16 \times 10^5$ (21.7%)	1.41×10^6 b $\pm 5.77 \times 10^4$ (4.1%)
Linalool	4.92×10^6 b ** $\pm 7.39 \times 10^5$ (15.0%)	1.39×10^6 c $\pm 3.75 \times 10^5$ (27.0%)	1.27×10^7 a *** $\pm 1.30 \times 10^6$ (10.3%)	2.80×10^6 c $\pm 3.30 \times 10^5$ (11.8%)	2.14×10^6 c * $\pm 3.98 \times 10^5$ (18.6%)	1.47×10^6 c $\pm 2.56 \times 10^5$ (17.5%)
α-Humulene	8.02×10^5 c $\pm 1.60 \times 10^5$ (19.9%)	4.56×10^5 c $\pm 8.97 \times 10^3$ (2.0%)	2.81×10^6 b ** $\pm 6.58 \times 10^5$ (23.4%)	2.78×10^5 c $\pm 6.31 \times 10^4$ (22.7%)	8.94×10^6 a *** $\pm 9.50 \times 10^5$ (10.6%)	3.31×10^5 c $\pm 9.89 \times 10^4$ (29.9%)
β-Citronellol	4.98×10^6 a $\pm 9.69 \times 10^5$ (19.4%)	4.38×10^6 a $\pm 3.99 \times 10^4$ (0.9%)	1.63×10^6 c ** $\pm 8.53 \times 10^4$ (5.2%)	1.21×10^6 c $\pm 1.73 \times 10^5$ (14.3%)	4.61×10^6 a *** $\pm 1.79 \times 10^5$ (3.9%)	3.35×10^6 b $\pm 1.96 \times 10^5$ (5.9%)
Geranyl acetone	1.44×10^6 a ** $\pm 2.21 \times 10^5$ (15.3%)	4.74×10^5 b $\pm 7.26 \times 10^4$ (15.3%)	1.37×10^6 a $\pm 1.97 \times 10^5$ (14.4%)	1.62×10^6 a $\pm 1.86 \times 10^5$ (11.5%)	7.04×10^5 b $\pm 2.38 \times 10^5$ (33.9%)	4.83×10^5 b $\pm 3.46 \times 10^4$ (7.2%)
Total average peak area of monoterpenes	*1.12×10^8 a ** *$\pm 4.32 \times 10^7$ (38.5%)*	*2.26×10^7 b* *$\pm 5.76 \times 10^5$ (2.5%)*	*8.23×10^7 a **** *$\pm 6.62 \times 10^6$ (8.0%)*	*1.77×10^7 b* *$\pm 3.02 \times 10^6$ (17.0%)*	*3.43×10^7 b *** *$\pm 3.15 \times 10^6$ (9.2%)*	*2.05×10^7 b* *$\pm 5.09 \times 10^6$ (24.8%)*
Sexquiterpens						
Caryophyllene	1.21×10^6 b * $\pm 2.29 \times 10^5$ (18.9%)	3.40×10^5 b $\pm 1.94 \times 10^5$ (57.1%)	2.19×10^7 a $\pm 1.94 \times 10^6$ (8.9%)	n.q.	9.33×10^5 b $\pm 2.30 \times 10^5$ (24.7%)	n.q.
Cis-Calamenene	1.39×10^6 c * $\pm 1.55 \times 10^5$ (11.1%)	1.93×10^6 c,b $\pm 1.96 \times 10^4$ (1.0%)	2.69×10^6 b $\pm 7.98 \times 10^5$ (29.7%)	2.02×10^6 c,b $\pm 2.78 \times 10^5$ (13.8%)	4.46×10^6 a *** $\pm 4.81 \times 10^5$ (10.8%)	2.35×10^6 c,b $\pm 3.39 \times 10^5$ (14.4%)
Δ-Cadinene	5.39×10^5 b * $\pm 5.39 \times 10^4$ (10.0%)	4.19×10^5 b $\pm 2.12 \times 10^4$ (5.1%)	1.03×10^6 b $\pm 4.68 \times 10^5$ (45.5%)	5.49×10^5 b $\pm 2.51 \times 10^5$ (45.6%)	2.12×10^6 a ** $\pm 5.38 \times 10^5$ (25.4%)	4.45×10^5 b $\pm 2.78 \times 10^4$ (6.2%)
Cadalene	9.04×10^5 a,b $\pm 2.74 \times 10^5$ (30.3%)	9.00×10^5 a,b $\pm 1.80 \times 10^4$ (2.0%)	9.33×10^5 a,b $\pm 2.91 \times 10^5$ (31.2%)	7.88×10^5 c,b $\pm 4.95 \times 10^4$ (6.3%)	1.18×10^6 a ** $\pm 4.30 \times 10^4$ (3.6%)	5.18×10^5 c $\pm 1.44 \times 10^5$ (27.7%)
Total average peak area of Sexquiterpens	*3.75×10^6 b* *$\pm 3.79 \times 10^5$ (10.1%)*	*3.59×10^6 b* *$\pm 1.78 \times 10^5$ (5.0%)*	*1.56×10^7 a ** *$\pm 1.12 \times 10^7$ (51.9%)*	*3.36×10^6 b* *$\pm 5.65 \times 10^5$ (16.8%)*	*8.10×10^6 a,b **** *$\pm 2.71 \times 10^5$ (3.3%)*	*3.31×10^6 b* *$\pm 4.83 \times 10^5$ (14.6%)*
Total average peak area	**1.16×10^8 a**	**2.62×10^7 b**	**9.79×10^7 a *****	**2.10×10^7 b**	**4.24×10^7 b ****	**2.38×10^7 b**
Furfuryl derivates						
Furfural	3.12×10^5 d *** $\pm 1.51 \times 10^4$ (4.8%)	1.56×10^5 d $\pm 6.35 \times 10^3$ (4.1%)	6.95×10^5 a *** $\pm 8.44 \times 10^4$ (12.2%)	1.34×10^5 d $\pm 1.61 \times 10^4$ (12.0%)	4.11×10^5 b * $\pm 8.35 \times 10^4$ (20.3%)	2.78×10^5 c $\pm 5.42 \times 10^4$ (19.5%)
2-Acetylfuran	n.q.	n.q.	1.47×10^6 b ** $\pm 6.46 \times 10^4$ (4.4%)	7.08×10^5 b $\pm 6.63 \times 10^4$ (9.4%)	3.58×10^6 a $\pm 3.89 \times 10^5$ (10.9%)	3.09×10^6 a $\pm 6.17 \times 10^5$ (20.0%)
2-Furanmethanol (Furfuryl alcohol)	2.60×10^5 c $\pm 6.47 \times 10^4$ (24.9%)	2.43×10^5 c $\pm 1.33 \times 10^3$ (0.5%)	5.51×10^5 b ** $\pm 5.19 \times 10^4$ (9.4%)	3.99×10^5 c $\pm 5.48 \times 10^4$ (13.7%)	1.26×10^6 a $\pm 1.05 \times 10^5$ (8.3%)	1.34×10^6 a $\pm 1.56 \times 10^5$ (11.6%)
Total average peak area	**5.72×10^5 c ***	**4.00×10^5 c**	**1.98×10^6 b ***	**8.87×10^5 c**	**5.25×10^6 a**	**4.71×10^6 a**
Pyrazines						
2-Methyl pyrazine	n.q.	1.88×10^5 b $\pm 8.02 \times 10^4$ (42.7%)	n.q.	5.51×10^5 b $\pm 1.67 \times 10^5$ (30.3%)	2.92×10^6 a $\pm 3.73 \times 10^5$ (12.8%)	2.75×10^6 a $\pm 3.96 \times 10^5$ (14.4%)
3-Ethyl-2,6-dimethyl-pyrazine	n.q.	1.25×10^5 d $\pm 3.78 \times 10^3$ (3.0%)	4.55×10^5 c * $\pm 7.71 \times 10^4$ (16.9%)	3.38×10^5 c $\pm 4.92 \times 10^4$ (14.6%)	7.97×10^5 a $\pm 1.43 \times 10^5$ (17.9%)	6.38×10^5 b $\pm 2.72 \times 10^4$ (4.3%)
Total average peak area	**(--)**	**3.13×10^5 b**	**4.55×10^5 b ****	**8.89×10^5 b**	**3.72×10^6 a**	**3.39×10^6 a**
Other compounds						
p-Allyl anisole	3.92×10^5 a $\pm 6.04 \times 10^4$ (15.4%)	n.q.	2.01×10^5 a $\pm 2.30 \times 10^4$ (11.4%)	n.q.	n.q.	n.q.
Eugenol	3.21×10^5 b $\pm 1.38 \times 10^5$ (43.1%)	6.45×10^5 b $\pm 1.43 \times 10^5$ (22.3%)	2.24×10^7 a $\pm 8.42 \times 10^6$ (37.5%)	n.q.	n.q.	n.q.
2-Acetylpyrrole	3.81×10^5 b * $\pm 1.90 \times 10^4$ (5.0%)	4.77×10^5 b $\pm 4.84 \times 10^4$ (10.2%)	7.38×10^5 b $\pm 8.42 \times 10^4$ (11.4%)	6.74×10^5 b $\pm 1.29 \times 10^5$ (19.2%)	1.56×10^6 a $\pm 1.41 \times 10^5$ (9.0%)	1.59×10^6 a $\pm 5.17 \times 10^5$ (32.5%)
Methoxy phenyl oxime	1.90×10^7 c *** $\pm 2.10 \times 10^6$ (11.0%)	4.31×10^7 a $\pm 2.76 \times 10^6$ (6.4%)	2.18×10^7 c ** $\pm 4.53 \times 10^6$ (20.8%)	3.45×10^7 b $\pm 2.03 \times 10^6$ (5.9%)	2.51×10^7 c $\pm 2.66 \times 10^6$ (10.6%)	2.25×10^7 c $\pm 4.88 \times 10^6$ (21.7%)
Acetoin	3.69×10^6 b * $\pm 4.64 \times 10^5$ (12.6%)	2.41×10^6 c $\pm 4.87 \times 10^5$ (20.2%)	2.51×10^6 c $\pm 2.80 \times 10^5$ (11.2%)	2.55×10^6 c $\pm 6.51 \times 10^5$ (25.5%)	1.88×10^6 c ** $\pm 7.11 \times 10^5$ (37.8%)	4.83×10^6 a $\pm 9.89 \times 10^5$ (20.5%)

(†) Values expressed in the average peak area of four replicates; (††) coefficient of variation (%); Ale—control Ale beer; Ale + Ch—Ale beer with cherry (*Prunus avium*) wood chips contact; Lager—control Lager beer; Lager + Ch—Lager beer with cherry (*Prunus avium*) wood chips contact; Porter—control Porter beer; Porter + Oak—Porter beer with oak (*Quercus petraea*) wood chips contact; n.q.—not quantifiable; (--) — not quantified; values with same letters for each volatile compound in line are not significantly different (Tukey's test, $p < 0.05$); the asterisks mark a significant difference between each beer group at different levels for each individual compound (*t*-Student's *t*-test): * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).

Values of 2-methyl-1-propanol (isobutyl alcohol) and other aliphatic alcohols (>C_6) were low, which agrees with the previously refereed papers [6,9], and Lager beers showed the lowest values. Moreover, the three craft beers showed high values of phenethyl ethanol, an aromatic alcohol that occurs widely in nature, is present in a variety of essential oils and fermented foods, and has a pleasant aroma with floral, fruity, and honey notes. In fermented beverages such as beer, this compound is formed from l-phenylalanine via the action of three enzymes via the Ehrlich pathway [50].

No significant effect of chip maceration on the total level of alcohols was observed. However, some differences were detected in some cases (Table 2). Thus, low peak area values of octanol and heptanol were observed in Porter and Lager beers macerated with chips compared with their respective control beers. These results could be associated with the possible adsorption of the alcohols by the wood and agree with those found by Del-Barrio-Galan et al. [51] in a red wine aged with oak wood chips. Furthermore, the possible extraction of alcohol from wood could explain the levels of 1-nonanol in macerated Ale and Lager beers. In fact, a few authors previously found 1-nonanol in oak and cherry woods [52]. It is also important to note that for these control beers, this alcohol was not quantified.

3.1.2. Esters

It is well known that esters have an important role in the aroma of fermented beverages. They are one of the main volatiles in beer [6] that provide fruity flowery pleasant notes, although high concentrations of these compounds can produce undesirable overly fruity aromas [9].

The main groups of detected volatile esters were ethyl esters and acetates. Hexanoic and decanoic ethyl esters, and ethyl, isoamyl, and phenylethyl acetate, were the compounds with the higher peak areas (Table 2). These results agree with others found in the literature, which established that the compounds cited before, together with the octanoic acid, are the main representative esters of beer [8,10]. Longer-chain fatty acid esters such as ethyl acetate esters (C_{13}–C_{22} long) are usually present at lower values in beers [6], a fact that avoids the risk of undesirable flavor occurring when their levels overpass their taste threshold [9].

In general, the maceration with wood chips produced a decrease in the ester levels. However, this tendency was variable among beers and volatile esters. The results seemed to show that minor ethyl esters (heptanoic acid, octanoic acid, nonanoic acid, tetradecanoic acid, *trans*-4-decenoic acid, 9-decenoic acid, undecanoic acid, benzoic acid, and diethyl succinate) were those more susceptible to be affected by maceration with chips, and this occurred especially in Ale beer (higher quantitative difference). In addition, *trans*-4-decenoic acid ethyl ester was only quantified in Ale and Lager beers not macerated with wood chips. These results agree with those found by Del-Barrio Galán et al. [51] in a red wine macerated with oak chips but contrasted with those described by Wyler et al. [15] who reported a significant increase in the ester values during the maturation of beers aged in oak barrels and with oak cubes for three months. Differences in the type of beer and in the conditions of maceration (time, type, and size of chips, etc.) together with adsorption phenomena could explain these differences. Coelho et al. [53] reported that wood, particularly oak, adsorbs compounds, especially esters.

3.1.3. Acids

Many organic and short-chain fatty acids have been reported in beers coming from diverse routes. However, they are quantitatively only minor constituents of beer, although they play an important role in beer taste and foam. Organic acids are directly involved in beer flavor, give acidity, but also contribute to flavor due to their participation in the formation of diverse volatiles (i.e., acetates, succinate, etc.). Free fatty acids also contribute to flavor mainly through their derivate esters.

The acids detected in the studied craft beers (Table 2) agree with the previous results found by Briggs et al. [49] who noted that beer acids, particularly C_4, C_6, and C_{10} acids, are predominant, deriving from fatty acid metabolism in yeast. In our work, the acids quantified at the highest levels for all craft beers were octanoic, decanoic, and hexanoic acids. This result partially confirms previous work by Dennenlöhr et al. [54] that indicates that hexanoic acid is one of the many volatile compounds present in beers.

Acetic acid is a common constituent of fermented beverages. However, the levels of this acid should be low because it contributes to unpleasant flavors. Thus, for Ale beers maceration with wood chips did not have a significant effect on this volatile acid content. However, for Lager and Porter beers after cherry and oak chips maceration, an increase in acetic acid was detected. This result can be explained by the fact that wood, especially after toasting, could contain a certain quantity of acetic acid [55].

3.1.4. Aldehydes

The aldehydes found in this work are mainly formed from lipid oxidation and during alcoholic fermentation, with acetaldehyde being the major beer aldehyde [56]. However, acetaldehyde was not detected in this study, probably due to its incorporation in the acetate's formation. Being bound to other compounds, obscuring them from instrumental detection, could also explain the detection of only three aldehydes in this work (octanal, nonanal, and decanal) [57]. The three aldehydes detected agree with previous studies. Ruvalcaba et al. [58] detected octanal and nonanal in 30 beer samples from different styles, while Alves et al. [8] detected nonanal in vestigial levels. Decanal was also detected in several rice beers by Lyu et al. [59], and together with nonanal in Lambic beer style [27].

Chip maceration produced significant changes only in particular cases, and opposite effects were observed. Therefore, while Lager beer macerated with cherry chips showed higher peak area values of octanal and decanal than the control beers, in Ale beers the chip maceration had a significant effect only on the nonanal level which decreased proximally twice (Table 2). According to Wyler et al. [15], the increase in aldehydes in beers can be partially indicative of the aging time because the increase in these compounds occurred during the beer maturation in oak barrels and in contact with oak chips. However, other researchers [60] also indicated that the extraction of aldehydes from oak barrels should also be considered, although they observed lower values of aldehydes in barrel-aged beers than in non-aged beers, explaining this fact based on the antioxidant role of malt melanoidins and barrel polyphenols.

3.1.5. Ketones

Diverse ketones are usually present in beers, with 2,3-butanedione and 2,3-pentanedione being the most noteworthy [56]. However, during maturation, they are reduced by rending acetoin and other compounds. Probably, this fact explains the presence of acetoin in the craft beers while no diacetyl (2,3-butanedione) was identified. However, acetoin is also formed during fermentation by the microbial activity of lactic acid bacteria and yeasts, and other authors have detected acetoin in beers [53].

In this study, two ketones were quantified (6-methyl-5-hepten-2-one and 2-undecanone) and maceration with chips did not produce any significant effect on the levels of these two ketones in Ale beer (Table 2). However, a significant reduction in the levels of ketones in Lager and Porter beers was detected. This fact could be associated with the absorption effect but also with some degradative effects of the wood and interaction between the ketones and wood components [61].

3.1.6. Terpenes

The ten monoterpenes and four sesquiterpenes identified in the studied beers have also been found in other beers and hops. According to several reports [2,5], around 80 terpenic compounds have been reported in the beer volatile compositions of an extensive range of beer types. Several factors determine the presence and content of terpenes in beers, with the characteristics of hops [2,35] and the hopping regimes used (kettle, late, or dry hopping) being the most notable. Furthermore, hop terpenes can be transformed in other terpenic compounds (such as geraniol in linalool and nerol also in linalool) by different mechanisms, such as biotransformation by yeasts [49,62].

Data from this study showed that, in general, β-phellandrene and DL-limonene were the compounds with the higher peak areas, although in Lager beer, linalool and caryophyllene showed similar values (Table 2). The results evidenced that wood chip maceration significantly reduced the levels of terpenes, with an intense decrease in the peak area values for many of the identified monoterpenes, especially in the case of Lager beer followed by Porter beer. Then, although previous works detected the presence of DL-limonene, α-terpineol, and linalool in oak and cherry woods [63], the obtained results pointed out that there was no extraction, and wood chips did not contribute positively to the terpene levels of the beers macerated with the chips.

3.1.7. Furfural, Pyrazines, and Other Compounds

The thermal process involved in beer manufacturing (mainly malt drying and toasting, wort boiling, and malt mashing) contributes to the formation of furfuryl derivates and pyrazines [64]. The Porter beer was richer in these kinds of compounds, mainly because of the use of a roasted black malt during the beer production which was submitted to a previous toasting process. Toasting gives rise to many aromatic compounds via pyrolysis and hydrothermolysis, which explains the fact that the more intense the heat treatment, the more new classes of odoriferous volatile substances (pyrazines and furfuryl derivates) and several phenols are formed [65]. Similar comments can apply to data of 2-acetylpyrrole. This compound derives from roasted malts [66], and the highest concentrations appear in malts produced at high temperatures. Other authors also found this compound in diverse types of beers [67,68].

In this study, maceration with chips did not increase the total levels of furfuryl compounds, and in Lager beers macerated with chips, their levels were significantly lower than in the control beers (Table 2). In the case of 2-acetylfuran, this compound was not even possible to quantify in Ale beer, indicating that no significant wood extraction occurred. This fact could be due to the low alcohol content of the beers being lower than those of other alcoholic beverages such as wine or spirits.

Concerning total levels of pyrazines, these were higher in Ale beer macerated with wood chips compared with the control Ale beer. In this case, the two pyrazines detected in the remaining beers (2-methyl pyrazine and 3-ethyl-2,6-dimethyl-pyrazine) were not quantified in the control Ale beer. This result agrees with those found by Coelho et al. [10], who reported 2-methyl-pyrazine in Ale beers aged in oak wood barrels. Furthermore, this compound also appeared in toasted cherry wood [14], which may justify its presence in Ale and Lager beer macerated with cherry chips. However, Kishimoto et al. [7], studying the components of the aroma in commercial Japanese Pilsner-type beers, also detected the presence of 3-ethyl-2,6-dimethyl-pyrazine in these beers produced without any wood contact.

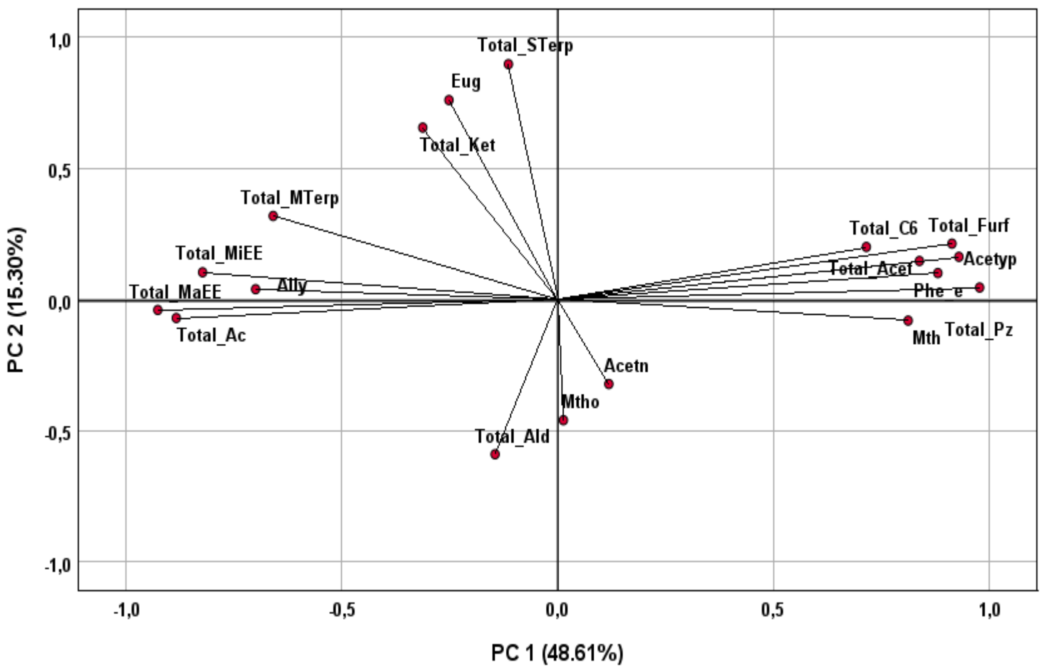
With respect to the three remaining volatile compounds: *p*-allyl anisole, eugenol, and methoxy-phenyl-oxime, a clear effect associated with wood chips maceration was not observed (Table 2). For *p*-allyl-anisole, this was exclusively found in Ale and Lager beers without cherry wood chip maceration, while eugenol was quantified in both Ale beers, having higher peak area values that have been detected in Ale beer macerated with cherry wood chips. The presence of eugenol in several types of wood used for beverage aging is well known, namely, in toasted oak and cherry woods [12]. Thus, the presence of eugenol has also been quantified in beers kept in contact with oak wood chips and barrels during the aging process [18]. However, several phenols, also including eugenol, come from yeast metabolism and are also present in beers without wood contact [59].

It could be expected that other well-known wood volatile compounds such as 4-ethylphenol, *cis* and *trans* whisky lactones, vanillin, syringol, etc., could be found in these beers as they have been described in other beers aged with wood fragments by other authors [15,18,53]. However, the concentrations of chips used in previous studies were much higher than those employed in this work: Wyler et al. [15] used 3 g/L; Sterckx et al. [18] used 5 g/L; and Coelho et al. [53] used 20 g/L and, at the same time, some of them applied a longer wood chip contact time. Furthermore, in the case of the beers studied by Sterckx et al. [18], where several wood volatile compounds were detected, their alcohol degree was 8.2% (v/v), which is almost twice the alcohol content of the craft beers elaborated in this work. Previously Garde-Cerdán et al. [69] reported the influence of alcohol content on the accumulation of several volatile wood compounds, which was more favored in wines with higher alcoholic content than in wines with lower alcohol content. Finally, temperature is also an important factor that induces wood compound extraction. Some authors reported the potential effect of higher temperatures on the extraction of wood compounds in wines [70]. Similarly, in beers analyzed by Coelho et al. [53], several volatile oak wood compounds were detected after storage at 40 °C. This is a much higher temperature than that used in this study. Considering these points, the low alcohol content of the beers and the low ratio of chips per liter, together with the short maceration time assayed in this study, explain the fact that no wood volatile compounds were detected in the studied craft beers.

3.2. PCA Applied to the Volatile Characterization of Craft Beers

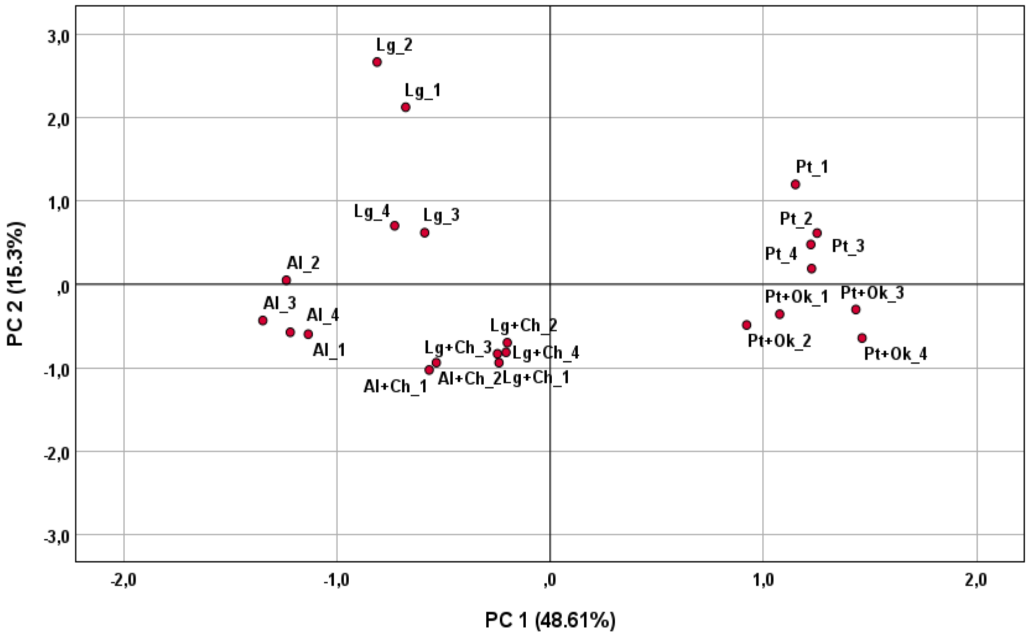
A principal component analysis (PCA) was used to better understand the global effect of chip maceration on the volatile profile of the craft beers. Considering the relatively low number of samples, the variables used in the PCA were the total values of the different subgroups (ethyl ester, acetates, monoterpenes, etc.) and the individual compounds (non-grouped) indicated in Table 2.

The main results obtained are graphically summarized (Figure 1). The weight of each variable in the first two principal component (PC) analyses noted that most of the variables (12 of 18) had stronger weight in the first component (PC 1) results than in the second (PC 2) results (Figure 1a), and an equal distribution of variables with positive and negative weight was observed in each PC result. In addition, the distribution of the beer samples in the plane defined via the first two principal component (PC) analyses, which explained 64% of the total variance, pointed out a complete separation of the samples. Furthermore, the PCA results indicated a high difference between Porter beer and Ale and Lager beers, which showed more similar profiles, especially after maceration with cherry chips (Figure 1b), which is more extensively commented on later.



(**a**)

Figure 1. *Cont.*



(**b**)

Figure 1. Graphical representation of PCA analysis. (**a**) Variable weights in the two first principal component (PC) results. Acet—acetates; Acetn—Acetoin; Acetyp—2-Acetylpyrrole; Ac—acids; Ald—Aldehydes; Ally—p-Allyl anisole; C6—>C6 alcohols; Eug—Eugenol; Furf—furfuryl derivates; Ket—ketones; MaEE—major ethyl esters; MiEE—minor ethyl esters; MTerp—monoterpenes; Mth—2-Methyl-1-propanol; Mtho—Methoxy phenyl oxime; Phe_e—Phenethyl ethanol; Pz—pyrazines; STerp—Sexquiterpenes; and (**b**) sample projections in the plane defined by PC 1 and PC 2. Al—Ale beer; Lg—Lager beer; Pt—Porter beer/Ch—cherry (Prunus avium) chips; and Ok—oak (Quercus petraea) chips.

Porter beer was characterized by a higher level of volatile compounds related to the thermal process of the malt used for the elaboration of this style of beer: pyrazines, pyrroles, and furfural derivates, but also by higher levels of phenethyl ethanol and acetates, while showing lower levels of acid, terpenes, and esters. Lager and Ale beer differed mainly in the levels of terpenes and ketones, being higher in the larger beers (higher values of PC 2), while Ale beers showed higher levels of ethyl esters but minor levels of acetates in the second (lower values of PC 1).

The Ale and Lager beer macerates with chips were grouped together in the plane defined by PC 1 and PC 2 (Figure 1b). They showed lower levels of the PC 2 component than their control beers, with this decrease being more important in the larger beers. Ale beers macerated with chips also showed higher levels of PC 1 than their control beers. This is a consequence of the loss of volatile compounds associated with wood maceration and the formation of acetoin, aldehydes, and 2-methyl-1-propanol. In the case of Porter beers, the minor modifications in their volatile compound profiles explain the absence of variation in the PC 1 values and the slight decrease in the PC 2 values (Figure 1b).

4. Conclusions

The three different craft beers used in this study after maceration for 30 days with cherry and oak wood chips showed some surprising significant differences in their volatile compound profiles. In fact, for most of the volatile groups quantified, beers without wood chip contact showed significantly higher peak area values. These results seem to suggest that maceration time and wood chip concentration were not enough to produce the desired increase in the flavor complexity through the increase in the volatile levels and by the incorporation of wood volatiles not present in original beers. The low extraction could also be associated with the relatively low alcohol values of the studied beers. However, it can be concluded that the outcomes of this study could be of practical interest to brewers, allowing them to make better use of different wood species and to have a perspective of how cherry and oak wood chips could contribute to volatile composition and beer aroma profile. Thus, further research involving different maceration times and wood chip concentrations will be necessary to improve the understanding of the potential impact of using wood chips on beer quality.

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