



# Bergamot and olive extracts as beer ingredients: their influence on nutraceutical and sensory properties

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## Abstract

*Citrus bergamia* and *Olea europaea* L. variety Carolea are accounted as niche functional food for their high content of bio active compounds. Their extracts were used as adjunct to produce two beers with different styles, Blanche and Weiss, rich in antioxidants for a pool of consumers interested in a healthy lifestyle. The nutraceutical properties of these two beers were compared to Blanche and Weiss without any addition to verify if the beers enriched with natural extracts changed their aromaticity, flavors, and functionality. The antioxidant activity changed in the order: blanche bergamot beer > Weiss olive beer > blanche basal beer > Weiss basal beer. The phenolic profile of bergamot beer was qualitatively and quantitatively the richest in bio-compounds. Pearson's correlation evidenced that total phenols contained in bergamot and olive beers were positively and significantly correlated with the antioxidant activities and precisely, with 2,2-diphenyl-1-picrylhydrazyl (DPPH) and total antioxidant capacity (TAC). Correlation data evidenced that the bergamot was the beer with the greatest antioxidant activity and bioactive compound amount. This study highlighted as the addition of these natural extracts together with the right productive process improved sensorial beer properties, satisfying consumer taste while potentially increasing the beneficial effects on human health.

**Keywords** Antioxidants · Craft beers · Flavonoids · Phenols · Sensory properties

## Introduction

Beer is one of the oldest most popular alcoholic beverages over the world [1], typically, produced with natural ingredients as barley (*Hordeum vulgare*), hops (*Humulus lupulus* L.), water, and yeast. In many cases, beers can be integrated with other cereals, sugars or natural herbal extracts called adjunct. Hops are multi-functional ingredients with preservative role which, release bitter compounds (humulones) [2] adding flavor, aroma, and bitterness to the beers. Beer is a drink that contains valuable nutritional compounds as carbohydrates, amino acids, minerals, vitamins, and polyphenols. About 30% of beer polyphenols come from hops, and 70% come from malts [3]. The antioxidants and polyphenols associated with a low alcoholic content determine the functional quality of the beers. Numerous researches evidenced that modest beer consumes can have anti-inflammatory and antioxidant properties [4, 5], with numerous human health benefits [6–8]. Currently, there is an increased interest of consumers for craft beers (CB), which are distinctly flavored, have a quality value unique and sensory properties details [9]. Craft beers are unpasteurized, unfiltered, without

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the addition of nitrogen or carbon dioxide under pressure [10], but with the addition of aromatic herbs, spices, fruit, honey, sugar, and coffee, that satisfy sensorial perception and improve their nutritional and functional values increasing the number of bio-compounds that can have beneficial impacts on one's health [11]. The goal of this manuscript was to develop beers using natural extracts from fruits accounted as niche functional food, bergamot (*Citrus bergamia*) and Olive (*Olea europaea* L. variety *Carolea*), whose beneficial properties depend on the specific geographic areas in which they grow (Grecanic costal area of Calabria Region, south Italy). Bergamot contains flavonoids and other beneficial components that contribute to its antioxidant, anti-inflammatory capacity and its ability to reduce cholesterol. Olives have important nutritional values depending on richness in monounsaturated fat, fiber and vitamin E together with the presence of several phytochemicals, mostly phenolic compounds that, acting as scavengers of reactive oxygen species, have the capability to protect from oxidative damage [12]. Extracts of Bergamot and Olive, rich in bioactive compounds, have been used as adjunct for personalizing a beer style and for producing functional beers, moderately alcoholic and rich in antioxidants, for a pool of consumers interested in a healthy lifestyle. In this manuscript, the quality of the craft beers enriched with bergamot or olive extract was compared to craft beers of the same typology (basal). Aroma beer fingerprints were also assessed to verify if the addition of extracts affected the aromaticity and flavor of the basal beers.

## Materials and methods

### Chemicals and reagents

Acetonitrile (ACN) HPLC grade, and formic acid were purchased from Sigma-Aldrich (Milan, Italy), Ultra-pure water was obtained using a Milli-Q system (Millipore, Milan, Italy). Flavonoids standard (Coumaric acid, 4-Hydroxybenzoic acid, Caffeic acid, Ethylgallate, Ferulic acid, Kampferol, Naringin, Protocatechuic acid, Syringic acid, Vanillic acid, Hesperidin, Sinensetin, Neoeriocitrin, (–) Epicatechin, Neohesperidin, Tangeretin, Chlorogenic acid and Nobiletin) was purchased from Extrasynthese (Genay Cedex, France). All the other chemicals were purchased from Sigma-Aldrich (Milan, Italy).

### Productive process

Brewing is an extremely complicated process involving highly complex chemical and biochemical reactions under highly variable process conditions. Therefore, both the compositions and the concentrations of the reducing substances

can change during the process even if the raw materials and the applied treatments are the same. Slight changes in structure, or even changes in shape, can alter the antioxidant activity of a compound. As it is known, beer shelf life and flavor are partially influenced by its antioxidant status [13]. To stabilize the productive process, we used 20 hectolitre Tanker EVO 2000 BBC Inox technological plant that allows to optimize production capacity while respecting quality. The new fully automatic brewhouse wisely combines technological research and the flexibility of the artisan process. Automation, in fact, allows the replication of tested recipes, optimizing production times and energy, allowing the brewer to give maximum expression to enhance the raw materials. Every detail has been carefully studied to minimize contact with oxygen from the malt grinding phase to the transfer to the fermentation tank. The whole system is also designed to be washed and sanitized automatically. Brewing plant has also cold chain system important to maintain the products during the stabilization of the beer. In the brewery, there is an automatic isobaric and under nitrogen bottling plant (Isobaric, Gai) to reduce at the minimum of the risks of beer oxidation. To produce the blanche, malt, barley, and oats were mixed in a well-established proportion, the hops were *Styrian Golding* and *Czech Saaz*. Extract of bergamot juice after terpene elimination was added to the blanche. Weiss was prepared using malt and barley and *Hallertau Hersbrucker* as hop. The extract from olive fruit of *Carolea* cultivar was added. The setting of the production process, the concentration of the ingredients used, and their mix ratio are covered by industrial secret. The fermentation batch was repeated three times for each typology of beer.

### Sample preparation

The present work analyzed and compared the chemical and sensory properties of two craft Beers produced in Calabria: Heraclea, Blanche with the addition of bergamot juice extract, not filtered and not pasteurized; Elais, Weiss with the addition of olive extract not filtered and not pasteurized. The two Calabrian beers have been compared to the Blanche and Weiss without any addition to verify if the beers enriched with natural extracts changed their aromaticity, flavors, and functionality.

The analysis has been carried out in triplicate for each batch and for each typology of Craft beer. The samples were degassed at 20 °C prior to testing, using magnetic stirrer until all gas has been released.

### Beer analytical methods

The methods for the analysis of the beers, and precisely alcohol, bitterness, color, pH, foam, haze, and shelf life are

described in Analytica EBC by European Brewery Convention [14, 15].

Beer pH was measured with pH-meter. The determination of alcohol content was performed by measuring the density of distillate from the degassed beer sample, which distillate is assumed to contain all the alcohol in the sample and nothing else except water. The alcohol content of beer is expressed in percentage (% v/v).

### Color determination

Color of the degassed beer was measured at 430 nm by a UV–Vis spectrophotometer according to the EBC method [14]. Color was expressed in EBC units and calculated according to the formula:  $C = A430 \cdot f \cdot 25$  where  $C$  gives the color (EBC),  $f$  is the dilution factor, and  $A430$  is the absorbance at 430 nm.

### Turbidity and bitterness assay

Beer turbidity was carried on by nephelometric method. The amount of turbidity is expressed in EBC units. The amount of turbidity was measured in NTU units and was expressed in EBC unit. Bitterness was measured using a spectrophotometer (UV–Vis) at 275 nm [14].

### Anti-oxidant compounds' determination

#### Total phenolic content (TP)

The method described by Singleton and Rossi [16] performed the TP analysis. The absorbance was measured at 725 nm. Quantification was carried out based on the standard curve of tannic acid, and the concentration of TP was expressed as tannic acid (TA) milligram per L of extract.

#### Total flavonoids' content (TF)

TF was determined by the colorimetric method described by Chang et al. [17] with modifications. The reaction between flavonoids and aluminum chloride forming the flavonoid– $Al^{3+}$  complex was determined at 510 nm. The results were expressed as milligram of quercetin (QE) per L of extract.

#### Total carbohydrates and ascorbic acid detection

Total carbohydrates were detected using the anthrone method with minor modifications [18]. Sugars react with the anthrone reagent under acidic conditions to yield a blue–green color. The samples were mixed with sulfuric acid and the anthrone reagent and then boiled until the reaction was completed. The solution was then cooled the

absorbance measured at 620 nm. There was a linear relationship between the absorbance and the amount of sugar present in the sample. This method determines both reducing and non-reducing sugars because of the presence of the strongly oxidizing sulfuric acid. Like the other methods, it is non-stoichiometric, and therefore, it is necessary to prepare a calibration curve using known glucose concentrations. For ascorbic acid determinations, the method reported in Muscolo et al. [18] was used.

### Anti-oxidant activity assays

The method reported in Muscolo et al. [18] was used to determine the 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH•) scavenging assay. DPPH• concentration in the cuvette has been chosen to give absorbance values of ~1.0. The reaction mixtures were composed of: 10 µL of each extract, 700 µL DPPH• and 95% ethanol brought to 1.0 mL. The change in absorbance of the violet solution was measured at 517 nm after 30 min of incubation at 37 °C. DPPH activity was expressed as µM of Trolox (T) using a calibration curve (1.0–50 µM T).

Total antioxidant capacity (TAC), as the oxygen radical absorbance capacity (ORAC) assay, which measures anti-oxidant inhibition of peroxy radical-induced oxidations and represents a measure of total antioxidant capacity, has been determined as reported in Papalia et al. [19].

### Polyphenols' profile determination

The beer samples were degassed by magnetic stirring (500 rpm) for 8 h and subsequently subjected to filtration through a 0.45 µm regenerated cell membrane filter (Aisino Corporation).

The polyphenolic profile of the beer samples was assessed by Ultra-High-Performance Liquid Chromatography system. The equipment consisted of a Photo-Diode Array detector (RP-UHPLC-DAD, Shimadzu, Milan, Italy), equipped with a column oven (CTO-20AC), an autosampler (SIL-30AC), an in-line degasser (DGU-20A5R), a communication module (CBM-20A), two parallel flow pumps with double piston (LC-30AD), and photodiode array detector (SPD-M30A).

Chromatographic separation was carried out with a Kinetex C18 50 mm × 3 mm × 1.7 µm d.p. column (Phenomenex), in addition, a Kinetex C18 guard column was used (Phenomenex). The analyses were conducted using the following optimized chromatographic conditions: water with 0.1% formic acid (mobile phase A), acetonitrile with 0.1% formic acid (mobile phase B), flow 0.6 mL/min, and oven temperature 40 °C.

Analysis was performed in gradient elution as follows: 1% B for 5 min, then a gradient 15 min from 1% to 30% of B and 7.5 min of higher gradient from 30% to 65% of B, and finally

washing and reconditioning of the system, with a separation time of the analytes considered of about 28 min.

The parameters for the photodiode array detector were: spectrum resolution 256, split width 8 nm, cell temperature, 40 °C, and sampling rate 40 Hz. Data acquisition was obtained in the range 190–400 nm and the chromatograms were acquired at the maximum absorbance of the compounds of interest.

## Sensory analysis

The ultra-rapid gas chromatographic analysis (UFGC) by odor analyzer called Heracles II (mod. Heracles II, Alpha MOS, Toulouse, France) was used for the analysis, using a detector system containing two short different polarity columns (MXT-5 a polar and MXT-1701 slightly polar) connected to 2 *flame ionization* detectors (FID) for a global fingerprint and a data acquisition and processing system (Alpha MOS proprietary software (Alpha Soft)).

It consisted of an Odorscanner headspace autosampler (mod. HS 100, CTC Analytics, Zwingen, Switzerland), to automate sampling and injection, a detector system containing two short different polarity metal columns working in parallel: a non-polar column (MXT-5: 5% diphenyl, 95% methylpolysiloxane) and a slightly polar column (MXT-1701: 14% cyanopropylphenyl, 86% methylpolysiloxane), length of 10 m, diameter of 180 µm (*Restek*), connected to two flame ionization detectors (FID1 and FID2) for a global fingerprint and a data acquisition and processing system AlphaSoft 12.4 software (Alpha MOS proprietary software).

Two chromatograms are obtained at the same time, allowing a well-defined identification of chemical compounds. The integrated solid adsorbent trap thermo-regulated by Peltier cooler (0–260 °C) achieves an efficient pre-concentration of light volatiles and shows a great sensitivity (in the pg range). With fast column heating rates (up to 600 °C/min), results are delivered within seconds and the analysis cycle time is from 5 to 9 min.

Heracles Analyzer provides a unique signature for each sample examined, through a chemical and/or olfactory fingerprint, quantifying specific molecules in complex matrices [20]. The response of each detector is converted into a chemical fingerprint by powerful software. For the calculation of Kovat's indices and the identification of volatile organic compounds, the alkane C6–C16 standard solution was used. Collected data were analyzed using Kovats Retention Index (RI) values, and specific compounds were identified by AroChemBase library of chemical compounds with name, formula, CAS number, molecular weight, Kovats retention Index, sensory attributes, and related bibliography, was used for confirming identification. The AroChembase (Alpha MOS, Toulouse, France) is an add-on module that can be used within the Heracles AlphaSoft 12.4 software.

It allows to pre-screen the chemical compounds and to give sensory features from the Heracles chromatograms.

To obtain maximum sensor response, the operating parameters were optimized as previously reported in Muscolo et al. [21] with modifications as follows: 1 mL of each sample diluted with water (1:5) was placed in a 10 mL glass vial, sealed with magnetic plugs. The vials were placed in the Heracles autosampler (Odor Scanner HS 100, Gerstel, Mülheim, Germany) for headspace generation leaving to equilibrate for approximately 20 min at 40 °C. Gas accumulated in the headspace of the sample was used for the analysis. Syringe pierced the silicone septum of the magnetic plug and for each sample, approximately, sampled 1 mL the headspace delivered at 125 µL/s by the autosampler to the injector at 270 °C. The 1 mL headspace aliquot was, before the chromatographic separation, adsorbed on a TENAX absorbent trap maintained at 40 °C for 30 s, while the carrier gas (H<sub>2</sub>) flowed (flow rate: 1 mL/min) through it to concentrate the analytes and to remove excess air and moisture. Desorption was obtained by increasing the temperature of the trap up to 240 °C in 30 s and the sample was injected. The thermal program started at 40 °C (held for 18 s) and increased up to 250 °C at 3 °C/s and held for 30 s. The total separation time was 118 s.

## Statistical analysis

Analysis of variance was carried out for all the data sets. One-way ANOVA with Tukey's Honestly. Powerful Statistical Analysis and Graphics Software for Windows 7 was used for all the statistical analyses. Effects were significant at  $p \leq 0.05$ .

## Results and discussion

The main factors characterizing beer are alcohol content, color bitterness, and variety and intensity of flavors. These characteristics are standardized and allow a uniform determination of the overall qualities of any beer. Data showed that all the beers had the same alcohol content. Conversely, significant differences were observed in color intensity, expressed as EBC, that is also a measure of beer turbidity and in IBU that is not a sensorial perceived beer parameter of bitterness, but it is rather the expression of the amount of iso-alpha acids presents, that not only plays an essential role in enhancing foam stability, but, as reported by Ano et al. [22], has a role in the suppression of neuro-inflammations and improvement of cognitive functions appearing useful for the prevention of dementia. Among the analyzed beers, the highest EBC value was observed for the Weiss, and the lowest one for Heraclea (bergamot extract) and Elais (olive extract). These data evidenced a less turbidity of both

Heraclea and Elais with respect to the basal Blanche and Weiss beers. The bitterness was the highest in the beers with the addition of the fruit extracts. The parameters of bergamot and olive beers were within the range of Blanche and Weiss category, respectively (Table 1). The pH average values of the analyzed beers were in the normal range of category to which they belong (between 3.8 and 4.7). pH is a really important parameter, not only because it conditions the beer flavor and taste, influencing its quality, but also because it works as a preservative creating an adverse environment for many pathogenic and food-spoilage microorganisms [23]. The addition of bergamot and olive extracts to Heraclea and Elais decreased the pH values, in respect to the Blanche and Weiss basal beers, respectively (Table 1). Heraclea and Elais had the same pH (4.2) and the same protein content (0.2 g/100 mL) (Table 2), and basal Weiss and Blanche had

a pH of 4.6 and a protein content of 0.6 g/100 mL (Tables 1, 2). The protein beer values should be close to zero, because proteins, binding to the polysaccharides, form insoluble complexes which cause turbidity, compromising beverage stability [24]. Data obtained evidenced a less clarity of basal Weiss and Blanche beers related to their protein content. All the beers analyzed did not contain cholesterol and fibers (Table 2). Total carbohydrates were a bit lower in the basal Weiss and Blanche than in Heraclea and Elais (Table 2), but in any case, lower than 3.3–4.4 g/100 mL range recommended in the literature [25]. The ash content ranged from 0.15% to 0.21% (Table 2). Data are in agreement with those reported by Alcázar et al. [26], who found the total ash values ranging from 0.061% to 0.158%. Vitamin C was significantly elevated in Heraclea and Elais, 20-fold greater than basal Blanche and Weiss. Vitamin C is a potent water-soluble antioxidant in humans, protecting tissues, lipids, and proteins from oxidative damages, and it is also a key regulator of immune function, cellular growth, and differentiation [27]. The DPPH and TAC activities were noticeable the highest in Heraclea and Elais beers as consequence of the greatest amount of antioxidant compounds present (Table 2). As expected, the lipid value was close to zero for all the beers, and this is relevant for the quality of beer, because the lipids act negatively on the formation of foam breaking the protein network [1]. The moisture values agreed with those recommended by Taylor [28], who stated that the minimum moisture percentage in beer has to be 90% (Table 2). The energy content (Kcal/100 mL) was lesser than the maximum limit allowed for commercial beers

**Table 1** Organoleptic properties of color (EBC), bitterness (IBU) alcohol by volume (%) of Heraclea (blanche with bergamot juice extract), basal Blanche, Elais (Weiss with olive extract), and basal Weiss

ID	pH	EBC	IBU	Alcohol
Heraclea	4.2 <sup>a</sup> ± 0.2	7.5 <sup>c</sup> ± 0.6	15 <sup>b</sup> ± 1.0	5%
Blanche	4.6 <sup>b</sup> ± 0.1	11 <sup>b</sup> ± 0.8	11 <sup>d</sup> ± 0.5	5%
Elais	4.2 <sup>a</sup> ± 0.1	8 <sup>c</sup> ± 0.4	17 <sup>a</sup> ± 0.5	5%
Weiss	4.6 <sup>b</sup> ± 0.2	17 <sup>a</sup> ± 0.7	13 <sup>c</sup> ± 0.7	5%

Data are the mean of three independent experiments ± standard errors  
\*Different letters, in the same row, indicate significant differences at  $p \leq 0.05$

**Table 2** Nutritional values of different craft beers

	Heraclea	Basal Blanche	Elais	Basal Weiss
Cholesterol	nd	nd	nd	nd
Fibre	nd	nd	nd	Nd
Ash	0.15 <sup>a</sup> ± 0.06	0.18 <sup>a</sup> ± 0.03	0.14 <sup>a</sup> ± 0.04	0.19 <sup>a</sup> ± 0.07
Moisture %	93.85 <sup>a</sup> ± 2	92.74 <sup>a</sup> ± 1.7	93.12 <sup>a</sup> ± 1.8	92.13 <sup>a</sup> ± 2
Protein	0.2 <sup>b</sup> ± 0.06	0.6 <sup>a</sup> ± 0.04	0.2 <sup>b</sup> ± 0.05	0.6 <sup>a</sup> ± 0.05
Carbohydrates	3.14 <sup>a</sup> ± 0.3	2.36 <sup>b</sup> ± 0.3	2.78 <sup>b</sup> ± 0.2	2.77 <sup>b</sup> ± 0.1
Phenols	468.49 <sup>b</sup> ± 11	356.89 <sup>d</sup> ± 10	530.14 <sup>a</sup> ± 15	435.03 <sup>c</sup> ± 16
Flavonoids	295.89 <sup>b</sup>	100.24 <sup>d</sup>	326.78 <sup>a</sup>	192.33 <sup>c</sup>
Flavonoids/protein	0.071 <sup>a</sup>	0.020 <sup>c</sup>	0.083 <sup>a</sup>	0.042 <sup>b</sup>
Vitamin C	30 <sup>a</sup> ± 1.43	1.5 <sup>c</sup> ± 0.36	20 <sup>b</sup> ± 1.76	1.6 <sup>c</sup> ± 0.25
DPPH	48.75 <sup>b</sup> ± 1.36	37.21 <sup>d</sup> ± 1.03	52.71 <sup>a</sup> ± 1.46	42.44 <sup>c</sup> ± 1.63
TAC	289 <sup>a</sup> ± 2.41	199.12 <sup>d</sup> ± 4.05	296 <sup>a</sup> ± 3.34	247.90 <sup>c</sup> ± 2.37
Lipids	nd	nd	nd	Nd
Energy	12.56 ± 1.3 <sup>b</sup>	11.44 ± 1.5 <sup>b</sup>	15.82 ± 1.5 <sup>a</sup>	12.88 ± 1.2 <sup>b</sup>

Heraclea (blanche beer with bergamot extract), basal Blanche, Elais (Weiss beer with olive extract), and basal Weiss. Protein (mg/100 mL), Carbohydrates (g/100 mL), Phenols (mg GAE L<sup>-1</sup>), flavonoids (mg Quercetin L<sup>-1</sup>), Vitamin C (mg/100 mL<sup>-1</sup>), 2,2-diphenyl-1-picrylhydrazyl (DPPH mg Trolox L<sup>-1</sup>), total antioxidant capacity (TAC mg Trolox L<sup>-1</sup>), and energy (Kcal/100 mL)

Data are the mean of three independent experiments ± standard errors

\*Different letters, in the same row, indicate significant differences at  $p \leq 0.05$



(35 kcal/100 mL) according to Decree No. 6.871, of June 4, 2009 [29]. Regarding bio-compounds, total polyphenols were the highest in Elais, followed by Heraclea (Table 2). Flavonoids had the same trend of polyphenols. The ratio of flavonoids/proteins was significantly the highest in the beers prepared with bergamot and olive extracts, evidencing a great binding interaction of some flavonoids with carrier proteins (Table 2) that can facilitate the absorption of flavonoids at intestinal level [30, 31].

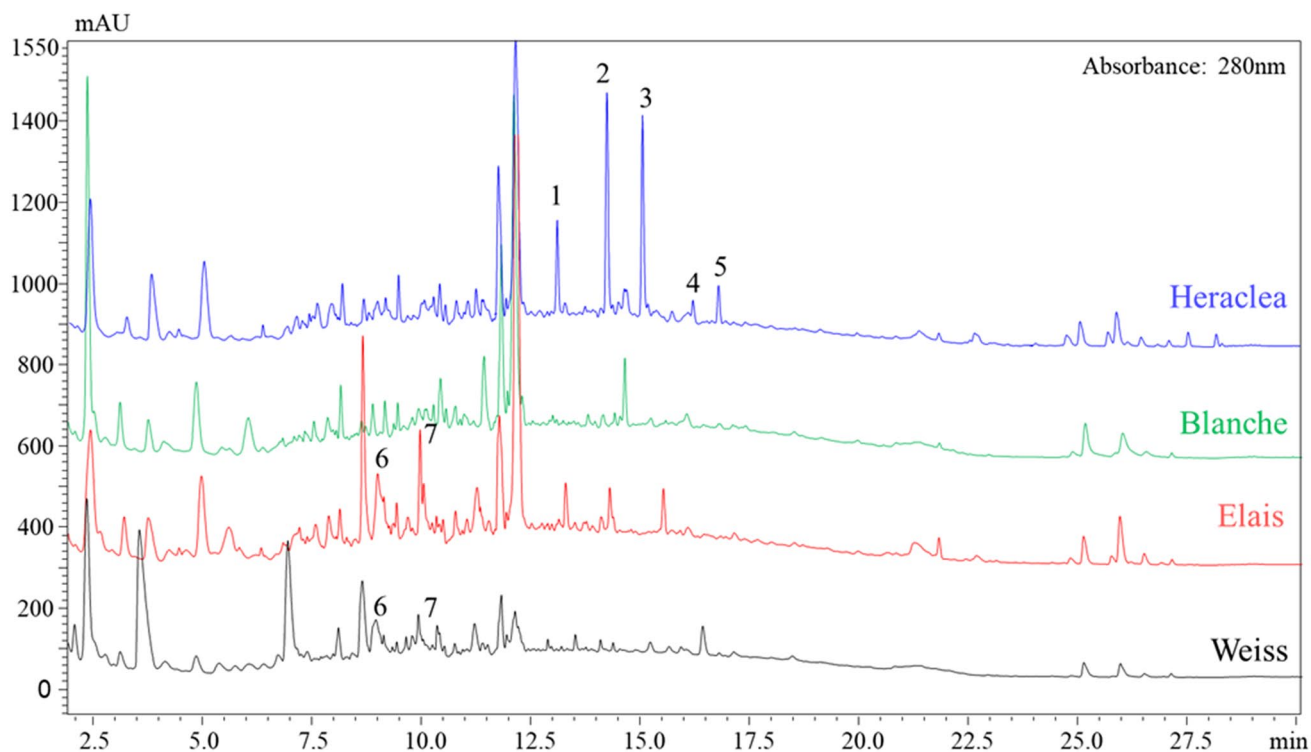
The chromatograms of the phenolic profiles of the analyzed beers evidenced compounds typical of beers (Fig. 1). The phenolic profile of Heraclea was qualitatively and quantitatively the richest in bio-compounds than the other beers. Peaks 1, 2, and 3 (Fig. 1) correspond to neohesperidin, naringin, and neohesperidin, respectively, molecules present in bergamot fruits in a very high quantity (Table 3). These bio-compounds possess various biological activities such as antidiabetic, antiatherogenic, antidepressant, immunomodulatory, antitumor, anti-inflammatory, DNA protective, hypolipidaemic, antioxidant, peroxisome proliferator-activated receptors (PPARs) [32], and memory improver [30, 33, 34]. Compounds 4 and 5 (Fig. 1), identified as Melitidin and Brutieridin [35, 36], are phenols present only in Bergamot. These molecules have a similar structure to statins (drugs that decrease fatty acids and cholesterol in the blood), and numerous studies reported their involvement in

the inhibition of hydroxy-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) [37], which in turn caused the decrease of fatty acids and cholesterol in human blood with hypo-cholesterolemic action [38, 39].

Comparing basal Blanche with Heraclea raised the absence of the chromatographic peaks 1, 2, 3, 4, and 5 (Fig. 1) and a very low total quantity of the quantified phenols 11.44 ng/ $\mu$ L (Blanche) and 43.53 ng/ $\mu$ L (Heraclea) (Table 3).

Elais had a more complex phenolic profile (Fig. 1) than the basal Weiss, and this difference was due to the addition of the olive extract rich in bio-compounds. Elais contained more total flavonoids and phenolic acids (7.36 ng/ $\mu$ L) than the beer belonging to its category (2.10 ng/ $\mu$ L). The differences could be principally related to the doubled amount of syringic acid and ethylgallate as emerges from Fig. 1 (chromatographic peaks 6 and 7) and Table 3. Considering the data obtained, Heraclea and Elais beers had a qualitative and quantitative profile of flavonoids superior to the examined beers of the respective category.

Pearson's correlation coefficient evidenced that total phenols contained in Heraclea and Elais were positively and significantly correlated with DPPH ( $r=0.829$  and  $0.866$ , respectively) and TAC ( $r=0.737$  and  $0.662$ , respectively), while only the Weiss positively correlated with (DPPH  $r=0.549$ ) even if at minor extent (Fig. 2A); Among the



**Fig. 1** Chromatogram profiles of beers: Heraclea (blanche with bergamot juice extract, blue), basal Blanche (green), Elais (Weiss with olive extract, red), and basal Weiss (black)

**Table 3** Polyphenolic content (ng/ $\mu$ L) of different craft beers

Compounds	Heraclea	SD	Blanche	SD	Elais	SD	Weiss	SD
Phenolic acids								
Coumaric acid	0.060 <sup>b*</sup>	$\pm 0.001$	0.044 <sup>c</sup>	$\pm 0.001$	0.086 <sup>a</sup>	$\pm 0.003$	<LOD	–
4-Hydroxybenzoic acid	0.516 <sup>a</sup>	$\pm 0.023$	0.507 <sup>b</sup>	$\pm 0.010$	0.542 <sup>a</sup>	$\pm 0.012$	<LOD	–
Caffeic acid	0.175 <sup>c</sup>	$\pm 0.027$	0.258 <sup>a</sup>	$\pm 0.012$	0.222 <sup>b</sup>	$\pm 0.010$	0.086 <sup>d</sup>	$\pm 0.005$
Chlorogenic acid	0.461 <sup>b</sup>	$\pm 0.019$	0.266 <sup>c</sup>	$\pm 0.002$	0.705 <sup>a</sup>	$\pm 0.078$	<LOD	–
Ferulic acid	0.359 <sup>b</sup>	$\pm 0.018$	1.766 <sup>a</sup>	$\pm 0.076$	0.052 <sup>c</sup>	$\pm 0.002$	<LOD	–
Protocatechuic acid	0.434 <sup>a</sup>	$\pm 0.018$	0.332 <sup>b</sup>	$\pm 0.012$	0.227 <sup>c</sup>	$\pm 0.014$	<LOD	–
Syringic acid	0.185 <sup>c</sup>	$\pm 0.001$	0.107 <sup>d</sup>	$\pm 0.001$	1.218 <sup>a</sup>	$\pm 0.002$	0.637 <sup>b</sup>	$\pm 0.016$
Vanillic acid	1.020 <sup>b</sup>	$\pm 0.380$	<LOD	–	1.238 <sup>a</sup>	$\pm 0.077$	<LOD	–
Ethyl gallate	<LOD	–	<LOD	–	2.791 <sup>a</sup>	$\pm 0.083$	1.378 <sup>b</sup>	$\pm 0.048$
Flavonoids								
Hesperidin	4.895 <sup>a</sup>	$\pm 0.074$	0.973 <sup>b</sup>	$\pm 0.160$	<LOD	–	<LOD	–
Sinensetin	0.052 <sup>b</sup>	$\pm 0.030$	0.092 <sup>a</sup>	$\pm 0.004$	<LOD	–	<LOD	–
Neoriocitrin	4.025 <sup>a</sup>	$\pm 0.031$	<LOD	–	<LOD	–	<LOD	–
(–) Epicatechin	1.768 <sup>a</sup>	$\pm 0.033$	<LOD	–	<LOD	–	<LOD	–
Neohesperidin	18.041 <sup>a</sup>	$\pm 0.136$	<LOD	–	<LOD	–	<LOD	–
Tangeretin	0.068 <sup>a</sup>	$\pm 0.005$	0.021 <sup>c</sup>	$\pm 0.001$	0.046 <sup>b</sup>	$\pm 0.003$	<LOD	–
Kampferol	0.113 <sup>b</sup>	$\pm 0.001$	<LOD	–	0.229 <sup>a</sup>	$\pm 0.001$	<LOD	–
Naringin	9.328 <sup>a</sup>	$\pm 0.216$	<LOD	–	<LOD	–	<LOD	–
Nobiletin	0.243 <sup>a</sup>	$\pm 0.015$	–	–	–	–	<LOD	–
Melitidin	0.633 <sup>a</sup>	$\pm 0.013$	<LOD	<LOD	<LOD	<LOD	<LOD	–
Butieridin	1.122 <sup>a</sup>	$\pm 0.016$	<LOD	<LOD	<LOD	<LOD	<LOD	–
Total	43.53		4.37		7.36		2.10	–

Heraclea (Italian blanche with bergamot juice extract), basal Blanche, Elais (Weiss with olive extract), and basal Weiss

Data are the mean of three independent experiments  $\pm$  standard errors

\*Different letters, in the same row, indicate significant differences at  $p \leq 0.05$

single phenolic acids, we observed different correlations depending on the amounts of single compounds. The best correlation with both antioxidant activities was observed for Heraclea followed by Elais. The less bioactive single phenolic acids appeared to be vanillic and chlorogenic acids and ethyl gallate (Fig. 2A). Total flavonoids contained in Heraclea and Elais positively correlated only with TAC ( $r=0.933$  and  $0.866$ , respectively) (Fig. 2B). Among the single flavonoids relived, melitidin, kaempferol, neoesperidin, hesperidin, and neoriocitrin, contained in Heraclea correlated both with DPPH and TAC (Fig. 2B). The correlation data evidenced a diversity of action of the single compounds belonging to the different classes. Considering these data, Heraclea was the beer with the greatest antioxidant activity and the greatest amount of bioactive and functional compounds followed in ranking by Elais, basal Blanche, and basal Weiss. The chemical structure of an antioxidant determines its intrinsic reactivity versus free radicals and other ROSs, influencing the antioxidant activity. Thus, the measure of antioxidant activity/capacity of food in general is essential for studying the efficiency of food antioxidants in preventing and treating the diseases related to oxidative stress like

diabetes, high blood pressure, preeclampsia, atherosclerosis, acute renal failure, Alzheimer's, and Parkinson's. Bergamot and olive extracts for their high polyphenol and flavonoid content have been tested in the numerous experimental and clinical studies, which demonstrated beneficial effects on human health and in particular showed their involvement in the inhibition of proliferation of many kinds of cancer cell lines including melanoma, colon, breast, squamous, leukemia, lung, prostate, colorectal, and hepatomas and neurodegenerative disease [40–44].

Aroma fingerprints (Fig. 3), extrapolated from the chromatographic profiles of beers and odor maps run with Heracles system [45], showed significant differences between Elais and Heraclea, compared to the basal beers. Elais and Heraclea had the greatest aromatic richness, due to the addition of bergamot and olive extracts which, in turn, increased the aromatic characteristics of the beer style, adding more intense aromatic nuances. These were mainly attributable to carbonyl compounds, in particular butanal in Elais and hexanal in Heraclea, that provided green, grassy, and pea-like flavors. Carbonyl compounds are normally low in beer, because yeasts, during the fermentation process, have the

a	DPPH				TAC			
	Heraclea	Blanche	Elais	Weiss	Heraclea	Blanche	Elais	Weiss
Coumaric acid	✗ -0,98	✓ 1	✗ -0,98	⚠ 0	✗ -0,84	⚠ 0,327	✓ 0,929	⚠ 0
4-Hydroxybenzoic acid	✓ 1	✗ -0,82	⚠ 0,327	⚠ 0	✓ 0,924	✗ -0,81	⚠ -0,14	⚠ 0
Caffeic acid	✓ 0,982	✗ -0,57	⚠ 0,327	✗ -0,65	✓ 0,836	✗ -0,96	⚠ -0,14	✓ 0,653
Chlorogenic acid	⚠ 0,277	✗ -0,81	✓ 0,866	⚠ 0	✓ 0,924	✗ -0,82	✗ -0,76	⚠ 0
Ferulic acid	✓ 1	✗ -0,87	✗ -0,65	⚠ 0	✓ 0,924	⚠ 0,177	✓ 0,786	
Protocatechuic acid	✓ 0,982	✓ 0,5	✓ 0,866	⚠ 0	✓ 0,98	✗ -0,65	✗ -0,76	
Syringic acid	✓ 0,997	✓ 0,397	✓ 0,866	✓ 0,693	✓ 0,953	✗ -0,74	✓ 0,756	✗ -0,69
Vanillic acid	⚠ -0,33	⚠ 0	✗ -0,91	⚠ 0	⚠ 0,058	⚠ 0	⚠ 0,3	⚠ 0
Ethyl gallate	⚠ 0	⚠ 0	✓ 0,933	✓ 0,378	⚠ 0	⚠ 0	✗ -0,94	✗ -1
Total phenols	✓ 0,829	⚠ 0,155	✓ 0,866	✓ 0,549	✓ 0,737	⚠ -0,29	✓ 0,662	✗ -1

b	DPPH				TAC			
	Heraclea	Blanche	Elais	Weiss	Heraclea	Blanche	Elais	Weiss
Hesperidin	✓ 0,822	⚠ -0,33	⚠ 0	⚠ 0	✓ 0,543	✗ -1	⚠ 0	⚠ 0
Sinensetin	⚠ 0,327	✗ -0,87	⚠ 0	⚠ 0	✓ 0,663	✗ -0,76	⚠ 0	⚠ 0
Neohesperidin	✓ 0,912	⚠ 0	⚠ 0	⚠ 0	✓ 0,687	⚠ 0	⚠ 0	⚠ 0
(-)Epicatechin	⚠ -0,33	⚠ 0	⚠ 0	⚠ 0	⚠ 0,058	⚠ 0	⚠ 0	⚠ 0
Neohesperidin	✓ 0,933	⚠ 0	⚠ 0	⚠ 0	✓ 0,726	⚠ 0	⚠ 0	⚠ 0
Tangeretin	✗ -0,98	✓ 0,5	✗ -0,5	⚠ 0	✗ -0,84	✗ -0,65	⚠ 0,327	⚠ 0
Kampferol	✓ 0,866	⚠ 0	✗ -0,98	⚠ 0	✓ 0,61	⚠ 0	✓ 1	⚠ 0
Naringin	⚠ 0,132	⚠ 0	⚠ 0	⚠ 0	✓ 0,5	⚠ 0	⚠ 0	⚠ 0
Nobiletin	✓ 0,904	⚠ 0	⚠ 0	⚠ 0	✓ 0,673	⚠ 0	⚠ 0	⚠ 0
Melitidin	✓ 0,993	⚠ 0	⚠ 0	⚠ 0	✓ 0,875	⚠ 0	⚠ 0	⚠ 0
Butieridin	✗ -0,87	⚠ 0	⚠ 0	⚠ 0	✗ -0,92	⚠ 0	⚠ 0	⚠ 0
Flavonoids	✗ -0,99	⚠ -0,33	✗ -0,5	✗ -0,87	✓ 0,933	✗ -0,98	✓ 0,866	✗ -1

**Fig. 2** Pearson's correlations ( $r$ ) between phenols (a), flavonoids (b), and antioxidant activities. The boxed dots show the significant correlations between values, and the color shows the level of correlation

(yellow-boxed dots  $p < 0.05$ , green-boxed dots  $p < 0.01$ ). The red dots indicate negative correlation

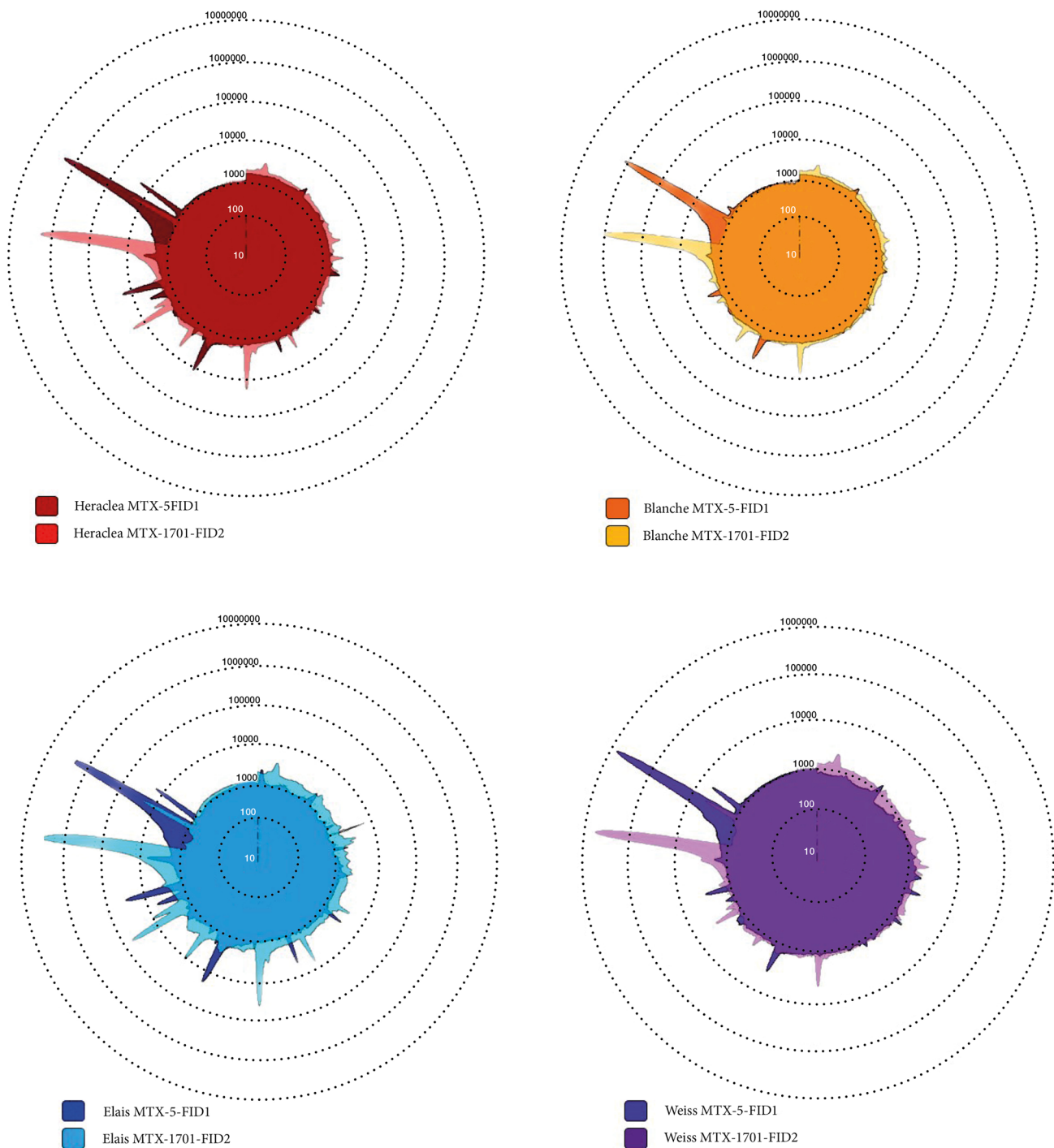
ability to completely remove aldehydes by reduction to alcohol counterparts. Their presence in the aromatic profile of Heraclea and Elais was therefore attributable to the added extracts [46].

Among the sensory markers, the presence of the two esters, butyl acetate and ethyl octanoate, connotes the aroma of Elais. The first compound is an acetate ester, the second is an ethyl ester in which the alcohol group is

ethanol, and the acid group is a medium-chain fatty acid. Ethyl octanoate, in particular, gives the sour apple aroma. Esters are volatile compounds impacting greatly aromaticity of beer and conferring, if in moderate quantities, a fruity-flowery aroma.

In Heraclea, in addition to the aforementioned two esters, 2-phenylethanol and 2-methyl-1-propanol alcohols were detected. This high content in alcohol compounds (also





**Fig. 3** Odor maps fingerprints of beers: Heraclea (blanche with bergamot juice extract), basal Blanche, Elais (Weiss with olive extract), and basal Weiss, with Heracles system equipped with two parallel non-polar column (MXT-5) and slightly polar column (MXT-1701):

**a** E MXT-5-FID1 + E MXT-1701-FID2; **b** EC MXT-5-FID1 + EC MXT-1701-FID2; **c** H MXT-5-FID1; **d** H MXT-1701-FID2 + HC MXT-5-FID1 + HC MXT-1701-FID2

known as fusel alcohols) could lead to a pungent smell and taste.

## Conclusion

In short, the production of these functional beers requires a combination between the type of product added and the step of addition. The addition of these kinds of fruit extracts to the brewing process at different steps proved to be attractive as the amount of phenols and bioactive compounds has been enhanced. This is reflected by the high phenolic and flavonoid content and antioxidant potential of *Elais* and *Heraclea* with respect to the basal beers of the respective category. Furthermore, this study evidenced that it is possible to add bioactive compounds without neglecting the sensory perception of the beers, that have rather showed an improvement in sensorial properties, important aspect in influencing the choices of consumers toward products that contribute to maintaining a better state of health satisfying the taste. The positive aspect is related to the possible health effects of these beers particularly rich in phenolic compounds that being potent scavengers may counteract the negative effects of reactive oxygen species produced from alcohol metabolism.

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**Data availability** The datasets used in the current study are available from the corresponding author on reasonable request.

## Declarations

**Conflict of interest** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Compliance with ethics requirements** The research did not involve human participants and/or animals, and no informed consent was necessary.

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