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# Comprehensive characterization of Chinese beers based on chemical composition, antioxidant activity and volatile metabolomics

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**Four different commercial beers in the Chinese market were compared and analyzed systematically, in order to provide more guidance for consumers. In this experimental study, various physicochemical parameters such as alcohol content, color, bitterness, total acidity, and carbohydrates were evaluated. The total phenolics content was determined using the Folin-ciocalteu method, while the total flavone and melanoidins were measured using colorimetric and spectrophotometric methods, respectively. The antioxidant capacity was determined by ORAC, DPPH and ABTS assays. Non-targeted metabolomics was used to analyze the composition and differences of volatile compounds in different beers. The results indicated significant physicochemical variations among the four different commercial beers. The higher the chroma of beer, the greater the content of active substances, and the corresponding antioxidant capacity in vitro was also stronger. The alcohol content of the four beers ranged from 4.23 to 7.54% (ABV), the color values of the four beers ranged from 4.8 to 141.5 EBC, and the bitterness ranged from 11.2 to 36.6 IBU. The total phenolics content varied between 159.10 mg/ L and 269.13 mg/ L, the total flavone content was in the range of 39.94 -144.59 mg/L, and the melanoidins content was in the range of 271.07-296.68 mg/L. The antioxidant activity ranged from 0.570 mmol TE/L to 0.873 mmol TE/L (ABTS), from 3.700 mmol TE/L to 26.73 mmol TE/L (ORAC), and from 26.12 to 86.72% (DPPH clearance rate). A total of 453 volatile compounds were detected in the four beers, primarily comprising terpenoids (21.24%), esters (19.47%), heterocyclic compound (14.16%), alcohol (9.96%) and hydrocarbons (9.96%), etc. Compared to Premium lager beer, the other three kinds of beers had unique and common metabolites, with only 9 common metabolites. The flavors of the differential metabolites were mainly green, floral, sweet, fruity, etc.**

**Keywords** Beer, Physicochemical parameters, Antioxidant activity, Volatile compounds, Differential metabolites, Flavor

Beer is a drink containing alcohol. It is brewed from malt, and among various types of alcohol beverage. It has a relatively low alcohol content but high nutritional content compared to other alcoholic beverages. Beer is made from natural ingredients, including malted cereals (most often barley), hops, yeast and water. Thanks to these, beer contains carbohydrates, proteins, minerals, vitamins, fibre and other substances, which is known as "liquid bread"[1]. As people's understanding and research of beer continue to deepen, they gradually discover that drinking beer in moderation does not have a significant impact on the body. In addition, drinking beer in moderation can be beneficial for cardiovascular diseases[2,3].

Malt is the primary source of carbohydrates, amino acids, proteins, and vitamins in beer. Additionally, malt components contain several compounds with antioxidant activity, namely phenolic compounds and melanoidins[4,5]. The levels of phenolic compounds and melanoidins affect the antioxidant activity and sensory characteristics of beer[6]. In beer, malt contributes about 70–80% of the total phenolic content (150 mg/L to 350 mg/L of gallic acid equivalents), while hops contribute about 30%[7,8]. The most commonly reported phenolic compounds include flavonoids (such as flavanols and flavonoids), hydroxycoumarins, phenolic acids, tannins, procyanidins, and aminophenolic compounds, all of which are associated with various biological effects[9].

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Phenolic compounds, acting as antioxidants, can prevent the oxidative degradation of beer and provide potential health benefits to consumers[10]. Research has shown that they exhibit strong antioxidant, estrogenic, anti-inflammatory, and anticancer activities[10–12]. Melanoidins are macroscopic, nitrogen-containing brown products produced through the Maillard reaction, which occurs during malt and brewing processes[13,14]. Melanoidins can exert antioxidant effects through various mechanisms: scavenging oxygen free radicals, eliminating reactive oxygen species, functioning as a reducing agent, and acting as a metal chelating agent[15].

With the development of economy and advancements in science and technology, people's choices for beer are becoming increasingly diverse, encompassing factors such as quality, taste, and brand. The flavor and taste of beer have always been significant factors influencing consumers' preference for products[16]. Depending on the brewing process and the selection of raw materials, there are many styles and types of beer. Parameters such as sweetness, bitterness, color, and alcohol content can affect consumers' choices[17]. Phenolic compounds can directly impact the characteristics of beer, primarily affecting its body, smoke taste, flavor, fullness, and astringency[18]. However, volatile compounds play a pivotal role in creating taste and aroma[19]. The sensory-active chemical substances in beer belong to various categories, such as esters, carbonyl compounds, alcohols, fatty acids, volatile phenols, furans, or terpenes[20]. The aroma characteristics of beer are one of the main factors for consumer to evaluate, making the analysis of volatile compound content a crucial aspect of product quality assessment. In addition, it is widely recognized that the physicochemical properties of beer are also essential for predicting the antioxidant activity of the final product[21].

China is one of the fastest-growing markets for beer. In 2023, the national beer industry achieved a total output of 37.89 million tons, marking a year-on-year increase of 0.8%. With the increasing consumption of beer in China, the selection of beer is becoming more diverse. However, to our knowledge, current research on the characterization of different types of beer in China is either limited to physical and chemical properties, individual active substances, or volatile substances. There is currently no comprehensive systematic comparison and analysis of the above characterization of beer. Therefore, this study will systematically compare different beers and conduct a comprehensive analysis of their differences, providing consumers with more theoretical basis for their choices and data support for the optimization and improvement of beer in the later stage.

## Materials and methods

### Samples

The present work was carried out on four kinds of different flavored beers purchased from local supermarket (Qingdao, China), including Premium lager beer, Original beer, India Pale Ale and Stout beer. The brand names were omitted. Premium lager beer and Original beer both belong to International Pale Lager, while the Original beer is an unfiltered pale lager that contains a certain amount of yeast. The India Pale Ale is English IPA, while the Stout beer belongs to Foreign Extra Stout with dark and intense. An aliquot of each type of beer (300 mL) was collected and degassed through sonication at 37 kHz for 10 min keeping the temperature between 15 ℃-20 ℃ (SB 5200DT, Scientz Biotechnology CO., LTD, NingBo, China). Immerse the degassed sample in liquid nitrogen for freezing, and freeze dry a portion for 48 h. Then store the untreated and freeze-dried materials at -20 ℃ until analysis is performed[21].

### Methods

*pH*

The pH value of beer was measured using Alalis PH 400 (Shanghai, China) according to GBT 4928 – 2008. At room temperature, take 50 mL of sample beer were taken and placed into a beaker. Then the electrode was insert into the sample solution, and waited for the pH to stabilize before taking a reading[21]. After measuring the sample, rinse the electrode with water as soon as possible and blot it dry. This process was done in triplicate.

*Color*

The color of the beer was determined according to the ASBC Beer-10 Color of Beer method. The absorbance of the beer was measured at 430 nm and 700 nm. The color of the beer was then calculated using the relevant conversion factor[22]. The measurements were performed in triplicate.

*Ethanol*

The the content of ethanol was examined by means of gas chromatography (GC) according to GB 5009.225–2016. Ethanol standard solutions: Six 100 mL volumetric flasks were used. Ethanol was added individually to each flask (flask 1: 2 mL, flask 2: 3 mL, flask 3: 4 mL, flask 4: 5 mL, flask 5: 6 mL, flask 6: 7 mL), and the volume of each flask was adjusted to the scale mark using distilled water. These solutions served as standards for preparing a calibration curve. In the next step, 10 mL of each respective beer sample (obtained as described in Sect. 2.1.) was transferred to another clean and dry 10 mL volumetric flask. After 0.5 mL of the internal standard nbutanol had been added to each sample, a thorough mixing was conducted. The FID conditions were as follows: column temperature of 200 ℃, gasification chamber and detector temperature of 240 ℃, carrier gas (high purity nitrogen) flow rate of 40 mL/min, hydrogen flow rate of 40 mL/min, and air flow of 500 mL/min. Using the chromatographic column (Perkin Elmer ED-624, 60 m × 0.25 mm) for detection, the conditions were as follows: the initial temperature of the column is 40 ℃, maintained for 5 min, heated at a rate of 3 ℃/min to 55 ℃, then heated at a rate of 10 ℃/min to 150 ℃, and finally heated at a rate of 40 ℃/min to 220 ℃, maintained for 5 min, carrier gas (high purity nitrogen) flow rate of 1.0 mL/min, hydrogen flow rate of 30 mL/min, and air flow of 350 mL/min[21].

Ethanol standards of different concentrations (10 mL) were also added to five 10 mL volumetric flasks and mixed with 0.5 mL n-butanol to each flask. Under the aforementioned chromatographic conditions, an injection 0.3 μL of working curves were plotted based on the ethanol concentrations. The alcohol content was based on

the ratio of peak areas (or peak height ratio) between the standard sample and the internal standard, or establish a corresponding regression equation[21]. Each sample was subjected to three parallel evaluations, and the average value was obtained for further analysis.

*Bitterness*

According to the EBC method, the bitterness content of beer was measured at 275 nm using a spectrophotometer (UV-5500, Shanghai metash Instruments Co., Ltd). A 10 mL sample of degassed beer, 0.5 mL of 6 M HCl and 20 mL of iso-octane were pipetted into a test tube and intensively mixed for 15 min. At room temperature, mixing was done with mechanical shaking (SHA-C, China). Subsequently, the samples were spun at 3000 rpm for 15 min, the supernatant fluid being aspirated, and the measurements taken at 275 nm in 10 mm quartz cuvettes. Pure iso-octane was used as blank. The bitterness of beer was calculated according to the following formula, where B denotes beer bitterness (IBU) and A denotes the absorbance at 275 nm[23].

$B = 50 * A275$.

*Total acidity*

Using phenolphthalein as an indicator for acid-base neutralization titration, the total titratable acidity values were calculated[21]. A 25 mL sample was added to a 250 mL conical flask, diluted to volume with carbon dioxide-free water, then shaken thoroughly and the filtrate was collected. A 250 mL Erlenmeyer flask was filled with 2–4 drops of phenolphthalein indicator solution and 25 mL of filtrate, which was titrated with 0.1 M NaOH solution until a pale pink hue was observed that lasted for 30 s. The volume of the NaOH standard titration solution was documented and the result was calculated.

*Determination of carbohydrate profile content*

High-Performance Liquid Chromatography (HPLC) was employed to inspect the sugar profile. Degassed and centrifuged (5500 rpm, 10 min) beer samples were diluted two-fold (1:1) with ultrapure water and filtered through nylon syringe filters with 0.22 µm pore size for chrom atographic vials. A Prominence liquid chromatography system (Waters, USA) was employed to analyse the samples, with a Rezex™ ROA-Organic Acid H + column (300 × 7.8 mm) from Phenomenex (Torrance, CA, USA). Measurement parameters were: sample volume of 20 µL, separation temperature of 60 ℃, mobile phase flow rate of 0.6 mL/min, mobile phase of 0.005 N $H_2SO_4$, detection temperature of 40 ℃. The concentration of dextrin (standard solution concentration of 50.0 g/L), maltose (standard solution concentration of 10.0 g/L), glucose (standard solution concentration of 5.0 g/L) and maltotriose (standard solution concentration of 20.0 g/L) was calibrated using Chromax 10.0 software (Pol-Lab, Wilkowice, Poland)[21]. All measurements were performed in triplicate.

*Total phenolics content (TPC)*

TPC was determined using the Folin-Ciocalteu method[6]. After blending 1 mL of the diluted sample (with 1mL of deionized water as the blank) with 2.5 mL of 0.1 M Folin phenol solution, the mixing was left to sit for 3 min. Subsequently, 2.5 mL of 7.5% $Na_2CO_3$ solution was blended well and incubated in the dark for 1 h. The absorbance of the solution was then measured at 760 nm. Finally, the TPC was calculated using the gallic acid standard curve (standard solution concentration of 0 mg/mL, 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4 mg/mL, 0.5 mg/mL).

*Total flavone content (TFC)*

A colorimetric method[24] was employed to ascertain TFC. A 2 mL aliquot of the diluted sample (2 mL deionized water as blank) and 0.5 mL of 5% $NaNO_2$ solution were blended in a well-balanced manner, leaving to stand for 6 min. Then, 4 mL of 4% NaOH was added, mixed well, and left to remain for 15 min. Finally, 70% methanol was added to bring the final volume to 10 mL. The absorbance of the solution was measured at 510 nm, and the TFC was calculated by means of the standard curve (standard solution concentration of 0 mg/mL, 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4 mg/mL, 0.5 mg/mL) of rutin.

*Melanoidins content*

Melanoidins were determined according to the following protocol[25]. A solution containing 0.05 M glucose and glycine in 100 mL of water was prepared. This solution was freeze-dried to constant weight, then baked at 125 ℃ for 2 h and then crushed into powder. Mixing 5 g of the powder with 200 mL of water, stirring at 4 ℃ for 1 h, the filtrate was then filtered. Subsequently, it was freeze-dried to a constant weight. A standard gradient of 0 to 10 mg/L melanin was prepared. For standards and samples, the absorbance was measured at 345 nm to generate a standard curve for the calculation of melanoidin content.

*ABTS free radical scavenging activity*

Using the Total Antioxidant Capacity Assay Kit with the ABTS method (Beyotime Biotechnology), ABTS was determined. To begin, a combination of ABTS and potassium persulfate ($K_2O_8S_2$) was formulated and left to incubate for 12 h (1:1, v/v). Afterwards, the combination was diluted 50-fold. After a thorough mixing of a sample of 0.01 mL diluted with 0.01 mL deionized water, and 0.2 mL of the ABTS diluted blend, it was incubated at 25 ℃ for 5 min. The absorbance was then measured at 734 nm[25]. The Trolox-ABTS standard curve (standard solution concentration of 0.15 mM, 0.3 mM, 0.6 mM, 0.9 mM, 1.2 mM, 1.5 mM) was used to calculate the ABTS.

*DPPH free radical scavenging activity*

DPPH was determined using DPPH free radical scavenging ability test kit (Beijing Solarbio Science & Technology Co., Ltd ). First, 100 µL beer sample solution and 900 µL extraction solution were shaked and mixed fully, then

centrifuged at 10,000 rpm for 10 min, aspirated the supernatant fluid. After combining 10 µL of the supernatant fluid (with 10 µL of extraction solution as a blank) with 190 µL of DPPH solution, the mixture was thoroughly blended and left to stand for 30 min. Subsequently, absorbance was measured at 515 nm. The standard curve (standard solution concentration of 0.03125 mg/mL, 0.0625 mg/mL, 0.125 mg/mL, 0.25 mg/mL, 0.3 mg/mL, 1 mg/mL) was used to calculate DPPH[21].

*ORAC*

A classic instrument for gauging the antioxidant potential of biomolecules from a range of specimens, the Oxygen Radical Antioxidant Capacity (ORAC) Assay is utilized in this research. The Oxygen Radical Antioxidant Capacity (ORAC) Activity Assay Kit (OxiSelect, Cell Biolabs, INC.) is also included. In each well of a solid black 96-well microplate, 25 µL of the diluted Antioxidant Standard or samples were mixed with 150 µL of the 1× Fluorescein Solution. After 30 min incubation in the dark at 37 ℃, 25 µL of the Free Radical Initiator Solution were added into each well using either a multichannel pipette or a plate reader liquid handling system. To guarantee homogeneity, the reaction mixture was blended thoroughly by pipetting. Subsequently, the sample and standard wells were read with a fluorescent microplate reader at 37 ℃, with an excitation wavelength of 480 nm and an emission wavelength of 520 nm. In intervals of 1 to 5 min, the wells were read, resulting in a total of 60 min[25]. The results were saved and calculated.

*Untargeted metabolomics analysis*

Sample preparation and treatment Four different flavored beers were purchased from a local supermarket. To facilitate degassing, an ultrasonic technique was employed at 4 ℃ for 10 min. Subsequently, the samples were filtered using a 0.22 µm PTFE filter. To preclude any potential enzyme reactions, 1 mL of the filtered liquid was quickly transferred to a 20 mL head-space vial (Agilent, Palo Alto, CA, USA) containing a NaCl solution (10 µg/mL). The vials were sealed using crimp-top caps with TFE-silicone headspace septa (Agilent). For solid-phase microextraction (SPME) analysis, each vial was incubated at 60 ℃ for 5 min, then a 120 µm DVB/CWR/PDMS fibre (Agilent) was exposed to the sample's headspace for 15 min at the same temperature[4].

GC-MS conditions After sampling, desorption of the VOCs from the fibre coating was carried out in the injection port of the GC apparatus (Model 8890; Agilent) at 250 ℃ for 5 min in the splitless mode[4]. An Agilent Model 8890 GC and a 7000 D mass spectrometer (Agilent) were employed to detect and quantify VOCs, with a DB-5MS (5% phenyl-polymethylsiloxane) capillary column measuring 30 m × 0.25 mm × 0.25 µm. At a linear velocity of 1.2 mL/min, helium was employed as the carrier gas. The injector temperature was maintained at 250 ℃, while the detector was kept at 280 ℃. The oven temperature was set to 40 ℃ (3.5 min), with increments from 10 °C/min to 100 ℃, 7 ℃/min to 180 ℃, 25 ℃/min to 280 ℃, and then remaining at that level for 5 min. Electron impact (EI) ionisation mode was used to record mass spectra at 70 eV. The quadrupole mass detector, ion source, and transfer line temperatures were respectively set to 150, 230, and 280 ℃. Ion monitoring (SIM) mode was chosen for the MS, and analytes were identified and quantified.

*Data analysis*

Statistical analysis of the mean ± standard deviation (SD) results of this work was conducted using SPSS V22.0 (SPSS Inc., Chicago, IL, USA). To identify any significant differences between treatment groups, a one-way ANOVA was performed. $p < 0.05$ was considered statistically significant[21].

## Results and discussion

### Physicochemical analysis of beer samples

The classification of beer in different styles is based on properties such as alcohol content, color, bitterness, pH, total acidity, flavor, and ingredients. Table 1 shows the physicochemical properties of several different types of beer (Premium lager beer, Original beer, India Pale Ale, Stout beer), related to ethanol content, color, bitterness, pH, total acidity and sugar content.

Ethanol, a crucial component of beer, can improve the flavor and aroma of beer depending on the substrate composition and type of strain employed[26]. The beer ABV is rarely more than 10% and most beers are in the

| | Premium lager beer | Original beer | India Pale Ale | Stout beer |
|---|---|---|---|---|
| Ethanol (ABV %) | 4.23 ± 0.015d | 5.42 ± 0.006c | 5.83 ± 0.010b | 7.54 ± 0.006a |
| Color (EBC) | 4.8 ± 0.058d | 7.8 ± 0.058c | 32.5 ± 0.100b | 141.5 ± 0.115a |
| Bitterness (IBU) | 11.2 ± 0.100d | 12.9 ± 0.058c | 36.6 ± 0.115a | 31.6 ± 0.208b |
| pH | 4.31 ± 0.020b | 4.32 ± 0.010b | 4.39 ± 0.015a | 4.34 ± 0.006b |
| Total acidity (mL/100mL) | 1.57 ± 0.010d | 1.78 ± 0.021c | 2.24 ± 0.012b | 2.29 ± 0.006a |
| Dextrin (g/L) | 17.99 ± 0.035d | 25.64 ± 0.042c | 30.76 ± 0.031b | 44.68 ± 0.021a |
| Maltotriose (g/L) | 4.46 ± 0.025d | 10.43 ± 0.015a | 6.65 ± 0.030c | 6.85 ± 0.021b |
| Maltose (g/L) | 0.94 ± 0.015c | 1.41 ± 0.006b | 0.52 ± 0.006d | 1.86 ± 0.010a |
| Glucose (g/L) | n.d. | n.d. | n.d. | n.d. |

**Table 1**. Physicochemical indexes of beer. *Values are expressed as mean ($n = 3$) ± standard deviation. Mean values with different letters (a, b, c, d) within the same line are statistically different (p-value < 0.05).

range of 3 to 6%[27]. According to Table 1, the ABV of the four different flavors of beer ranged significantly from 4.23 to 7.54%. Stout beer has the highest beer ABV (7.54%), while Premium lager beer has the lowest beer ABV (4.23%). Previous studies have demonstrated that the alcohol present in beers can exert neuroprotective effects, potentially due to signal transduction activation processes involving reactive oxygen species (ROS), several crucial protein kinases, and increased levels of heat shock proteins. In addition, the presence of alcohol can also protect against various heart diseases[21].

The bitterness mainly derived from hops, a small portion from amino acids, polyphenols, and other components. The color is related to the malt, hops, and saccharification steps used[21]. Beer IBUs typically range between 5 and 120, and the popular use of higher quantities of more bitter hops in beers leads to higher IBU levels[21]. Among the four different flavors of beer, the bitterness of Premium lager beer is the lowest (11.2 IBU). Compared to Premium lager beer, the bitterness of India Pale Ale and Stout beer has significantly been increased, respectively 36.6 IBU and 31.6 IBU. There are also significant differences in color. Color, a crucial sensory parameter, represents the first qualitative attribute perceived by consumers, thus being crucial in product acceptance[28]. The color values range from 4.8 EBC to 141.5 EBC. Stout beer has the highest color value, followed by India Pale Ale, and Premium lager beer has the lowest color value. It has been shown that for beers, darker brown colors are usually associated with stronger, or more bitter, tastes/flavors[29], but not absolutely. This result is, in part, in accordance with our results which demonstrated that the India Pale Ale and Stout beer showed high values for color and bitterness.

The pH values of the four types of beer are between 4.31 and 4.39, with little significant difference. These results are in accordance with Granato et al. (2011), who reported pH values from 4.13 to 4.97[30]. The total acidity content of different beers ranges from 1.57 mg/100 mL to 2.29 mg/100 mL (Table 1), with Premium lager beer having the least amount. The pH and total acidity are important criteria for brewers due to their influence on the sensory attributes, biological and chemical stability.

The main components of beer are water, alcohol, carbohydrates composed of fermentable sugars and oligosaccharides[1,31], and minerals. Fermented sugars are beneficial for the aromatic flavor of beer, and carbohydrates with more than 4 sugar units can enhance the sensory quality of beer, which is beneficial to the beer body and contributes to the taste[21]. From the results, it can be seen that the content of dextrin in each type of beer is the highest, ranging from 17.99 g/L to 44.68 g/L, followed by the content of maltotriose, ranging from 4.46 g/L to 6.85 g/L. The content of maltose is the lowest, ranging from 0.52 g/L-1.86 g/L. But no glucose content was detected in any of the tested beers. Perhaps due to the fact that glucose is primarily used by brewing yeast, the final product usually does not contain this sugar or contains a small amount.

## Bioactive substances

In recent years, the nutritional value of beer has been increasing due to its high content of antioxidant compounds and low ethanol content[32]. The production of reactive oxygen species or nitrogen species (ROS/RNS) can lead to oxidative stress, which is counteracted by the use of antioxidants, which are essential compounds for sustaining our health. Antioxidants can also act in different ways, such as clearing free radicals, inhibiting oxidase, chelating metal ions, etc[33].

The main antioxidant compounds in beer are phenolic compounds and melanoids (formed in the Maillard reaction (MR))[34,35]. In regard to polyphenols, their characteristic are the presence of one or more phenolic groups in their structure, which can reduce reactive oxygen species and various organic substrates and minerals. Research has shown that phenolic compounds in beer can help lower blood pressure, increase the concentration of nitric oxide in plasma, and reduce the risk of cardiovascular disease[36]. Polyphenols can also be anti-inflammatory, antioxidant, cholesterol lowering, and prevent the oxidation of low-density lipoprotein[37], as they can block free radicals that can oxidize body fat[38]. Polyphenols are considered as preventive agents for cancer[39–41]. In addition, they can also induce positive changes in the gut microbiota, which are associated with improvements in menopausal women[42] and arthritis patients[43].

So far, more than 50 polyphenolic compounds have been identified in beer, of which 75–80% come from malt and 15–25% from hops[44]. These compounds improve the benefit and acceptance of beer, affect flavor and product stability, and contribute to the overall antioxidant activity of beer[30].

For traditional beers, the total phenolic content has been reported to range from 150 mg/L to 350 mg/L of gallic acid equivalents (GAE)[45]. In this study, TPC varied between 159.10 ± 0.62 mg/L and 269.13 ± 0.74 mg/L (Table 2). In beers, Stout beer, India Pale Ale and Original beer showed significantly higher values for TPC (269.13 ± 0.74 mg/L, 260.43 ± 0.83 mg/L and 218.13 ± 0.95 mg/L, respectively). The lowest value was observed in Premium lager beer (159.10 ± 0.62 mg/L). Comparing our results with other studies, TPC values are basically consistent. For example, García-Guzmán et al. (2018) also observed that the highest total phenolic content indices were obtained in stout beers[46]. Granato et al. (2011) studied 29 beers (11 brown ale and 18 lager) and

| | Total phenolic content (mg/L) | Flavonoids (mg/L) | Melanoidins (mg/L) |
|---|---|---|---|
| Premium lager beer | 159.10 ± 0.62d | 39.94 ± 2.11c | 271.07 ± 0.76c |
| Original beer | 218.13 ± 0.95c | 139.44 ± 2.82a | 288.03 ± 3.34b |
| India Pale Ale | 260.43 ± 0.83b | 111.37 ± 2.11b | 293.84 ± 1.82a |
| Stout beer | 269.13 ± 0.74a | 144.59 ± 3.99a | 296.68 ± 0.48a |

**Table 2**. Content of bioactive substances. *Values are expressed as mean ($n = 3$) ± standard deviation. Mean values with different letters (a, b, c, d) within the same column are statistically different (p-value < 0.05).

TPC ranged from 119.96 to 525.93 GAE mg/L[30]. Zhao et al. (2010) analyzed 34 commercial beer samples and found values varying from 152.01 GAE mg/L to 339.12 GAE mg/L[47]. These differences can be explained by beers with high original gravity and with more dark/brown color, which tends to be associated with an increased value of phenolic compounds[48].

The flavonoids compounds are a kind of phenolic compounds. They have at least two phenolic rings[49]. The total flavonoids content of beers (range 39.94-144.59 mg/L) (Table 2) was higher in respect to that of conventional beers (range 52–73 mg/L)[50]. Among the four beers, the highest flavonoids content was measured in Stout beer, followed by Original beer, and India Pale Ale. Premium lager beer exhibited the lowest flavonoids content. The differences likely due to the content of the wort (most obviously), variations in the barley and hop variety, growing conditions, brewing methodology, and the style of beer.

Melanoidins are macromolecular, nitrogenous and brown products of Maillard reactions, which are formed during the malting and brewing process[13,14]. Regarding melanoidins, several works have shown that these compounds can not only affect the color, flavor and body of beer, but also have a certain effect on health. Some studies have shown that melanoids have antioxidant, antibacterial, antihypertensive, antiallergic and prebiotic properties[51]. Melanoidins also show the ability to bind metal ions such as $Fe^{2+}$-ions[52], and are considered to be an antimutagenic and tumor growth inhibitory compound[53,54]. Melanoidins can also protect DNA damage by ROS, and dark beer has a stronger effect than blonde beer, because dark beer is rich in melanoidins[14].

In this study, the results showed that the content of melanoidin in beers ranged from 271.07 mg/L to 296.68 mg/L. Among them, the Stout beer has the highest melanoidin content (296.68 mg/L), followed by India Pale Ale (293.84 mg/L), which had no significant difference with Stout beer ($P > 0.05$). Premium lager beer has the lowest melanoidin content (271.07 mg/L). The change trend of melanoidin content is the same as that of TPC. According to published studies, the melanoidin content of the different beers would be: Black lager > blonde ale[25,55]. Also generally, black lager beer has a higher melanoidin content than blond ale beer, since black lager beers are brewed from a malt which is more toasted than in blond ale beers[56]. Research shows that our results are consistent with the above results.

## Antioxidative activity

Compounds with antioxidant activity contained in beer, such as polyphenols, flavonoids and melanin, endow beer with antioxidant activity. Research is being developed to show there is consistent evidence in the literature of the antioxidant protection of compounds present in beer[8,57]. The DPPH, ABTS, ORAC, FRAP, TRAP, ·OH methods are those that are routinely used to measure the antioxidant activity in beers because of their better sensitivity, convenience, and short determination time[35].

Generally speaking, dark beers have higher antioxidant activity[58]. The fact that dark beers have a high antioxidant activity may be due to the use of speciality malts such as caramel malts or malts of different colors. During the mashing and boiling process, different Maillard compounds with antioxidant activities are produced[59].

The antioxidant capacity of the four beer samples was evaluated and the results are presented in Fig. 1. Using the ABTS method, the results obtained revealed that Stout beer has the highest capacity (0.873 ± 0.002 mmol TE/L), significantly different from all the other samples; but the difference between India Pale Ale and Original beer was not significant ($p > 0.05$) for India Pale Ale (0.814 ± 0.008 mmol TE/L vs. 0.778 ± 0.011 mmol TE/L, respectively). In contrast, Premium lager beer showed the lowest antioxidant capacity(0.570 ± 0.025 mmol TE/L). According to Zhao et al. (2010) the values obtained for ABTS that in the range from 0.55 mmol TE/L to 1.95 mmol TE/L, were clearly similar to those obtained in the present study[47]. Pereira et al. (2020) also evaluated the antioxidant capacity of different beers and obtained values (1.568–1.737 mmol TE/L) much higher than those obtained in the present study[60]. These differences may be caused by different formulations during beer production[61].

A similar trend outlined above was also observed for the data generated using the ORAC assay: indeed, a strong significant correlation was found between these beers ($p < 0.05$). The ORAC values of the four beers to be tested rang from 3.700 mmol TE/L to 26.726 mmol TE/L. Similarly, Stout beer has a significantly highest antioxidant capacity (26.726 ± 2.944 mmol TE/L), followed by India Pale Ale (23.038 ± 0.474 mmol TE/L). Premium lager beer also showed the lowest ORAC value (3.700 ± 0.316 mmol TE/L).

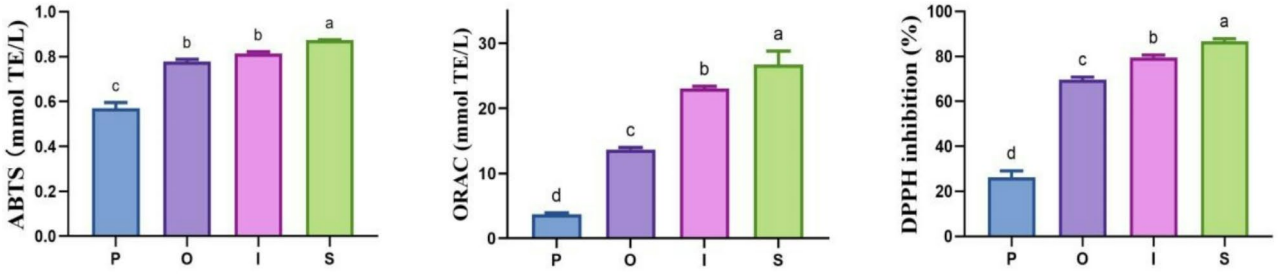


**Fig. 1**. Antioxidant activity in tested beers. P—Premium lager beer; O—Original beer; I—India Pale Ale, S-Stout beer. Values are expressed as mean ($n = 3$) ± standard deviation. Mean values with different letters (a, b, c, d) are statistically different (p-value < 0.05).

The DPPH radical scavenging activity of the beer samples was 26.116 ± 3.040% to 86.722 ± 1.099% (Fig. 1). Similarly, there are significant differences in DPPH radical scavenging activity between different beers, with Stout beer being the highest and Premium lager beer the lowest. Antioxidants with DPPH radical scavenging activity can provide hydrogen to free radicals, especially those generated by lipid peroxides or hydroperoxides, which are the main propagators of lipid chain autoxidation reactions, forming non radical substances, thereby inhibiting further lipid peroxidation[47]. Beer with higher DPPH radical scavenging activity may have enhanced flavor stability, because beer staling may occur due to oxidation to form trans-2-nonenal and other saturated and unsaturated aldehydes[47].

Studies have indicated a powerful link between the antioxidant compounds present in beer and its antioxidant activity. The amount of certain phenolic compounds determines the biological activity of alcoholic beverages with respect to antioxidant activity[62]. A significant correlation was found between antioxidant activity and total flavonoids content[50,63]. Our findings revealed a significant correlation between the polyphenol and melanoidin content of beer and the antioxidant capacity tested by ABTS, ORAC, and DPPH methods. This was further corroborated by (2013)[25], which found positive correlations between melanoidin content in beers and antioxidant capacity.

## Volatile organic compounds in beers

A total of 453 VOCs were identified in the four kinds of beers by non-targeted volatile omics methods. The VOCs could be broadly classified into 15 categories, including terpenoids, ester, heterocyclic compound, alcohol, hydrocarbons, ketone, aldehyde, aromatics, acid, nitrogen compounds, amine, phenol, sulfur compounds, ether and other compounds (Table 3). Among them, the categories with the highest number of VOCs were terpenoids (96, 21.20%), followed by esters (91, 20.10%) and heterocyclic compound (64, 14.10%). Alcohol ranked the fourth (48, 10.60%). In addition, of the 453 VOCs, four were not detected in the Premium lager beer and Stout beer, one was not detected in the Original beer and two were unique the India Pale Ale.

Analysis of the volatile compounds identified in the four kinds of beers revealed a high degree of commonality, with 446 out of 453 compounds shared across all four varieties. The top 50 compounds include well-known aroma-active substances such as Phenylethyl Alcohol (fruity, rose, sweet aroma); Acetic acid, 2-phenylethyl ester (floral, rose, sweet, honey, fruity aroma); 1-Butanol, 3-methyl- (whiskey, malty, burnt); 1-Butanol, 2-methyl- (malty, fruity); Octanoic acid, ethyl ester (fruity, banana, waxy) and Hexanoic acid, ethyl ester (fruity, apple). These compounds are likely responsible for the foundational aroma characteristics of the beers.

The group of compounds ranked between 50th and 200th includes substances such as Decanoic acid, ethyl ester (fruity, waxy, sweet); Octanoic acid (sweaty, cheese); Hexanoic acid (sour, rose, fatty); Benzeneethanol, 4-hydroxy- (floral, mild, phenolic); Octanoic acid, methyl ester (fruity, citrus) and Dodecanoic acid, ethyl ester (sweet, waxy, floral). These compounds contribute to secondary aroma notes, enhancing the complexity and depth of the beer's flavor profile. Additionally, lower-ranked common compounds, such as Butanoic acid, 2-methyl-, 3-methylbutyl ester (sweet, fruity); 3-cyclopentyl-1-propanol; Hexadecanoic acid, ethyl ester (mild, waxy, fruity) and Tetradecanoic acid, ethyl ester (sweet, waxy), further enrich the aromatic diversity of the beers.

Among the top ten VOCs with the highest content in each beer, there were eight common components. These include: *Hydrocarbon*: 2-Methyl-7-exo-vinylbicyclo[4.2.0]oct-1(2)-ene (earthy, herbal); *Alcohols*: Phenylethyl Alcohol (fruity, rose, sweet aroma); 1-Butanol, 3-methyl- (whiskey, malty, burnt); 1-Butanol, 2-methyl- (malty, fruity) and 2-Furanmethanol (caramel, burnt sugar); *Heterocyclic Compound*: Pyrazine, 2-methoxy-3-(2-methylpropyl)- (nutty, earthy); *Ester*: Acetic acid, 2-phenylethyl ester (floral, rose, sweet, honey, fruity); *Amine*: Ethenamine, N-methylene- (subtle nitrogenous sharpness).

| Class I | Number | Percentage |
|---|---|---|
| Acid | 13 | 2.90% |
| Alcohol | 48 | 10.60% |
| Aldehyde | 26 | 5.70% |
| Amine | 6 | 1.30% |
| Aromatics | 14 | 3.10% |
| Ester | 91 | 20.10% |
| Ether | 1 | 0.20% |
| Heterocyclic compound | 64 | 14.10% |
| Hydrocarbons | 45 | 9.90% |
| Ketone | 27 | 6.00% |
| Nitrogen compounds | 7 | 1.50% |
| Others | 3 | 0.70% |
| Phenol | 6 | 1.30% |
| Sulfur compounds | 6 | 1.30% |
| Terpenoids | 96 | 21.20% |

**Table 3**. Volatile compounds category composition.

## Differential VOCs in the different beers

In order to further reveal the differences of volatile metabolites among samples of different beers, fold change (FC) value was used to judge the change trend of the different volatile compounds. Differential VOCs (DVOCs) were screened out between two different groups of samples under the following condition: |Log2 fold change|≥2.0 or ≤0.5. If the difference in metabolites between the control group and the experimental group is more than 2 times or less than 0.5, it is considered significant.

*O vs. P* The results showed that there were 56 different volatile compounds between Original beer and Premium lager beer. Intriguingly, Original beer exhibited an up-regulation in 55 of these compounds compared to Premium lager beer (Fig. 2). Notable up-regulated compounds include Hexadecanoic acid, ethyl ester; Nonanoic acid, ethyl ester; Butyl caprylate; Longiforenaldehyde; and Tetradecanoic acid, ethyl ester, among others. Only one compound, 2-((3,3-dimethyloxiran-2-yl)methyl)-3-methylfuran, belonging to the heterocyclic compound class, was down-regulated. Table 4 showcases the top 20 metabolites with the most significant differential fold changes. Among the 55 up-regulated compounds, esters dominate with 19 species, accounting for 34.5% of the total. Terpenoids follow closely with 13 species, representing 23.6% of the up-regulated compounds. Alcohols rank third, with 7 species comprising 12.7% of the total. Heterocyclic compounds and hydrocarbons make up the remaining 29.2%, indicating their lesser abundance among the up-regulated compounds.

*I vs. P* The analysis between India Pale Ale and Premium lager beer has uncovered a fascinating array of 96 significant volatile compounds, as depicted in Fig. 2. Notably, 73 of these compounds were up-regulated, while 23 were down-regulated. The metabolite results of the top 20 fold change are neatly presented in Table 4. Among the 73 up-regulated compounds, the top ones were TriSulfur compounds, dipropyl; 2,6-Octadienoic acid, 3,7-dimethyl-, methyl ester; Toluene; Ethanone, 1-(1 H-pyrrol-2-yl)- and so on. Terpenoids dominated this group with 18 species, accounting for 24.7% of the up-regulated compounds, closely followed by esters with 16 species, representing 21.9%. Conversely, among the 23 down-regulated compounds, Dodecanoic acid, ethyl ester; Ethyl 9-decenoate; and Butanoic acid, octyl ester ranked highly. In this group, esters were the most prevalent with 10 species, accounting for 43.5% of the down-regulated compounds, followed by terpenoids, which comprised 13.0% of the total.

*S vs. P* Between Stout beer and Premium lager beer, 67 differential volatile compounds were discovered (41 up-regulated, 26 down-regulated) (Fig. 2). The metabolite results of the top 20 fold change are also shown in Table 4. Among the 41 up-regulated compounds, the top ranked ones were Phenol, 4-ethyl-2-methoxy-; Ethanone, 1- (1 h-pyrrol-2-yl) -; Toluene et al. Heterocyclic compound has the most species, with 11 species accounting for 26.8%; and the second is Ester, with 8 species, accounting for 19.5%. Similarly, among the 26 down-regulated compounds, 2-buten-1-one, 1- (2,6,6-trimethyl-1,3-cyclohexadien-1-yl) -, (E) -; 2,2'-ethylidenebis (5-methylfuran); Hexanoic acid, 2-phenylethyl ester, et al. ranked high. Among them, the most species are Terpenoids, with 10 species accounting for 38.5%.

In this study, the Wayne diagram was employed as a visual tool to elucidate the common and unique metabolites across various comparison groups, as depicted in Fig. 3. Additionally, a dynamic distribution map was utilized to highlight the differences in volatile content among different groups, also presented in Fig. 3. Among them, the unique substances in the I vs. P group were the most, with 46 species, while the unique substances in the O vs. P group were the least, with only 23 species. It is worth mentioning that there were nine substances that were present in all comparison groups. These included three heterocyclic compounds: 5-Isoxazolecarboxylic acid,

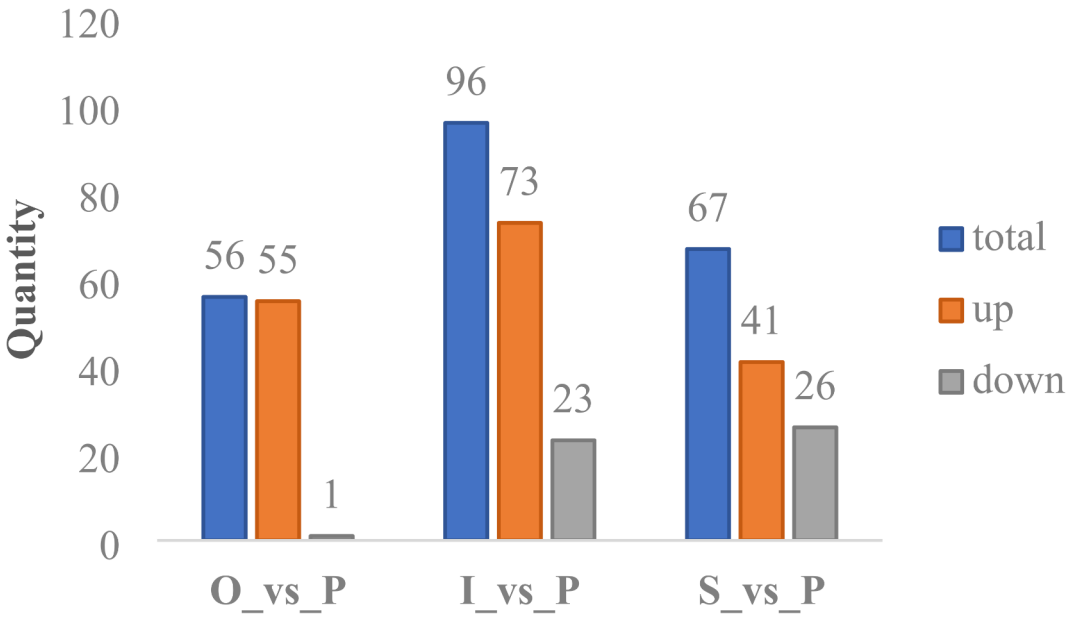


**Fig. 2**. Statistics of volatile compounds between control and experimental groups. P—Premium lager beer; O—Original beer; I—India Pale Ale, S-Stout beer.

| Group | Index | Compounds | Class | $Log_2FC^a$ | RI | Type |
|---|---|---|---|---|---|---|
| O_vs_P | KMW0709 | Hexadecanoic acid, ethyl ester | Ester | 3.90 | 1986.28 | Up |
| | XMW0614 | Nonanoic acid, ethyl ester | Ester | 3.73 | 1294.92 | Up |
| | KMW0534 | Butyl caprylate | Ester | 3.56 | 1385.82 | Up |
| | XMW1038 | Longifolenaldehyde | Terpenoids | 3.43 | 1592.66 | Up |
| | KMW0676 | Tetradecanoic acid, ethyl ester | Ester | 3.41 | 1793.71 | Up |
| | NMW0167 | Resorcinol monoacetate | Ester | 3.28 | 1385.82 | Up |
| | KMW0552 | (-)-.beta.-Bourbonene | Terpenoids | 3.15 | 1385.82 | Up |
| | XMW1097 | Hexanoic acid, 3-hexenyl ester | Ester | 3.11 | 1385.82 | Up |
| | KMW0594 | Dodecanoic acid, ethyl ester | Ester | 3.08 | 1592.66 | Up |
| | XMW0755 | m-Camphorene | Terpenoids | 3.06 | 1964.75 | Up |
| | WMW0071 | TriSulfur compounds, dipropyl | Sulfur compounds | 2.73 | 1324.42 | Up |
| | KMW0687 | 1 H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4. beta.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]- | Terpenoids | 2.70 | 1592.66 | Up |
| | WMW0217 | 2-Octenal, 2-butyl- | Alcohol | 2.39 | 1385.82 | Up |
| | XMW1098 | Butanoic acid, octyl ester | Ester | 2.37 | 1393.42 | Up |
| | XMW0618 | Ethyl 9-decenoate | Ester | 2.35 | 1393.42 | Up |
| | w42 | 2-Ethyl-1-dodecanol | Alcohol | 2.27 | 1592.66 | Up |
| | KMW0372 | Butanoic acid, hexyl ester | Ester | 2.13 | 1195.02 | Up |
| | XMW0608 | Ethyl 4-acetoxybutanoate | Ester | 2.11 | 1195.02 | Up |
| | KMW0537 | Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, (1.alpha.,2.beta.,4.beta.)- | Terpenoids | 2.08 | 1393.42 | Up |
| | KMW0464 | Octanoic Acid | Acid | 2.05 | 1195.02 | Up |
| I_vs_P | WMW0071 | TriSulfur compounds, dipropyl | Sulfur compounds | 6.54 | 1324.42 | Up |
| | XMW0693 | 2,6-Octadienoic acid, 3,7-dimethyl-, methyl ester | Ester | 5.51 | 1323.97 | Up |
| | KMW0060 | Toluene | Aromatics | 4.39 | 754.34 | Up |
| | KMW0240 | Ethanone, 1-(1 H-pyrrol-2-yl)- | Heterocyclic compound | 4.32 | 1067.78 | Up |
| | XMW0246 | OxiranecarboxAldehyde, 3-methyl-3-(4-methyl-3-pentenyl)- | Aldehyde | 4.28 | 1227.05 | Up |
| | NMW0091 | 8-Azabicyclo[3.2.1]octan-3-ol, 8-methyl-, endo- | Heterocyclic compound | 4.25 | 1227.49 | Up |
| | D355 | 2,6-Octadienenitrile, 3,7-dimethyl-, (Z)- | Nitrogen compounds | 4.02 | 1227.49 | Up |
| | XMW0764 | (S)-(-)-(4-Isopropenyl-1-cyclohexenyl)methanol | Terpenoids | 3.95 | 1323.97 | Up |
| | KMW0460 | Geraniol | Terpenoids | 3.66 | 1254.01 | Up |
| | NMW0070 | 1,2-Propanedione, 1-phenyl- | Ketone | 3.44 | 1193.27 | Up |
| | NMW0075 | Cyclohexanol, 1-methyl-4-(1-methylethylidene)- | Terpenoids | 3.36 | 1192.84 | Up |
| | KMW0070 | Benzene, 1,3-dimethyl- | Aromatics | 3.14 | 866.12 | Up |
| | NMW0122 | Geranyl formate | Ester | 2.76 | 1300.32 | Up |
| | XMW1448 | 3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran | Heterocyclic compound | 2.63 | 1193.27 | Up |
| | D94 | Benzenemethanethiol | Sulfur compounds | 2.51 | 1098.24 | Up |
| | D372 | 2-Hepten-1-ol, (E)- | Alcohol | 2.50 | 981.28 | Up |
| | NMW0071 | L-.alpha.-Terpineol | Terpenoids | 2.49 | 1195.02 | Up |
| | XMW1098 | Butanoic acid, octyl ester | Ester | -2.45 | 1393.42 | Down |
| | XMW0618 | Ethyl 9-decenoate | Ester | -2.61 | 1393.42 | Down |
| | KMW0594 | Dodecanoic acid, ethyl ester | Ester | -2.65 | 1592.66 | Down |

Continued

| Group | Index | Compounds | Class | $Log_2FC^a$ | RI | Type |
|---|---|---|---|---|---|---|
| S_vs_P | KMW0469 | Phenol, 4-ethyl-2-methoxy- | Phenol | 4.35 | 1283.86 | Up |
| | KMW0240 | Ethanone, 1-(1 H-pyrrol-2-yl)- | Heterocyclic compound | 3.93 | 1067.78 | Up |
| | KMW0060 | Toluene | Aromatics | 2.86 | 754.34 | Up |
| | WMW0058 | 5-Hepten-2-ol, 6-methyl- | Alcohol | 2.51 | 998.29 | Up |
| | KMW0709 | Hexadecanoic acid, ethyl ester | Ester | 2.31 | 1986.28 | Up |
| | XMW1038 | Longifolenaldehyde | Terpenoids | 2.16 | 1592.66 | Up |
| | D372 | 2-Hepten-1-ol, (E)- | Alcohol | 1.79 | 981.28 | Up |
| | WMW0046 | trans-2-Undecen-1-ol | Alcohol | 1.77 | 1361.81 | Up |
| | KMW0271 | Phenol, 3-methyl- | Phenol | 1.65 | 1079.70 | Up |
| | KMW0219 | 1-Hexanol, 2-ethyl- | Alcohol | 1.62 | 1028.01 | Up |
| | XMW0517 | Thiophene, 3-ethyl- | Heterocyclic compound | 1.58 | 874.78 | Up |
| | XMW0794 | 1,5-Cyclooctadiene, 3-(1-methyl-2-propenyl)- | Hydrocarbons | 1.53 | 1219.15 | Up |
| | XMW1468 | (2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl)-methanol | Alcohol | -1.52 | 1270.70 | Down |
| | KMW0446 | Citral | Terpenoids | -1.53 | 1270.70 | Down |
| | KMW0456 | 1-Decanol | Alcohol | -1.70 | 1270.70 | Down |
| | XMW0856 | 1-Butanone, 2-hydroxy-1-phenyl- | Ketone | -1.75 | 1388.50 | Down |
| | XMW1413 | Hexathiane | Sulfur compounds | -1.84 | 1507.36 | Down |
| | XMW0094 | Hexanoic acid, 2-phenylethyl ester | Ester | -1.87 | 1651.20 | Down |
| | XMW0174 | 2,2'-Ethylidenebis(5-methylfuran) | Heterocyclic compound | -2.06 | 1388.50 | Down |
| | KMW0526 | 2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-, (E)- | Terpenoids | -2.25 | 1388.06 | Down |

**Table 4**. Top 20 DVOCs with high Log2FC in each comparison of samples. Index: MW ID; [a] $Log_2FC$: Log base 2 of the fold change.

4,5-dihydro-5-methyl-, methyl ester, (R)-; 2-((3,3-Dimethyloxiran-2-yl)methyl)-3-methylfuran; and Ethanone, 1-(1 H-pyrrol-2-yl)-. Furthermore, two aromatics, two esters (Dodecanoic acid, ethyl ester and Hexanoic acid, 2-phenylethyl ester), one alcohol (2-Hepten-1-ol, (E)-), and one terpenoid [1,4-Methanoazulen-3-ol, decahydro-1,5,5,8a-tetramethyl-, [1 S-(1α,3β,3aβ,4α,8aβ)]-] were also identified across all groups.

## Flavor analysis of differential volatile compounds

The primary volatile chemicals found in alcoholic beverages are ethanol and carbon dioxide. Additionally, other compounds such as esters, alcohols, terpenoids, and heterocyclic compounds play a pivotal role in crafting the distinct aroma of beer[64].

Higher alcohols and esters are particularly significant as they contribute to the beer's complexity and its fruity, floral aroma. The formation of esters primarily occurs through esterase-catalyzed reactions, involving glucose and amino acid-derived alcohols and acids, during microbial metabolism[65]. Specifically, ethyl acetate, ethyl hexanoate, and caprylate lend a fruity and floral note to beer[66]. On a general overview, esters and aldehydes are often associated with a green aroma, while aldehydes and furanones are highly correlated with a sweet aroma[67]. Pyrazines and pyrroles impart typical notes of nuts, bread, and a burnt aroma[67]. Phenols, which are often described as spicy and woody, can contribute to the aroma and flavor of malt[67]. However, it's essential to note that these are general descriptions based on averages, and the ultimate taste of beer can vary depending on the brewing process and yeast strains employed.

In this study, we analyzed the aroma compounds present in various beer types. Our analysis identified several flavor substances, including alcohols, esters, terpenoids, ketones, aldehydes, and phenols. These compounds, due to their abundance and low threshold, significantly enhance the flavor and nutritional profile of beer[68], thereby playing a crucial role in improving its overall quality[69].

The results revealed that, in comparison to Premium lager beer, Original beer contained 30 aroma-related compounds, with esters being the most abundant (13), followed by terpenoids. The primary aroma descriptors for Original beer were green, fruity, waxy, and sweet, among others (Fig. 4). Notably, 12 compounds contributed to the green aroma, in conjunction with esters and aldehydes, such as Butanoic acid, hexyl ester; Hexanoic acid, butyl ester; and Tri-sulfur compounds, dipropyl. The compounds primarily associated with a fruity aroma were esters, including Hexanoic acid, butyl ester; Butanoic acid, 3-hexenyl ester, (Z)-; and Ethyl 9-decenoate, among others.

Similarly, the aroma profile of India Pale Ale (IPA) revealed an impressive 57 aroma-related compounds, with esters remaining the most prevalent, closely followed by terpenoids. The primary aroma descriptors for IPA were green, floral, sweet, and fruity, among others. Terpenoids and esters were the primary compounds contributing to the floral aroma. When compared to Premium lager beer, IPA contains a higher concentration of terpenoids, which explains its predominant floral aroma alongside the green notes.

There were 41 aroma related compounds in Stout beer compared with Premium lager beer, with alcohols being the most abundant, followed by esters and terpenoids. The variation in the content and composition of volatile substances results in distinct flavor profiles. Stout beer is characterized by a sweet, floral, green, and

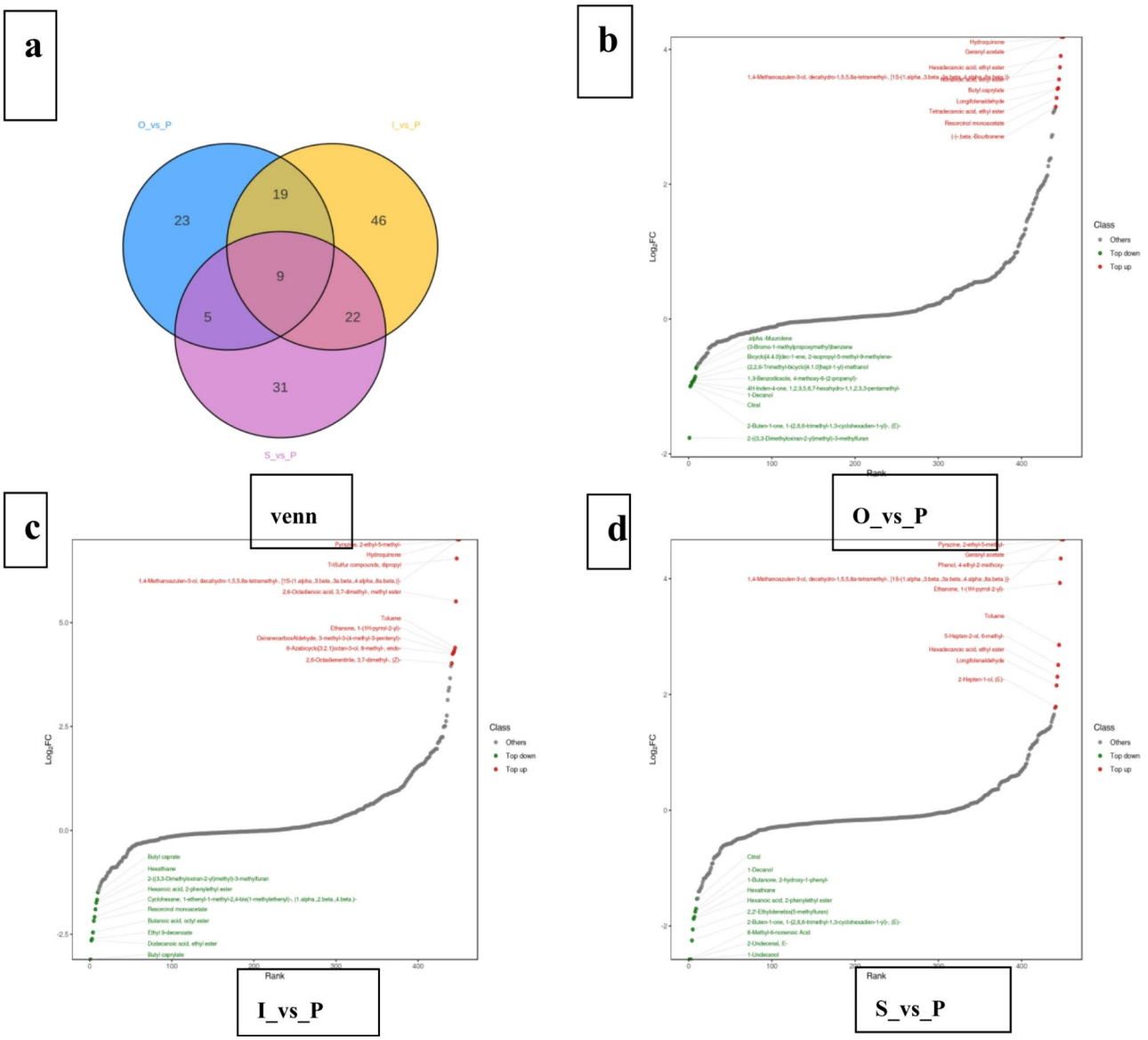


**Fig. 3**. (**a**) VENN diagram of 155 differential volatile compounds in P, O, I, S samples. (**b**) Dynamic distribution map of volatile content difference (O_vs_P). (**c**) Dynamic distribution map of volatile content difference (I_vs_P). (**d**) Dynamic distribution map of volatile content difference (S_vs_P). The green point represents the substances in the top 10 down regulation, and the red point represents the substances in the top 10 up regulation.

fruity aroma. The compounds that most significantly contribute to its sweet aroma include esters, alcohols, aromatics, and acids.

## Conclusions

This study indicates that four different flavors of beer have different Characterization. The alcohol content of the four beers ranged significantly from 4.23 to 7.54%. Among the four different flavors of beer, the bitterness of Premium lager beer is the lowest. There are also significant differences in color, total acidity, and carbohydrates.

The research conducted has demonstrated that beer with varying physicochemical properties exhibits considerable differences in its antioxidant activity content and volatile organic compound composition. Notably, Stout beer emerged with the highest concentration of active ingredients, including total polyphenols, melanoidins, and flavonoids, which are its primary components. Furthermore, Stout beer exhibited the strongest antioxidant capacity in vitro. Our findings revealed a significant increase in polyphenol and melanoidin content in beer, accompanied by enhanced antioxidant activity as assessed by ABTS, ORAC, and DPPH tests.

Non-targeted volatile omics analysis revealed that terpenoids, esters, heterocyclic compounds, alcohols, and hydrocarbons constitute the primary volatile substances present in the four distinct types of beer. The identified common volatile compounds include Phenylethyl Alcohol; Acetic acid, 2-phenylethyl ester; 1-Butanol, 3-methyl-;

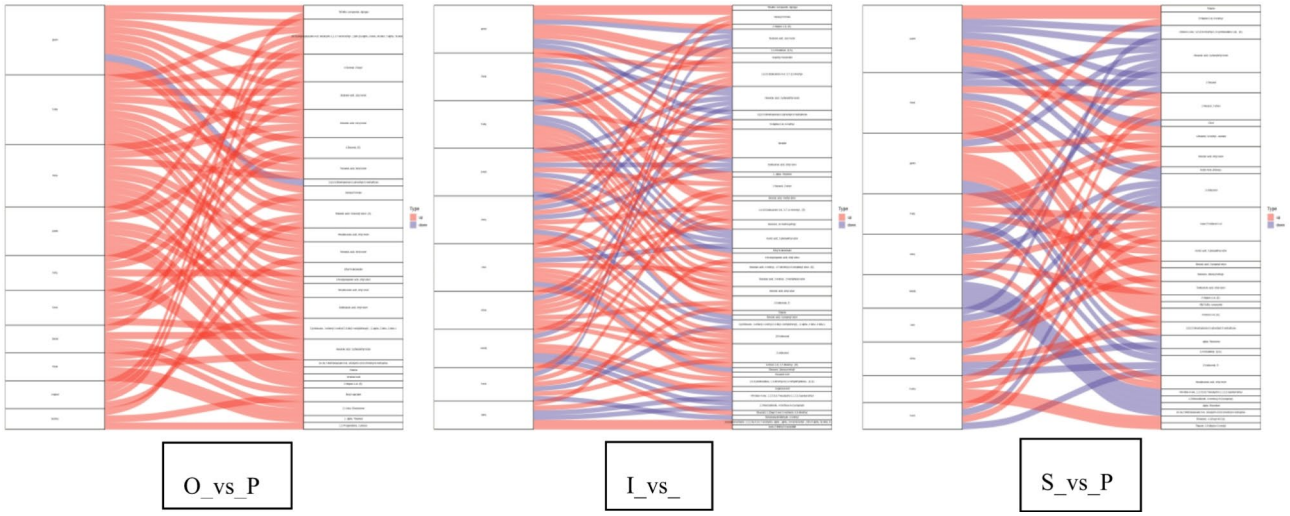


**Fig. 4**. Analysis of sensory flavor characteristics of differential group.

1-Butanol, 2-methyl-; Octanoic acid, ethyl ester; Hexanoic acid, ethyl ester; Decanoic acid, ethyl ester; Octanoic acid; Hexanoic acid; Benzeneethanol, 4-hydroxy-; Octanoic acid, methyl ester; and Dodecanoic acid, ethyl ester, among others. These compounds collectively contribute to the intricate aroma profile of the samples under investigation. By utilizing the fold change (FC) value to evaluate the trends in volatile compound variations, we identified nine substances that were common across all comparison groups. These include compounds such as 5-isoxazolecarboxylic acid, 4,5-dihydro-5-methyl -, methyl ester, (R)-; 2-((3,3-dimethyloxiran-2-yl)methyl)-3-methylfuran; Ethanone, 1-(1 H-pyrrol-2-yl)-; Dodecanoic acid, ethyl ester; Hexanoic acid, 2-phenylethyl ester; and 2-Hepten-1-ol, (E)-. The diverse flavors observed in different beers can be attributed to the varying types and concentrations of volatile compounds they contain. However, the four types of beer examined in this study generally share significant flavors, primarily characterized as green, floral, sweet, and fruity.

The research undertaken has indeed unveiled that beers possessing diverse physicochemical properties exhibit varying antioxidant capacities and flavors. This significant finding offers valuable theoretical insights for consumers when making their selections and for manufacturers during product production. It is noteworthy that our study focused solely on beers from a specific region, Qingdao, in China. Hence, further research exploring the characteristics of beers from diverse regions or countries is imperative to enhance beer quality and refine its flavor profiles. Such an endeavor would undoubtedly contribute to the broader understanding and appreciation of beer worldwide.

## Data availability

Data Availability Statement: Data is contained within the article. Changwei Wang should be contacted if someone wants to request the data from this study.

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## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

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