Effects of dandelion addition on antioxidant property, sensory characteristics and inhibitory activity against xanthine oxidase of beer

Jiangqi Yao, Zhiyuan Ma, Yuxuan Wang, Yutang Wang, Lijun Sun **, Xuebo Liu *

College of Food Science and Engineering, Northwest A&F University, Yangling 712100, PR China

ARTICLE INFO

Editor name: Alejandro G. Marangoni

Keywords: Craft beer Dandelion Chicoric acid Antioxidant activity Polyphenols Xanthine oxidase

ABSTRACT

The effects of dandelion addition (DA) on the physiochemical properties, antioxidant activity, inhibitory activity against xanthine oxidase (XOD) and flavor of craft beer were investigated. It was found that DA changed the pH value, total acid content, thiobarbituric-acid-value, sugar content and color of beer, and increased the contents of total polyphenols and flavonoids and thus the antioxidant activity of beer. HPLC analysis showed that DA provided beer with chlorogenic, caffeic, ferulic, and chicoric acid, contributing to the inhibition activity against XOD that is a key enzyme in uric acid production. GC-MS analysis showed that 3-methyl-1-butanol, isopentyl acetate and ethyl caprylate were main aroma components of all samples. Although DA introduced the special aroma component of azulene, it did not significantly affect the appearance, bubble, aroma and taste evaluation of beer. Conclusively, DA potentially improved the beer properties of antioxidant and inhibition of uric acid production without changing its sensory characteristics.

1. Introduction

Beer is also known as liquid bread, excessive intake can easily lead to excess energy and obesity, damage the liver and kidneys, and affect cardiovascular health. The global production of beer has been experimenting with a steady and robust increasing trend in the last decade, establishing it in the top rank of the most consumed and popular alcoholic beverages (Vieira et al., 2020). It is produced by the alcoholic fermentation of yeast and converted sugars contained in malt wort mainly into ethanol and carbon dioxide (Kawa-Rygielska et al., 2019). Moreover, it is the third-largest consumed beverage in the world after water and tea, and has a rich source of many nutrients, such as vitamins, minerals, carbohydrates, amino acids, and bioactive compounds. The phenolic compounds in beer originate mainly from malt (70-80%) and to a smaller extent from hops (Calleminen and Collin, 2009; Tan et al., 2021), which substantially contributes to the color, taste, and stability of beer. Phenolic acids are rapidly absorbed in the human intestine and are mainly present in the blood in the form of glucuronic acid compounds and sulfate conjugates ( Ducruet et al., 2017).

Craft beer first emerged in the United States in the 1970s. Compared with the traditional industrial beer, craft beer has a fine selection, rich taste, mellow taste, and more full-bodied, which has become the main representative of the high-end beer market. “Craft beer” is defined as the beer made by a brewery having a Tobacco Tax and Trade Bureau (TTB) Brewer’s notice that is small (fewer than 6 M barrels per year), and independent (less than 25% owned by a non-craft brewer alcohol beverage industry member) (Baiano, 2021; Salazar et al., 2021). Based on the current demand for functional food and health, the addition of fruits and plants has become the main trend in the development of craft beer, which can not only bring more flavor but also improve the nutritional value of beer.

However, the heavy use of malt can cause excessive purines in beer, which are eventually metabolized to uric acid in the body. In addition, alcohol is easy to accumulate lactic acid in the body, inhibit the excretion of uric acid by the kidneys, destroy the balance between uric acid production and excretion, and result in the accumulation of uric acid and the formation of stones or gout. At the same time, excessive production or insufficient excretion of uric acid caused by excessive consumption of beer leads to an increase in serum uric acid levels, which can lead to hyperuricemia, it is directly related to an increase in XOD activity, which is closely related to the level of uric acid in the body (Ghallab et al., 2021; Honda and Masuda, 2016). Because the increase of XOD activity will promote the decomposition of purine into uric acid in the human body (Mehmood et al., 2019). Moreover, the increase of XOD

* Corresponding author. College of Food Science and Engineering, Northwest A&F University, Yangling 712100, Shaanxi, PR China.
** Corresponding author. College of Food Science and Engineering, Northwest A&F University, Yangling 712100, Shaanxi, PR China.

E-mail addresses: lijunsun@nwafu.edu.cn (L. Sun), xueboliu@nwafu.edu.cn (X. Liu).

https://doi.org/10.1016/j.crfs.2022.05.008
Received 25 February 2022; Received in revised form 30 April 2022; Accepted 19 May 2022
Available online 30 May 2022
2665-9271/© 2022 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
activity has been associated with oxidative stress and the development of other metabolic diseases, such as respiratory syndrome, viral infection, hepatitis, inflammation, ischemia-reperfusion, cancer, and aging (Ahmed et al., 2018; Fatima et al., 2018). Due to the crucial role of XOD in the generation of uric acid, suppressing XOD activity has become the main treatment strategy for hyperuricemia and gout, and is also the best defense against the harmful oxidative damage associated with the accumulation of free radicals.

Dandelion (Taraxacum spp.) is a perennial herb grown throughout the world especially in the warmer temperature zones of the Northern Hemisphere, which has most abundant phenolic compounds are hydroxycinnamic acid derivatives, especially chiecoric acid, chlorogenic acid and caffeic acid (Chen et al., 2012; Kenny et al., 2015; Williams et al., 1996). Although dandelion is considered a weed, it is known to treat a variety of ailments, such as heartburn, indigestion, anorexia, hepatitis, spleen cancer, liver cancer, etc, and without any side effects (Rehman et al., 2017).

At present, the treatment of HUA mostly relies on chemical drugs, such as allopurinol, febuxostat, phenbromarone and indomethacin. However, the use of these drugs has side effects, which limits their clinical application. Meanwhile, the research on beer with low purge content has been studied for a long time in China, the preparation method could affect the original flavor and taste evaluation of beer, and there are no mature methods to reduce the purine contents in beer at present. Moreover, beer is one of the most consumed beverages in China, and the way of banning or restricting drinking beer is not accepted generally. Therefore, in order not to affect the taste of beer and slow down the increase of uric acid after drinking beer, the ingredients that are applied to produce beer need to be modified. Chlorogenic acid, chlorogenic acid and flavonoids are the main biological active components in dandelion (Chen et al., 2012; Kenny et al., 2015). All these phytochemicals have uric acid lowering effects (Zhou et al., 2021), which can alleviate the symptoms of a variety of diseases, such as heartburn, indigestion, anorexia, hepatitis, spleen diseases and liver cancer, with almost no side effects (Rehman et al., 2017). The preparation of dandelion beer has been reported, which mainly focuses on its acceptability to consumers instead of its nutritional values (Hayward et al., 2019). Meanwhile, there are few reports on functional craft beers in recent years, and most of them evaluated the contents of bioactive ingredients and antioxidant activity in vitro. Therefore, the preparation of a functional craft beer with unique taste and potential to delay the rise of uric acid has a promising market prospect.

The present study aimed to develop a craft beer with DA, resulting in a beer with high bioactive compounds concentration, high antioxidant capacity, high XOD inhibitory activity, as well as desirable sensory characteristics.

2. Materials and methods

2.1. Materials

2.1.1. The raw materials

Dried dandelion leaves were acquired from Zhenyuantang Chinese medicinal materials Co., Ltd (Haozhou, Anhui, China) and kept in the original packaging in a dry place until use. Barley malt and wheat malt were supplied by Shandong Haiyue Malt Co., Ltd (Yantai, Shandong, China). Hops (Magluman and Sax) were obtained from Yakima Valley (Washington, USA). Saccharomyces cerevisiae yeasts (WB-06) were purchased from Fermendez Yeast Co., Ltd (Belgium).

2.1.2. Reagents and standards

Folin-Ciocalteau reagent, 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH, 98%) were purchased from Macklin Co., Ltd (Shanghai, China). 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, 98%), 2-octanol, XOD (5U) and xanthine were obtained from solarbio Biotechnology Co., Ltd (Beijing, China). Chlorogenic acid, caffeic acid, ferulic acid, and chiecoric acid were provided by Shanghai yuanye Biotechnology Co., Ltd (Shanghai, China). Ethyl acetate and methanol were supplied by Zhiyuan Chemical Reagent Co., Ltd (Tianjin, China). Gallic acid (>98%) was acquired from Kamiou Chemical Reagent Co., Ltd (Tianjin, China). Rutin was purchased from Sinopharm Chemical Reagent Co., Ltd (Beijing, China). Reagent water were ultra-pure and deionized water.

2.2. Beer processing technology

The beer processing technology was optimized based on the design method of the experimental team in the early stage.

2.2.1. Experimental design optimization

In order to design the best beer production parameters, the indexes of beer production were optimized before the experiment. The optimization factors were broken particle size, malt addition ratio, hops addition time, hops addition amount, yeast addition amount, and fermentation time. Raw material addition ratio according to Table S1 and Table S2.

2.2.2. Brewing process

In this study, the wort was prepared in the following manner (all equipment had been sterilized before use): First, the wheat malt and Australian barley malt were selected and mixed in 1:1, and crushed by a JHF-250A high-speed multi-functional grinder to a particle size of 80 mesh. Secondly, the water was heated to a temperature of 50 °C (measured in real time by thermometer), a quarter of the water volume of malt flour were poured into saccharifying bucket and stirred for 30 min. Next, the temperature was increased to 62 °C at a rate of 1 °C per minute for 90 min, rose to 72 °C for 10 min, and then maintained at 78 °C ± 0.5 for 10 min. The wort concentration was measured using a handheld meter and controlled at 11.5 °Bx. After the saccharification stage, the wort were divided into 6 lots of 2 L each. All beer lots were boiled at 100 °C for 1 h, the ratio of 0.6% hops were added in the boiling process (Magluman: Sax = 1:2), different doses of dried dandelion leaves and other additives were added in the wort 45 min after boiling, and the dried dandelion leaves used were gently crushed by hand (about 20 mesh). After boiling, the wort were cooled to room temperature, Saccharomyces cerevisiae yeasts Fermentis WB-06 were added to all six lots, primary fermentation lasted 7 days at 18 °C and the secondary one 14 days at 20 °C. The detailed production flow chart can be seen in Fig. S1. After the secondary fermentation, the beer samples were stored at 4 °C for 1–2 h. Then, the flocculated yeast was remove.

2.3. Physical and chemical analysis

2.3.1. Beer analysis

The pH value was determined by portable pH meter, the total acid content was determined by titration method and sugar content was measured with a hand-held sugar meter.

2.3.2. TBA

5 mL degassed beer were mixed with 2 mL 0.33% thiobarbituric acid solution of 50% acetic acid evenly, then it was heated accurately in a water bath at 60 °C for 60 min and cooled rapidly, in which the absorbances of beer samples were measured at 530 nm, followed by adding 2 mL water in the same treatment to make blank control. The TBA value of beer was represented by the absorption value.

2.3.3. Colorimetric analysis

The color of beer samples was determined by CS-820 Spectrophotometric colorimeter (Hangzhou, China) as described by Musso et al. (2016), and the color difference between them was compared and analyzed. The colorimeter whiteboard color was used as the standard, and the luminance index L* and color index A* (red) and B* (yellow) of all samples were measured. Each sample was randomly selected 6 times
to read out L*, A* and B* values.

2.4. Analysis of bioactive compounds

2.4.1. Determination of total polyphenol content

Total polyphenol content (TPC) was determined using Folin-Ciocalteu (F-C) Spectrophotometric method as described previously (Bertuzzi et al., 2020) after slight modification. Briefly, 0.2 mL beer samples were mixed with 2 mL Folin-Ciocalteu (F-C) diluted 10 times. After 5 min of the reaction, 2 mL 7.5% NaCO₃ aqueous solution and 5.5 mL deionized water were added to the mixture. After the mixing for 60 min at room temperature, the absorbance at 765 nm was measured using an ultraviolet spectrophotometer and substituted into the standard curve to calculate the total polyphenols content of the samples to be tested. The absorbance value was converted to gallic acid equivalent (GAE) mg/L beer through a calibration curve obtained with standard gallic acid in water in the range 50–700 mg/L. The data expressed the mean from triplicate values.

2.4.2. Determination of total flavonoid content

Total flavonoids content was measured by using a modified aluminum chloride colorimetric assay according to Yang et al. (2019). In short, rutin standard solution (0.00, 0.50, 1.00, 2.00, 3.00, 4.00 mL) were prepared and placed in a 10 mL volumetric flask, 60% ethanol solution were added to bring the volume of solution to 5 mL. 0.3 mL 5% sodium nitrite were added and shook well. After 6 min for reaction, 0.3 mL of 10% aluminum nitrate solution were added and stood for 6 min. Then, 4 mL of 4% sodium hydroxide solution were added, and then 60% ethanol were added to make the volume constant. After 15 min for reaction, and the absorbance was measured at 510 nm with an ultraviolet spectrophotometer.

Use concentration as the abscissa and absorbance as the ordinate to draw a standard curve. 1 mL beer sample was taken in 10 mL volumetric flask, and determined the flavonoid concentration in the dandelion craft beer according to the above standard curve method, and calculated the flavonoid content. The data were expressed in milligrams of rutin equivalent (RE) per liter of beer. The data expressed the mean from triplicate values.

2.4.3. Determination of phenolic acids in beer by high performance liquid chromatography (HPLC)

High performance liquid chromatography (HPLC) was used for the quantitative analysis of chlorogenic acid, caffeeic acid, ferulic acid, and chicoric acid. The beer samples were analyzed using HPLC system (Shimadzu Corp., Japan) with an Agilent Eclipse XDB-C18 column (5 μm; 4.6 mm × 150 mm; Phenomenex, CA, USA). According to Nardini and Garaguso (2020) and modified on this basis. 20 mL beer sample were extracted with ethyl acetate three times, and concentrated in a vacuum at 40 °C until dry. Finally, the dry residue was dissolved in 2 mL methanol, filtered by 0.45 μm membrane, and the corresponding phenolic acid contents were determined. 10 μL beer samples were analyzed on an Agilent Eclipse XDB-C18 column (5 μm; 4.6 mm × 150 mm; Phenomenex, CA, USA) film thickness. The mobile phase was delivered in gradient mode at a constant flow rate of 1.0 mL/min. Mobile phase A consists of 0.1% aqueous phosphoric acid, Mobile phase B consists of acetonitrile. Using chlorogenic acid, caffeeic acid, ferulic acid, and chicoric acid as reference materials, quantitative analysis of peak area was carried out. The phenolic acids contents of dandelion craft beer were calculated by the external standard method and the corresponding correction curve was drawn by the peak area method. All samples should be analyzed in triplicate.

2.5. Volatile substance composition analysis

2.5.1. Electronic nose analysis

The E-nose (PEN3 Airsense, Schwerin, Germany) was used to tentatively estimate the aroma profile similarity between all beer samples. E-nose showed the good capacity of differentiating beer samples mainly through W1C, W5S, W3C, W6S, W5C, W1S, W1W, W2S, W2W, and W3C sensors (S1-10). The cycle time of measurement was the 60 s, and the injection flow was 10–400 mL/min. The flow control and sampling system were built in. The dandelion craft beers were degummed and diluted 20 times, and then 5 mL was absorbed into a headspace bottle and sealed with a lid. Analysis conditions are as follows: manual injection, heating temperature 50 °C, heating time 5 min, carrier gas flow 150 mL/s, injection volume 1500 μL, injection rate 1500 μL/s, data acquisition time 1 min, time delay 6 min. Each sample was tested 10 times in parallel.

2.5.2. Volatile compounds analysis

Determination of flavor substances in beer by GC-MS (GCMS-QP2010 Ultra, Island ferry, Japanese). 2-octanol was used as an internal standard. 6 mL sample and 1.5 g NaCl were added, and 20 μL 20 mg/mL 2-octanol standard solution were added into a 20 mL headspace bottle. In addition, the actual mass concentration of 2-octanol in the sample is 66.7 μg/mL and measure after capping and sealing. The unknown compounds were searched by computer and matched with the National Institute of Standards and Technology, NIST, and WileyLibrary databases. Results with SI greater than 85 were retained, and the flavor substances obtained were the content relative to the internal standard.

Chromatographic conditions: chromatomic column is DB-1 ms woolen tube column (60 m × 0.25 mm × 0.25 μm); the initial column temperature was 40 °C; the inlet temperature was 230 °C; The temperature was programmed to 409 °C for 3 min, then ramped at 4 °C/min to 120 °C followed by an increase of 6 °C/min to 240 °C and held for 9 min. The carrier gas was helium (He) with a flow rate of 1.0 mL/min; splitless injection. Mass spectrometry conditions: Ion source temperature was 230 °C, electric power mode was EI, ionization voltage was 70 eV, mass scanning range was 35–400 amu. The carrier gas was helium with a constant flow rate of 1 mL/min. The injection port was maintained at 260 °C in non-split mode, while the transmission line and ion source were maintained at 260 °C and 240 °C, respectively. The oven temperature program was started at 40 °C for 2 min, then increased at 4 °C/min to 250 °C, and finally kept at 250 °C for 5 min. The total GC run time was approximately 60 min.

2.6. Antioxidant activity

2.6.1. DPPH assay

The free radical scavenging activity was determined by DPPH assay, in accordance with well-established procedures and modify it slightly (Humia et al., 2020). The blank control group was composed of 2 mL DPPH and 200 μL anhydrous ethanol. The sample group was composed of 2 mL DPPH and 200 μL beer sample solution. The sample control group was composed of 2 mL anhydrous ethanol and 200 μL beer sample solution. After 30 min in the dark at room temperature, the supernatant was centrifuged and the absorbance value was measured at 517 nm using a microplate analyzer. All measurements were made in triplicate at room temperature and without light.

2.6.2. ABTS assay

The antioxidant activity of beer was determined using the ABTS assay and some modifications were made (Neto et al., 2017). The anti-oxidants present in the sample scavenge ABTS free radicals and reduce the blue color of the solution. The ABTS solution were prepared by reacting ABTS diammonium salt at a concentration of 7 mmol/L with 2.45 mmol/L potassium persulfate at room temperature for 16 h. 200 μL ABTS solution and 20 μL deionized water were added into the blank test well. 200 μL deionized water and 20 μL beer were added into the control test well. 200 μL ABTS solution and 20 μL beer were added into the sample test well, shook well and stood for 5 min at 25 °C. The absorbance was measured at 734 nm. Each sample was tested in triplicate.
2.7. XOD inhibitory activity in vitro

Under aerobic conditions, the formation of uric acid was measured at 290 nm using xanthine as the substrate with slight modifications (Ghalab et al., 2021). Briefly, the reaction mixture was prepared in such a way that it contains 50 μL of test solution together with 50 μL of XOD solution (0.1 units/mL) and determined in a 96-well plate. The assay mixture was pre-incubated at 37 °C for 15 min. Then, 60 μL of substrate solution (0.2 mM xanthine) were added and placed in the incubator set at 37 °C. After 30 min for reaction, 40 μL 0.5 M HCl were added to terminate the reaction. Sodium phosphate buffer (200 mM, pH 7.4) were used as negative control and allopolyp was set as the positive control. The absorbance at 290 nm was measured using microplate reader. The XOD inhibitory activity was calculated using the following equation:

\[ \% \text{ inhibition} = 1 - \left[ \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}}} \right] \times 100 \]

Where Abs\text{control} means absorbance of the negative control sample; Abs\text{sample} means absorbance of the sample; Abs\text{blank} means absorbance of the blank sample.

2.8. Sensory analysis

The sensory characteristics of dandelion craft beer were evaluated by 10 volunteers from Northwest A&F University (including regular drinkers and non-drinkers). Four main sensory properties of appearance, bubble, aroma and taste (GB/T 4928–2008) were selected to characterize the sensory properties of beer samples. The sensory tests were conducted in the Pioneer Park laboratory of Northwest A&F University. The sample size provided to each participant was 20 mL, and all samples were provided at a refrigerator temperature of 4 °C. A cup of gurgling water was provided to the testers and 5–10 min break was allowed between two sample evaluations to avoid alcohol-induced taste fatigue. All evaluations were conducted separately, and 5–10 min break was allowed between two sample evaluations to avoid alcohol-induced taste fatigue.

2.9. Statistical analysis

The standard deviation (SD) and mean value of all data were determined and expressed as mean ± SD. All data were analyzed for significance using SPSS 20.0. Data were evaluated by ANOVA, while numerical means were compared at p < 0.05 by Tukey’s HSD test. For chemical composition analysis, three samples from each treatment were analyzed, and all analyses were performed in triplicate. All data were analyzed by ANOVA and HSD test of Tukey at p < 0.05.

3. Results and discussion

3.1. Effects of DA on physicochemical analysis of beer

The main physicochemical properties of beer are shown in Table 1. It was found that the pH value of craft beer with DA was significantly higher (p < 0.05) than the blank sample group (beer-1) and commercial beers, which might be due to the more polyphenol contents brought by

| Sample | pH   | Total acid (g/100mL) | TBA (°Brix) | Sugar (°Brix) | L*  | a*  | b*  | c*  | Total Phenolic Content (mg GAE/L) | Total Flavonoids Content (mg RE/L) | Chlorogenic acid (mg/L) | Caffeic acid (mg/L) | Ferulic acid (mg/L) | Chlorogenic acid (mg/L) |
|--------|------|---------------------|------------|---------------|-----|-----|-----|-----|-------------------------------|-------------------------------|------------------|------------------|------------------|-------------------|
| 1      | 4.22 | 4.29 ± 0.06        | ±           | ±             | ±   | ±   | ±   | ±   | 241.46 ± 6.25                | 192.58 ± 4.47                 | 8.58 ± 0.06       | 2.28 ± 0.05       | 6.78 ± 0.15       | nd                |
|        | ±    | ±                   | ±           | ±             | ±   | ±   | ±   | ±   | 0.01bc                        | 0.09bc                        | 0.17a            | 0.55abc           | 0.06c            | 1.18bc            |
| 2      | 4.31 | 4.64 ± 0.19         | ±           | ±             | ±   | ±   | ±   | ±   | 255.00 ± 5.07                | 247.76 ± 0.48                 | 112.50 ± 0.01     | 8.34 ± 0.12       | 8.95 ± 1.15       | 115.16            |
|        | ±    | ±                   | ±           | ±             | ±   | ±   | ±   | ±   | 0.01a                         | 0.12b                         | 0.05c            | 0.47b            | 0.19bc           | 0.01bc            |
| 3      | 4.32 | 4.81 ± 0.04         | ±           | ±             | ±   | ±   | ±   | ±   | 260.83 ± 3.44                | 309.76 ± 3.25                 | 226.13 ± 0.23     | 12.13 ± 0.02      | 13.19 ± 0.02      | 255.56            |
|        | ±    | ±                   | ±           | ±             | ±   | ±   | ±   | ±   | 0.02e                         | 0.01bc                        | 0.03bc           | 0.65bc           | 0.65bc           | 0.65bc            |
| 4      | 4.32 | 5.15 ± 0.12         | ±           | ±             | ±   | ±   | ±   | ±   | 299.67 ± 7.06                | 493.21 ± 5.94                 | 518.57 ± 18.48    | 29.25 ± 1.20      | 15.78 ± 0.01      | 581.56            |
|        | ±    | ±                   | ±           | ±             | ±   | ±   | ±   | ±   | 0.04a                         | 0.19c                         | 0.16b            | 0.02f            | 0.26b            | 0.27f             |
| 5      | 4.31 | 4.47 ± 0.069        | ±           | ±             | ±   | ±   | ±   | ±   | 255.71 ± 6.25                | 254.64 ± 5.89                 | 116.44 ± 0.04     | 8.57 ± 0.15       | 10.66 ± 0.15      | 131.56            |
|        | ±    | ±                   | ±           | ±             | ±   | ±   | ±   | ±   | 0.05a                         | 0.16a                         | 0.53bc           | 0.05c            | 0.81b            | 0.81b             |
| 6      | 4.35 | 4.87 ± 0.03         | ±           | ±             | ±   | ±   | ±   | ±   | 257.92 ± 2.50                | 306.12 ± 4.73                 | 215.50 ± 3.25     | 12.88 ± 0.50      | 14.22 ± 0.50      | 256.21            |
|        | ±    | ±                   | ±           | ±             | ±   | ±   | ±   | ±   | 0.01a                         | 0.00ed                        | 0.15e            | 0.06d            | 1.34ed           | 1.33ed            |
| 7      | 3.82 | 1.69 ± 0.03         | ±           | ±             | ±   | ±   | ±   | ±   | 123.46 ± 3.83                | 108.70 ± 2.37                 | 15.56 ± 0.01      | 12.05 ± 0.01      | 14.56 ± 0.01      | 10.56 ± 0.01      |
|        | ±    | ±                   | ±           | ±             | ±   | ±   | ±   | ±   | 0.01e                         | 0.11f                         | 0.20bc           | 0.01f            | 0.04f            | 0.04f             |
|        | ±    | ±                   | ±           | ±             | ±   | ±   | ±   | ±   | 0.02d                         | 0.00f                         | 0.19c            | 0.64a            | 0.06d            | 0.67f             |
| 9      | 4.25 | 2.27 ± 0.10         | ±           | ±             | ±   | ±   | ±   | ±   | 290.54 ± 2.91                | 139.75 ± 1.20                 | 10.85 ± 0.02      | 9.88 ± 0.01       | 9.88 ± 0.01       | 10.85 ± 0.02      |
|        | ±    | ±                   | ±           | ±             | ±   | ±   | ±   | ±   | 0.02b                         | 0.01f                         | 0.13d            | 0.49bc           | 0.03f            | 1.32bc            |
| 10     | 4.18 | 1.98 ± 0.11         | ±           | ±             | ±   | ±   | ±   | ±   | 285.84 ± 4.25                | 28.73 ± 7.02                  | 17.17 ± 0.19      | 17.17 ± 0.19      | 17.17 ± 0.19      | 17.17 ± 0.19      |
|        | ±    | ±                   | ±           | ±             | ±   | ±   | ±   | ±   | 0.04ed                        | 0.02f                         | 0.27b            | 0.03f            | 0.51e            | 0.50f             |

L*: lightness; a*: greenness/redness and b*: blueness/yellowness. Results are expressed as mean ± standard deviation. Different letters (a, b, c etc.) in the column indicate significant differences between values at p < 0.05.

A: TBA stands for aging.
1: GAE: Gallic acid eq.
2: RE: Rutinum eq.
3: Not detected.

930
dandelion and the more total acid content produced by the interaction of protein, and also related to the raw materials, brewing processes, and DA. The total acid content in beers mainly came from the original total acid in malt and the acid produced by biochemical reaction during saccharification. It was observed that the total acid content of craft beer with DA was higher than commercial beers, because DA introduced more organic acids to beer, and with the increase of DA, the contents of organic acids in beer increased accordingly, because dandelion is rich in organic acids, such as chicoric acid, chlorogenic acid, caffeic acid, etc. (Xue et al., 2017).

Beer aging is a complex phenomenon involving several degradation and formation of chemical reactions that affect the sensory profiles of the product (Mutz et al., 2020). So far, the thiobarbituric acid method (TBA) has been normally used to detect beer aging in China. It was found that the TBA value in beer gradually decreased with the increase of DA, because dandelion contains a variety of monophenols, which has a certain influence on the aging of beer (Martinez-Perinan et al., 2011). The color parameters of beer are shown in Table 1. L* represents the comprehensive value of brightness and whiteness of the product, the positive value of A* represents redness, and the positive value of B* represents yellowness. It was observed that the whiteness of dandelion craft beer was lower than commercial beers, while the redness and yellowness were higher. This might be caused by the increase in the contents of dandelion extract and polyphenols due to the high temperature and formation of melanoidins resulting from the Maillard reaction that decides the color of beer to a large extent (Pieczonka et al., 2021).

### 3.2. Effects of DA on the contents of total polyphenols (GAE), total flavonoids, and phenolic acids in beer

Polyphenols are mainly derived from malt and hops, which affect the color, taste, bitterness, and other characteristics of beer (Kawa-Rygielska et al., 2019). The total polyphenols content of beer samples is shown in Table 1. It was found that the total polyphenols content of the blank sample group (beer-1) without dandelion was significantly higher than that of the commercial beers (p < 0.05), indicating that the raw materials, brewing processes, and DA had a direct effect on the polyphenols in beer (Humia et al., 2020). Because barley and wheat are used more as ingredients in craft beer than industrial beers, which are rich in polyphenols, it made more polyphenols precipitated out and existed in the beer in free form, which was consistent with the research results of Sanna and Pretti (2015). It was found that the contents of total polyphenols in beer-1 without DA was the lowest, which was statistically different from that of other beer sample groups with DA, and the result showed that the content of polyphenols was concentration-dependent with a dependency correlation of 96.4% (R² = 0.9644). The contents of total polyphenols in beer-4 reached 299.67 ± 7.06 mgGAE/L, which was significantly higher than the other sample groups (p < 0.05). Martinez-Perinan et al. (2011) reported that polyphenols had an indirect and positive role in beer aging, and the TBA value was positively correlated with the contents of polyphenols (Martinez-Perinan et al., 2011). This might be the reason why beer-4 had the lowest TBA value and the best stability as shown in Table 1. In addition, phenolic compounds also play an important role in the antioxidant capacity, flavor, and colloidal stability of beer (Cecconetti et al., 2019). The detection range of the contents of total flavonoids in all groups were 192.58–493.21 mgRE/L. The contents of total flavonoids in beer-4 were the highest and statistically significant (p < 0.05), and it was consistent with the result of total polyphenols content. It was found that the contents of total flavonoids were higher than total polyphenols with DA of beer, it might be because the total flavonoids content in DA was higher than total polyphenols (hot water extraction was beneficial for the precipitation of flavonoids). In addition, the contents of total flavonoids in the blank sample group (beer-1) were significantly higher than that in the control group, which was also consistent with the finding of total polyphenols. Thus, we concluded that DA to the traditional brewing process can effectively dissolve its functional phenolic components.

Williams et al. (1996) identified chicoric acid, chlorogenic acid, and caffeic acid in dandelion. It has also been found that chicoric acid is one of many active ingredients (alkaloids, caffeic acid derivatives, polysaccharides, and glycoproteins), which possess a variety of functional properties, including antioxidant, antiviral, anti-inflammatory, and uric-lowering activities (Ding et al., 2019; Lee and Scagel, 2010; Lu et al., 2018; Xiao et al., 2013). Therefore, we took chicoric acid as the main research object of dandelion craft beer, and selected the three major phenolic acids (chlorogenic acid, caffeic acid, ferulic acid) for HPLC analysis. The chromatograms of the four phenolic acids in dandelion craft beer are shown in Fig. 1A and Fig. 1B. It was found that the four phenolic acids could be effectively separated by treatment and were all present in dandelion craft beer. The HPLC chromatograms of the other beer sample groups are shown in Fig. S3. As shown in Table 1, it was observed that DA provided abundant phenolic acids to craft beer, including chlorogenic acid (112.50–518.57 mg/L), caffeic acid (8.34–29.25 mg/L), ferulic acid (8.95–15.78 mg/L), and chicoric acid (115.15–581.56 mg/L), increasing with the increase of DA amount. The contents of ferulic acid were higher than commercial beers, while caffeic acid and chicoric acid were not detected, this is because ferulic acid is the most abundant free phenolic acid in beer (Ducruet et al., 2017). It was found that DA contained unique bioactive components of chicoric acid, and the relationship was directly proportional to the concentration of dandelion. The contents of chlorogenic acid and chicoric acid were higher in beer-4 than other beer samples, which were 518.57 mg/L and 581.56 mg/L, respectively.

### 3.3. Antioxidant activity by DPPH and ABTS free radical scavenging assay

The antioxidant activity of beer is mainly attributed to the contents of phenolic compounds (Gorinstein et al., 2007). The DPPH and ABTS free radical scavenging activities of beer samples are shown in Table 2. All beer samples exhibited potential DPPH and ABTS free radical scavenging activities at the tested concentrations. It was found that beer-4 had the highest free radical scavenging activity (with both the DPPH and ABTS free radical scavenging abilities reached more than 90%) and were statistically different compared with other groups (p < 0.05). Next, the antioxidant capacity between beer-2 and beer-5, and between beer-3 and beer-6 were not statistically significant (p > 0.05), which indicates that the addition of bitter flower and tangerine peel did not affect the antioxidant capacity of beer. There were studies reporting that dietary polyphenols have strong antioxidant activity, anti-inflammatory, allergic, antiviral/antibacterial, anti-mutagenic/anticancer properties, as well as protective effects against various diseases (Liu et al., 2021; Zhang et al., 2014). As shown in Tables 1 and 2, it was found that DA increased the contents of total polyphenols, flavonoids and phenolic acids, thus the antioxidant activity of beer, which also confirmed our experimental results. Sample beer groups (beer-1 to beer-6) showed higher antioxidant capacity than control beer groups (beer-7 to beer-10), which might be related to the fact that the raw materials of industrial beer are mainly composed of corn and rice (Humia et al., 2020). This is consistent with the findings of Humia et al. (2020). At the same time, the two kinds of commercial craft beer were investigated, the results showed that the antioxidant capacity of the blank control group (beer-1) than beer-9 and beer-10 slightly high but not statistically significant, and the sample group (beer-2 to beer-6) had a significantly higher the antioxidant capacity of commercial craft beers, it directly related to DA. Studies have pointed out that beer is a good source of antioxidants, its antioxidant composition depends not only on the raw materials, but also on the production technology (Jurková et al., 2012). DA could make beer possess strong antioxidant capacity, and it is a suitable additive in beer production.
3.4. XOD inhibitory activity in vitro

XOD is abundant in the liver and plays an important role in purine nucleotide metabolism, which is closely related to metabolic diseases such as gout and hyperuricemia. It is the rate limiting enzyme that catalyzes the formation of uric acid, and it can catalyze hypoxanthine to xanthine and further catalyze xanthine to uric acid (Ahmed et al., 2018; Nile et al., 2016). Studies have shown that many kinds of flavonoids and

Fig. 1. HPLC analysis of four main phenolic acid content and GC-MS analysis of aroma components in beer samples. (A). Chromatograms of chlorogenic acids, caffeic acids, ferulic acids and chicoric acids for separation. (B). Chromatograms of four phenolic acids in craft beer with 20 g/L dandelion as an example. (C). Total ions chromatogram of flavor compounds in craft beer samples by GC-MS. (D). Heat maps of the main volatile components.
beers. Through the determination of XOD inhibitory activity of all beer samples, it was found that traditional commercial beer did not have XOD inhibitory activity against XOD, while the craft beer supplemented with dandelion had a certain effect of XOD inhibitory activity (Fig. 2A), which could increase with the increase of DA amount, thus beer-4 had the highest XOD inhibitory activity (Fig. 2A). 

Phenolic substances have inhibitory activity on XOD (Masuoka and Kubo, 2018). Thus, the phenolic and flavonoid constituents may play an important role in the inhibition against XOD, and these XOD inhibitors are commonly used against the treatment and curing of inflammatory diseases and gouty arthritis (Nile et al., 2016). Allopurinol is a XOD inhibitor, which can inhibit the breakdown of xanthine into uric acid in the body, but it has serious side effects. Thus, it is a meaningful study to find a new substitute without side effects, and can provide better health care effect and inhibit the production of uric acid content after drinking beer. 

Through the determination of XOD inhibitory activity of all beer samples, it was found that traditional commercial beer did not have the inhibitory activity against XOD, while the craft beer supplemented with dandelion had a certain effect of XOD inhibitory activity (Fig. 2A), which could increase with the increase of DA amount, thus beer-4 had the strongest inhibition ability by 65.16%. Compared with beer-2, beer-5 (orange peel addition) improved the inhibition ability slightly, but there was no statistical significance (p > 0.05), the results were consistent with the phenolic acid contents in Table 1. This is because DA provided beer with chlorogenic, caffeic, ferulic, and chicoric acid, contributing to the inhibitory activity against XOD, which is consistent with the research results of Zhou et al. (2021). As shown in Fig. 2B, it was found that the four phenolic acids had the inhibitory activity against XOD, the results were similar to previous studies (Gawlik-Dzik et al., 2017; Manzanilla and Robles, 2022; Wang et al., 2021; Wan et al., 2019). It also confirmed that the phenolic acid components brought by dandelion contributed to the inhibitory activity against XOD. The results showed that the product potentially provides beer consumers with a certain ability to resist the risk of high uric acid.

The inhibitory activity against XOD of allopurinol was determined as shown in Fig. S2. It was found that it had a good the inhibitory activity against XOD. Our results showed that this product had some pharmacological potential, but it need to be further improved in terms of pharmacological action.

3.5. Analysis of flavor active substances

SPME-GC-MS was used to analyze the volatile aroma spectrum of all beer samples. Total ions chromatogram is shown in Fig. 1C and volatile aroma compounds concentrations (μg/L) diagram as shown in Table 3. It was found that a total of 54 volatile compounds were detected, including 8 alcohols, 3 aldehydes, 3 acids, 26 esters, 4 ketones, and 10 other compounds. Higher alcohols, esters, and fatty acids were classified as fermentation aroma compounds. As shown in Table 3 and Fig. 1D, the contents of isopentyl acetate were the highest among all beer samples, and its contents of beer treatment groups (beer-1 to beer-6) were significantly higher than commercial beers, ranging from 590.49 to 1782.03 μg/L, especially which in beer-4 was significantly increased compared with the other groups (p < 0.05). It was found that 3-methyl-1-butanol, isopentyl acetate, and ethyl caprylate were the main components of all beer samples, and azulene was only found in the beer with DA, which was a unique component of composite plants. The content of azulene in beer-4 was 5.51 μg/L, which was statistically different from that in other groups (p < 0.05), indicating that DA could bring the special aroma component. Among alcohol compounds, the content of 3-methyl-1-butanol was higher and followed by phenyl ethanol. Alcohols were mainly derived from the metabolism of yeast during fermentation, also came from the decomposition of glycoside precursors and esters. As an important component of the higher alcohol in beer, benzene ethanol gives beer fullness of aroma and taste, increases the coordination of beer and constitutes the main aroma of the wine body. Among ester compounds, a moderate amount of volatile ester compounds was not only beneficial to the flavor and aroma coordination, but also had a certain masking effect on aging substances in beer. Dandelion contains a certain amount of ester compounds, which improves the content of ester compounds in beer, further contributing the change of TBA substances in Table 1. Among aldehydes compounds, acetaldehyde was the most abundant volatile aldehyde in beer, excessive content gives the beer strong grass flavor and shorten the shelf life. The OVA of acetaldehyde and decanal were both greater than 1, indicating that these aldehydes play an important role in the smell of beer. In the presence of oxygen, heat treatment promotes the peroxidation and decomposition of unsaturated fatty acids. The degradation products of oleic acid usually include heptanal, nonanal, 2-decanal, and heptane, while the decomposition of linoleic acid yields pentanal, hexanal, 1-pentanol, decanal, 2-heptanone, and octanoic acid. Decanal, 2-heptanone, and octanoic acid had been identified among these compounds, decanal had a citrus and floral aroma, while 2-heptanone provided a fruit-like aroma and could be used as a spice ingredient, and octanoic acid had a fruity aroma after being diluted. What needs special attention here was the integrated experience that in other groups (p < 0.05), indicating that DA could bring the special aroma component. Among alcohol compounds, the content of 3-methyl-1-butanol was higher and followed by phenyl ethanol. Alcohols were mainly derived from the metabolism of yeast during fermentation, also came from the decomposition of glycoside precursors and esters. As an important component of the higher alcohol in beer, benzene ethanol gives beer fullness of aroma and taste, increases the coordination of beer and constitutes the main aroma of the wine body. Among ester compounds, a moderate amount of volatile ester compounds was not only beneficial to the flavor and aroma coordination, but also had a certain masking effect on aging substances in beer. Dandelion contains a certain amount of ester compounds, which improves the content of ester compounds in beer, further contributing the change of TBA substances in Table 1. Among aldehydes compounds, acetaldehyde was the most abundant volatile aldehyde in beer, excessive content gives the beer strong grass flavor and shorten the shelf life. The OVA of acetaldehyde and decanal were both greater than 1, indicating that these aldehydes play an important role in the smell of beer. In the presence of oxygen, heat treatment promotes the peroxidation and decomposition of unsaturated fatty acids. The degradation products of oleic acid usually include heptanal, nonanal, 2-decanal, and heptane, while the decomposition of linoleic acid yields pentanal, hexanal, 1-pentanol, decanal, 2-heptanone, and octanoic acid. Decanal, 2-heptanone, and octanoic acid had been identified among these compounds, decanal had a citrus and floral aroma, while 2-heptanone provided a fruit-like aroma and could be used as a spice ingredient, and octanoic acid had a fruity aroma after being diluted. What needs special attention here was the integrated experience.

Table 2
Antioxidant activity of all beer sample groups.

| Sample | DPPH (%) | ABTS (%) |
|--------|----------|----------|
| 1      | 70.18 ± 1.31<sup>a</sup> | 63.12 ± 0.39<sup>cd</sup> |
| 2      | 75.41 ± 0.87<sup>c</sup>  | 66.73 ± 1.76<sup>c</sup>  |
| 3      | 85.29 ± 1.67<sup>b</sup>  | 75.79 ± 0.87<sup>b</sup>  |
| 4      | 95.68 ± 0.21<sup>a</sup>  | 88.24 ± 2.62<sup>b</sup>  |
| 5      | 75.39 ± 2.96<sup>c</sup>  | 68.78 ± 1.25<sup>c</sup>  |
| 6      | 85.91 ± 1.22<sup>b</sup>  | 77.60 ± 4.88<sup>b</sup>  |
| 7      | 54.70 ± 1.73<sup>b</sup>  | 59.29 ± 6.64<sup>d</sup>  |
| 8      | 64.29 ± 1.36<sup>d</sup>  | 63.01 ± 0.63<sup>cd</sup> |
| 9      | 68.47 ± 1.96<sup>b</sup>  | 67.18 ± 2.13<sup>c</sup>  |
| 10     | 65.72 ± 0.69<sup>c</sup>  | 64.14 ± 2.26<sup>c</sup>  |

Results are expressed as mean ± standard deviation. Different letters (a, b, c etc.) in the column indicate significant differences between values at p < 0.05.

<sup>a</sup> 1, 1-diphenyl-2-picrylhydrazyl.
<sup>b</sup> 2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid).

![Fig. 2](https://example.com/fig2.png)

Inhibitory activity of xanthine oxidase in (A) beer supplemented with dandelion and (B) four phenolic acids. Data are expressed as the means ± SE (n = 3).
| NO. | Compounds       | CAS number | R£ | Odor threshold (g/L) | OAV | Concentration (µg/L) |
|-----|-----------------|------------|----|----------------------|-----|---------------------|
|     |                 |            |    |                      |     | 1                   |
|     |                 |            |    |                      |     | 2                   |
|     |                 |            |    |                      |     | 3                   |
|     |                 |            |    |                      |     | 4                   |
|     |                 |            |    |                      |     | 5                   |
|     |                 |            |    |                      |     | 6                   |
|     |                 |            |    |                      |     | 7                   |
|     |                 |            |    |                      |     | 8                   |
|     |                 |            |    |                      |     | 9                   |
|     |                 |            |    |                      |     | 10                  |

**Alcohols**

1. Ethanol 64-17-5 463 10000 <0.1 752.5 ± 1017.20 ± 925.52 ± 1022.34 ± 1104.48 ± 948.36 ± 859.88 ± 835.12 ± 988.76 ± 757.65 ±
2. 3-Methyl-1-Butanol 123-51-3 697 40000 <0.1 921.48 ± 1267.24 ± 1191.85 ± 1328.40 ± 1294.04 ± 1129.17 ± 850.46 ± 529.12 ± 668.73 ± 562.31 ±
3. 2-Methyl-1-Butanol 137-32-6 697 1200 <0.1 284.85 ± 353.21 ± 373.81 ± 378.03 ± 374.58 ± 333.38 ± 309.48 ± 215.15 ± 204.15 ± 206.25 ±
4. 1-Heptanol 111-70-6 960 425 <0.1 8.35 ± 1.10 ± 12.86 ± 8.16 ± 17.40 ± 12.04 ± 16.17 ± 17.10 ± 18.78 ± 21.22 ±

**Aldehydes**

9. Acetaldehyde 75-07-0 408 0.2 >1 36.60 ± 48.00 ± 49.49 ± 23.08 ± 48.83 ± 36.96 ± 27.02 ± 2.28 ± 10.67 ± 7.63 ±
10. 3-Methyl-Butanal 590-86-3 643 1 >1 2.47 ± 0.31 ± 3.84 ± 1.48 ± 2.48 ± 3.11 ± 4.24 ± 2.89 ± 1.35 ± 0.84 ±
11. Decanal 112-54-9 1402 0.3 >1 6.26 ± 1.95 ± 5.80 ± 2.19 ± 6.08 ± 4.97 ± 7.29 ± 12.17 ± 5.47 ± 5.02 ±

**Acids**

12. Octanoic acid 124-07-2 1173 1000 <0.1 59.99 ± 85.76 ± 83.37 ± 156.80 ± 82.70 ± 36.95 ± 38.18 ± 38.36 ± 36.64 ± 40.79 ±
13. Hexanoic acid 124-07-1 974 2000 <0.1 10.29 ± 20.16 ± 20.84 ± 40.36 ± 16.18 ± 14.76 ± 20.08 ± 11.01 ± 14.38 ± 10.12 ±
14. Acetic acid 64-19-7 576 20000 <0.1 4.50 ± 3.99 ± 3.68 ± 4.76 ± 6.30 ± 13.09 ± 7.12 ± 4.23 ± 1.11 ± 3.75 ±

**Esters**

15. Ethyl Acetate 141-78-1 586 870 >0.1 271.60 ± 325.08 ± 306.31 ± 341.84 ± 375.39 ± 307.89 ± 242.92 ± 208.69 ± 266.90 ± 106.59 ±
16. Ethyl Propanoate 105-37-6 686 7 >0.1 24.48 ± 21.98 ± 19.65 ± 23.56 ± 23.47 ± 19.43 ± 9.45 ± 4.54 ± 2.88 ± 4.09 ±
17. n-Propyl acetate 109-60-4 686 2700 >0.1 10.90 ± 10.16 ± 10.08 ± 12.17 ± 13.85 ± 11.60 ± 9.22 ± 3.00 ± 5.47 ± 4.35 ±
18. Isobutyl acetate 110-19-0 721 25 >0.1 15.38 ± 16.36 ± 18.66 ± 18.73 ± 18.63 ± 19.44 ± 13.63 ± 7.05 ± 3.13 ± 4.80 ±
19. Ethyl butanoate 105-54-4 785 18 >1 36.24 ± 37.99 ± 39.88 ± 43.66 ± 43.34 ± 34.33 ± 23.85 ± 25.24 ± 27.62 ± 27.04 ±
20. Isopentyl acetate 122-92-2 820 93 >1 1377.86 ± 1458.95 ± 1655.63 ± 1782.03 ± 1643.69 ± 1593.60 ± 1180.12 ± 933.97 ± 1231.19 ± 590.49 ±
21. 2-Methylbutyl Acetate 624-41-9 820 8 >1 122.86 ± 197.48 ± 250.26 ± 247.23 ± 212.0 ± 224.09 ± 173.28 ± 130.06 ± 191.30 ± 103.17 ±
22. Ethyl valerate 539-82-2 884 27 >0.1 1.99 ± 0.69 ± 1.26 ± 1.68 ± 1.48 ± 1.25 ± 1.37 ± 1.31 ± 0.88 ± 0.91 ±
23. Pentyl acetate 628-63-7 884 9 >0.1 4.78 ± 4.16 ± 8.76 ± 6.24 ± 2.28 ± 1.86 ±
24. Hexyl acetate 142-92-7 984 1.8 >1 14.45 ± 13.42 ± 21.35 ± 18.45 ± 17.15 ± 15.63 ± 8.74 ± 3.17 ± 9.74 ± 12.41 ±

(continued on next page)
| NO. | Compounds                | CAS number | RI |
|-----|--------------------------|------------|----|
| 25  | Ethyl hexanoate          | 123-66-0   | 55 |
| 26  | Methyl octanoate         | 111-11-5   | 200|
| 27  | Ethyl caprylate          | 106-32-1   | 12 |
| 28  | 2-Ethylphenyl octanoate  | 103-45-7   | 1259|
| 29  | Propyl octanoate         | 624-13-7   | 1282|
| 30  | Ethyl nonanoate          | 1262-3150  | 1261|
| 31  | Ethyl caprate            | 110-38-3   | 1122|
| 32  | Isoamyl Decanoate        | 2306-91-4  | 1615|
| 33  | 7-Octenoic acid, ethyl ester | 35194-38-8 | 1173|
| 34  | Ethyl palmitate          | 628-97-7   | 1978|
| 35  | Ethyl heptanoate         | 106-30-9   | 1083|
| 36  | Ethyl 9-decanoate        | 67233-91-4 | 1371|
| 37  | 2-Methylbutyl octanoate  | 67121-38-5 | 0   |
| 38  | Octyl acetate            | 112-14-1   | 47  |
| 39  | Isoamyl caprylate        | 2035-99-6  | 1417|
| Ketones                      |            |     |
| 40  | Acetone                  | 67-64-1    | 455|
| 41  | 2-Heptanone              | 110-43-3   | 853|
| 42  | 2-Nonanone               | 821-55-6   | 1052|
| 43  | 2-Octanone               | 111-13-7   | 952|
| Others                       |            |     |
| 44  | Ethylbenzene             | 100-41-4   | 893|
| 45  | Dimethyldecane           | 6141-1285  | 1704|
| 46  | Tetradecane              | 629-59-4   | 1413|
| 47  | 2,6,10-Trimethyldecane   | 3891-99-4  | 260|
| 48  | 2-Fluoropropene          | 1184-60-7  | 2739|
| 49  | Carbon dioxide           | 124-38-9   | 2109|

| Concentration (μg/L) | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    |
|----------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Ethyl hexanoate      | 438.76 ± 502.62 | 677.92 ± 700.14 | 516.49 ± 453.67 | 268.98 ± 321.32 | 225.13 ± 290.65 |
| Methyl octanoate     | 83.11 ± 92.73 | 115.16 ± 52.40 | 49.15 ± 79.04 | 160.10 ± 67.99 | 84.30 ± 50.16 |
| Ethyl caprylate      | 3.62 ± 3.05 | 1.51 | 7.14 ± 0.86 | 1.10 | 0.43 ± 0.40 | 0.33 |
| 2-Ethylphenyl octanoate | 64.94 ± 112.30 | 125.52 ± 164.47 | 149.17 ± 115.81 | 99.79 ± 61.21 | 77.24 ± 56.51 |
| Propyl octanoate     | 5.36 ± 0.90 | 2.79 ± 1.36 | 2.66 ± 1.52 | 2.87 ± 1.76 | 4.79 ± 1.15 |
| Ethyl nonanoate      | 6.69 ± 4.94 | 3.81 ± 2.62 | 3.45 ± 1.93 | 1.83 ± 1.54 | 1.58 ± 1.50 |
| Ethyl caprate        | 324.92 ± 254.56 | 232.21 ± 268.51 | 76.17 ± 139.34 | 95.81 ± 92.88 |
| Isoamyl Decanoate    | 6.3 ± 6.66 | 2.77 ± 0.84 | 4.23 ± 2.99 | 2.22 ± 1.34 | 7.04 |
| Octyl acetate        | 6.32 ± 1.84 | 6.47 ± 3.05 | 6.61 ± 7.13 | 8.55 ± 4.77 | 5.25 |
| Ethyl palmitate      | 5.81 ± 3.97 | 2.31 ± 0.93 | 4.98 ± 7.13 | 5.75 ± 5.69 | 3.42 |
| Ethyl heptanoate     | 5.80 ± 0.58 | 4.00 ± 1.70 | 5.06 ± 4.45 | 4.18 ± 4.70 | 3.08 ± 3.08 | 2.37 ± 2.84 |
| Ethyl 9-decanoate    | 138.44 ± 136.92 | 131.16 ± 138.92 | 133.32 ± 117.15 | 81.20 ± 35.06 | 24.89 ± 33.62 |
| 2-Methylbutyl octanoate | 81.25 ± 30.76 | 32.41 ± 36.39 | 28.47 ± 22.31 | 57.49 ± 40.59 | 12.99 ± 25.97 |
| Octyl acetate        | 4.81 ± 4.13 | 3.72 ± 2.96 | 5.57 ± 5.42 | 2.25 ± 3.94 | 6.77 ± 5.04 |
| Isoamyl caprylate    | 11.01 ± 10.84 | 11.81 ± 11.56 | 10.40 ± 9.41 | 6.87 ± 4.66 |
| Acetone              | 36.17 ± 22.32 | 65.32 ± 61.63 | 61.62 ± 26.05 | 33.18 ± 24.56 |
| 2-Heptanone          | 3.44 ± 2.45 | 3.33 ± 1.73 | 3.37 ± 2.66 | 2.85 ± 1.66 | 1.29 ± 0.66 |
| 2-Nonanone           | 3.00 ± 0.96 | 2.83 ± 0.79 | 2.94 ± 2.03 | 1.70 ± 2.72 | 0.62 |
| 2-Octanone           | 19.73 ± 21.09 | 15.60 ± 17.60 | 11.90 ± 12.49 | 11.12 ± 10.54 | 12.40 ± 16.87 |

| Concentration (μg/L) | 1           | 2           | 3           | 4           | 5           | 6           | 7           | 8           | 9           | 10          |
|----------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Ethylbenzene         | 2.59 ± 2.42 | 2.39 ± 2.79 | 2.05 ± 3.05 | 2.62 ± 2.02 | 2.52 ± 1.52 |
| Dimethyldecane       | 21.91 ± 18.66 | 19.38 ± 17.91 | 12.67 ± 13.99 | 5.35 ± 9.14 | 7.44 ± 6.48 | 7.17 |
| Tetradecane          | 8.30 ± 15.53 | 11.19 ± 4.39 | 6.45 ± 7.16 | 1.51 ± 2.90 | 2.31 ± 1.59 |
| 2,6,10-Trimethyldecane | 5.42 ± 1.84 | 3.28 ± 2.44 | 3.99 ± 2.60 | 2.60 ± 1.98 | 2.84 ± 1.98 | 1.54 |
| 2-Fluoropropene      | 46.44 ± 36.38 | 37.52 ± 34.78 | 39.99 ± 37.17 | 12.70 ± 17.85 | 14.30 ± 23.46 | 25.68 ± 17.22 |
| Carbon dioxide       | 10.98 ± 11.21 | 11.79 ± 15.36 | 12.70 ± 17.85 | 13.04 ± 23.46 | 25.68 ± 17.22 |
| Heneicosane          | 1.99 ± 3.81 | 2.14 ± 8.53 | 1.36 ± 3.99 | 3.06 ± 13.72 | 13.13 ± 6.61 | 3.81 ± 2.08 |
Table 3 continued

| NO. | Compounds       | CAS number | RI       | OAV Concentration (μg/L) | SD       |
|-----|----------------|------------|----------|--------------------------|----------|
| 51  | Stryene         | 109-42-2   | 883      | < 0.1                    | 0.04     |
| 52  | o-Xylene        | 95-47-6    | 997      | 2.51 ± 1.65              | 0.46     |
| 53  | Anthole         | 275-51-1   | 1069     | < 0.1                    | 0.04     |
| 54  | D-Limonene      | 5989-5     | 1018     | 20.9 ± 0.57              | 0.44     |

Results are expressed as mean ± standard deviation. Different letters (a, b, c etc.) in the column indicate significant differences between values at p < 0.05.

3.6. Sensory analysis

3.6.1. Electronic nose and principal component analysis (PCA) of beer samples

The sensor response data of the electronic nose (PEN3, Germany) were collected in 60 s during the measurement process, and the radar map as shown in Fig. 3A. It was found that the signals of S2 and S6 sensors in all beer samples were generally higher than other sensor values, and there were different among samples, which indicates that the relative contents of nitrogen oxides and alcohol compounds in beer aroma components were higher than other ingredients, and its contents of dandelion craft beer were generally higher than commercial beers. While the response of S7 and S8 sensors were relatively weak, indicating that the beer samples might have lower abundances of terpenes, alcohols and aromatic compounds. Although the signal strength of S5, S4, S9 sensors to the samples were lower, there still had difference in the signal strength between the samples, which might be due to the difference between aromatics and hydrocarbons (Li et al., 2017). Secondly, it was found that with the increase of DA amount, the signal strength of each sensor will also increase, which indicated that DA had a certain effect on the beer aroma. Next, the sensor value of beer-5 was slightly higher than beer-2, and the sensor value of beer-6 with bitter flower addition was slightly higher than beer-5 without it, which explained that the addition of tangerine peel and bitter flower would appropriately increase the flavor of beer and make it more mellow in taste.

PCA constitutes a statistical tool used to explain differentiation between samples and to extract information from the variables that mainly affects the sample spatial distribution. The PCA are shown in Fig. 3B. It was observed that PC1 and PC2 represented 83.10% and 12.10% of the total variance, respectively, due to the cumulative variance contribution rate of the first two principal components reached 95.20%, which indicates they are sufficient to explain the total variance in the dataset. In Fig. 3B, it can be observed that grouping of the samples was clearly apparent in the bi-plot of PCA, with beer 1–6 being located to the right of the X-axis, whereas the beer 7–10 were located to the left of the X-axis, it was found that there were significant differences in the flavor components between the experimental beers and commercial beers. Particularly, beer-7 stayed away from other groups, and this phenomenon may result from differences in brewing ingredients and processes. In addition, the diagram also suggested that beer-1 was deviated from beer 2–6, it can be inferred that DA had a certain effect on the aroma composition. To sum up, DA brought azulene had significant influence on the aroma of beers, making them different from the commercial beers, which may have implications for consumer preferences.

3.6.2. Sensory evaluation

On the basis of comprehensive consideration of personal preferences, through the sensory analysis of appearance, bubble, aroma, and taste evaluation of beer, the sensory evaluation radar chart is shown in Fig. 3C. In terms of appearance, the contents of polyphenols in dandelion may have some influence on beer, which makes the color of beer-4 darker than beer-2, which is less transparent and affects the visual impression of consumers, because the color is the first sensory contact between consumers and beer, it is crucial for building up interest in the product. The beer-2 had higher mean notes of color intensity in appearance. These beers were visually suggested with more amber color and clarity. It has been reported that polyphenols interact with proteins to have better foaming ability, so the foaming performance of beer-4 is
the highest among the sample group of beers. Meanwhile, dandelion contains a special bitter ingredient sesquiterpene lactone, which greatly affected the taste of beer and resulted in a lower score of beer-4. However, The unique aroma and taste of dandelion bring great possibilities to the craft beer market. The total preference score of beer-5 was the highest and significantly different from the other groups \((p < 0.05)\), which indicated that the addition of orange peel brought certain taste preferences to consumers without changing its physicochemical indicators. To sum up, beer-5 gave consumers the most impression and had the highest acceptance in all other beer samples, beer-4 had most the bitter taste and foam richness, DA brought health function to the beer while other substances addition could bring certain taste comfort to beer.

4. Conclusions

More and more scientific reports showing that moderate beer consumption has beneficial effects on the human immune system. However, commercial beers contained only a very small amounts of phenolic acids and flavonoids; therefore, we intended to supply dandelion to enhance the bioactive ingredients in beer. It was found that craft beer supplemented with dandelion could change the pH value, total acid content, TBA value, sugar content and color, and obtained more total polyphenols (299.67 mgGAE/L), total flavonoids (493.21 mgRE/L), and phenolic acids, especially chlorogenic acid (518.57 mg/L), chicoric acid (581.56 mg/L) and other functional factors with a variety of bioactive ingredients, which were ultimately affected by DA amount. In addition, the increase in the contents of these bioactive ingredients enhanced the antioxidant activity, the inhibitory activity against XOD, and DA brought the special aroma component of azulene, but had no significant effect on the appearance, bubble, aroma, and taste evaluation of beer. The results provided useful information for future research on the development of craft beers with inhibition of uric acid production. In the range of product acceptability, DA did not have significant effects on the sensor characteristics of the beer. However, in order to improve the contents of bioactive components and inhibitory activity against XOD in beers in practical applications, it is necessary to increase the amount of DA, which would bring unpleasant bitterness and reduce the acceptability of sensory analysis of beer samples. The problem regarding how to reduce the unpleasant pleasure caused by bitterness needs to be further shed light on. In this study, we proposed a functional craft beer with the inhibitory activity against xanthine oxidase, and the potential in slowing down uric acid elevation. With the increase of beer consumption year by year, it may gradually cater to consumers regarding the taste pursuit and health need. Also, it provides a novel idea for creation of a kind of functional craft beer in the future.

CRediT authorship contribution statement

Jiangqi Yao: Conceptualization, Methodology, Data curation, Software, Visualization, Investigation, Writing – original draft, Supervision, Writing – review & editing. Zhiyuan Ma: Conceptualization, Methodology, Formal analysis, Investigation, Project administration, Supervision. Yuxuan Wang: Investigation, Methodology. Yutang Wang:
Investigation, Methodology, Visualization. Lijun Sun: Resources, Project administration, Writing – review & editing. Xuebo Liu: Resources, Funding acquisition, Supervision.

Declaration of competing 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.

Acknowledgements

Innovative Talent Promotion Program-Technology Innovation Team of Shaanxi (2019TD-006), and Shaanxi Keypoint Research and Development Program (No. 2021NY-125). The authors thank Jingyan Li (College of Food Science and Engineering, Northwest A&F University, China) for her precious help in the detection of phenolic acids by HPLC system (Shimadzu Corp., Japan).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crsf.2022.05.008.

References

Ahmed, Q.U., Alhassan, A.M., Khatib, A., Shah, S.A.A., Hasam, M.M., Sarian, M.N., 2018. Antiradical and Xanthine Oxidase inhibitory activity evaluations of averrhoa bilimbi leaves and tentative identification of bioactive constituents through LC-QTOF-MS/MS and molecular docking approach. Antioxidants 7 (10), 157–153. https://doi.org/10.3390/antiox7100137.

Baiano, A., 2021. Craft beer: an overview. Compr. Rev. Food Sci. Food Saf. 20 (2), 1829–1856. https://doi.org/10.1111/1541-4337.12693.

Bertuzzi, T., Malازي, A., Chiari, S., Donadini, G., Roni, F., Spigno, G., 2020. Targeted healthy compounds in small and large-scale brewed beers. Food Chem. 310, 125935 https://doi.org/10.1016/j.foodchem.2018.12.053.

Cecconari, D., Sileoni, V., Marconcini, O., De Francesc, G., Lee, E.G., Perretti, G., 2019. Speciality rice optimization and improvement of rice milk beer aspect and aroma. Lebensm. Wiss. Technol. 99, 299–305. https://doi.org/10.1016/j.lwt.2019.08.060.

Chen, H.J., Inbaraj, B.S., Chen, B.H., 2012. Determination of phenolic acids and flavonoids in Taraxacum formosanum Kitam by liquid chromatography-tandem mass spectrometry coupled with post-column derivation technique. Int. J. Mol. Sci. 13 (1), 260–285. https://doi.org/10.3390/ijms13010026.

Ding, H., G.X.X., Cheng, H., Yu, Q.L., Li, D., 2019. Chiricoid acid alleviates lipopolysaccharide-induced acute lung injury in mice through anti-inflammatory and antioxidant activities. Int. Immunopharmac. 66, 169–176. https://doi.org/10.1016/j.intimp.2019.04.042.

Ducrut, J., Rebanque, F., Dierssen, S., Konisinska-Gagnazzo, A., Hettier, I., Andlauer, W., 2017. Amber ale beer enriched with goji berries:The effect on bioactive compound content and sensorial properties. Food Chem. 226, 104–118. https://doi.org/10.1016/j.foodchem.2017.01.047.

Fatima, I., Zafar, H., Khan, K.M., Saad, S.M., Javaid, S., Perveen, S., Choudhary, M.I., 2018. Synthesis, molecular docking and xanthine oxidase inhibitory activity of 5-aryl-H-tetrazoles. Bioorg. Chem. 79, 201–211. https://doi.org/10.1016/j.bioorg.2018.04.021.

Gawkli-Dziki, U., Dziki, D., Šwićewska, M., Nowak, R., 2017. Mechanism of action and interaction between coffeeine oxidase inhibitors derived from natural sources of chogenic and ferric acids. Food Chem. 225, 138–145. https://doi.org/10.1016/j.foodchem.2017.01.016.

Ghalib, D.S., Mohyeldin, M.M., Shawkly, E., Merwally, A.M., Ibrahim, R.S., 2021. Chemical profiling of Egyptian propolis and determination of its xanthine oxidase inhibitory properties using UPLC-MS/MS and chemometrics. Lebensm. Wiss. Technol. 136, 110298 https://doi.org/10.1016/j.lwt.2021.110298.

Gorinstein, S., Caspi, A., Libman, I., Leontowicz, H., Leontowicz, M., Tashma, Z., Katrich, E., Iafratevski, Z., Traktenberg, S., 2007. Bioactivity of beer and its influence on human metabolism. Int. J. Food Sci. Nutr. 58 (2), 94–107. https://doi.org/10.1080/09637480601108661.

Hayward, L., Wedel, A., McWeeny, M.B., 2019. Acceptability of beer produced with dandelion, nettle, and sage. Int. J. Food Sci. Technol. 18, 100180 https://doi.org/10.1111/1365-2672.13203.

Honda, S., Masuda, T., 2016. Identification of pyrogallol in the ethyl acetate-soluble part of coffee as the main contributor to its Xanthine Oxidase inhibitory activity. J. Agric. Food Chem. 64 (41), 7743–7749. https://doi.org/10.1021/acs.jafc.6b03539.
hyperuricemia rats. J. Funct. Foods 57, 150–156. https://doi.org/10.1016/j.jff.2019.03.038.

Wang, Q., Lin, B.F., Li, Z.F., Su, J., Feng, Y.L., Gu, J.Y., 2021. Cichoric acid ameliorates monosodium urate-induced inflammatory response by reducing NLRP3 inflammasome activation via inhibition of NF-κB signaling pathway. Evid. Based Complementary Altern. Med. 2021, 8668527 https://doi.org/10.1155/2021/8668527.

Williams, C.A., Goldstone, F., Greenham, J., 1996. Flavonoids, cinnamic acids and coumarins from the different tissues and medicinal preparations of Taraxacum officinale. Phytochemistry 42, 121–127. https://doi.org/10.1016/0031-9422(95)00865-5.

Xiao, H.F., Xie, G., Wang, J.W., Hou, X.F., Wang, X., Wu, W.Q., Liu, X.B., 2013. Chicoric acid prevents obesity by attenuating hepatic steatosis, inflammation and oxidative stress in high-fat diet-fed mice. Food Res. Int. 54 (1), 345–353. https://doi.org/10.1016/j.foodres.2013.07.035.

Xue, Y.S., Zhang, S.M., Du, M., Zhu, M.J., 2017. Dandelion extract suppresses reactive oxidative species and inflammasome in intestinal epithelial cells. J. Funct. Foods 29, 10–18. https://doi.org/10.1016/j.jff.2016.11.032.

Yang, J.Y., Kim, G.B., Chae, J.S., Kan, H., Kim, S.S., Hwang, K.S., Lee, B.H., Yu, S., Moon, S., Park, B., Bae, M.A., Shin, D.S., 2019. Antioxidant and anti-inflammatory effects of an ethanol fraction from the Schisandra chinensis buillon hot water extract fermented using Lactobacillus paracasei subsp. tolerans. Food Sci. Biotechnol. 28 (6), 1759-1767. https://doi.org/10.1007/s11006-019-00626-4.

Zhang, X.C., Chen, F., Wang, M.F., 2014. Antioxidant and antiglycation activity of selected dietary polyphenols in a cookie model. J. Agric. Food Chem. 62 (7), 1643-1648. https://doi.org/10.1021/jf4045827.

Zhou, X.F., Zhang, B.W., Zhao, X.L., Lin, Y.X., Wang, J., Wang, X.W., Hu, N., Wang, S., 2021. Chlorogenic acid supplementation ameliorates hyperuricemia, relieves renal inflammation, and modulates intestinal homeostasis. Food Funct. 12 (12), 5637-5649. https://doi.org/10.1039/d0fo03199b.