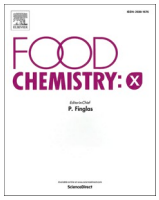
Food Chemistry: X 21 (2024) 101193

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Effects of tea addition on antioxidant capacity, volatiles, and sensory quality of beer

Jianhang Wu [a,b], Ye Zhang [b], Ran Qiu [c], Li Li [b,*], Xuyan Zong [a,b,*]

[a] Liquor Brewing Biotechnology and Application Key Laboratory of Sichuan Province, Sichuan University of Science and Engineering, Yibin 644000, Sichuan, China
[b] College of Bioengineering, Sichuan University of Science and Engineering, Yibin 644000, Sichuan, China
[c] China Resources Snow Breweries Co., Ltd, Bei Jing 100000, China

ARTICLE INFO

Keywords:
Beer
Green tea
HS-SPME-GC–MS
Antioxidant capacity
Volatile flavor components

ABSTRACT

Green tea has great potential to enhance the quality of beer. In this study, green tea was added at different stages of beer brewing, and evaluated the antioxidant capacity, volatile components, as well as sensory quality. The results showed that the addition of green tea during the start of boiling has great potential for application, and the green tea beer (GTB) had remarkable antioxidant properties (ABTS radical scavenging ability, 8.67 mmol TE/L; DPPH radical scavenging ability, 3.97 mmol TE/L; reducing power, 3.28 mmol TE/L), and an excellent sensory quality (acceptance, 6.09/9). HPLC analysis indicated that the principal phenolics in GTB were catechin and caffeic acid, in addition, the relative amounts of ferulic acid, gallic acid can be used to differentiate between GTB and beer. HS-SPME-GC–MS analyses showed that ethyl caprylate, ethyl nonanoate, ethyl caprate, linalool, and phenethyl alcohol were potentially significant for the aroma profile of GTB.

1. Introduction

Beer is the most widely consumed alcoholic beverage in the world, with a refreshing taste and attractive aroma. However, the ageing of beer in storage leads to a deterioration of flavor, resulting in “aged flavors”, such as the typical “cardboard aroma” of lagers (Lehnhardt, Gast, & Becker, 2019). The freshness of the beer is a key factor in consumer decision-making, with beers that have been stored for shorter periods of time more likely to be favored by consumers as it means fewer sensory deficiencies (Paternoster et al., 2020). But there are still many underdeveloped and inaccessible regions around the world, which still don’t have local brewing companies and need to supply beer from other regions after long logistical journeys, this leads to inevitable flavor degradation and reduced drinkability during transport and storage (Jaskula-Goiris et al., 2019). Furthermore, there are a number of well-known craft beer brewers who produce excellent quality beer, but most of them are not in a position to produce across borders or across regions, consumers are unable to taste the beer at its best after a long international transport journey. Therefore, delaying the ageing of beer remains a common challenge in the beer industry today.

Despite brewers working for a long time to reduce the oxygen content of packaged beers to prolong flavor stability, even oxygen levels as low as 0.1 mg/L were ineffective in curbing flavor spoilage of beers. (Barnette & Shellhammer, 2019). In contrast, the enhancement of endogenous antioxidant power in beer to slow down the deterioration of beer flavor has been extensively demonstrated (Yang & Gao, 2021). Phenolic compounds are the principal component of endogenous antioxidants in beer, they effectively inhibit the production of reactive oxygen radicals, as well as the removal of metal ions and thus the production of hydroxyl radicals (Zhao, Chen, Lu, & Zhao, 2010). Traditionally, barley malt (70 %–80 %) and hops (20 %–30 %) provide the phenolic compounds in the beer (De Francesco et al., 2020). As a result, brewers choose specific varieties and regions of barley malt and hops, then optimized the brewing process to enhance the beer’s antioxidant capacity (Zhao, 2015). Moreover, the prevalence of craft beer has led to the introduction into the beer industry of numerous ingredients that were previously not considered for use in beer brewing, such as some fruits and some herbs, which typically have a distinctive and fascinating flavor, as well as an abundance of biologically active components (Yang & Gao, 2021). This not only adds to the style of the beer, but also provides another strategy for slowing down the aging of the beer.

Tea (*Camellia sinensis*), an aromatic beverage, especially green tea, is widely grown and consumed in China. Green tea is rich in phenolic

* Corresponding authors at: College of Bioengineering, Sichuan University of Science and Engineering, Yibin 644000, Sichuan, China.
E-mail addresses: wujianhang1996@163.com (J. Wu), 1211312023@qq.com (Y. Zhang), qiuran001@126.com (R. Qiu), lili@suse.edu.cn (L. Li), zongxuyan@suse.edu.cn (X. Zong).

https://doi.org/10.1016/j.fochx.2024.101193
Received 29 November 2023; Received in revised form 19 January 2024; Accepted 2 February 2024
Available online 4 February 2024

compounds (account for about 42 % of the dry weight of green tea extract with excellent antioxidant and antibacterial properties) and other bioactive substances, with antioxidant and cardiovascular disease risk-reduction properties (Fraga, Croft, Kennedy, & Tomas-Barberan, 2019; Tang et al., 2019). Green tea has been reported to have a potential positive impact in enriching beer flavor as well as enhancing beer flavor stability (Chen et al., 2023; Rong et al., 2016). The extraction of components from green tea is affected by several factors, and the addition of green tea at different stages of beer brewing will result in differences in extraction temperature, extraction time, and composition of the extraction fluid, leading to significant differences in the quality of the final beer (Liu et al., 2021). Hence, there is an urgent need to elucidate which stage is more suitable for the addition of green tea.

This paper aimed to evaluate the protective effect of the addition of green tea on beer stability and quality, furthermore, indicated the most promising ways of green tea addition. Green tea was added at different brewing stages (boiling, cooling, and fermentation), evaluating the antioxidant properties, phenolic compounds, volatile flavor compounds, and sensory quality of green tea beer (GTB) to provide theoretical references to the production of GTB, which is of great significance for the promotion of the development of tea beer.

2. Materials and methods

2.1. Materials

Hulless barley malt, Yongchang County Smit Wine Co., Ltd.; Saaz hops: East China Brewing Materials Co., Ltd; Green tea, Pingshan Ming Zhu Tea Industry Co., Ltd; BF16 Saccharomyces cerevisiae (viable count: $\geq 6 \times 10^9$ cfu/g), Angel Yeast Co., Ltd.;

2.2. Beer brewing

Crushed hulless barley malt (1 kg) was mixed with 5 L of hot water (35 °C) for mashing (35 °C 30 min, 50 °C 50 min, 65 °C 60 min, 78 °C 10 min). A filtration was done after the end of the mashing, boiling the filtered wort for 1 h (hopping, 1 g/L), using stainless steel cooling coils to cool the wort after the boiling, cooling to room temperature with sterile water to adjust the soluble solids content to 14 °P. The wort was transferred to a fermentation bucket and added yeast (1 g/L) and then fermented in a temperature-adjustable climatic chamber (12 °C, the suitable temperature for yeast). Then the beer was bottled after 14 d of fermentation (soluble solids content at about 4 °P), and then continued to ferment for 14 d after replenishment of sugar. At the end of the fermentation, the temperature of the climatic chamber was adjusted to 4 °C to store the beer for subsequent analysis.

The experimentation (Fig. S1) included a beer without tea (Control) and 4 beers with green tea (3 g/L) added in different times: T_1: start of boiling; T_2: 10 min before the end of boiling; T_3 at cooling (temperature reduced to 80 °C); T_4 at the begging of fermentation (added with yeasts).

2.3. Physicochemical analysis

The physicochemical parameters of wort and beer analyzed in this study included pH, Plato, free amino nitrogen (FAN), and reducing sugar (RS). Turbidity, ethanol, real extract, and real fermentation degree (RDF) were also analyzed for beer.

pH was measured by a PHS-25 pH meter (INESA, China); Plato was measured by a hand-held refractometer (LICHEN, China); turbidity was measured by WGZ-200 turbidity meter (INESA, China); ethanol and real extract were measured using the density bottle method and the RDF is calculated by ethanol and real extract.

FAN was determined by ninhydrin-based methods. 2 mL of diluted sample (diluted 10 times) and 1 mL ninhydrin dilution solution are mixed well in a 25 mL colorimetric tube; cover the stopper with a boiling water bath for 15 min and then quickly cool to room temperature; add 5 mL of 2 g/L potassium iodate dilution and mix well, determine the absorbance with UV–vis spectrophotometer at 570 nm. The same method was used to determine the absorbance of the glycine standard solution. The FAN concentration was calculated from the ratio of the sample's absorbance to the standard's absorbance, considering the dilution factor (Zdaniewicz, Pater, Hrabia, Dulinski, & Cioch-Skoneczny, 2020).

The RS content was determined by 3,5-dinitrosalicylic acid (DNS) method. 1 mL of diluted sample (diluted 100 times) and 1 mL of deionized water, and 1.5 mL of DNS reagent are mixed well in a colorimetric tube; after boiling water bath for 5 min, remove it quickly and cool it to room temperature in a cold water bath, and then fix the volume with deionized water to 25 mL, shake it well and then measure its absorbance with a UV–vis spectrophotometer (wavelength of 540 nm) with a blank group (2 mL of deionized water and 1.5 mL of DNS reagent) for zeroing instruments. The reducing sugar content was calculated with reference to the glucose standard curve (Park, Park, Lee, Lee, & Lee, 2019).

2.4. Bioactive substances and antioxidant capacity

The bioactive substances of wort and beer analyzed in this study included total phenolic content (TPC), total flavonoid content (TFC), beta-glucan content (BGC); antioxidant capacity including DPPH radical scavenging ability (DSA), ABTS radical scavenging ability (ASA), reducing power (RP). These indicators were determined using the UV–visible spectrophotometer colorimetric method, with reference to the method of Zong et al. and with minor optimization (Zong et al., 2023). The absorbance was measured at a specific wavelength after the sample has been processed, then the concentration of bioactive substances and antioxidant capacity were calculated from the standard curve (considering the dilution factor), the content of bioactive substances were expressed as mg/L and the antioxidant capacity were expressed as mmol Trolox value/L (mmol TE/L).

2.5. HS-SPME-GC–MS

The volatile components of beers were analyzed using headspace solid phase microextraction (HS-SPME) combined with Thermo Fisher TRACE 1310 gas chromatography (GC) with Thermo Fisher TSQ 8000 mass spectrometry (MS) (Humia et al., 2020).

The beer sample was degassed; 10 mL of degassed beer sample was taken in a 20 mL headspace flask containing 3.2 g NaCl (supersaturated), equilibrated at 40 °C for 10 min, and extraction with a 50/30 um Divinylbenzene/carboxy/polydimethylsiloxane (DVB/CAR/PDMS) solid phase microextraction head at 40 °C for 30 min. The fiber is inserted into the injection port of gas chromatography and resolve at 250 °C for 6 min. The GC procedure was as follows: 40 °C 5 min, 3.5 °C/min increase to 120 °C and 5 °C/min increase to 215 °C for 10 min. The MS detector was at 70 eV in electron impact mode, ion source temperature: 230 °C; quadrupole temperature: 150 °C; scan range: 35–300 amu.

The relative content of each volatile compound was calculated by the ratio of the volatile compound's peak area to the internal standard's (2-Ethylbutyric acid) peak area.

2.6. HPLC

Sample preparation: Take 50 mL of filtered and degassed beer and 10 g of NaCl in a dispensing funnel, extract with 50 mL, 40 mL, and 40 mL of ethyl acetate for three times successively, collect the organic phase and evaporate to dryness at 40 °C under vacuum, then redissolve it in 4 mL of methanol, and then filter it through 0.45 μm micropore filter membrane.

HPLC conditions: COSMOSIL C18 column (4.6 × 250 mm, 5 μm), 0.1 % acetic acid–water solution (A_1), and 0.1 % acetic acid–methanol

Table 1
Physicochemical properties of worts and beers.

		T_1	T_2	T_3	T_4	Control
Wort	Plato (%)	14.0 ± 0.0^a	14.0 ± 0.0^a	14.0 ± 0.0^a	14.0 ± 0.0^a	14.0 ± 0.0^a
	pH	5.47 ± 0.00^a	5.43 ± 0.00^b	5.38 ± 0.00^c	5.24 ± 0.00^d	5.24 ± 0.00^d
	FAN (mg/L)	142.12 ± 1.38^a	144.28 ± 1.97^a	139.16 ± 1.18^b	128.91 ± 0.39^c	128.32 ± 2.56^c
	Reducing sugar (g/100 mL)	10.55 ± 0.08^b	11.15 ± 0.10^a	11.14 ± 0.01^a	10.62 ± 0.15^b	10.32 ± 0.11^c
Beer	Ethanol (% vol)	4.62 ± 0.06^a	4.66 ± 0.03^a	4.64 ± 0.02^a	4.10 ± 0.02^b	4.02 ± 0.04^b
	Real extract (%)	4.59 ± 0.03^c	4.73 ± 0.04^b	5.03 ± 0.02^a	4.69 ± 0.05^b	4.53 ± 0.03^c
	RDF (%)	67.52 ± 0.20^a	67.06 ± 0.11^a	65.59 ± 0.15^b	64.36 ± 0.23^c	64.72 ± 0.41^c
	pH	4.62 ± 0.00^b	4.68 ± 0.00^a	4.59 ± 0.00^c	4.52 ± 0.00^d	4.50 ± 0.00^e
	FAN (mg/L)	27.32 ± 0.04^b	27.24 ± 0.51^b	29.25 ± 0.16^a	25.74 ± 0.12^c	29.29 ± 0.35^a
	Reducing sugar (g/100 mL)	1.90 ± 0.00^c	1.97 ± 0.01^a	1.93 ± 0.02^b	1.99 ± 0.01^a	1.75 ± 0.01^d
	Turbidity (NTU)	7.78 ± 0.02^b	7.48 ± 0.03^b	7.17 ± 0.16^b	30.64 ± 7.62^a	11.66 ± 1.71^b

Note: T_1, means added tea at the start of boiling; T_2, means added tea at the end of boiling; T_3, means added tea at the cooling process; T_4, means added tea at the fermentation process; Control, means brewing beer without tea. Different letters in the shoulders of the same row indicate significant differences ($p < 0.05$).

solution (B_1). The gradient elution program was 0 min, 5 % B_1; 15 min, 20 % B_1; 35 min, 40 % B_1; 42 min, 65 % B_1; 50 min, 80 % B_1; 52 min, 5 % B_1; 60 min, 5 % B_1. The flow rate was 1 mL/min, the column temperature was 30 °C, and the injection volume was 10 μL. The external standard method performed the quantification, and the average value was obtained by repeating each sample three times (Rahman, Liang, Eskin, Eck, & Thiyam-Hollander, 2020).

2.7. Sensory analysis

The beer's general acceptance of each attribute was assessed on a nine-point hedonic scale: malt aroma, tasted, bitterness, tea flavor, foam, and acceptance of the beer. Thirty-two students trained in beer tasting were invited to take part in the test, who are also regular beer drinkers, aged between 20 and 30. Beer samples were provided at 4 °C for 50 mL. The panelists were asked to complete a nine-point hedonic scale, where 1 means extremely averse; 5 means neither like nor dislike; and 9 means like very much (Conti-Silva & de Souza-Borges, 2019).

2.8. Statistical analysis

Each experiment was carried out in triplicate and data were analyzed using IBM SPSS version 26 (SPSS Inc., Chicago, IL, USA), and all data were expressed as mean ± standard deviation (SD). Origin 9.0 software (Hampton, MA, USA) was used for graphical processing and PCA analysis. Pearson correlation test was used to determine the correlation between means. Statistical significance was declared at $p < 0.05$.

3. Results and discussion

3.1. Physicochemical analysis

The physicochemical properties of wort and beer are shown in Table 1. All wort was adjusted to 14°P before fermentation to ensure that the wort had the same soluble solids content. The FAN content of wort was significantly elevated by the addition of tea, especially when added at the boiling stage (T_1, 142.12 mg/L; T_2, 144.28 mg/L), which was attributed to the fact that the rich amino acids in tea were extracted during the boiling process (Damiani et al., 2019). There was no significant difference between T_4 (128.91 mg/L) and Control (128.31 mg/L), which was to be expected since T_4 did not have tea added at this point. Tea addition had a slight effect on the reducing sugar content of the wort, and the differences in reducing sugar content in wort can be attributed to experimental error. The ethanol content of GTB was higher than Control, maybe implying that some substances extracted from the tea promoted the ethanol metabolism of the yeast, which enabled the green tea barley beer to have a better RDF (T_1, 67.52 %), or it may be attributed to experimental error. The green tea barley beer also had a higher real extract, which was attributed to the extraction of polysaccharides from the tea by the wort. Beer has a pH between 4.53 and 5.03, which is suitable for yeast growth. The turbidity of T_4 was significantly higher than that of the other beers, perhaps due to the release of a large number of phenolic compounds from the green tea during fermentation, which complexed with the proteins and other macromolecules in the beer, resulting in abiotic turbidity (Jongberg, Andersen, & Lund, 2020).

Table 2
Bioactive substances in worts and beers.

Samples	Wort			Beer		
	TPC (mg/L)	TFC (mg/L)	BGC (mg/L)	TPC (mg/L)	TFC (mg/L)	BGC (mg/L)
T_1	638.04 ± 11.17^a	389.93 ± 2.67^a	244.70 ± 1.00^a	584.97 ± 2.79^b	275.93 ± 2.00^b	316.45 ± 2.25^a
T_2	633.35 ± 3.07^b	375.93 ± 2.00^b	243.20 ± 2.50^a	539.16 ± 5.59^c	256.27 ± 7.00^c	281.20 ± 3.00^b
T_3	441.40 ± 8.38^c	266.27 ± 3.67^c	221.45 ± 2.75^b	359.55 ± 3.07^d	147.60 ± 3.00^d	237.45 ± 2.75^e
T_4	362.63 ± 5.59^d	173.93 ± 3.33^d	200.45 ± 1.25^c	630.78 ± 5.59^a	296.27 ± 2.33^a	267.20 ± 2.00^c
Control	359.27 ± 3.91^d	170.27 ± 5.67^d	199.70 ± 1.25^c	265.14 ± 5.31^e	98.93 ± 4.00^e	253.70 ± 2.50^d

Note: T_1, means added tea at the start of boiling; T_2, means added tea at the end of boiling; T_3, means added tea at the cooling process; T_4, means added tea at the fermentation process; Control, means brewing beer without tea. Different letters in the shoulders of the same column indicate significant differences ($p < 0.05$).

3.2. Bioactive substances

Polyphenols, flavonoids, and β-glucan are important bioactive components in beer, and the determination of TPC, TFC, and BGC allows a preliminary characterization of the content of bioactive substances in wort and beer (Zong et al., 2023). The results in Table 2 indicate that adding tea can significantly enhance the bioactive content of wort and beer.

For wort, T_1 had the highest bioactive content with TPC of 638.04 mg/L, TFC of 389.93 mg/L, and BGC of 244.70 mg/L, which were similar to T_2 and significantly higher than T_3 (TPC, 441.40 mg/L; TFC, 266.27 mg/L; BGC, 221.45 mg/L) due to the shorter extraction time of T_3. T_1 showed a 78 % increase in TPC, 129 % increase in TFC, and 22 % increase in BGC compared to Control, which is an encouraging result, and it shows again that the application of tea will bring a non-negligible increase in the bioactives content of wort.

After fermentation and storage, the TPC and TFC in beer decreased markedly, with TPC decreasing by up to 18 % (T_3) and TFC decreasing by up to 44 % (T_3), which resulted from oxidative degradation and polyphenol adsorption by yeast during fermentation and storage (Nguela, Vernhet, Julien-Ortiz, Sieczkowski, & Mouret, 2019; Ribeiro et al., 2019). Autolysis of yeast occurs in the later stages of fermentation, which results in the release of β-glucan from the yeast cell wall and a significant increase in BGC (Puligundla, Mok, & Park, 2020; Wang et al., 2019). Notably, the TPC in the beer of T_4 was 630.78 mg/L, which is

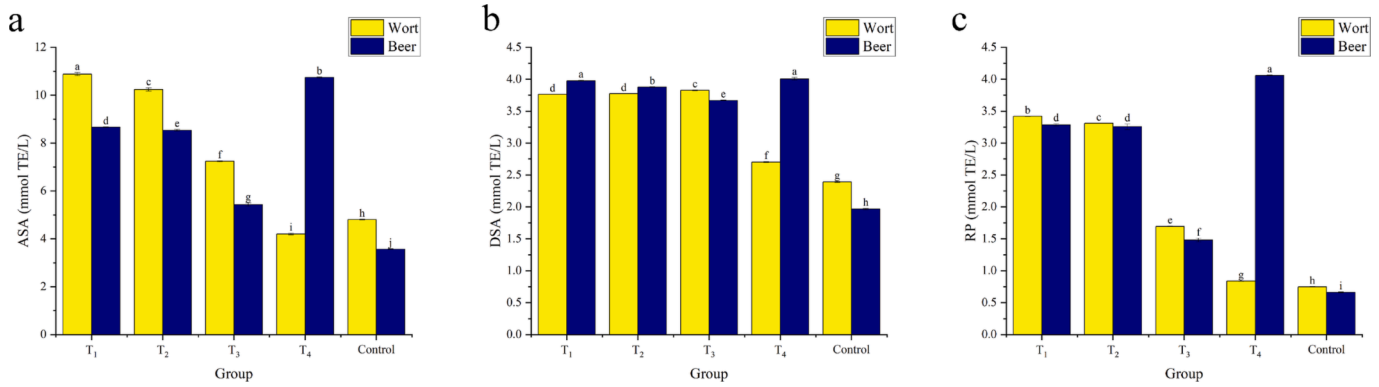


Fig. 1. Antioxidant capacity of worts and beers. a, ASA of worts and beers; **b**, DSA of worts and beers; **c**, RP of worts and beers. Standard deviation is indicated by error bars. T_1, means added tea at the start of boiling; T_2, means added tea at the end of boiling; T_3, means added tea at the cooling process; T_4, means added tea at the fermentation process; Control, means brewing beer without tea. Samples indicated with different characters are significantly different ($p < 0.05$).

Table 3
Individual phenolic contents of beers.

Phenolic compounds (mg/L)	Group				
	T_1	T_2	T_3	T_4	Control
Ferulic acid	9.78 ± 1.78^{a}	5.07 ± 0.49^{bc}	6.59 ± 1.25^{bc}	7.56 ± 1.75^{ab}	4.89 ± 0.31^{c}
Epicatechin	4.10 ± 0.49^{a}	1.76 ± 0.30^{b}	0.82 ± 0.10^{c}	0.69 ± 0.10^{c}	0.53 ± 0.12^{c}
Syringic acid	15.20 ± 2.23^{a}	11.66 ± 2.88^{a}	5.70 ± 1.08^{b}	12.55 ± 1.43^{a}	0.68 ± 0.18^{c}
p-Coumaric acid	3.00 ± 0.31^{a}	1.16 ± 0.17^{b}	1.12 ± 0.04^{b}	1.13 ± 0.14^{b}	0.67 ± 0.08^{c}
Catechin	34.15 ± 2.34^{b}	41.79 ± 3.81^{b}	15.50 ± 0.89^{c}	95.97 ± 10.26^{a}	2.11 ± 0.21^{d}
Caffeic acid	110.42 ± 6.39^{a}	69.28 ± 7.24^{b}	15.10 ± 1.58^{c}	119.04 ± 5.98^{a}	0.36 ± 0.01^{d}
Rutin	14.28 ± 1.67^{b}	15.81 ± 1.03^{b}	25.13 ± 1.23^{a}	6.56 ± 0.59^{c}	3.19 ± 0.33^{d}
Gallic acid	12.09 ± 1.34^{a}	7.78 ± 1.54^{d}	9.24 ± 0.60 ^{cd}	11.54 ± 0.35^{ab}	9.90 ± 0.28^{bc}
Vanillic acid	3.98 ± 0.44^{a}	1.15 ± 0.13^{b}	1.01 ± 0.07^{b}	1.03 ± 0.26^{b}	0.67 ± 0.08^{b}
Protocatechuic acid	10.04 ± 1.91^{b}	8.22 ± 1.36^{b}	4.07 ± 0.37^{c}	13.39 ± 0.96^{a}	0.57 ± 0.06^{d}
Chlorogenic acid	3.25 ± 0.55^{a}	1.07 ± 0.31^{b}	0.98 ± 0.10^{b}	0.90 ± 0.15^{b}	0.82 ± 0.02^{b}
Total	220.28	164.74	85.27	270.35	24.39

Note: T_1, means added tea at the start of boiling; T_2, means added tea at the end of boiling; T_3, means added tea at the cooling process; T_4, means added tea at the fermentation process; Control, means brewing beer without tea. Different letters in the shoulders of the same row indicate significant differences ($p < 0.05$).

similar to that in the wort of T_1, which may indicate that the temperature is not the main reason for the variation of TPC.

3.3. Antioxidant capacity

ASA, DSA, and RP are commonly used to characterize the antioxidant capacity of wort and beer, which is illustrated in Fig. 1 for wort and beer. Adding green tea significantly enhances the antioxidant capacity of wort and beer. The ASA is reduced considerably after fermentation and storage (Fig. 1a). The wort of T_1 had the highest ASA of 10.88 mmoL TE/L, which was attributed to the higher phenolic content of T_1 wort. The changes in ASA of wort and beer are consistent with the changes in TPC in Table 2. Many previous studies have shown a significant positive correlation between phenolic content and antioxidant properties in beer, which could also explain the variation in RP (Fig. 1c) (Humia et al., 2019; Nardini & Garaguso, 2020). The addition of green tea also enhanced the DSA of wort and beer (Fig. 1b). Unlike ASA, the DSA of T_1, T_2, and T_3 did not show this significant difference, varying slightly from 3.76 mmol TE/L (T_1) to 3.83 mmol TE/L (T_3), in spite of the significant difference in the content of bioactive in the wort. DSA did not increase with increasing TPC, suggesting that TPC cannot be directly used to characterize the antioxidant capacity of wort or beer. Similar conclusions were reported by Zhao et al. as different phenolics do not have the same power to contribute to the antioxidant properties of beer (Zhao et al., 2010).

In conclusion, the addition of green tea had a promising enhancement of the antioxidant capacity of wort and beer, with the highest enhancement of 201 %, 103 %, and 515 % for beer ASA, DSA, and RP, and for enhancing antioxidant properties of beer, T_4 was the most effective, followed by T_1, T_2, T_3.

3.4. Individual phenolics

Ferulic acid, epicatechin, syringic acid, p-coumaric acid, catechin, caffeic acid, rutin, gallic acid, vanillic acid, protocatechuic acid, and chlorogenic acid are common phenolic compounds in beer, and changes in their content profoundly affect the organoleptic quality and sensory stability of beer (Carvalho & Guido, 2022). The content of phenolics in the five types of beer was analyzed by HPLC (Table 3). The lowest content of phenolic compounds was found in the control group (24.39 mg/L) and it contained 14.79 mg/L of ferulic acid and gallic acid, which accounted for 60 % of the total. Ferulic acid and gallic acid are the most reported phenolic compounds in beer, generally account for more than 50 % of the total content, which is consistent with the results of the present study (Zhao et al., 2010). Therefore, it seems that the relative amounts of ferulic acid and gallic acid can be used to distinguish between GTB and beer. In addition, added green tea significantly increased the phenolic compounds content, in which the content of syringic acid (15.20 mg/L, T_1), catechin (95.97 mg/L, T_4), caffeic acid (119.04 mg/L, T_4), rutin (25.13 mg/L, T_3), and protocatechuic acid (13.39 mg/L, T_4) were considerably increased. The main phenolic compounds in GTB were caffeic acid, catechin, and rutin, which was accounted for more than 65 %. Due to the production of ethanol during fermentation and the significant extension of time of the extraction, the highest content of phenolic compounds was found at T_4 (270.35 mg/L). However, this was still lower than the TPC determined by the folin-phenol method, and the

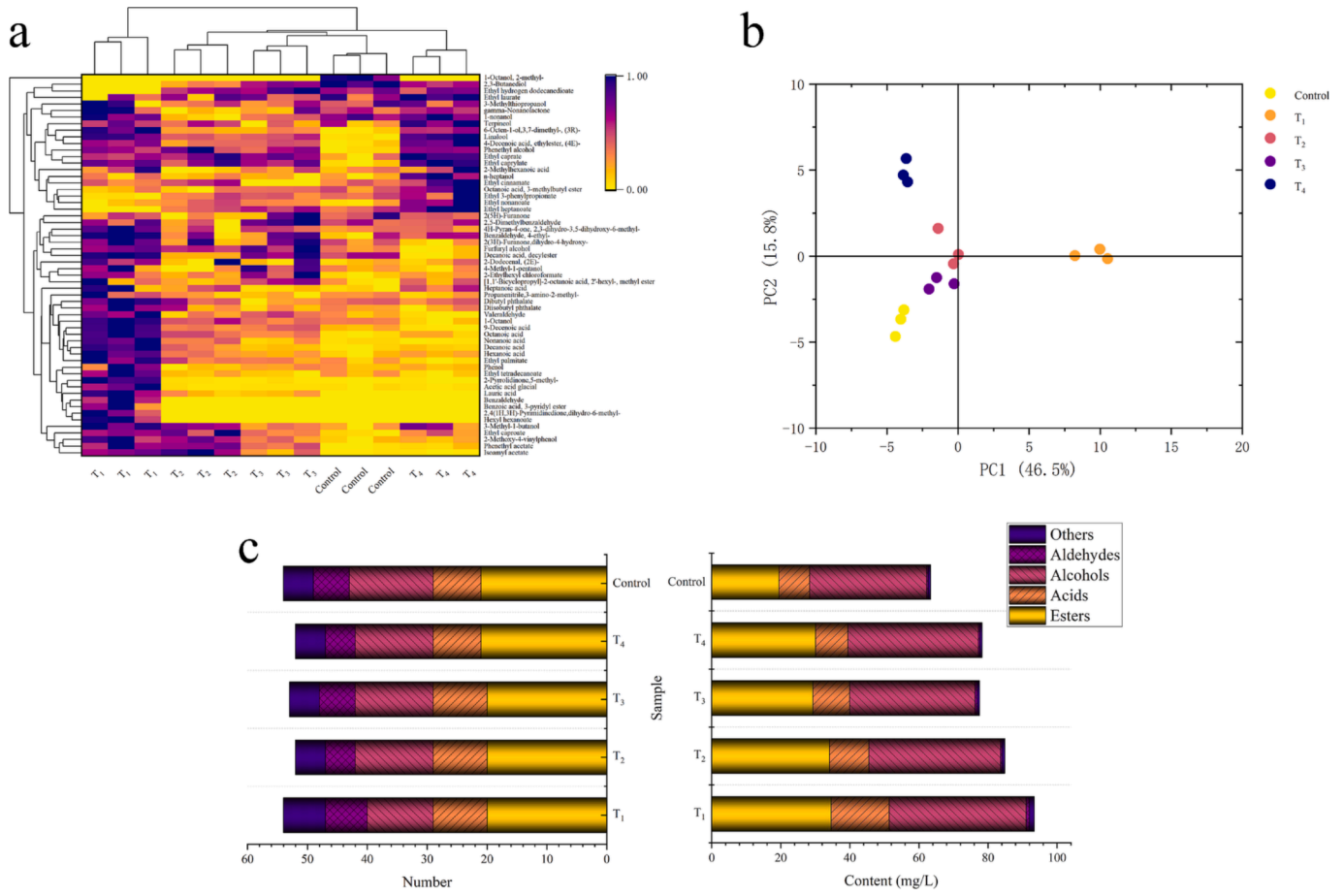


Fig. 2. Volatiles analysis of beers. a, heat map clustering analysis of volatile flavor substances; **b**, PCA analysis of different beers; **c**, number, and content of volatile flavor substances in different beers. T_1, means added tea at the start of boiling; T_2, means added tea at the end of boiling; T_3, means added tea at the cooling process; T_4, means added tea at the fermentation process; Control, means brewing beer without tea.

difference in the assay principle led to this discrepancy, with the HPLC method having a higher precision despite the faster rate of determination by the folin-phenol method.

Further analyses revealed that both hot water and aqueous ethanol solution were effective in extracting phenolic compounds from green tea, and the time of extraction of hot water was the key factor affecting the extraction rate of green tea phenolic compounds. The contents of epicatechin, syringic acid, caffeic acid, protocatechuic acid, and chlorogenic acid were significantly reduced after the time was shortened. In addition, the highest content of catechins was found in T_4, indicated that the aqueous ethanol solution was more advantageous for the extraction of catechins from green tea, and on the contrary, the hot water was more suitable for the extraction of epicatechin, coumaric acid, rutin, vanillic acid, and chlorogenic acid.

3.5. *Volatile flavor substances*

The volatile flavor substances of beer were analyzed by HS-SPME-GC–MS, and a total of 58 flavor substances were resolved, including 23 esters, 9 acids, 13 alcohols, 6 aldehydes, and 7 other volatile flavoring substances were detected (Table S1).

Esters are common flavor substances in beer and are the most perceptible flavor substances, they have floral and fruity aromas when in the correct concentration (Holt, Miks, de Carvalho, Foulquie-Moreno, & Thevelein, 2019). T_1 was the most abundant in esters, with the higher contents of isoamyl acetate (9.460 mg/L), ethyl hexanoate (2.503 mg/L), ethyl caprylate (13.683 mg/L), ethyl caprate (2.203 mg/L), 4-Decenoic acid, ethylester, (4E)- (2.873 mg/L) and phenethyl acetate (5.037 mg/L). Volatile acids in beer are very important in balancing the taste of beer and providing a refreshing taste (Giannetti, Boccacci Mariani, Torrelli, & Marini, 2019). They are mainly low molecular weight organic acids produced by yeast metabolism, some of which combine with alcohols to form esters, and some are retained in the beer to enrich the taste. Volatile acids in beers are mainly hexanoic acid (1.457–2.650 mg/L) and octanoic acid (6.107–9.237 mg/L). Volatile acids in T_1 are the most abundant. Higher alcohols are one of the by-products of yeast metabolism, which can also give beer a pleasant floral and fruity aroma at the appropriate concentration (Holt et al., 2019). The primary alcohols in GTB are 3-methyl-1-butanol (19.030–22.153 mg/L) and phenethyl alcohol (14.970–15.520 mg/L). Aldehydes and other volatile components are lower in GTB, but aldehydes have a lower flavor threshold, and the presence of small amounts of aldehydes can enhance the complexity and layering of beer flavors (Lehnhardt et al., 2019).

The alcohol-ester ratio can be used as an important indicator for evaluating beer's drinkability. When the alcohol-ester ratio of beer is higher than 4, it will lead to poor beer taste and dizziness or headache more quickly after drinking (Cui et al., 2021). GTB has a lower alcohol-ester ratio than beer, indicating that green tea can improve the drinking quality of beer.

Overall, it seems that the five types of beers have slight differences in the kinds of volatile components but significant differences in their contents (Fig. 2c), with T_1 having the highest content of volatile components and Control having the lowest content of volatile components. The principal flavor components in GTB were esters, acids, and alcohols, which accounted for more than 90 % of the content of volatile components.

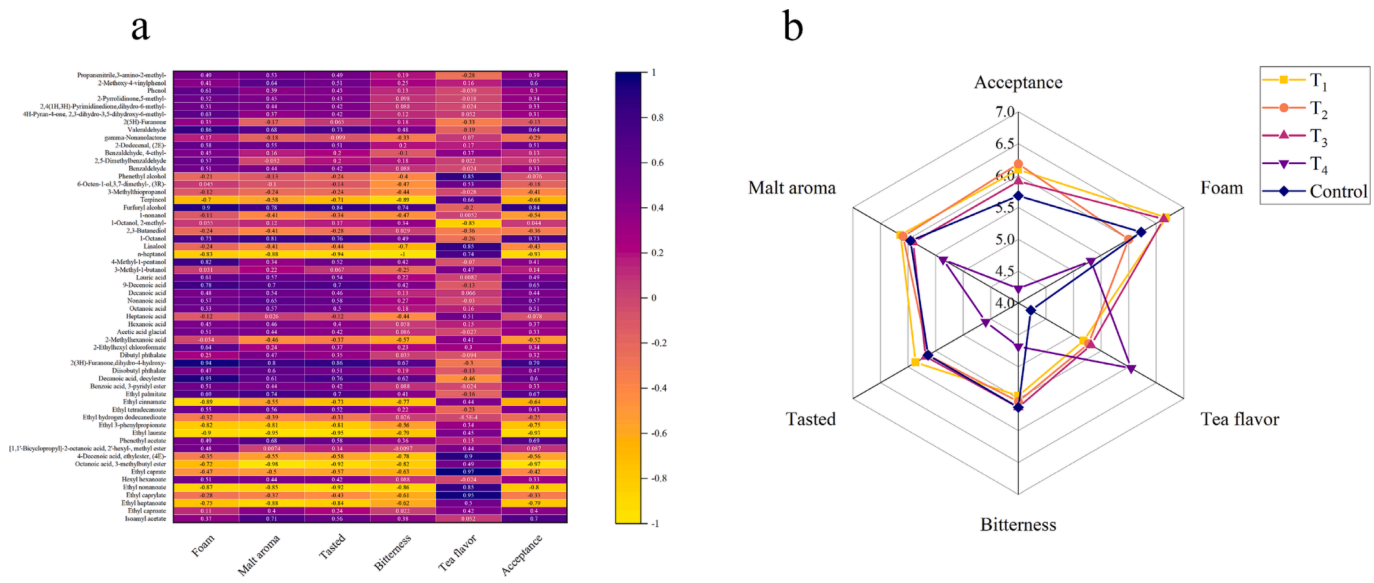


Fig. 3. Sensory analysis of beers. a, correlation analysis of volatile flavor substances and sensory indicators; **b**, radar charts for sensory evaluation. T_1, means added tea at the start of boiling; T_2, means added tea at the end of boiling; T_3, means added tea at the cooling process; T_4, means added tea at the fermentation process; Control, means brewing beer without tea.

Heat map clustering analysis was used to analyses the differences in volatile flavor substances in different beers (Fig. 2a), and the heat maps show the increase or decrease in volatile flavor substances in GTB compared to beer. In addition, the clustering results differentiated the five different beers sufficiently, which indicates that there are not only different volatile flavor substance profiles between tea addition beer and no tea addition beer but also statistically significant differences in volatile flavor substance profiles between different tea addition methods.

To further differentiate between the compounds in beer and GTB, PCA analysis was performed on the GC–MS data (Fig. 2b). The first and second principal components accounted for 46.5 % (PC 1) and 15.8 % (PC 2) of the total variation, respectively. Scatter plots of the samples showed a better separation between the different beers, with T_1 in the first quadrant, T_2 and T_4 in the second quadrant, and T_3 and Control in the third quadrant.

3.6. *Sensory analysis*

A 9-point hedonic scale was used for sensory analysis of the beers (Fig. 3b). Acceptance for T_1, T_2, T_3, and Control were all higher than 5.5, T_1, T_2, and T_3 had better acceptance than Control; T_1, T_2, and T_3 had satisfactory performance in malt aroma, tasted, bitterness, and tea flavor, but T_2 had a decrease in foam relative to Control; T_4 had the lowest scores for malt aroma, tasted, bitterness, and foam but the highest score for tea flavor; Control had higher scores for malt aroma, tasted, and bitterness, but the lowest score for tea flavor. The acceptance of the beers, in descending order, were T_2, T_1, T_3, Control, and T_4. On balance, T_1 may be the best way to add green tea.

Analysis of the correlation between sensory scores and volatiles (Fig. 3a) and the correlation coefficients of tea aroma with ethyl caprylate, ethyl nonanoate, ethyl caprate, 4-decenoic acid, ethyl ester, (4E)-, linalool and phenethyl alcohol were higher than 0.8. The correlation coefficients of malt aroma with furfuryl alcohol (0.78), 1-octanol (0.81), and 2(3H)-furanone, dihydro-4-hydroxy- (0.80) were high. This is not a complete overlap with the 14 characterized volatile flavor compounds reported in the literature, which again demonstrates that the flavor compounds of green tea and beer are not simply superimposed in GTB (Wang et al., 2020). According to the correlation coefficients, tea flavor's characteristic volatile flavor substances were obviously different from those of other sensory indicators, and esters mainly reflected the differences.

4. Conclusion

This study analyzed the effect of adding green tea in different brewing stages on the antioxidant capacity and flavor of beer. The results showed that the addition of green tea in beer brewing can effectively enhance the antioxidant capacity and enrich the flavor of beer. The GTB produced by different tea addition methods was significantly different in terms of bioactive substance content, antioxidant capacity, phenolic composition, flavor composition, and sensory quality, and added during boiling (T_1) is a tea addition method worth considering for its excellent antioxidant capacity (ASA increased by 142 %, DSA by 102 % and RP by 397 %) and abundant volatile flavor compounds (total volatile contents: 93.409 mg/L) as well as satisfactory sensory scores (acceptance, 6.09/9).

CRediT authorship contribution statement

Jianhang Wu: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Ye Zhang:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Ran Qiu:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Li Li:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Xuyan Zong:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

Data availability

The datasets generated during and analyzed during the current study

are available from the corresponding author on reasonable request.

Acknowledgment

This work was supported by the Key Lab of Aromatic Plant Resources Exploitation and Utilization in Sichuan Higher Education (23XLY02).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101193.

References

Barnette, B. M., & Shellhammer, T. H. (2019). Evaluating the impact of dissolved oxygen and aging on dry-hopped aroma stability in beer. *Journal of the American Society of Brewing Chemists, 77*(3), 179–187. https://doi.org/10.1080/03610470.2019.1603002

Carvalho, D. O., & Guido, L. F. (2022). A review on the fate of phenolic compounds during malting and brewing: Technological strategies and beer styles. *Food Chemistry, 372*, 14. https://doi.org/10.1016/j.foodchem.2021.131093

Chen, D. Q., Zou, C., Huang, Y. B., Zhu, X., Contursi, P., Yin, J. F., & Xu, Y. Q. (2023). Adding functional properties to beer with jasmine tea extract. *Frontiers in Nutrition, 10*. https://doi.org/10.3389/fnut.2023.1109109

Conti-Silva, A. C., & de Souza-Borges, P. K. (2019). Sensory characteristics, brand and probiotic claim on the overall liking of commercial probiotic fermented milks: Which one is more relevant? *Food Research International, 116*, 184–189. https://doi.org/10.1016/j.foodres.2018.08.011

Cui, D. Y., Ge, J. L., Song, Y. M., Feng, P. P., Lin, L. C., Guo, L. Y., & Zhang, C. Y. (2021). Regulating the ratio of higher alcohols to esters by simultaneously overexpressing ATF1 and deleting BAT2 in brewer's yeast Saccharomyces pastorianus. *Food Bioscience, 43*, 9. https://doi.org/10.1016/j.fbio.2021.101231

Damiani, E., Carloni, P., Rocchetti, G., Senizza, B., Tiano, L., Joubert, E., & Lucini, L. (2019). Impact of cold versus hot brewing on the phenolic profile and antioxidant capacity of rooibos (Aspalathus linearis) herbal tea. *Antioxidants, 8*(10), 19. https://doi.org/10.3390/antiox8100499

De Francesco, G., Bravi, E., Sanarica, E., Marconi, O., Cappelletti, F., & Perretti, G. (2020). Effect of addition of different phenolic-rich extracts on beer flavour stability. *Foods, 9*(11). https://doi.org/10.3390/foods9111638

Fraga, C. G., Croft, K. D., Kennedy, D. O., & Tomas-Barberan, F. A. (2019). The effects of polyphenols and other bioactives on human health. *Food & Function, 10*(2), 514–528. https://doi.org/10.1039/c8fo01997e

Giannetti, V., Boccacci Mariani, M., Torrelli, P., & Marini, F. (2019). Flavour component analysis by HS-SPME/GC–MS and chemometric modeling to characterize Pilsner-style Lager craft beers. *Microchemical Journal, 149*, Article 103991. https://doi.org/10.1016/j.microc.2019.103991

Holt, S., Miks, M. H., de Carvalho, B. T., Foulquie-Moreno, M. R., & Thevelein, J. M. (2019). The molecular biology of fruity and floral aromas in beer and other alcoholic beverages. *Fems Microbiology Reviews, 43*(3), 193–222. https://doi.org/10.1093/femsre/fuy041

Humia, B. V., Santos, K. S., Barbosa, A. M., Sawata, M., Mendonca, M. D., & Padilha, F. F. (2019). Beer molecules and its sensory and biological properties: A review. *Molecules, 24*(8), 19. https://doi.org/10.3390/molecules24081568

Humia, B. V., Santos, K. S., Schneider, J. K., Leal, I. L., Barreto, G. D., Batista, T., & Padilha, F. F. (2020). Physicochemical and sensory profile of Beauregard sweet potato beer. *Food Chemistry, 312*, 8. https://doi.org/10.1016/j.foodchem.2019.126087

Jaskula-Goiris, B., De Causmaecker, B., De Rouck, G., Aerts, G., Paternoster, A., Braet, J., & De Cooman, L. (2019). Influence of transport and storage conditions on beer quality and flavour stability. *Journal of the Institute of Brewing, 125*(1), 60–68. https://doi.org/10.1002/jib.535

Jongberg, S., Andersen, M. L., & Lund, M. N. (2020). Characterisation of protein-polyphenol interactions in beer during forced aging. *Journal of the Institute of Brewing, 126*(4), 371–381. https://doi.org/10.1002/jib.623

Lehnhardt, F., Gast, M., & Becker, T. (2019). Forced into aging: Analytical prediction of the flavor-stability of lager beer. A review. *Critical Reviews in Food Science and Nutrition, 59*(16), 2642–2653. https://doi.org/10.1080/10408398.2018.1462761

Liu, H. C., Xu, Y. J., Wen, J., An, K. J., Wu, J. J., Yu, Y. S., & Guo, M. H. (2021). A comparative study of aromatic characterization of Yingde Black Tea infusions in different steeping temperatures. *Lwt-Food Science and Technology, 143*. https://doi.org/10.1016/j.lwt.2021.110860

Nardini, M., & Garaguso, I. (2020). Characterization of bioactive compounds and antioxidant activity of fruit beers. *Food Chemistry, 305*, 9. https://doi.org/10.1016/j.foodchem.2019.125437

Nguela, J. M., Vernhet, A., Julien-Ortiz, A., Sieczkowski, N., & Mouret, J. R. (2019). Effect of grape must polyphenols on yeast metabolism during alcoholic fermentation. *Food Research International, 121*, 161–175. https://doi.org/10.1016/j.foodres.2019.03.038

Park, J. W., Park, K. G., Lee, N. Y., Lee, J. H., & Lee, J. W. (2019). Enhanced extraction of reducing sugars from fruit of Hovenia dulcis with treatment of cellulase and sequential production of ethanol and acetic acid containing ampelopsin from extracted reducing sugars. *Industrial Crops and Products, 139*. https://doi.org/10.1016/j.indcrop.2019.111522

Paternoster, A., Jaskula-Goiris, B., Buyse, J., Perkisas, T., Springael, J., Braet, J., & De Cooman, L. (2020). The relationship between flavour instability, preference and drinkability of fresh and aged beer. *Journal of the Institute of Brewing, 126*(1), 59–66. https://doi.org/10.1002/jib.582

Puligundla, P., Mok, C., & Park, S. (2020). Advances in the valorization of spent brewer's yeast. *Innovative Food Science & Emerging Technologies, 62*, 8. https://doi.org/10.1016/j.ifset.2020.102350

Rahman, M. J., Liang, J. B., Eskin, N. A. M., Eck, P., & Thiyam-Hollander, U. (2020). Identification of hydroxycinnamic acid derivatives of selected Canadian and foreign commercial beer extracts and determination of their antioxidant properties. *Lwt-Food Science and Technology, 122*, 8. https://doi.org/10.1016/j.lwt.2020.109021

Ribeiro, V. R., Maciel, G. M., Fachi, M. M., Pontarolo, R., Fernandes, I. D. A., Stafussa, A. P., & Haminiuk, C. W. I. (2019). Improvement of phenolic compound bioaccessibility from yerba mate (Ilex paraguariensis) extracts after biosorption on Saccharomyces cerevisiae. *Food Research International, 126*, 10. https://doi.org/10.1016/j.foodres.2019.108623

Rong, L., Peng, L.-J., Ho, C.-T., Yan, S.-H., Meurens, M., Zhang, Z.-Z., & Ling, T.-J. (2016). Brewing and volatiles analysis of three tea beers indicate a potential interaction between tea components and lager yeast. *Food Chemistry, 197*, 161–167. https://doi.org/10.1016/j.foodchem.2015.10.088

Tang, G. Y., Meng, X., Gan, R. Y., Zhao, C. N., Liu, Q., Feng, Y. B., & Li, H. B. (2019). Health functions and related molecular mechanisms of tea components: An update review. *International Journal of Molecular Sciences, 20*(24), 38. https://doi.org/10.3390/ijms20246196

Wang, J. J., Ding, H. J., Zheng, F. Y., Li, Y. X., Liu, C. F., Niu, C. T., & Li, Q. (2019). Physiological changes of beer brewer's yeast during serial beer fermentation. *Journal of the American Society of Brewing Chemists, 77*(1), 10–20. https://doi.org/10.1080/03610470.2018.1546030

Wang, M. Q., Ma, W. J., Shi, J., Zhu, Y., Lin, Z., & Lv, H. P. (2020). Characterization of the key aroma compounds in Longjing tea using stir bar sorptive extraction (SBSE) combined with gas chromatography-mass spectrometry (GC-MS), gas chromatography-olfactometry (GC-O), odor activity value (OAV), and aroma recombination. *Food Research International, 130*, 11. https://doi.org/10.1016/j.foodres.2019.108908

Yang, D. S., & Gao, X. (2021). Research progress on the antioxidant biological activity of beer and strategy for applications. *Trends in Food Science & Technology, 110*, 754–764. https://doi.org/10.1016/j.tifs.2021.02.048

Zdaniewicz, M., Pater, A., Hrabia, O., Dulinski, R., & Cioch-Skoneczny, M. (2020). Tritordeum malt: An innovative raw material for beer production. *Journal of Cereal Science, 96*, 8. https://doi.org/10.1016/j.jcs.2020.103095

Zhao, H., Chen, W., Lu, J., & Zhao, M. (2010). Phenolic profiles and antioxidant activities of commercial beers. *Food Chemistry, 119*(3), 1150–1158. https://doi.org/10.1016/j.foodchem.2009.08.028

Zhao, H. (2015). Chapter 64 – Effects of Processing Stages on the Profile of Phenolic Compounds in Beer. In V. Preedy (Ed.), *Processing and Impact on Active Components in Food* (pp. 533-539). San Diego: Academic Press. https://doi.org/10.1016/B978-0-12-404699-3.00064-0.

Zong, X. Y., Wu, J. H., Chen, Z. Y., He, L. H., Wen, J. J., & Li, L. (2023). Impact of Qingke (hulless barley) application on antioxidant capacity and flavor compounds of beer. *Journal of Cereal Science, 109*, 6. https://doi.org/10.1016/j.jcs.2022.103624