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Research Article

New beer type produced by using bioactive compound - rich materials as an alternatives of hops

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Abstract

Beer is one of the most popular beverages in the world, so developing new flavors and increasing nutritional value in beer production is critical. In the study green tea, rosemary and grape pomace were used as alternatives to hops. The total phenolic and flavonoid content of beers obtained by adding green tea and rosemary was found to be higher than that of those made with hops ($p < 0.05$). The discussed values of beer made with black grape pomace were similar to those produced with hops. The green tea and rosemary beers had higher antioxidant activity than hop-based beers, but black grape pomace beers had lower antioxidant activity than hop-based beers ($p < 0.05$). According to our data, the use of plants rich in bioactive components could be used as an alternative to hops resulted in a significant enrichment both qualitatively and quantitatively and improved the properties of the final beer. Our results also demonstrated that there is a strong relation between the amount of bioactive components of beer with different plant-based origins and their antioxidant activity values and consequently, with their properties.

Keywords

beer, green tea, rosemary, grape pomace, hop, total phenolic, total flavonoid

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Introduction

Beer is one of the most well-known, popular consumed alcoholic beverages in the world. The main raw materials of beer are considered to be water, malt/non-malt grains, yeast and hops. The most important process of beer production is the alcohol fermentation by which glucose is converted to ethanol and carbon dioxide and new flavour compounds are obtained (Yang and Gao 2021).

Beer was flavoured with various spices and herbs until the 8th century. To increase the alcohol content instead of sugar mainly was used honey. Among the most used raw materials were juniper and ginger. After the discovery of using hops in brewing in the eighth century, brewing took on a whole new meaning. The keen aroma and bitterness of the beer are attributable to the added hops. The resin in the cones allows additional flavour (bitterness, aroma) to the beer. There are two types of acid groups in hop resin, hard (alpha) and soft (beta) ones. Considering alpha acids, humulone is quite important, while for the beta acids, lupulon is very prominent. Alpha and beta acids are essential constituents of hops that significantly influence the flavour, aroma and stability of beer. In beer, alpha acids are responsible for bitterness, whereas beta acids contribute to its general aroma and taste (Oladokun et al. 2017; Machado et al. 2019; Silva et al. 2019).

In some studies, different ingredients were added in addition to hops at the beginning of fermentation in order to develop new beer varieties. For example, by adding different fruits (cherry, raspberry, peach, apricot, grape, plum, orange and apple) during the fermentation process were examined the total polyphenolic and total flavonoid contents of produced beers. Antioxidant activity, total polyphenols and total flavonoids content were determined to be considerably higher in most of the fruit beers in respect to conventional, no-fruit beers. The cherries beers were exhibited to have the highest values, followed by grape, plum and orange beers (Nardini and Garaguso 2020). In the other study was evaluated the effect of adding different foods (in presence of hops) on the nutritional quality of beer. Seven different beers that were commercially available and made by adding natural foods during the fermentation were evaluated for their total phenolic, total flavonoids contents and

antioxidant activity (Nardini and Foddai 2020). In some Muslim countries, flavour compounds (peach, apple) have been added to non-alcoholic beers and malt beverages at the final stage of the production of non-alcoholic beer or malt beverage with different flavours. Low-alcohol beers were produced by ending the fermentation process or by applying cold fermentation. In these methods, the lack of aroma substances formed during the fermentation stage were compensated for by the aroma substances added in the final stage (Akbari - Adreagani 2018).

Beers with original extracts of 14°P and 16°P produced with the addition of blueberries did not significantly affect the mouthfeel or body, nor did it alter the protein fractions and concentrations (Nedyalkov et al. 2023) demonstrating the effects of fruits on beer characteristics.

In another study, researchers investigated the benefits of using the carqueja plant instead of hops. It was used at five different concentrations (1 to 5 g.L⁻¹) during production of craft lager beer. The results emphasized that the use of carqueja instead of hops did not have any negative effects on the physicochemical properties of produced beers. The results of the sensory analyses of beers produced with carqueja were found to be so similar to commercial beers (Schuina et al. 2020).

In our study, green tea, rosemary and black grape pomace were chosen as alternative materials to hops because of their antioxidant and antimicrobial properties. All of these materials possess high antimicrobial and antioxidant activities like hops (Gouveia et al. 2016; Rashidi et al. 2017; Peixoto et al. 2018; Elmi et al. 2019; Mira-Sanchez et al. 2020; Rodrigues Silva et al. 2021; Li et al. 2021; Chowdary et al. 2021).

Tea (*Camellia sinensis*) has been an integral part of human culture for millennia and continues to be a staple beverage in numerous countries. The predominant bioactive compounds found in tea are referred to as tea polyphenols. This group encompass a variety of classes, including catechins, flavonoids, anthocyanins and phenolic acids, which constitute approximately 15% to 35% of the dry weight of tea. Among these, catechins represent the most significant category, accounting for approximately 70% of the total tea polyphenols, thereby serving as the central active constituents.

The flavonoid compounds present in tea primarily exist in the forms of flavonols, flavanols and anthocyanins. Additionally, fresh tea contains various phenolic acids, which may exist independently or combine with sugar molecules to form hydrolysable tannins. The primary tea polyphenols can be classified into four main types: (-)-epigallocatechin gallate (EGCG), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC) and (-)-epicatechin (EC) (Rusak et al. 2008; Yang et al. 2014; Yan et al. 2020; He et al. 2020; Luo et al. 2023).

Originating from the Mediterranean region, rosemary (*Rosmarinus officinalis*) has demonstrated remarkable adaptability, allowing it to be cultivated in various climates worldwide. This plant is particularly rich in bioactive phytochemical compounds, with polyphenols being one of its most significant constituents.

Rosemary extract contains a wide array of polyphenolic compounds including phenolic terpenes, flavonoids and various phenolic acids. The exact composition of these extracts varies depending on the extraction method employed. Among the identified antioxidant constituents, the phenolic diterpenes, carnosic acid and carnosol are the most potent, accounting for over 90% of the antioxidant activity attributed to rosemary extract. In addition to these major compounds other phenolic constituents such as rosmanol, epirosmanol, isorosmanol, rosmadial, rosmaridiphenol, and rosmariquinone also contribute to its antioxidative properties.

Rosemary extract may also contain triterpenes and triterpenic acids such as betulin, amyrin, betulinic acid, oleanic acid and ursolic acid. The flavonoid profile includes flavones and flavonols such as apigenin, genkwanin, luteolin, hispidulin, rutin, kaempferol, naringin, hesperetin, apigenin-7-O-glucoside, and quercetin. Additionally, various phenolic acids are present in rosemary extract, including rosmarinic acid, caffeic acid, chlorogenic acid, coumaric acid, p-coumaric acid, ferulic acid, vanillic acid, syringic acid, homovanillic acid, and p-hydroxybenzoic acid, among others (Sotelo-Fe'líx et al. 2002; Borrás-Linares et al. 2014; Bozin et al. 2007; Namal Senanayake 2018; Aziz et al. 2022; Raffo et al. 2023).

Grape pomace (GP) is an abundant source of polyphenolic compounds, including flavonols (e.g., quercetin, myricetin, kaempferol, and their glycosides), anthocyanins such as malvidin 3-O-glucoside, and phenolic acids (e.g., caffeic acid, p-coumaric acid, among others). Generally, GP polyphenols are categorized into three primary classes based on the number of aromatic rings attached to hydroxyl groups in their structure: (1) phenolic acids, including hydroxybenzoic and hydroxycinnamic acids; (2) flavonoids, which encompass anthoxanthins, anthocyanins, leucoxanthins and flavonoidal alkaloids; (3) nonflavonoids, such as stilbenes, tannins, coumarins and neolignans. Factors such as soil conditions, temperature, cultivation methods, grape variety, and geographical origin significantly influence the chemical composition of GP, the optimization of traditional extraction techniques has ensured the concentration of polyphenols and the preservation of their potential bioactivities, including antioxidant, antimicrobial and anti-inflammatory properties (Kalli et al. 2018; Peixoto et al. 2018; Chakka and Babu 2022; Almanza-Oliveros et al. 2024).

The importance of using natural preservatives during beer production was indicated in other study, also (Tumbariski et al. 2023).

The results of studies demonstrated that the antioxidant activity, total polyphenols content were determined to be considerably higher in most of the fruit beers in respect to conventional, no-fruit hops containing beers.

So, the objectives of this study included producing beers with elevated antioxidant properties and unique aromas and flavours. Specifically, the study aimed to assess the distinct flavour, aroma and astringency of green tea beer, attributed to the catechin group and the unique flavour and aroma of rosemary beer, attributed to the rosmarinic acid group. Considering the properties of chosen materials, by using substituting plants rich in bioactive components as black grape pomace, rosemary and green tea as an alternative of hops were aimed to create a new type of beers for consumers seeking functional beers with new flavour attributes.

Materials and Methods

Materials. The barley malt wort, yeast (*Saccharomyces pastorianus*) and hop were kindly supplied by Efes Pilsen Brewery. The barley malt wort and yeast were transported to the laboratory in pasteurized glass containers. Hop cones were transported to the lab in airtight and watertight package. The green tea sample used in the study was obtained from the Rize, Turkey-based company CAYKUR (Organic Emerald Green Tea). The rosemary sample was obtained from the KÜTAŞ agricultural products factory operating in İzmir, Turkey. The botanical identification of both samples were done in mentioned companies. The sample of black grape pomace was produced from grapes obtained from Migros chain stores in İzmir, Turkey. The botanical identification of grape samples was done by supplying company. The grapes were separated, washed with water and crushed with a laboratory blender (Waring 8011 EB) and the pulp obtained was dried in a trial dryer at 50°C for 10 h by modifying the methods used in other studies (Casazza et al. 2016; Monteiro et al. 2021). After drying, the grape pulp was crushed to particle size of 0.5 µm and turned into powder form. A sample of commercial beer with 5% alcohol content, was selected as considering the most consumable and bought from Migros chain stores from Turkey.

Methods. Beer production was by done following several steps: the wort was divided into 4 pots in quantities of 3 L and left to boil (initial temperature: 15.6°C). Hops, green tea, rosemary and black grape pomace were added at the beginning of boiling (6.5 g) (two thirds of total dosages: 10 g) and at 10 min before the end of boiling (one third of total dosages) (3.5 g). At the end of the boiling period, the boiled wort in the pot was quickly cooled by immersion in an ice water bath (20°C). The amount of 1g of yeast (*Saccharomyces pastorianus*) (1.5×10^8 cfu.mL⁻¹) was added to 20 mL of boiled and cooled (20°C) water and 10 mL wort allowing propagation of yeasts for 20 min. After rehydration of cultures the inoculation to 3 L pot was done aseptically. The procedure was done for each pot. The control of anaerobic conditions was created by using airlock attached bottles (Emirleroglu 2020). The production process was monitored hydrometrically (Alla 0.990-0.1160 Beer Hydrometer). The final bottled products (Fig.1) were subsequently stored in an air-

conditioned room maintained at 15°C. Fermentation lasted 23 days. The wort gravity measurements were employed as indicators of the alcoholic strength of the beer and to assess the completion of fermentation. To preserve the bioactive components within the beer samples, no filtration was applied.



Figure 1. Beer samples produced by different materials used as an alternatives of hops

Main beer analyses. The main physicochemical parameters of beers as original gravity (EBC Methods 9.43.1), density (EBC Method 1.6), alcohol content (EBC Methods 9.43.2), pH (EBC Method 1.5), total acidity (EBC Method 9.2.1 and 9.2.4) and bitterness (EBC Method 9.8), were performed according to the European Brewery Convention methodology (EBC 2010).

Total phenolic content of beers. The total phenolic content was measured using the Folin-Ciocalteu method, as described by Gujral et al. 2012; Chobdar Rahim et al. 2021), with slight modifications. Initially, beer or plant extracts were diluted in distilled water at a 1:10 (v/v) ratio. As the next, the amount of 0.5 mL of the Folin–Ciocalteu reagent was added to 0.1 mL of diluted extract. After 2 min allowing the reaction to be completed, the amount of 2 mL of sodium carbonate (7% w/v) was put on. The last volume was adjusted to 7.5 mL by distilled water. Obtained solution was placed in dark for 1 h. The absorbances were determined at 760 nm using a spectrophotometer in Shimadzu 1240 (Japan). Different concentrations (50-600 mg.L⁻¹) of gallic acid (Merck, G7384) were prepared for drawing the calibration graph. The calibration curve of the gallic acid was used for calculations of total phenols amounts (GAE mg.L⁻¹). All measurements were performed against blanc sample prepared with distilled water.

Total flavonoid contents of beers. The total flavonoid concentrations were measured and evaluated by using aluminum chloride colorimetric method proposed by other studies (Singleton and

Rossi 1965; Zhishen et al. 1999; Liü et al. 2009; Chobdar Rahim et al. 2021). Shortly, 250 µL sample solution was combined with 1.50 mL distilled water and 75 µL of 5% sodium nitrite and the mixture was kept for 5 min. Subsequently, 150 µL of 10% AlCl₃ and 125 µL of 1M NaOH were added and waited for 10 min. The absorbances were determined at 510 nm. The standard curve was evaluated by using +/(-) catechin (50-600 mg.L⁻¹) (Merck C1788) and total flavonoid contents were expressed as catechin mg.L⁻¹ equivalents. During the measurements, distilled water was used as a blank.

Antioxidant capacity of beers. The antioxidant capacity of beers and plants samples were determined according to the DPPH (2,2-diphenyl-1-picrylhydrazyl) (Merck D9132) radical reducing activity method (Singleton and Rossi 1965; Brand-Williams et al. 1995; Perales-Sánchez et al. 2014; Chobdar Rahim et al. 2021). The concentration of 6×10⁻⁵ M DPPH was prepared by using methanol as solvent. The quantities of 4.9 mL of methanolic DPPH solution was added to 0.1 mL of sample. The solution was stored in the dark for 30 minutes. The calibration curve of (1-5 mM) Trolox (Merck 53188-07-1) was prepared for analysis and the absorbance at 517 nm was recorded by using spectrophotometer (Shimadzu 1240). The results were calculated as mg Trolox equivalents (Trolox mM.L⁻¹).

The ABTS⁺ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) (Merck 30931-67-0) radical scavenging activity was performed according to Re et al. (1999). For this purpose were used ABTS⁺ radical cation for ABTS⁺ stock solution (7mM). The solution was obtained by mixing with 2.45 mM potassium persulfate solution and incubating the mixture in the dark at room temperature for 12-16 h. Before measurements, the stock solution was diluted with distilled water until it provided an absorbance value of 0.700 at 734 nm. After addition of 2 mL of ABTS⁺ solution to 0.1 mL of extract, it was stored in the dark for 6 min and the absorbance was measured at 734 nm (Shimadzu 1240). Regarding the ABTS⁺ test, a Trolox calibration curve was drawn and the results were calculated as (Trolox mM.L⁻¹). Distilled water was used as a blank during measurements.

Sensory analyses of beers. The sensory panel were made up with 80 untrained evaluators, with a gender

split of 40% male and 60% female and an average age of 25. They were given samples in monadic form, with three-digit codes and randomly assigned to prevent bias. The experimental design consisted of complete blocks, that is, all evaluators analyzed the samples using the scales presented below. The evaluators were asked to drink a glass of room-temperature water and rinse their mouths before each evaluation. The samples were evaluated by analyzing the following sensorial attributes: aroma, flavor, color, bitterness and overall acceptance using a structured hedonic scale of nine points (9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely) (Meilgaard et al. 2016).

Statistical evaluation of beers. In the study, the design experiment was conducted by using 3 parallel random plots with 2 replicates were used (n=6). Statistical analyses were performed by using one-way analysis of variance (ANOVA) and Duncan's multiple range tests of SPSS software (version 25.0, SPSS Inc, Chicago, USA). Significant levels were based on the confidence level at range of 95% (p<0.05).

Results and Discussion

The Original Gravity (OG) values of all beer samples are closely aligned, indicating that the plant-based additives do not have a significant effect on OG. This minimal difference is attributed to the amount of fermentable sugars present at the onset of the fermentation process. Green tea, rosemary, and grape pomace do not produce a notable difference in OG (p<0.05) (Table 1). The alcohol values of produced beers were determined to be between 5.12 – 7.87% (p<0.05). The original gravity values obtained in this study are consistent with the results reported in previous research (Schuina et al. 2020; Guan et al. 2023). Regarding Alcohol Content (AC), the grape pomace beer exhibits the highest value at 7.87%, which can be attributed to the abundance of fermentable sugars such as glucose and fructose found in grape pomace. These sugars are utilized more efficiently by yeast for alcohol production. Additionally, the rosemary beer shows a high alcohol content of 7.61%, suggesting that rosemary may possess bioactive components that contribute to the fermentation

process. Conversely, the alcohol content of the green tea-infused beer is lower at 5.90%. This phenomenon may be explained by the presence of polyphenols, particularly epigallocatechin gallate (EGCG) and catechins in green tea, which could inhibit yeast activity (Table 1). In a study, beer varieties were produced by adding green tea in 4 stages of beer making (pre-boiling, post-boiling, cooling stage and pre-fermentation). The alcohol content of the beer varieties produced varied between 4.10 and 4.66% (Wu et al. 2024). The examining of Total Acidity (TA) values demonstrated that the green tea-infused beer possesses the highest acidity at 1.621 g.L⁻¹, likely due to the acidic nature of green tea. The increase in acidic components may limit yeast activity, resulting in lower alcohol production. Lower acidity levels are observed in the grape pomace and rosemary samples. The pH values among the beer samples are quite similar, averaging around 3.9 (P > 0.05). Although pH has a significant impact on yeast

fermentation, the additives used in this study do not appear to cause substantial changes in pH. In terms of Color (EBC) values, the grape pomace-infused beer has the highest color value at 16.244 EBC, attributed to the presence of natural color pigments such as anthocyanins in grape skins and pomace. Although the rosemary and green tea-infused beers exhibit lighter colors, certain compounds found in these plants, including rosmarinic acid and flavonoids, may cause slight variations in color (p<0.05). Analyzing the bitterness (IBU) values, the grape pomace beer shows the lowest bitterness at 6.521 IBU. This may indicate that the sugars and polyphenols, particularly resveratrol and quercetin in grape pomace, mask the bitterness. In contrast, the standard (8.733 IBU) and rosemary beers (8.443 IBU) exhibit more pronounced bitter profiles, likely due to the bioactive components present in rosemary that can contribute to increased bitterness (p<0.05) (Table 1).

Table 1. Standard analyses of beers with green tea, rosemary, grape pomace, standard and commercial samples

Samples	OG*	AC	pH	TC	Color	Bitterness
Standard (3 g.L ⁻¹)	11.77 ^{1***} (0.003)**	6.56 ² (0.47)	3.92 ¹ (0.95)	1.51 ¹ (0.14)	10.81 ² (0.22)	8.73 ³ (0.32)
Green Tea (3 g.L ⁻¹)	11.82 ¹ (0.001)	5.90 ¹ (0.20)	3.88 ¹ (0.14)	1.62 ¹ (0.23)	10.13 ² (0.18)	7.61 ² (0.22)
Rosemary (3 g.L ⁻¹)	11.68 ¹ (0.001)	7.61 ³ (0.34)	3.95 ¹ (0.15)	1.41 ¹ (0.15)	12.52 ³ (0.24)	8.44 ³ (0.41)
Grape Pomace (3 g.L ⁻¹)	11.65 ¹ (0.001)	7.87 ³ (0.34)	3.97 ¹ (0.16)	1.36 ¹ (0.05)	16.24 ⁴ (0.20)	6.52 ¹ (0.23)
Commercial	-	5.12 ¹ (0.28)	3.90 ¹ (0.21)	1.29 ¹ (0.07)	5.31 ¹ (0.21)	7.42 ² (0.38)

*OG: original gravity (°P), AC: alcohol content (°abv), TA: Total acidity (g.L⁻¹) (Lactic acid). Color: (EBC), B: Bitterness (IBU).

**Average (standard deviation).

*** Different numbers in each column; indicates a statistical difference between the values (P < 0.05)

Total phenolic content, total flavonoid content and antioxidant activity values of beers produced with different plants. The total phenolic content, total flavonoid content and antioxidant activity values of beer samples produced with different plants are presented in Table 2.

The results presented in the table clearly demonstrate the impact of botanical additives on the phenolic and flavonoid content of the beer samples (p<0.05). The green tea beer exhibited the highest

total phenolic content (TPC) at 1562.50 mg gallic acid.L⁻¹, which can be attributed to the presence of polyphenols such as epigallocatechin gallate (EGCG) and other catechins found in green tea. This high value of TPC was also reflected to the high total flavonoid content (83.69 mg catechin. L⁻¹), contributing to the beer's superior antioxidant activity (DPPH: 3.80 mM Trolox.L⁻¹; ABTS: 4.61 mM Trolox.L⁻¹).

The rosemary beer ranged as samples with the second highest value of TPC (1338.74 mg gallic Acid.L⁻¹), likely due to the presence of bioactive compounds such as rosmarinic acid, carnolic acid and carnolol, known for their potent antioxidant properties. The flavonoid content (50.56 mg catechin.L⁻¹) in rosemary beer also supported its notable antioxidant capacity (DPPH: 3.72 mM Trolox.L⁻¹; ABTS: 4.49 mM Trolox.L⁻¹).

In contrast, the grape pomace beer displayed a slightly lower phenolic content (730.12 mg gallic acid.L⁻¹), despite grape pomace being rich in polyphenols like proanthocyanidins and anthocyanins. This phenolic and flavonoid content (14.47 mg catechin.L⁻¹) resulted in comparatively reduced antioxidant activity (DPPH: 2.26 mM Trolox.L⁻¹; ABTS: 3.02 mM Trolox.L⁻¹) in the grape pomace beer. The main reason of this could be related to the used grape variety of pomace.

The commercial beer had the lowest value of TPC (427.00 mg gallic Acid.L⁻¹) and flavonoid content (9.73 mg catechin.L⁻¹), which corresponded to its low antioxidant activity (DPPH: 1.78 mM Trolox.L⁻¹; ABTS: 2.43 mM Trolox.L⁻¹). These findings highlight the significant influence of botanical additives on the antioxidant capacity of the beer, primarily driven by their phenolic and flavonoid content.

In a different study, the green tea was incorporated at four distinct stages of beer production: pre-boiling, post-boiling, cooling, and the beginning of fermentation. The resulting beers exhibited variations in total phenolic content (359-630 mg.L⁻¹), total flavonoid content (147-296 mg.L⁻¹), DPPH radical scavenging activity (3-11 mmol Trolox equivalent.L⁻¹) and ABTS radical scavenging activity (1.50-4.00 mmol Trolox equivalent.L⁻¹), highlighting the time impact of green tea addition on the bioactive properties of the final product (Wu et al. 2024). Ten types of

craft beer were produced by adding different kinds of green tea during brewing and their antioxidant activity and phenolic profiles were evaluated. The results showed remarkable variations in antioxidant activity and antioxidative compound contents, which were considerably higher in green tea beers than in non-tea beer ($p < 0.05$) (Yang et al. 2023).

In our study, green tea and other elements were added at the beginning of the boiling stage. As a result of this process, an increase in total phenolic content was observed, while a decrease in flavonoid content and antioxidant activity values (according to DPPH and ABTS results) were noted. These differences suggest that the boiling stage may enhance the extraction of phenolic compounds while leading to the degradation of some flavonoids and a reduction in overall antioxidant activity.

The adverse effects of heat treatment and changes during the fermentation process regarding the stability and integrity of bioactive components were emphasized in most studies (Nguela et al. 2019; Ribeiro et al. 2019; Gao et al. 2022). It was indicated also that changes in the amount of bioactive compounds in plants can be observed depending on the growing conditions in different latitudes, season, day length, light quality and temperature (Jaakola and Hohtola 2010).

The total phenolic content of beers was in the same order of magnitude of those reported in previous studies (Nardini and Ghiselli 2004; Piazzon et al. 2010; Meneses et al. 2013; Nardini and Garaguso 2020; De Francesco et al. 2020; Maia et al. 2020).

The amount of flavonoids obtained in this research was similar with finding of many studies (Piazzon et al. 2010; Andreou et al. 2018; Di Sotto et al. 2018; Samadi and Raunf Ford 2020; Rodrigues et al. 202; Monterio et al. 2021; Arruda et al. 2021).

Table 2. The total phenolic content, total flavonoids content and antioxidant activities of beers

Samples	TPC, mg Gallic Acid.L ⁻¹	TFC, mg Catechin.L ⁻¹	DPPH, mM Trolox.L ⁻¹	ABTS ⁺ , mM Trolox.L ⁻¹
Standard beer	895.00 ± 7.18 ^{*3**}	17.63 ± 1.93 ³	2.45 ± 0.13 ³	3.28 ± 0.14 ³
Green tea beer	1562.50 ± 39.95 ⁵	83.69 ± 2.17 ⁵	3.80 ± 0.05 ⁴	4.61 ± 0.12 ⁴
Rosemary beer	1338.74 ± 5.11 ⁴	50.56 ± 0.94 ⁴	3.72 ± 0.12 ⁴	4.49 ± 0.13 ⁴
Grape pomace beer	730.12 ± 13.18 ²	14.47 ± 0.75 ²	2.26 ± 0.09 ²	3.02 ± 0.14 ²
Commercial beer	427.00 ± 5.08 ¹	9.73 ± 0.14 ¹	1.78 ± 0.16 ¹	2.43 ± 0.09 ¹

*Standard deviation

**Different numbers in each column; indicates a statistical difference between the values ($p < 0.05$).

In a study in which white grape pomace were incorporated during beer production were obtained concentration of phenolic compounds ranging from 321.584 mg of gallic acid equivalent (GAE).L⁻¹ to 501.459 mg GAE.L⁻¹. The antioxidant activity of the beers containing white grape pomace, assessed through ABTS⁺, DPPH[•], and FRAP assays, demonstrated a significant enhancement (Gasinski et al. 2022).

The study related to using of wine pomace during beer production was done by valorization of different grape origin, namely with adding must of gas (CO₂ or N₂) macerated red cv. Gamay Teinturer grape and adding pomace of red cv. Aleatico grape dehydrated, a variety used in Italy to produce sweet wine. According to the results, gas-macerated grape musts beers showed higher alcohol content, higher polyphenol content and antioxidant activity than control beer. The addition of Aleatico dehydrated grape pomace provided less polyphenol content but a prominent aromatic nuance (floral) due to the use of Aleatico grape. According to the authors, the high volatile acidity could be due to a bacteria rich grape material (must and pomace) and the scarce presence and instability of the foam due, probably, to the high amount of added grape matrices. Comparing the results with outcomes of our study demonstrated the importance of used grape variety

of wine pomace, used quantity and the time of addition (Mastrangelo et al. 2023).

The antioxidant activity values of the beers produced in this study were found to exhibit some differences with those reported in previous research (Tafulo et al. 2010; Moreira et al. 2013; Polak et al. 2013; Nardini and Garaguso 2020). Variations in antioxidant activity among different studies can be attributed to several factors, including the specific types and varieties of herbs used, the chemical composition of the soil where these herbs were cultivated, environmental conditions, the yeast strains employed (such as Lager or Ale), the duration of the boiling process, the timing of herb and fruit additions, and various methodological differences. In our study, a strong correlation was determined between total phenolic compounds and antioxidant activity, measured both by the DPPH and ABTS methods ($r = 0.9497$, for DPPH assay; $r = 0.9539$, for ABTS assay). Regarding the total flavonoid content, a strong correlation was determined between antioxidant activity evaluated by DPPH and ABTS methods ($r = 0.8534$ for DPPH assay; $r = 0.8351$, ABTS assay (Fig. 2). In the literature review, no resources were found that examined the relationship between bioactive components and antioxidants in beer produced by materials that are used in our design experiment.

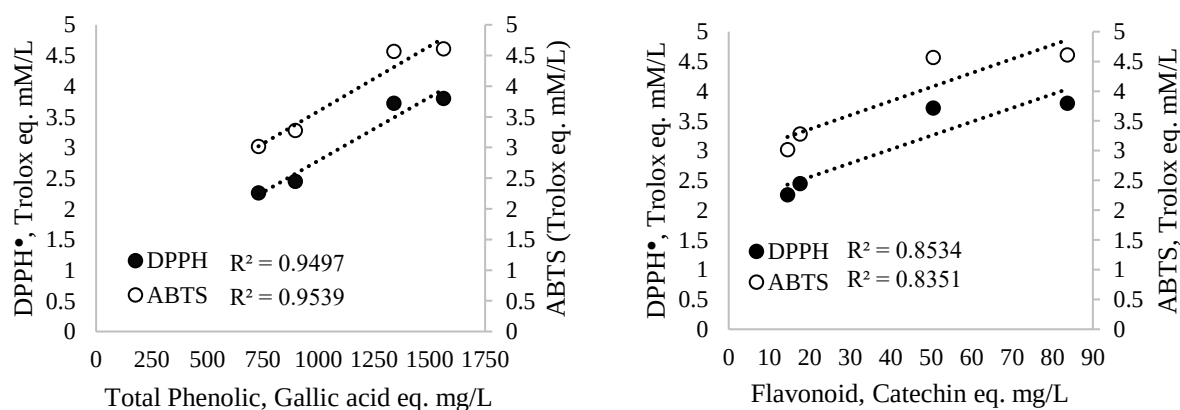


Figure 2. Relationship between the beer content of total phenolic and flavonoids and antioxidant activity, measured by both DPPH (●) and ABTS (○) assays.

Sensory analysis. The results of sensory evaluation are presented in Table 3. Considering the overall acceptance, the beer samples produced with green tea and rosemary were scored with higher values than commercial and standard beers. However, the sample of beer made with grape pomace were scored with lower values than standard and commercial beers. The overall acceptance values were evaluated to be so similar

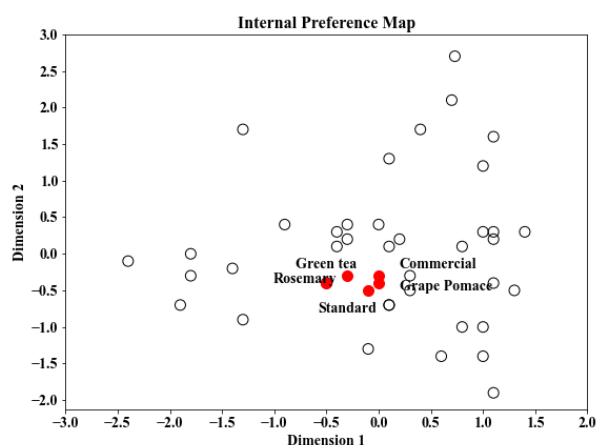
with that of other studies (Araújo et al. 2003; Ribeiro et al. 2007; Schuina et al. 2020; Wu et al. 2024).

The beer sample made with rosemary received the highest score and was selected as (7.60) the most liked one ($p < 0.05$) (Fig. 3). Regarding the bitterness, panelists evaluated all samples with scores between 6 and 7 (like moderately) points.

Table 3. Sensory acceptance of beers with green tea, rosemary and grape pomace

Samples	Color	Aroma	Flavor	Bitterness	Overall Acceptance
Standard	6.47 ± 0.65 ^{a***}	5.85 ± 0.65 ^a	5.72 ± 0.82 ^b	6.40 ± 0.75 ^a	6.41 ± 0.74 ^{ab}
Green tea	6.38 ± 0.70 ^a	6.83 ± 0.66 ^c	6.47 ± 0.82 ^c	6.45 ± 0.74 ^a	6.55 ± 0.96 ^b
Rosemary	6.48 ± 0.63 ^a	7.33 ± 0.74 ^d	6.91 ± 0.69 ^d	6.42 ± 0.74 ^a	7.60 ± 0.60 ^c
Grape pomace	6.46 ± 0.61 ^a	5.92 ± 0.56 ^a	5.31 ± 0.68 ^a	6.41 ± 0.75 ^a	6.43 ± 0.43 ^a
Commercial	6.37 ± 0.65 ^a	6.21 ± 0.68 ^b	5.73 ± 0.70 ^b	6.41 ± 0.75 ^a	6.36 ± 0.48 ^{ab}

*Standard deviation

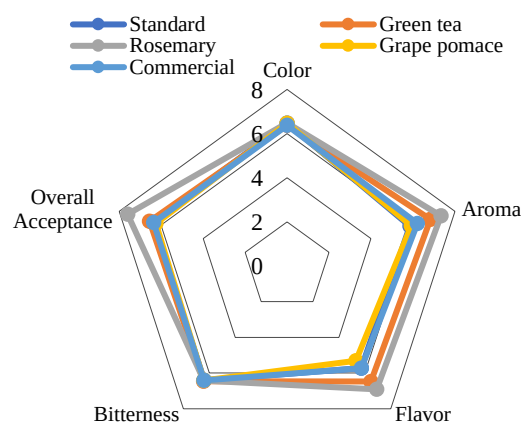
Different numbers in each column; indicates a statistical difference between the values ($P < 0.05$).Figure 3.** Internal preference map for the overall acceptance of beers produced with green tea, rosemary, grape pomace and standard (hops) and commercial ones

There was no statistically significant difference among commercial, standard beers and the beer samples made with green tea, rosemary and grape pulp ($p < 0.05$).

In terms of flavor and aroma, samples produced with green tea, rosemary and grape pomace were determined to be statistically significantly different from standard and commercial ones ($P < 0.05$) (Fig. 4). The differences in the color of the produced beers were determined to be not statistically significant.

A sensory gene taste analyses of beers produced with five different medicinal and aromatic plants (*Melissae folium*, *Thymi herba*, *Juniperi fructus*, *Urticae radix* and *Lupuli strobuli*) were evaluated by hedonic scale; 1 = dislike strongly, 9 = like extremely. Beer sample produced with lemon balm plant extract was scored with the highest value

during the panelists' evaluation (Dordevic et al. 2016). In other study, fresh and sterile cherry and plum puree were added to the beer at the fermentation stage and their effects on the sensory profile was evaluated. The differences observed in the sensory analyses did not affect the acceptability of the beers (Fanari et al. 2020). In another study was added broccoli to American amber ale type beer. The results of produced beer samples confirmed an acceptable sensory effect after 150 days of storage (Abellán et al. 2021).

**Figure 4.** Panelists' average score for beers samples

Considering the results of all data, the green tea and rosemary beers had better properties than hop-based beers ($p < 0.05$). So, the use of plants rich in bioactive components could be used as an alternative to hops bringing new and better functional properties of the final beer. The results emphasized that there is a strong relation between the amount of bioactive components of beer with different plant-based origins and their antioxidant activity values and consequently, with their functional properties.

Conclusions

According to the findings of our investigation, used green tea (*Camellia sinensis*) and rosemary (*Salvia rosmarinus*) plants could be considered as potential substitutes for studied hop type and concentrations for production of new beers. Higher levels of bioactive compounds and antioxidant activity have been obtained in beers produced by using rosemary and green tea compared to beer produced with selected type of hops at determined concentrations. Future studies on the enhancement of beers regarding the comparison with different type of hops replacement may make use of these findings.

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