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Research Article

Effect of the Addition of Propolis Extract on Bioactive Compounds and Antioxidant Activity of Craft Beer

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Antioxidant-rich foods and beverages play an essential role in the prevention of diseases. This study assessed the influence of the addition of ethanolic extract of propolis (EEP) to beer at different concentrations (0.05, 0.15, and 0.25 g/L). Total phenolic content (TPC) and total flavonoid content (TFC) were determined. Antioxidant activity (AA) was evaluated by radical scavenging activity (DPPH and ABTS) and reducing power (FRAP). The addition of EEP in beer resulted in a linear increase in the TPC with values of 4.5%, 16.7%, and 26.7% above a control (no EEP added; 242 mg gallic acid equivalent/L). A similar increase was observed with TFC values 16.0%, 49.7%, and 59.2% above the control (16.9 mg quercetin equivalent/L). The FRAP assay indicated linear increases in AA relative to control with values of 1555, 1705, and 1892 μmol Trolox equivalent/L following EEP additions. The incorporation of EEP resulted in increases in the bioactive compounds and AA in beer without altering the physicochemical parameters of golden ale beer. The results indicate a promising use of propolis extract as a functional ingredient in beer.

1. Introduction

Bioactive phenolic compounds are widely distributed in nature, are abundant in fruits, vegetables, and cereals crops, and represent the highest contribution of antioxidants in the diet [1–5]. Numerous studies have associated the consumption of foods rich in bioactive compounds, such as phenolics, with the prevention of cardiovascular diseases, certain types of cancer, and other diseases related to aging [6]. It reported that the positive association of a moderate intake of alcoholic beverages with a low risk for cardiovascular disease may be linked to polyphenol content and the associated antioxidant activities [4]. Several studies describe the effects of acute ingestion of beer, dealcoholized beer, and ethanol on the increase of plasma antioxidant and anticoagulant activities and a positive effect on plasma lipid levels of healthy humans [4, 7–9].

Antioxidants are widely used in the food industry to prevent or delay the oxidation of fats and oils. The global trend to avoid or reduce the use of synthetic additives, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), has resulted in a need to identify natural alternative sources of food antioxidants [10]. Therefore, there is a growing interest in the use of natural antioxidants in the food industry, not only for application as preservatives but also for the potential benefits to human health.

Propolis is a natural product that has a large potential as a functional food additive due to a diversity of bioactive compounds with many functional properties (e.g., antioxidant, antibacterial, anticancer, antifungal, anti-inflammatory, antiviral, and anticancer activities) [11–14]. It is an adhesive and resinous natural product, collected by bees *(Apis mellifera)* from harvested exudates of plant, leaves, and buds, which are mixed with pollen, wax, and the salivary enzyme β-glucosidase [14, 15]. The chemical composition of propolis varies depending on the season, geographical, and botanical origins, and the mechanisms used for collection of the material [12, 16]. More than 300 compounds, such as phenolic acids and esters, flavonoids (flavones, flavonones, flavonols,

dihydroflavonols, and chalcones), terpenes, amino acids, caffeic acid phenyl esters, aromatic aldehydes and alcohols, fatty acids, stilbenes, and steroids have been identified in propolis [17–19]. Variability in propolis composition makes functional application and quality control challenging [20–22]. In general, propolis is composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, and 5% pollen, and various other substances, including organic debris [23].

Beer is a product rich in polyphenols and bioactive compounds due to the raw materials used in brewing such as barley malt and hops [24–26]. However, during the mashing and boiling stages some phenolic compounds could be removed via fining, filtration, or oxidation [27]. Reduction or degradation of phenolic components can also occur during fermentation, maturation, bottling, and storage [28–30]. Moreover, during the storage of beer, colloidal haze can form due to the interaction between polyphenols, proteins, and polysaccharides [28, 29, 31]. The negative impact associated with malt and hop polyphenols on the colloidal stability of beer can be minimized by clarification processes using fining agents such as carrageenan, silica gel, or polyvinylpolypyrrolidone (PVPP) [32, 33]. However, these clarification practices may also reduce the bioactive compounds and the potential antioxidant activity of the beer. For that reason, this study investigates the potential for increasing the bioactive compounds (phenolic and flavonoid contents) and antioxidant activity of a craft beer by adding propolis extract during cold maturation without affecting the physicochemical parameters.

2. Materials and Methods

2.1. Chemicals. All reagents referenced within were of analytical quality. Quercetin was obtained from Fluka (Bio Chem), and Folin-Ciocalteu's reagent, methanol, and ethanol were obtained from Merck. The 2,4,6-Tri(2-pyridyl)-S-triazine (TPTZ), gallic acid, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich.

2.2. Ethanolic Extract of Propolis (EEP). Raw propolis samples were collected from beehives located in the Valparaíso, Chile (V region). The propolis was frozen (−20°C) and ground in a chilled grinder to produce small amounts of pulverized crude propolis. The EEP was prepared using the conditions described by Gregoris and Stevanato [17]. The extracts of propolis were prepared by periodic agitation by a magnetic stir bar in ethanol at 20°C in the dark for 24 h to concentrations of 0.05, 0.15, and 0.25 g/L [34]. The solution was centrifuged (Thermo IEC HNS-II, USA) at 4000 rpm for 5 min and filtered through Whatman N° 1 filter paper. The filtrate was used as the EEP and stored at −18°C until incorporation into beer.

2.3. Brewing and Ethanolic Extract of Propolis (EEP) Addition. A 30 L batch of golden ale was brewed in triplicate. The grain bill for the recipe was comprised of 93.5% of pale malt, 1.1% of caramel malt, and 5.4% of flaked oats (Wyermann Malzfabrik GmbH, Bamberg, Germany). An infusion mash at 67°C was performed for 60 min after mixing the milled gains with hot water at a grist : water ratio of 1 : 3 (w/v). Reverse osmosis water and tap water were mixed to achieve the water chemistry consistent with the beer style. Phosphoric acid was added to the sparge water to a pH of 5.2. The sparge continued and wort collected in the boil kettle until the desired specify gravity was achieved. The wort was boiled for 60 min; hop dosage was performed after 5 and 15 min of boiling with Cascade pellets (GeCorp, Santiago Chile), and 1 min prior to the end of boiling with Hallertauer-Magnum pellets (GeCorp). The separation of the hot break was carried out by whirlpool for 15 min followed by a 20 min rest for settling. The wort was cooled and moved into a temperature-controlled cylindrical-conical fermenter. The original gravity (OG) of the wort was 1.047. Dry ale yeast (Nottingham, Lallemand, Austria) was pitched with a rate of 15–20 × 10^6 yeast cells/mL, with a viability of 95%.

Primary fermentation was conducted at 20°C for 7 days. The green beer was transferred to 5 L stainless steel kegs, stabilized by PVPP (Polyclar, Ashland, USA), and stored at 5°C for 10 days for maturation. After 2 days of cold maturation, three different concentrations of EEP were added to the beer (0.05, 0.15, and 0.25 g/L) and were coded of following: B/EEP0.05, B/EEP0.15, and B/EEP0.25, respectively. A control sample was prepared as the golden ale beer without EEP addition. Following maturation, 6.5 g/L of dextrose was added and a secondary fermentation was conducted in-bottle for 2 weeks at room temperature.

2.4. Physicochemical Parameters of Beer Samples. Beers were analyzed using a Densitometer and NIR Alcolyzer (DMA 4500, Anton Paar, GmbH, USA) for original extract, real extract, and alcohol content. Standard beer analyses such as total acidity, pH, color, and bitterness were carried out according to ASBC methods beer-8, beer-9, beer-10, and beer-23 [35]. All samples were homogenized and degassed with in an ultrasonic water bath (10 min) at room temperature to remove the remaining CO_2.

2.5. Bioactive Compounds Present in Beer Samples

2.5.1. Total Phenolic Content (TPC) Determination. The TPC of beer was determined according to the Folin-Ciocalteu spectrophotometric method described by Zhao et al. [36], with slight modifications. Briefly, 0.5 mL of diluted beer sample was mixed with 2.5 mL of Folin-Ciocalteu (0.1 N) phenol reagent and incubated for 5 min. Then, 2 mL of Na_2CO_3 (7.5%) solution was added, and the final volume was made up to 10 mL with deionized water. After 1 h of reaction at room temperature, the absorbance at 760 nm was determined using a spectrophotometer 6715 UV/Vis Jenway (Dunmow, England). The measurement was compared to a calibration standard curve of gallic acid in the concentration range between 0.01 and 1 mg/mL ($R^2 = 0.9977$). Results were expressed as milligrams of gallic acid equivalents (GAE) per liter of beer (mg GAE/L).

Table 1: Physicochemical parameters of beer control (without EEP) and beer with added ethanolic extract of propolis (B/EEP) at different concentrations (0.05, 0.15, and 0.25 g/L).

Parameter	Beer	Beer treated with EEP (g/L)		
	Control	B/EEP 0.05	B/EEP 0.15	B/EEP 0.25
Original gravity (OG)	1.046 ± 0.001			
Final gravity (FG)	1.005 ± 0.002[a]	1.005 ± 0.002[a]	1.006 ± 0.002[a]	1.006 ± 0.002[a]
Alcohol (%, v/v)	5.17 ± 0.17[a]	5.18 ± 0.16[a]	5.15 ± 0.17[a]	5.18 ± 0.16[a]
Color (EBC)	13 ± 3[a]	13 ± 2[a]	13 ± 4[a]	13 ± 5[a]
Bitterness units (BU)	42 ± 1[a]	42 ± 1[a]	42 ± 2[a]	42 ± 1[a]
pH	4.18 ± 0.03[a]	4.06 ± 0.05[b]	4.02 ± 0.07[b]	4.05 ± 0.06[b]
Total acidity (lactic acid, g/L)	0.161 ± 0.001[a]	0.162 ± 0.000[a,b]	0.165 ± 0.000[a,b]	0.167 ± 0.001[b]

*Values marked with the same letter do not show significant differences ($p < 0.05$) for the same parameter.

2.5.2. Total Flavonoid Content (TFC) Determination. The TFC of beer was determined using aluminium chloride ($AlCl_3$) solution in methanol as described by Pai et al. [37]. Briefly, 1.5 mL $AlCl_3$ solution (2%) was added to 1.5 mL dealcoholized beer. The samples were incubated at 30°C for 10 min and the absorbance was determined at 368 nm using a spectrophotometer 6715 UV/Vis Jenway (Dunmow, England). The measurement was compared to a calibration standard curve of quercetin in the concentration range between 0.01 and 0.15 mg/mL ($R^2 = 0.9982$). Results were expressed as milligrams of quercetin equivalents (QE) per liter of beer (mg QE/L).

2.6. Antioxidant Activity

2.6.1. DPPH Radical Scavenging Activity. DPPH radical scavenging activity of beer was determined according to the method described by Tafulo et al. [38] A solution of DPPH (0.19 mmol) was prepared in a 2 : 1 (v/v) mixture of methanol and aqueous sodium acetate (0.1 mol); 2.8 mL of this solution and 0.2 mL of beer sample were mixed. The discoloration of the DPPH radical was measured at 517 nm using a spectrophotometer 6715 UV/Vis Jenway (Dunmow, England); the solution had been allowed to incubate in the dark for 30 min. The Trolox calibration standard curve (0.1–25 μmol, $R^2 = 0.9986$) was plotted as a function of the percentage of DPPH radical scavenging activity. The results were expressed as millimoles of Trolox equivalents (TE) per liter of beer (mmol TE/L).

2.6.2. ABTS Radical Cation Scavenging Activity. ABTS radical scavenging activity of beer samples was determined according to the method described by Zhao et al. [36]. ABTS was dissolved in water to a concentration of 7 mmol/L. The ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mmol/L potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The ABTS radical cation solution was diluted and equilibrated at 30°C. An aliquot of each beer sample was mixed with 2.9 mL of diluted ABTS radical cation solution. After incubation for 20 min, the absorbance at 734 nm by using a spectrophotometer 6715 UV/Vis Jenway (Dunmow, England) was measured. The Trolox calibration standard curve (0.1–20 μmol, $R^2 = 0.9989$) was plotted as a function of the percentage of ABTS radical cation scavenging activity. The results were expressed as mmol TE/L of beer.

2.6.3. FRAP Reducing Power. The total antioxidant activity of the beer samples was measured by the ferric reducing antioxidant power (FRAP) assay described by Piazzon et al. [8] and Can et al. [39]. Briefly, the FRAP reagent contained 5 mL of a TPTZ solution (10 mmol) in HCl (40 mM) plus 5 mL of $FeCl_3$ (20 mmol) and 50 mL of acetate buffer (0.3 mol/L, pH 3.6). The working FRAP reagent was prepared daily. An aliquot (100 μL) of each beer sample was mixed with 3 mL of FRAP reagent; the mixture was incubated for 10 min at 37°C, and the absorbance was measured at 593 nm using a Jenway 6800 UV/VIS spectrophotometer (Dunmow, England). A blank containing FRAP reagent and water was included for analysis. The difference between sample absorbance and blank absorbance was used to calculate the FRAP value. The reducing capacity of the beer tested was calculated with reference to the reaction signal given by Fe^{2+}, on the basis of the Trolox calibration standard curve (20–800 μmol, $R^2 = 0.9981$). The antioxidant activity of the beer was expressed as micromoles of Trolox equivalents (TE) per liter of beer (μmol TE/L).

3. Statistical Analysis

All the analyses were performed in triplicate and the data are presented as arithmetic mean and standard deviation. Analysis of variance (ANOVA) was performed using Statgraphics XV (USA); a $p < 0.05$ was considered to be statistically significant.

4. Results and Discussions

4.1. Physicochemical Parameters. The original and final gravities, alcohol content, color, bitterness units, pH, and total acidity of golden ale beer are presented in Table 1. The results showed that the addition of ethanolic extract of propolis (EEP) did not affect the physicochemical parameters compared to the experimental control. All of the analytical parameters were within the ranges according to recipe and beer style.

Table 2: Results obtained for bioactive compounds (total phenolic content, TPC, and total flavonoids content, TFC) and antioxidant activities (DPPH, ABTS, and FRAP assays) for ethanolic extracts of propolis (B/EEP) at the concentrations used.

Extracts	Bioactive compounds		Antioxidant activity		
	TPC (mg GAE/L)	TFC (mg QE/L)	DPPH (mmol TE/mL)	ABTS (mmol TE/mL)	FRAP (μmol TE/mL)
EEP 0.05	21.3 ± 1.0^{a}	59 ± 4^{a}	0.014 ± 0.001^{a}	0.079 ± 0.002^{a}	0.206 ± 0.002^{a}
EEP 0.15	33.5 ± 6.7^{b}	104 ± 24^{b}	0.029 ± 0.002^{b}	0.114 ± 0.002^{b}	0.412 ± 0.005^{b}
EEP 0.25	66.3 ± 1.4^{c}	148 ± 6^{c}	0.044 ± 0.001^{c}	0.149 ± 0.003^{c}	0.801 ± 0.002^{c}

*Values marked with the same letter do not show significant differences ($p < 0.05$).

Table 3: Results obtained for bioactive compounds (total phenolic content, TPC, and total flavonoids content, TFC) for beer control (without EEP) and beer with added ethanolic extract of propolis (B/EEP) at different concentrations (0.05, 0.15, and 0.25 g/L).

	Bioactive compounds	
	TPC (mg GAE/L)	TFC (mg QE/L)
Control	242.0 ± 21.2^{a}	16.9 ± 2.2^{a}
B/EEP 0.05	253.0 ± 19.8^{a}	19.6 ± 2.2^{a}
B/EEP 0.15	282.5 ± 28.9^{ab}	25.3 ± 1.0^{b}
B/EEP 0.25	306.5 ± 45.9^{b}	26.9 ± 2.7^{b}

*Values marked with the same letter do not show significant differences ($p < 0.05$).

4.2. Bioactive Compounds

4.2.1. Ethanolic Extract of Propolis (EEP). The bioactive compounds, expressed as TPC and TFC, increased with the concentration of propolis in the extract (Table 2). The antioxidant activity also increased with respect to concentration of propolis in the EEP (Table 2). This is consistent with a direct relationship between the presence of bioactive compounds and antioxidant activity [13, 40]. Propolis has been reported to contain ca. 300 compounds that are considered biologically active. Compounds such as coumaric acid, cinnamic acid, and caffeic acid are frequently reported in literature [14, 19].

4.2.2. Beer Samples with Incorporation of EEP. The results obtained for bioactive compounds in the beer samples are shown in Table 3. The total phenolic contents of the beer samples enriched with EEP ranged from 253.0 to 306.5 mg GAE/L, whereas the total flavonoid content ranged from 19.6 to 26.9 mg QE/L. These results were significantly higher than the beer control (242 mg GAE/L and 16.9 mg QE/L, resp.). The concentration of bioactive compounds (TPC and TFC) was found to increase linearly relative to EEP additions. Diverse studies have analyzed the presence of bioactive compounds in commercial beer samples. Lugasi [41] reported the TPC in lager beers ranging from 270 to 470 mg GAE/L and TPC for darker beers (from heavily roasted grains) ranging from 380 to 600 mg GAE/L. Piazzon et al. [8] evaluated several types of dealcoholized and bock-styles of beers for TPC and found values of 366 and 875 mg GAE/L, respectively. Another study by Zhao et al. [36] analyzed 34 Chinese commercial beers and showed TPC that varied from 152 mg GAE/L (Reeb beer) to 339.1 mg GAE/L (Carlsberg). Granato et al. [9] evaluated 29 different types of Brazilian lager and brown ale beers. The results obtained for TPC ranged from 119.9 to 525.9 mg GAE/L and the TFC varied from 23.1 to 171.3 mg catechin equivalents/L; the highest values of bioactive compounds were obtained in the brown ales. A similar result was found by Lugasi [41] indicating that darker beers contain high concentrations than lighter lager beers; reported TPC ranged from 380 to 600 mg/L in European commercial lagers and dark beers, respectively. Oroian et al. [42] evaluated the TPC for 10 commercial beers with values from 61.4 to 361.4 mg GAE/L. Pai et al. [37] investigated the TPC and TFC for Indian commercial beers; the results obtained in this study ranged from 160 to 620 mg GAE/L and 14 to 379 mg QE/L, respectively. Moura-Nunes et al. [43] evaluated the presence of individual phenolic compounds in different types and styles of Brazilian commercial beers using HPLC. The TPC was determined as a sum of the individual concentrations and the resulting concentrations ranged from 4.6 to 28.3 mg/L.

Đorđević et al. [34] determined the total phenolic content and antioxidant activity of lager beer samples after incorporation of different extracts of medical plants (e.g., *Melissae folium, Thymi herba, Juniperi fructus, Urticae radix*, and *Lupuli strobuli*). The results showed that the TPC was highest in beers following the addition of thyme (384.2 mg GAE/L), juniper (365.4 mg GAE/L), and lemon balm (363.1 mg GAE/L), representing increases of 37.1, 30.4, and 30%, respectively, compared to the commercial lager beer control (without extracts, 280.3 mg GAE/L).

The phenolic content and antioxidant activity of beer depend on the quality and quantity of starting materials and on the conditions of process (malting and brewing steps) [9, 43]. The flavonoids content varies as a result of barley and hop varieties, growing conditions, brewing methodology, and the style of beer [37]. These compounds impact the color, taste, flavor, stability, and shelf-life of the beer [8, 37, 41, 42]. The ingredients in beer, such as barley malt and hops, are all rich in polyphenol compounds. For example, barley has 1.2–1.5 g/kg polyphenols, while fresh hops contain roughly 700 mg/kg quercetin and 550 mg/kg kaempferol [41]. About 20–30% of beer polyphenols originate from hops, whereas 70–80% is malt derived [9].

Studies have reported the steps most critical for changes in bioactive compounds (polyphenols) and antioxidant activity during beer production include filtration and clarification,

Table 4: Results obtained for antioxidant activity (DPPH, ABTS, and FRAP assay) for beer control (without EEP) and beer with added ethanolic extract of propolis (B/EEP) at different concentrations (0.05, 0.15, and 0.25 g/L).

	Antioxidant activity		
	DPPH (mmol TE/L)	ABTS (mmol TE/L)	FRAP (μmol TE/L)
Control	0.533 ± 0.156^{a}	0.629 ± 0.038^{a}	1415.0 ± 241.8^{a}
B/EEP 0.05	0.530 ± 0.164^{a}	0.705 ± 0.123^{ab}	1555.0 ± 175.4^{ab}
B/EEP 0.15	0.491 ± 0.096^{a}	0.687 ± 0.094^{ab}	1705.0 ± 131.5^{bc}
B/EEP 0.25	0.576 ± 0.1782^{a}	0.808 ± 0.197^{b}	1892.5 ± 251.0^{c}

*Values marked with the same letter do not show significant differences ($p < 0.05$).

boiling, fermentation, and maturation [27]. Li et al. [3] evaluated the evolution of the TPC during storage. The content was reduced in the first three months and by the end of storage (6 months) was approximately 18.6% lower than the starting concentration. This behavior was attributed to the oxidation of phenolic compounds by free radicals and polymerization with proteins [44]. The oxidation of polyphenols can lead to enhanced protein-polyphenol interactions and the formation of a colloidal haze or instability [9]. Baltas et al. [45] demonstrated that some phenolic compounds present in the propolis extract could be useful to enzyme inhibition (e.g., urease, xanthine oxidase, and acetylcholinesterase) which can be related to the shelf-life of food and beverages.

Beers with high phenolic and antioxidant contents exhibit higher quality, more stable sensory properties such as flavor and aroma, increased foam stability, and longer shelf-life in comparison to beer with lower antioxidant activity [8]. Flavonoids, as primary antioxidants, are thought to act as free radical acceptors and chain breakers [9, 41].

4.3. Antioxidant Activity. The results obtained for antioxidant activities of beer samples analyzed by different methods are shown in Table 4. The beer samples with propolis extract added at the highest level (B/EEP 0.25) exhibited a slight increase (0.576 mmol TE/L, not significant) in values of DPPH activity relative to the control (0.533 mmol TE/L). No increase in AA was observed for the lower concentrations B/EEP 0.05 (0.530 mmol TE/L) and B/EEP 0.15 (0.491 mmol TE/L). Beer with higher DPPH radical scavenging activity is important to beer flavor stability because beer staling is generally considered associated with the formation of trans-2-nonenal and other saturated and unsaturated aldehydes due to lipid oxidation [36].

The ABTS assay showed a significant increase in antioxidant activity for all the beers with added EEP relative to the control; the highest scavenging activity of radicals was found in samples with the highest addition of extract (B/EEP 0.25). This beer showed a value of 0.808 mmol TE/L; an increase of approximately 28.5% in comparison to the beer control (0.629 mmol TE/L). Beer with a higher ABTS radical cation scavenging activity may stabilize active oxygen radicals resulting in improved flavor stability [36]. Hydrogen-donating ability is an index of the primary antioxidant activity of molecules. Primary or chain-breaking antioxidants donate hydrogen to free radicals, particularly the lipid hydroperoxide radicals that are the major propagators of autoxidation of fats. This conversion leads to nonradical species, therefore inhibiting the propagation phase of lipid peroxidation [41].

The results obtained for the scavenging activity assays (DPPH and ABTS) are in agreement with other studies. For example, Zhao et al. [36] showed values of DPPH and ABTS radical scavenging activities for 34 Chinese commercial beers. These beers showed activities ranging from 0.24 to 1.35 mmol TE/L and 0.55 to 1.95 mmol TE/L, respectively.

The results obtained using the FRAP method are also given in Table 4. This assay showed a consistent increase in antioxidant activity with increasing concentration of added EEP. The beer samples with the highest concentration of EPP (B/EEP 0.25; value of 1892.5 μmol TE/L) resulted in the highest values of reducing power (an increase of 33.7% over the beer control; 1415 μmol TE/L). Similar results were published by Đorđević et al. [34] who determined the antioxidant activity using the DPPH and FRAP methods in lager beer samples after incorporation of different extracts of medical plants. The same extracts that increased the TPC (thyme, juniper, and lemon balm) resulted in an increase in antioxidant activity. These results support the relationship between bioactive compounds and antioxidant activity. Correlations were drawn between bioactive compounds (phenol and flavonoid content) and antioxidant activities. A direct, positive correlation was exhibited between TPC and FRAP values. A linear relationship was derived (FRAP antioxidant activity $= 6.676 \times \text{TPC} - 167.4$) with a high correlation coefficient ($R^2 = 0.943$) similar to results published by Piazzon et al. [8] who observed that the antioxidant capacity of commercial beers measured by the FRAP method was remarkably different depending on beer type, ranging from 1525 μmol Fe^{+2}/L (dealcoholized beer) to 4663 μmol Fe^{+2}/L (bock beer). Similarly, Moura-Nunes et al. [43] observed a range from 810 μmol Fe^{+2}/L (dealcoholized beer) to 6370 μmol Fe^{+2}/L (lager beer) after analyzing different types and styles of Brazilian beers using the FRAP method. The antioxidant activity was also determined using the ABTS assay; the results obtained showed values ranging from 0.40 to 3.02 mmol TE/L, in line with those observed in this study.

The reducing power can be interpreted as an index of secondary antioxidant activity. Secondary or preventive antioxidants can reduce the rate of chain initiation in the lipid peroxidation process or can react with the products

of lipid peroxidation. This conversion leads to more stable nonradical, nondeleterious products [41].

5. Conclusions

This study highlights the promising potential for the development and use of propolis extract as an additional source of bioactive compounds and increase in antioxidant activity in beer. The incorporation of propolis extract in beer may help reduce oxidation and fortify the phenolic content that is typically reduced during the boiling, filtration, bottling, and storage stages of the brewing process. This has potential promise to improve the stability and shelf-life of commercial beers without the incorporation of artificial preservatives.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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