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Impact of the Addition of Botanical Ingredients on the Physicochemical Properties, Polyphenolic Content, and Antioxidant Activity of Craft Beers

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Abstract

Background: The incorporation of botanical ingredients into craft beer has emerged as a promising strategy to enhance nutritional value and expand its sensory diversity. Thus, this review aims to discuss the impact of adding botanical extracts on the physicochemical properties, phenolic content, and antioxidant potential of craft beers. Methods: A narrative review was conducted using PubMed, Science Direct, Web of Science, and b-on databases, with the keywords 'craft beer', 'physicochemical properties', 'polyphenolic content', and 'antioxidant activity'. Results: The incorporation of botanical ingredients into beers modified the physicochemical parameters, total phenolic content (TPC), and antioxidant activity. These effects varied according to the type of matrix, concentration, timing of addition, beer style, and brewing conditions. Overall, an increase in beer TPC and antioxidant activity was observed. However, higher TPC can present technological challenges, as phenolic–protein interaction may lead to turbidity. Conversely, enhanced antioxidant potential contributes to oxidative stability and extends the shelf-life of beer. Conclusions: Future studies should validate the current results, explore new bioactive matrices, and evaluate variables that ensure the functional quality of beer. Practical applications under real production conditions should also be prioritized to guarantee effective functional benefits without compromising the stability and sensory acceptance of craft beer.

Keywords: craft beer; botanical ingredients; physicochemical properties; phenolic content; antioxidant activity

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1. Introduction

Craft beer is produced from malt, water, hops, and yeast (high-quality raw materials) in small quantities (production of less than 6 million barrels/year in the United States; less than 200,000 hectoliters/year in Italy; and less than 5000 hectoliters/year in the United Kingdom) in independent breweries using traditional techniques [1–5]. In general, the absence of pasteurization and microfiltration steps in the brewing process is associated with higher total phenolic content (TPC) and greater antioxidant potential compared to industrial beers [6,7].

In recent years, craft beers have been the focus of both technical and creative innovation, allowing the incorporation of ingredients such as algae, medicinal plants, mushrooms, fruits, and other ingredients like coffee and propolis. These additions impart unique organoleptic characteristics, as well as potential nutritional and functional benefits [8–17].

Additionally, due to storage time, the antioxidant potential of craft beers tends to decline. Consequently, there is growing interest in enhancing its antioxidant activity by adding botanical ingredients that help mitigate the effects of free radicals [18]. Free radicals are highly unstable atoms, molecules, or ions characterized by the presence of unpaired electrons, which makes them highly reactive [19]. They can derive from oxygen (reactive oxygen species—ROS), nitrogen (reactive nitrogen species—RNS), and sulfur (reactive sulfur species—RSS) [19].

Despite being more expensive, due to the quality of the raw materials, innovation, rigorous production process, and tax rates, craft beers attract consumers because of their perceived higher quality and authenticity compared to industrial beers [20–25]. This positions the craft beer sector as an innovation area, where bioactive compounds are conveyed through craft beer, linking the beer market to health.

Some important bioactive compounds, such as polyphenols, present in beer can be reduced due to the filtration and clarification processes, low-quality raw materials, and storage conditions. It is known that the flavor of beer can become unstable after being bottled and stored, changing with time [26]. Therefore, the addition of botanical ingredients or plant-based compounds (e.g., mango, persimmon fruit, olive extract, and bergamot juice) can improve the health and organoleptic properties of manufactured beer [9,27–29]. The use of fruits as additives imparts natural sweetness, acidity, and complex aromas to craft beer [27]. Also, the use of agro-industrial by-products in the production of beer has been explored [30].

Borsa et al. [31] specifically compiled the impact of adding botanical ingredients to craft beer in terms of TPC and antioxidant activity. Also, Matrella et al. [27] explored the incorporation of bergamot juice and olive extract as functional ingredients in craft beer and their effects on antioxidant activity and cellular oxidative stress. However, more studies are needed to understand the impact of adding botanical ingredients on physicochemical parameters (color, pH, bitterness, acidity, and alcohol content), TPC, and antioxidant activity. This work could encourage new research about the nutraceutical value of beers enriched with botanical ingredients.

2. Materials and Methods

A narrative review was carried out between April and June 2025, using the PubMed, Science Direct, Web of Science, and b-on databases, with the keywords 'craft beer', 'physicochemical properties', 'polyphenolic content', and 'antioxidant activity' combined using the Boolean operator 'AND' (Figure 1). Research articles that addressed the effects of incorporating botanical extracts into craft beers, with a focus on their physicochemical properties, phenolic content, and antioxidant potential were included. Articles in English and Portuguese with no time restrictions were considered. Review articles, studies that did not evaluate the incorporation of botanical ingredients into craft beer, those that did not include the defined keywords, and studies that analyzed production conditions were excluded.

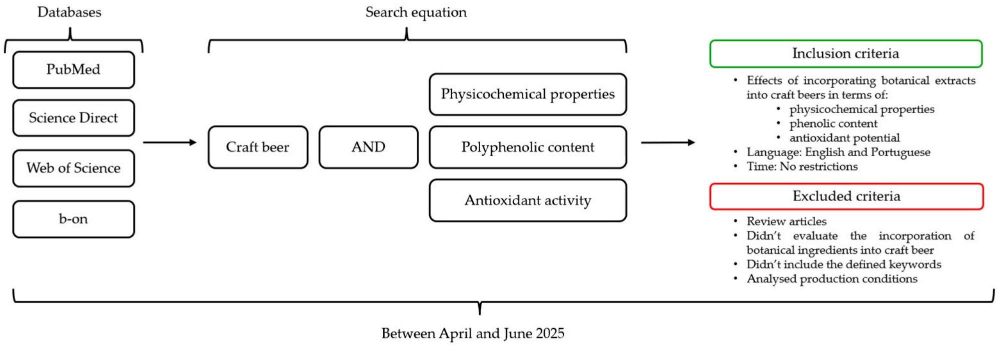

Figure 1. Summary scheme of the research strategy.

3. Physicochemical Properties

The incorporation of botanical extracts into craft beers induces changes in physicochemical parameters such as color, pH, bitterness, acidity, and alcohol content (Tables 1 and 2). Understanding its variation according to the type of extract is essential given the potential impact on product stability.

3.1. Physical Properties

Color

The color of beer is a parameter influenced by multiple factors, including the type and quantity of the malts used, boiling time, beer style, and the raw materials incorporated [32]. In general, lighter malts give beer a pale color, while darker malts (caramelized and/or roasted) result in darker beers [33]. The color can be expressed in European Brewery Convention (EBC) units or in Standard Reference Method (SRM) units, ranging from 4.5 to 1550 EBC [33–35].

The results presented in Table 1 indicate that most extracts intensified the color of the beer compared to the control. This effect was observed with the addition of natural basil pepper leaves [36], saskatoon berries [37], mushrooms [10], elderberry added to Blonde Ale and Catharina Sour beers [38], huaimi [39], olive leaf extract at concentrations of 1.0% and 2.0% [40], pitaya [41], kamchatka berry and haskap fruit [42], red rice from Bahia [35], and purple grape pomace [43].

Table 1. Impact of adding botanical ingredients on the physical characteristics of beer.

Ingredients/Stage of Incorporation/Beer Style	Color (EBC Scale)	Reference
Basil pepper (*Ocimum selloi*): aqueous extract (0.05 and 0.1%) and *in natura* (0.1 and 0.5%), added before and after the fermentation stage in Pilsner-style beer.	Addition of *in natura* leaves: color of the beers more intense. Addition of the aqueous extract: similar color to the control.	[36]
Bergamot (*Citrus bergamia*), juice; olive fruit (*Olea europaea*) extract, variety Carolea, added in Blanche-style and Weiss-style beer.	Significant decrease in color after adding bergamot to Blanche beer (7.5 ± 0.6 EBC) and olive to Weiss beer compared to the control (without bergamot, 4.6 ± 0.1; without olive, 4.6 ± 0.2), $p < 0.05$.	[44]
Coffee bagasse (1, 5, and 10 mg/mL), added during the maturation stage in Stout-style beer.	No significant differences between the control and the beer after the addition of coffee bagasse.	[9]

Table 1. *Cont.*

Ingredients/Stage of Incorporation/Beer Style	Color (EBC Scale)	Reference
Elderberry (*Sambucus nigra*): dried fruit (20 g/L), added in the boiling stage and during maturation in Blonde Ale-style and Catharina Sour-style beer.	Blonde Ale beer: Increase in color after the addition of elderberry in the boiling phase (17 EBC) and during maturation (29 EBC) compared to control beer (8 EBC). Catharina Sour beer: Increase in color after adding elderberry in the boiling phase (17 EBC) and during maturation (21 EBC) compared to control beer (7 EBC).	[38]
Guava (*Campomanesia adamantium*) fruits and aqueous leaf extract (0.1%), added after the fermentation stage in Pilsner–style beer.	Change in beer color after adding guava fruit (14.09 ± 0.64 EBC) and leaf extract (14.05 ± 0.71 EBC). Color of the control: 14.01 ± 0.77 EBC).	[45]
Huaimi (*Flos Sophorae Immaturus*) varieties: Cyan and Golden (5 g/L), added in the wort saccharification phase, 10 min after the start of wort boiling, 10 min before the end of wort boiling, before the start of primary fermentation.	Significant increase in color of beer after adding huaimi (13.22 ± 0.02 to 16.17 ± 0.05 EBC) compared to control (12.32 ± 0.04 EBC), $p < 0.05$.	[39]
Jambu (*Acmella oleracea*): alcoholic extract of flowers 5 and 7.5 mL/L), added in the bottling process in Pilsner-style beer.	No color change. Beer with 5.0 mL/L and 7.5 mL/L showed a color value of 2.20 ± 0.5 EBC and 2.20 ± 0.4 EBC, respectively. Control: 2.20 ± 0.5 EBC.	[46]
Kamchatka berry (*Lonicera caerulea* var. kamtschatica) 'Duet' and 'Aurora' variety and haskap (*Lonicera caerulea* var. emphyllocalyx) 'Lori' and 'Willa' variety fruit, added after 7 days of fermentation in Wheat-style beer.	Significant increase in color of beer after adding kamchatka berry and haskap fruit (28.4 ± 0.2 to 31.5 ± 0.5 EBC) compared to control (22.4 ± 0.4 EBC), $p < 0.05$.	[42]
Lemongrass (*Cymbopogon citratus*) (1, 2.5, and 5%), added on day 7 of fermentation in Wheat-style beer.	Significant decrease in color after adding lemongrass (16.9 ± 0.7 to 21.5 ± 0.5 EBC) compared to control (22.7 ± 0.6 EBC), $p < 0.05$.	[47]
Mushrooms (*Pleurotus eryngii*) (5 and 10 g/L), added before and after the fermentation stage.	Significant increase in color of beer after adding mushrooms (5.63 ± 0.34 to 11.85 ± 0.55 EBC) compared to control (3.82 ± 0.12 EBC), $p < 0.05$. The addition of 5 g/L before the fermentation stage showed no significant compared to control.	[10]
Olive (*Olea europaea*): leaf extract (0.5, 1, and 2%), added during the maturation stage in Light-style beer.	Significant increase in color of beer after adding 1% and 2% (15.0 EBC and 17.0 EBC, respectively) compared to control (9.0 EBC), $p < 0.05$.	[40]
Pine (*Pinus sylvestris*) shoot extract (15 g/L) added in the boiling stage in Wheat-style beer.	No significant differences between the control and the beer after the addition of pine shoots.	[48]
Pitaya (*Hylocereus polyrhizus*): pulp (5, 10 and 20% *v*/*v*), added in the beginning of the fermentation stage in Witbier-style beer.	Addition of pitaya significantly decreased the brightness of the beer compared to control, $p < 0.05$. Addition of pitaya significantly increased the green–red chroma of the beer compared to control, $p < 0.05$.	[41]
Propolis: ethanolic extract (0.05, 0.15, and 0.25 g/L), added during the maturation stage in Golden Ale-style beer.	No significant differences between the control and beer with propolis.	[17]

Table 1. *Cont.*

Ingredients/Stage of Incorporation/Beer Style	Color (EBC Scale)	Reference
Purple grape pomace (1, 5 and 10% *w*/*w*), added before fermentation.	Significant increase in color after adding purple grape pomace (19.41 ± 0.03 to 22.50 ± 0.03 EBC) compared to control (14.99 ± 0.03 EBC), $p < 0.05$.	[43]
Red rice (*Oryza sativa*) from Bahia and Paraíba (3 Kg/35 L), added in the mashing stage.	Significant increase in color of beer after adding Bahia rice (43.1 ± 0.03 EBC); and significant decrease in color after adding Paraíba rice (27.0 ± 0.05 EBC) compared to control (34.2 ± 0.05 EBC), $p < 0.05$.	[35]
Freeze-dried cape gooseberry dried cape gooseberry (*Physalis peruviana*) (20, 40, and 60 g/L), added after reaching 75% of the fermentation.	Significant increase in color after adding purple gooseberry (11.76 ± 0.04 to 21.46 ± 0.09 EBC) compared to control (7.07 ± 0.05 EBC), $p < 0.05$.	[49]
Saskatoon berry (*Amelanchier alnifolia*) of the 'Thiessen' and 'Honeywood' species with and without ozonated treatment, added on day 7 of fermentation.	Significant increase in color of beer after adding saskatoon berry (23.1 ± 0.0 to 26.9 ± 0.1 EBC) compared to control (20.1 ± 0.3 EBC), $p < 0.05$.	[37]
Turmeric (*Curcuma longa*), black pepper (*Piper nigrun*) and aromatic hops (*Humulus lupulus*) (1, 1.5, and 5 g/mL, respectively), added during maturation (turmeric and black pepper) or the final boiling stage (aromatic hops) in Red Ale-style. Spices incorporated alone and in combination.	No significant differences between the control and the beer after the addition of spices.	[15]
Umatola (*Parastrephia lucida*) dry leaves (0.1, 0.5, 1 and 5%), added during the maturation stage in Aumaita Porter-style beer.	No significant differences between the control and the beer after the addition of 1% umatola.	[50]

EBC: European Brewery Convention.

Thus, the impact of compounds on beer coloring depends on the incorporated matrix, the respective concentration, and composition of colored compounds, as well as the timing of addition and the fermentation conditions (such as temperature and pH) [51].

The incorporation of active ingredients from day 7 of fermentation in the studies by Belcar et al. [42] and Gorzelany et al. [37] intensified the color of the beer, but in the study by Belcar & Gorzelany. [47], the addition of lemongrass reduced it. The same inconsistency was observed for the incorporation of botanical ingredients in the maturation stage in the studies by Ullo et al., [17] Bustos et al. [50], and Nunes Filho et al. [15], where the incorporation of propolis, umatola, and spices did not significantly alter the color of the product, while the incorporation of olive leaf extract [40] intensified it in relation to the control beer. The incorporation of ingredients before fermentation in the studies performed by Cirlincione et al. [10] and Luz et al. [43] (mushroom *Pleurotus eryngii* and purple grape pomace, respectively) at the beginning of fermentation (pitaya) [41] and mashing stage (gooseberry) [49] seems to have increased the color of the beer. Finally, incorporation at the boiling stage (pine shoots) [48] did not significantly alter the color of the beer.

The change in the color of beers after the addition of botanical ingredients, compared to control beers, may result from the presence of anthocyanins, betalains, β-carotene, the formation of melanoidins from Maillard reactions, the possible addition of artificial colorings, and the potential oxidation reactions of polyphenols. [41,51–53]. In the study conducted by Luz et al. [43], the addition of purple grape pomace significantly increased the anthocyanin content (30.34 ± 0.32 mg of cyanidin-3-glucoside/L) compared to the control (3.76 ± 0.32 mg of cyanidin-3-glucoside/L). Santana et al. [35] justified the change in beer color after the incorporation of red rice from Bahia by the contribution of proanthocyanins and carotenoids to the matrix. Also, in the study conducted by Da Costa et al. [41], the intensification of the color of the beer after the addition of pitaya is pointed out by the authors due to the presence of betalain in the fruit, although its concentration has not been determined. Finally, the formation of melanoidins resulted from Maillard reactions (between amino acids and sugars), due to their dark color, may be responsible for the intensification of beer color [54].

Factors such as the instability of coloring compounds, sensitivity to temperature, pH, oxygen, light, enzymes, proteins, and ions may explain the decrease in beer coloring [48].

3.2. Chemical Properties

3.2.1. pH

pH is an important parameter in the biological, chemical, and sensory stability of beer [32,55]. This parameter influences yeast growth and metabolism, fermentation speed, alcohol synthesis, and susceptibility to bacterial contamination [47,56]. Its value varies throughout the beer manufacturing process, preferably ranging between 4.3 and 4.6, ensuring beer quality in terms of organoleptic balance and stability [32,57]. An increase in pH may indicate yeast autolysis and microbiological instability, resulting in beer deterioration [32]. It can also reduce the oxidative process and, with that, the formation of compounds that can interfere with the flavor of the beer [41]. However, pH values below 4.2 are undesirable, as they can interfere with yeast metabolism, despite favoring resistance to microbial spoilage and increasing colloidal and foam stability [32,39]. It is worth noting the contribution of the fermentation process to the decrease in pH, as yeasts consume free amino nitrogen and excrete acetic acid and other organic acids. Thus, environments that promote yeast activity can contribute to an increase in acidic compounds in beer and, consequently, to a decrease in pH [57].

The incorporation of some ingredients in beers caused changes in pH. These changes were influenced by the composition and concentration of the matrix or extract, its physical state (fresh vs. dry extract), the timing of its addition, and the beer style (Table 2).

An increase in pH compared to the control was observed with the addition of the following ingredients: dandelion [53], jambu flower [46], mushrooms [10], elderberry and Catharina Sour beer [38], pitaya 20% v/v, guava fruit in aqueous leaf extract [45], and red rice from Bahia [35]. It should be noted that the pH increase observed in the studies mentioned occurred for values close to 4.6, in agreement with the ideal pH of beer.

However, the addition of lemongrass [47], propolis [17], saskatoon berry [37], elderberry and Blond Ale beer [38], pine shoots [48], huaimi [39], bergamot [44], extract fruit olive [44], kamchatka berry [42], haskap fruit [42], purple grape pomace 10% w/w [43], and dried cape gooseberry [49] reduced the pH compared to the control. In the studies by Zhang et al. [39], Belcar et al. [42], and Belcar & Gorzelanv [47], the control beers had a pH above 4.6, so the added matrices may have contributed to increased stability.

The addition of elderberry to beers of two distinct styles (Blonde Ale-style and Catharina Sour-style) highlights the influence of the control beer on pH [38].

Despite the interstudy heterogeneity, it can be observed that the addition of matrices on day 7 of fermentation caused a decrease in pH compared to the control beer [37,42,47]. In contrast, the addition of matrices in the maturation cap did not alter the pH compared to the respective control beers [15,40,50], with the exception of the study by Ulloa et al. [17], where the addition of propolis decreased the pH. The addition of ingredients before fermentation has opposite effects on pH depending on the matrix, as the incorporation of mushrooms [10] and pitaya [41] increase the pH, while the addition of purple grape pomace [43] reduced it.

Table 2. Impact of adding botanical ingredients on the chemical characteristics of beer.

Ingredients/Stage of Incorporation/Beer Style	pH	Bitterness	Acidity	Alcohol Content	Reference
Basil pepper (*Ocimum selloi*): aqueous extract (0.05 and 0.1%) and *in natura* (0.1 and 0.5%), added before and after the fermentation stage in Pilsner-style beer.	N.D.	N.D.	N.D.	Significant increase in alcohol content with the addition of *in natura* leaves (7.78 ± 0.08% v/v to 8.50 ± 0.09% v/v and the aqueous extract (7.20 ± 0.08% v/v to 7.55 ± 0.09% v/v) compared to control (7.57 ± 0.12% v/v), $p < 0.05$.	[36]
Bergamot (*Citrus bergamia*), juice; olive fruit (*Olea europaea*) extract, variety Carolea, added in Blanche-style and Weiss-style beer.	Significant decrease in pH after adding bergamot to Blanche beer (4.2 ± 0.2) and olive to Weiss beer (4.2 ± 0.1)	Significant increase in bitterness after adding bergamot to Blanche beer (15 ± 1.0 IBU) and olive to Weiss beer (8 ± 0.4 EBC) compared to control (without bergamot, 11 ± 0.8 EBC; without olive, 17 ± 0.7 EBC), $p < 0.05$. (17 ± 0.5 IBU) compared to control (without bergamot, 11 ± 0.5 EBC; without olive, 13 ± 0.7 EBC), $p < 0.05$.	N.D.	No changes in alcohol content after the addition of bergamot or olive compared to the control.	[44]
Dandelion (*Taraxacum* spp.) (5, 10 and 20 mg/mL), added 45 min after boiling.	Significant increase in pH after adding dandelion (4.31 ± 0.01 to 4.32 ± 0.02) compared to control (4.22 ± 0.01), $p < 0.05$.	N.D.	Significant increase in acidity after adding dandelion (4.64 ± 0.19 to 5.15 ± 0.12 g/100 mL) compared to control (4.29 ± 0.06 g/100 mL), $p < 0.05$.	N.D.	[53]
Elderberry (*Sambucus nigra*): dried fruit (20 g/L), added in the boiling stage and during maturation in Blonde Ale-style and Catharina Sour-style beer.	Blonde Ale beer: decrease in pH after adding elderberry in the boiling stage (4.2) and during maturation (4.3) compared to control beer (4.4). Catharina Sour beer: increase in pH after adding elderberry in the boiling stage (3.8) and during maturation (3.4) compared to control (3.3).	Blonde Ale: Decreased bitterness after the addition of elderberry in the boiling phase (11 IBU) and during maturation (15 IBU) compared to control beer (16 IBU). Catharina Sour beer: Increased bitterness after the addition of elderberry in the boiling stage (6 IBU), compared to control (4 IBU) When added during the maturation stage, the same amount of bitterness was obtained (4 IBU).	N.D.	Blonde Ale: Increase in alcohol content after the addition of elderberry in the boiling phase (6.1% v/v) and during maturation (6.0% v/v) compared to control beer (5.4% v/v). Catharina Sour beer: Increase in alcohol content after the addition of elderberry in the boiling phase (5.7% v/v) and during maturation (5.6% v/v) compared to control beer (5.4% v/v).	[38]
Guava (*Campomanesia adamantium*) fruits and aqueous leaf extract (0.1%), added after the fermentation stage in Pilsner-style beer.	Increase in beer pH after adding guava fruit (4.71 ± 0.08) and leaf extract (4.60 ± 0.10), compared to control (4.51 ± 0.11).	N.D.	N.D.	The alcohol content increased after the addition of guava fruit (5.69 ± 0.12% v/v) and leaf extract (5.23 ± 0.11% v/v) compared to control (5.11 ± 0.09% v/v).	[45]

Table 2. *Cont.*

Ingredients/Stage of Incorporation/Beer Style	pH	Bitterness	Acidity	Alcohol Content	Reference
Huaimi (*Flos Sophorae Immaturus*) varieties: Cyan and Golden (5 g/L), added in the wort saccharification phase, 10 min after the start of wort boiling, 10 min before the end of wort boiling, before the start of primary fermentation.	Significant decrease in pH after adding huaimi (4.55 ± 0.01 to 4.74 ± 0.01) compared to control (4.80 ± 0.01), $p < 0.05$.	N.D.	Significant increase in acidity of beer after adding huaimi (2.62 ± 0.08 to 2.97 ± 0.04 mL/100 mL) compared to control (2.48 ± 0.09 mL/100 mL), $p < 0.05$.	Significant increase in alcohol content after adding huaimi (5.09 ± 0.01 to 6.06 ± 0.01% *v*/*v*) compared to control (4.96 ± 0.01% *v*/*v*) $p < 0.05$.	[39]
Jambu (*Acmella oleracea*): alcoholic extract of flowers 5 and 7.5 mL/L), added in the bottling process in Pilsner-style beer.	Increase in pH with the addition of 5 mL/L of extract (4.18 ± 0.003) and 7.5 mL/L of extract (4.25 ± 0.002) compared to control (4.10 ± 0.002).	No change in bitterness. Beer with 5.0 mL/L and 7.5 mL/L showed a bitterness value of 29.50 ± 0.5 IBU and 29.50 ± 0.8 IBU, respectively. Control: 29.50 ± 0.7 IBU.	N.D.	No alcohol content change. Beer with 5.0 mL/L and 7.5 mL/L showed an alcohol content value of 2.20 ± 0.012% *v*/*v* and 2.20 ± 0.012% *v*/*v*, respectively. Control: 2.50 ± 0.016% *v*/*v*.	[46]
Kamchatka berry (*Lonicera caerulea* var. kamtschatica) 'Duet' and 'Aurora' variety and haskap (*Lonicera caerulea* var. emphyllocalyx) 'Lori' and 'Willa' variety fruit, added after 7 days of fermentation in Wheat-style beer.	Significant decrease in pH after adding kamchatka berry and haskap fruit (4.01 ± 0.06 to 4.19 ± 0.07) compared to control (4.83 ± 0.02), $p < 0.05$.	Significant decrease in bitterness of beer after adding kamchatka berry and haskap fruit (11.1 ± 0.1 to 13.5 ± 0.5 IBU) compared to control (15.4 ± 0.2 EBC), $p < 0.05$.	Significant increase in acidity of beer after adding kamchatka berry and haskap fruit (3.33 ± 0.03 to 4.18 ± 0.04 M NaOH/100 cm^3) compared to control (2.71 ± 0.01 M NaOH/100 cm^3), $p < 0.05$.	Significant increase in alcohol content after adding kamchatka berry and haskap fruit (3.72 ± 0.08 to 4.36 ± 0.04% *v*/*v*) compared to the control (3.40 ± 0.10% *v*/*v*), $p < 0.05$.	[42]
Lemongrass (*Cymbopogon citratus*) (1, 2.5, and 5%), added on day 7 of fermentation in Wheat-style beer.	Significant decrease in pH after adding lemongrass (4.52 ± 0.02 to 4.68 ± 0.03) compared to control (4.79 ± 0.04), $p < 0.05$.	Significant increase in bitterness after adding lemongrass (16.1 ± 0.1 to 18.5 ± 0.5 IBU) compared to control (14.4 ± 0.3 IBU), $p < 0.05$.	Significant increase in acidity after adding lemongrass (4.36 ± 0.06 to 5.38 ± 0.08 M NaOH/100 mL) compared to control beer (3.82 ± 0.05 M NaOH/100 mL), $p < 0.05$.	Significant decrease in alcohol content after adding lemongrass (3.28 ± 0.07 to 4.00 ± 0.10% *v*/*v*) compared to control beer (4.73 ± 0.05% *v*/*v*), $p < 0.05$.	[47]
Mushrooms (*Pleurotus eryngii*) (5 and 10 g/L), added before and after the fermentation stage.	Significant increase in pH after adding mushrooms (4.26 ± 0.02 to 4.44 ± 0.04) compared to control (4.13 ± 0.04), $p < 0.05$.	N.D.	N.D.	Significant increase in alcohol content after adding mushrooms (4.28 ± 0.01 to 5.37 ± 0.01% *v*/*v*) compared to control (4.14 ± 0.01% *v*/*v*), $p < 0.05$. The addition of 5 g/L before the fermentation stage showed no significant differences compared to control.	[10]
Olive (*Olea europaea*): leaf extract (0.5, 1, and 2%), added during the maturation stage in Light-style beer.	No significant differences between the control and the beer after the addition of olive.	No significant differences between the control and the beer after the addition of olive.	No significant differences between the control and the beer after the addition of olive.	No significant differences were found with the addition of olive compared to control.	[40]

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Table 2. *Cont.*

Ingredients/Stage of Incorporation/Beer Style	pH	Bitterness	Acidity	Alcohol Content	Reference
Pine (*Pinus sylvestris*) shoot extract (15 g/L) added in the boiling stage in Wheat-style beer.	Significant decrease in pH after adding pine shoots (4.31 ± 0.01) compared to (4.31 ± 0.00), $p < 0.05$.	Significant increase in bitterness of beer after adding pine shoots (17.36 ± 0.09 IBU) compared to control (16.94 ± 0.42 IBU), $p < 0.05$.	Significant decrease in acidity of beer after adding pine shoots (2.46 ± 0.03 M NaOH/ 100 mL) compared to control (2.70 ± 0.03 M NaOH/ 100 mL), $p < 0.05$.	Significant increase in alcohol content after adding pine shoots (4.90 ± 0.07% v/v) compared to control (4.27 ± 0.06% v/v), $p < 0.05$.	[48]
Pitaya (*Hylocereus polyrhizus*): pulp (5, 10 and 20% v/v), added in the beginning of the fermentation stage in Witbier-style beer.	Significant increase in pH after adding pitaya 20% v/v (4.52 ± 0.01) compared to control (4.38 ± 0.04), $p < 0.05$.	N.D.	No significant differences between the control and the beer after the addition of pitaya.	Significant increase in alcohol content after adding pitaya 10% v/v and 20% v/v (4.55 ± 0.70% v/v and 4.55 ± 0.60% v/v, respectively) compared to control (4.42 ± 0.00% v/v) $p < 0.05$.	[41]
Propolis: ethanolic extract (0.05, 0.15, and 0.25 g/L), added during the maturation stage in Golden Ale-style beer.	Significant decrease in pH after adding propolis (4.02 ± 0.07 to 4.06 ± 0.05) compared to control (4.18 ± 0.03), $p < 0.05$.	No significant differences between the control and beer with propolis.	Significant increase in acidity after adding 0.25 g/L propolis (0.167 ± 0.001 g lactic acid/L) compared to control (0.161 ± 0.001 g lactic acid/L), $p < 0.05$.	No significant differences between the control and beer with propolis.	[17]
Purple grape pomace (1, 5 and 10% w/w), added before fermentation.	Significant decrease in pH after adding 10% w/w purple grape pomace (3.94 ± 0.05) compared to control beer (4.08 ± 0.06), $p < 0.05$.	Significant decrease in bitterness after adding 5% and 10% w/w of grape pomace (15.00 ± 0.43 IBU and 16.66 ± 0.84 IBU, respectively) compared to control (28.57 ± 1.50 IBU), $p < 0.05$.	Significant decrease in acidity after adding 10% w/w purple grape pomace (3.13 ± 0.17% M NaOH) compared to control (2.56 ± 0.31% M NaOH), $p < 0.05$.	Increase in alcohol content after the addition of purple grape pomace (5.43% v/v to 5.5% v/v) compared to control (3.8% v/v).	[43]
Red rice (*Oryza sativa*) from Bahia and Paraíba (3 Kg/35 L), added in the mashing stage.	Significant increase in pH after adding Bahia rice (4.39 ± 0.01) compared to control (4.31 ± 0.01), $p < 0.05$.	N.D.	Significant increase in acidity of beer after adding Paraíba rice (0.13 ± 0.03 acetic acid w/v) compared to control (0.1 ± 0.02 acetic acid w/v), $p < 0.05$.	Significant increase in alcohol content after adding red rice from Bahia and Paraíba (5.9 ± 0.13 and 5.7 ± 0.18 °Gl, respectively) compared to control (4.8 ± 0.11 °Gl).	[35]
Freeze-dried cape gooseberry (*Physalis peruviana*) (20, 40, and 60 g/L), added after reaching 75% of the fermentation.	Significant decrease in pH after adding gooseberry (3.81 ± 0.02 to 3.93 ± 0.02) compared to control (4.15 ± 0.01), $p < 0.05$.	Significant decrease in bitterness of beer after adding gooseberry (6.66 ± 0.28 to 10.25 ± 0.83 IBU) compared to control (11.62 ± 0.08 IBU), $p < 0.05$.	Significant increase in acidity of beer after adding gooseberry (0.35 ± 0.01 to 0.73 ± 0.02% citric acid) compared to control (0.22 ± 0.01% citric acid), $p < 0.05$.	Significant increase in alcohol content after adding gooseberry (5.13 ± 0.08 to 6.13 ± 0.06% v/v) compared to the control (4.24 ± 0.04% v/v), $p < 0.05$.	[49]

Table 2. *Cont.*

Ingredients/Stage of Incorporation/Beer Style	pH	Bitterness	Acidity	Alcohol Content	Reference
Saskatoon berry (*Amelanchier alnifolia*) of the 'Thiessen' and 'Honeywood' species with and without ozonated treatment, added on day 7 of fermentation.	Significant decrease in pH after adding saskatoon berry (4.40 ± 0.10 to 4.47 ± 0.03) compared to control (4.54 ± 0.06), $p < 0.05$.	No significant differences between the control and the beer after the addition of saskatoon berry.	Significant increase in acidity after adding saskatoon berry (3.55 ± 0.05 to 4.22 ± 0.03 M NaOH/100 mL) compared to control (3.46 ± 0.06 M NaOH/100 mL), $p < 0.05$.	Significant decrease in alcohol content after adding saskatoon berry (3.21 ± 0.01 to 3.56 ± 0.10% *v*/*v*) compared to control (4.18 ± 0.04% *v*/*v*), $p < 0.05$.	[37]
Seriguela (*Spondias purpurea*) (3 and 6%) and orange peel (*Citrus sinensis*) (0.3, and 6%), added in the beginning of the fermentation stage.	No significant differences between control and beer after the addition of seriguela and orange peel.	N.D.	N.D.	No significant differences between the control and the beer addition of seriguela and orange peel. No significant differences between the control and the beer after addition of seriguela and orange peel.	[58]
Turmeric (*Curcuma longa*), black pepper (*Piper nigrun*) and aromatic hops (*Humulus lupulus*) (1, 1.5, and 5 g/mL, respectively), added during maturation (turmeric and black pepper) or the final boiling stage (aromatic hops) in Red Ale-style. Spices incorporated alone and in combination.	No significant differences between the control and the beer after the addition of spices.	Significant increase in bitterness after adding spices (29.91 ± 1.48 to 68.90 ± 1.09 IBU) compared to control (15.0 ± 1.00 IBU), $p < 0.05$.	N.D.	No significant differences between the control and the beer after the addition of spices.	[15]
Umatola (*Parastrephia lucida*) dry leaves (0.1, 0.5, 1 and 5%), added during the maturation stage in Aumaita Porter-style beer.	No differences between the control and the beer after the addition of 1% umatola.	Significant increase in bitterness of beer after adding umatola (34.95 ± 0.50 to 41.45 ± 1.12 IBU) compared to control (34.12 ± 0.54 IBU), $p < 0.05$.	No significant differences between the control and the beer after the addition of 1% umatola.	Control beer, beer with 0.5 and 1% of umatola: alcohol content of 5.2% *v*/*v*. Beer with 0.1 and 5% of umatola: alcohol content of 5.3% *v*/*v*.	[50]

°Gl: Degrees Gay-Lussac. IBU: International Bitterness. NaOH: Sodium hydroxide. N.D.: No Data.

Although a pH higher than 4.6 may be associated with microbiological instability, in the study performed by Belcar & Gorzelany [47], both the control and beers with addition of 1% *m*/*v* and 2.5% *m*/*v* of lemongrass showed pH values above 4.6. The authors found that the addition of the extract reduced the microbiological load of the beers, which was attributed to its antimicrobial potential, due to the presence of geraniol and α- and β-citral in its composition [47].

The addition of ingredients containing organic acids (tartaric, malic, citric, and acetic acids) and phenolic acids (gallic, ferulic, caffeic, *p*-coumaric, vanillic, chicoric, and chlorogenic acids) may explain the tendency toward a lower pH compared to the control, as observed with the addition of gooseberry and pine shoots [48,49]. The physicochemical composition is influenced by geographical characteristics (such as climate and soil), as well as harvest, storage, and processing conditions.

Finally, there is a tendency for less impact on pH when dry extracts such as olive leaves, umatola, and propolis are incorporated into beer [17,40,50], compared to fresh fruits and vegetables, such as kamchatka berry and olives [42,44], which may be correlated with the higher free acid content in these matrices.

3.2.2. Bitterness

The bitterness of beers is usually expressed in International Bitterness Units (IBU), which can vary between 0–100 IBU [59]. This parameter is essentially determined by hops, given their α-acid and β-acid composition (1 IBU = 1 mg of isomerized α-acids/L) [59–61]. However, the polyphenols present in malt and hops can also contribute to the bitterness of beer [60]. Factors such as the quality and nature of the raw materials, the fermentation process, manufacturing techniques and interactions between the different constituents of beer are decisive for the total bitterness of beer [60].

The addition of lemongrass [47], black pepper and aromatic hop [15], elderberry-added Catharina Sour beer [38], pine shoots [48], bergamot [44], olive fruit extract [44], and dry umatola leaves [50] increased the bitterness of the beer compared to the control beer (Table 2). The incorporation of elderberry-added Blond Ale beer [38], kamchatka berry, and haskap fruit [42]; purple grape pomace 5% *w*/*w* and 10% *w*/*w* [43]; and dried cape gooseberry [49] reduce the bitterness of beer.

The impact of matrices or extracts on the bitterness of beers depends on their composition in bitter compounds, their concentration, and the stage of incorporation, as well as the beer style. The addition of fruity matrices containing sugars in their composition appears to attenuate the bitterness imparted by hops when compared to the control [42]. In the study conducted by Machado et al. [38], the addition of elderberry to Blond Ale beer reduced its bitterness, whereas for Catherina Sour beer, it increased the bitterness. In both cases, the incorporation occurred during the boiling stage. However, the incorporation of elderberry during the maturation stage did not result in any changes in the bitterness of Blonde Ale beer. The same is true in the studies of Cappelin et al. [40] and Ulloa et al. [17], where the incorporation of olive leaf extract and propolis did not significantly alter the bitterness in comparison to the respective control beers. By contrast, the incorporation of spices [15] and umatola [50] significantly increased bitterness ($p < 0.05$). The effect of incorporating matrices on day 7 of fermentation varied depending on the botanical ingredient, with bitterness increasing with the incorporation of lemongrass [47], decreased by kamchatka berry [42], and without significant changes with the addition of saskatoon berry [37].

In the study performed by Cappelin et al. [40], bitterness is associated with the presence of oleuropein, a glycoside characteristic of olive leaves that is characterized by bitterness and astringency.

The impact of hops on bitterness is evident in the study performed by Nunes Filho et al. [15], given that bitterness is higher in beer where aromatic hops are added alone (52.59 ± 1.05 IBU) or in combination with other spices (turmeric and hop = 47.59 ± 1.29 IBU; black pepper and hop = 61.38 ± 1.22 IBU; turmeric, black pepper, and hop = 68.90 ± 1.09 IBU) compared to control (15.0 ± 1.00 IBU).

3.2.3. Acidity

Acidity influences the sensory perception of beer and is determined by the organic acids dissolved in it, which contribute to lower pH [62,63]. Monitoring acidity is crucial, as its increase may indicate bacterial contamination, making it a critical parameter for quality control [63].

According to the results compiled in Table 2, the incorporation of matrices or extracts into beers generally resulted in an increase in beer acidity compared to the control. This effect was observed with the incorporation of lemongrass [47], dandelion [53], propolis [17], saskatoon berry [37], huaimi [39], kamchatka berry and haskap fruit [42], red rice from Paraíba [35], purple grape pomace 10% [43], and dried cape gooseberry [49].

The increase in acidity may be associated with a rise in organic acids (tartaric, malic, citric, and acetic acids) after the incorporation of the extracts, as well as their impact on the fermentation stage, since yeast metabolism culminates in the synthesis of organic acids, such as acetic acid [41,57]. In the study performed by Cappelin et al. [40], the incorporation of olive leaf extract resulted in an increase in organic acids, with acetic acid being the predominant one. The authors caution that an excessive increase in its concentration may result in an unpleasant taste. Also, the increased acidity of the beer in the study performed by Yao et al. [53] after the addition of dandelion may be due to the presence of phenolic acids (ascorbic acid, chlorogenic acid, and caffeic acid). Parameters such as raw material composition, incorporated concentration, beer style, and manufacturing process can influence the acidity of the final beer [41].

Finally, it should be noted that, in most beers, after the addition of matrices or extracts (e.g., lemongrass and propolis) that resulted in a decrease in pH, an increase in acidity was observed [17,47].

3.2.4. Alcohol Content

The alcohol content of commercially available beers varies between 4–6% (v/v), reaching up to 14% (v/v) or even containing no alcohol at all (non-alcoholic beers) [34,64,65]. It is formed during the fermentation stage, when yeasts transform the sugars in the wort into carbon dioxide, ethanol, and volatile compounds [64,66]. Thus, the alcohol content depends directly on the initial concentration of fermentable sugars, the type and viability of the yeast, oxygenation, and fermentation conditions, such as temperature, time of inoculation, and physicochemical parameters [64,67].

According to the results described in Table 2, the addition of various matrices or extracts resulted in an increase in alcohol content, namely, through the addition of basil pepper *in natura* leaves [36], mushrooms at 10 g/L [10], elderberry-added Blond Ale beer and Catherina Sour beer [38], pine shoots [48], huaimi [39], pitaya of concentration 10% v/v and 20% v/v umatola 0.1 and 5.0% [50], guava fruits and aqueous extract of leaves [45], kamchatka berry and haskap fruit [42], red rice from Bahia and Paraíba [35], purple grape pomace [43], and dried cape gooseberry [49]. The increase in alcohol content is attributed, in most studies, to the presence of fermentable sugars in the matrices or extracts incorporated into the beer and, consequently, to the increase in the degree of fermentation [39–41,46].

It should be noted that a lower degree of fermentation may suggest the presence of residual fermentable sugars in the wort, which could increase susceptibility to microbiological contamination [39]. In addition, increased alcohol content is also associated with the presence of compounds that promote the metabolism, viability, and proliferation of yeasts [41,48]. The addition of sucrose during the manufacturing process, as reported in some studies, may contribute to an increase in fermentable sugar content [36,38,43,68,69].

The addition of lemongrass [47], aqueous extract basil pepper [36], and saskatoon berry [37] decreased the alcohol percentage in beers compared to the control.

The possible impact of the extraction process and the lack of selectivity for the extraction of fermentable sugars in the dry extracts of olive extract leaves [40], jambu flower extract [46], and basil pepper aqueous extract [36] may explain the lower presence of fermentable sugars and, consequently, the maintenance/decrease in alcohol content compared to the control. In addition the physicochemical changes resulting from the addition of a matrix or extract to beer may hinder the development and proliferation of yeasts, requiring a period of adaptation, which may lead to the death of weakened yeast cells [48].

The studies performed by da Silva et al. [46] and Cappelin et al. [40] produced beer with lower alcohol content by replacing part of the malt with malt bagasse and using the non-enzymatic mashing technique, respectively.

Beers with dandelion were effective in inhibiting the xanthine oxidase enzyme compared to control, $p < 0.05$. This may represent a biological advantage for beer, as the presence of alcohol reduces the renal excretion of lactic acid and purines, which are associated with the formation of kidney stones and/or gout syndrome due to increased xanthine oxidase activity [53].

Finally, it should be noted that the variation in alcohol content depends on the matrices or extracts incorporated, their concentration and composition in reducing sugars, the phase of incorporation, yeast viability, fermentation conditions, brewing process/techniques, interactions between raw materials, and beer style.

3.3. Polyphenolic Content and Antioxidant Activity

Phenolic compounds are characterized by their antioxidant potential and are directly correlated with the functionality of beers [70]. Considering the raw materials used in beer production, it is estimated that around 80% of polyphenols derive from malt, while 20% come from hops [61]. Thus, the addition of other bioactive ingredients during the brewing process has the potential to modify the polyphenolic content and, consequently, the antioxidant and functional profile of the beer

3.3.1. Polyphenolic Content

The analysis of Table 3 shows that most of the active ingredients incorporated into beers resulted in an increase in TPC, with the exception of the addition of lemongrass at 1 and 5% [42], pitaya at 10% *v*/*v* [41], and red rice from Bahia [35], which reduced the TPC. In this sense, the impact of adding botanical ingredients depends on the matrix added and its concentration, the moment of addition, the quality of the raw materials (variability inherent to the conditions of cultivation, harvest, storage, and processing), the fermentation process condition, and the beer style [17,46].

Table 3. Impact of addition of botanical ingredients to beers in terms of total phenolic content (TPC).

Ingredients/Stage of Incorporation/Beer Style	Total Phenolic Content (TPC)	Reference
Basil pepper (*Ocimum selloi*): aqueous extract (0.05% and 0.1%) and *in natura* (0.1% and 0.5%), added before and after the fermentation stage in Pilsner-style beer.	Increase after adding basil pepper (359.0 ± 2.8 to 371.9 ± 1.9 µg GAE/mL) compared to control (291.2 ± 4.0 µg GAE/mL).	[36]
Bergamot (*Citrus bergamia*), juice; olive fruit (*Olea europaea*) extract, variety Carolea, in Blanch-style and Eiss-style beer.	Significant increase after adding bergamot (468.49 ± 11 mg GAE/mL) and olive (530.14 ± 15 mg GAE/mL) compared to control (without bergamot, 356.89 ± 10 mg GAE/mL; without olive, 435.03 ± 16 mg GAE/mL), $p < 0.05$.	[44]
Coffee bagasse (1, 5, and 10 mg/mL), added during the maturation stage in Stout-style beer.	Significant increase after adding coffee bagasse (115.01 ± 1.95 to 537.130 ± 7.24 mg GAE/ g DW) compared to control (13.26 ± 0.94 mg GAE/g DW), $p < 0.05$. The levels of catechin, epigallocatechin gallate, epicatechin and *p*-coumaric acid increased in relation to the control beer.	[9]
Dandelion (*Taraxacum* spp.) (5, 10 and 20 mg/mL), added 45 min after boiling.	Increase after adding dandelion (255.00 ± 5.07 to 299.67 ± 7.06 mg/ GAE/L) compared to control (241.46 ± 6.25 mg GAE/L), $p < 0.05$. Increased content of chlorogenic acid, caffeic acid, ferulic acid and chicoric acid.	[53]

Table 3. *Cont.*

Ingredients/Stage of Incorporation/Beer Style	Total Phenolic Content (TPC)	Reference
Guava (*Campomanesia adamantium*) fruits and aqueous leaf extract (0.1%), added after the fermentation stage in Pilsner-style beer.	Increase after adding guava fruit and aqueous leaf extract (384.9 ± 4.3 and 314.7 ± 4.1 µg GAE/mL, respectively) compared to control (256.3 ± 2.9 µg GAE/mL).	[45]
Huaimi (*Flos Sophorae Immaturus*) varieties: Cyan and Golden (5 g/L), added in the wort saccharification phase, 10 min after the start of wort boiling, 10 min before the end of wort boiling, before the start of primary fermentation	Significant increase after the addition of huaimi compared to control, $p < 0.05$.	[39]
Jambu (*Acmella oleracea*): alcoholic extract of flowers 5.0 and 7.5 mL/L), added in the bottling process in Pilsner-style beer.	Increase after adding 5.0 mL/L (226.89 ± 2.63 mg GAE/mL) and 7.5 mL/L of extract (229.11 ± 3.62 mg GAE/mL), compared to control (203.34 ± 1.52 mg GAE/L), $p < 0.05$.	[46]
Kamchatka berry (*Lonicera caerulea* var. kamtschatica) 'Duet' and 'Aurora' variety and haskap (*Lonicera caerulea* var. emphyllocalyx) 'Lori" and 'Willa' variety fruit, added after 7 days of fermentation in Wheat-style beer.	Significant increase after adding kamchatka berry and haskap fruit (180.3 ± 0.3 to 276.3 ± 0.3 mg GAE/L) compared to control (134.0 ± 0.2 mg GAE/L), $p < 0.05$.	[42]
Lemongrass (*Cymbopogon citratus*) (1, 2.5, and 5%), added on day 7 of fermentation in Wheat-style beer.	TPC after adding 1 and 5% lemongrass: significant decrease (230 ± 0.8 and 182.0 ± 0.5 mg GAE/L, respectively) compared to control (248.2 ± 0.5 mg GAE/L), $p < 0.05$. TPC after adding 2.5% lemongrass: significant increase (264.7 ± 0.06 mg GAE/L) compared to control, $p < 0.05$.	[47]
Olive (*Olea europaea*): leaf extract (0.5, 1, and 2%), added during the maturation stage in Light-style beer.	Significant increase after adding olive extract (234.53 to 437.4 mg GAE/mL) compared to control (171.09 mg GAE/mL), $p < 0.05$.	[40]
Pine (*Pinus sylvestris*) shoot extract (15 g/L) added in the boiling stage in Wheat-style beer.	TPC after main fermentation: increase after adding pine shoots after main fermentation (332.85 ± 37.35 µg/g) compared to control (87.34 ± 17.96 µg/g). TPC after one month of storage: beer with pine shoots (384.71 ± 45.68 µg/g) is higher than the control (82.12 ± 14.81 µg/g).	[48]
Pitaya (*Hylocereus polyrhizus*): pulp (5, 10 and 20% *v*/*v*), added in the beginning of the fermentation stage in Witbier-style beer.	TPC after adding pitaya 10% *v*/*v*: significant decrease (409.82 ± 5.99 mg GAE/mL) compared to control (502.17 ± 5.49 mg GAE/mL), $p < 0.05$. TPC after adding pitaya 5 and 20% *v*/*v*: non-significant change compared to control.	[41]
Propolis: ethanolic extract (0.05, 0.15, and 0.25 g/L), added during the maturation stage in Golden Ale-style beer.	Significant increase after adding propolis 0.25 g/L (306.5 ± 45.9 mg GAE/L) compared to control beer (242.0 ± 21.2 mg GAE/L), $p < 0.05$. Non-significant increase after adding propolis 0.05 and 0.15 g/L compared to control.	[17]
Purple grape pomace (1, 5 and 10% *w*/*w*), added before fermentation.	Significant increase after adding purple grape pomace (270.17 ± 4.71 to 308.10 ± 2.0 mg GAE/L) compared to control (181.56 ± 1.48 mg GAE/L), $p < 0.05$.	[43]
Red rice (*Oryza sativa*) from Bahia and Paraíba (3 Kg/35 L), added in the mashing stage.	TPC after adding red rice from Bahia: decreased (812 ± 37.35 mg GAE/100 g) compared to control (873 ± 39.30 mg GAE/100 g). TPC after adding red rice from Paraíba: significant increase (967 ± 45.46 mg GAE/100 g), compared to control (873 ± 39.30 mg GAE/100 g).	[35]
Freeze-dried cape gooseberry (*Physalis peruviana*) (20, 40, and 60 g/L), added after reaching 75% of fermentation.	Significant increase in after adding gooseberry (248.98 ± 8.70 to318.62 ± 6.58 mg GAE/L) compared to control (216.62 ± 5.18 mg GAE/L), $p < 0.05$.	[49]

Table 3. *Cont.*

Ingredients/Stage of Incorporation/Beer Style	Total Phenolic Content (TPC)	Reference
Saskatoon berry (*Amelanchier alnifolia*) of the 'Thiessen' and 'Honeywood' species with and without ozonated treatment, added on day 7 of fermentation.	TPC after adding non-ozonated and ozonated saskatoon berry: significant increase (8.57 ± 0.69 to 9.16 ± 0.40 mg GAE/L and 7.37 ± 0.05 to 8.10 ± 0.34 mg GAE/L, respectively) compared to control (3.22 ± 0.08 mg GAE/L), $p < 0.05$.	[37]
Seriguela (*Spondias purpurea*) (3 and 6%) and orange peel (*Citrus sinensis*) (0.3, and 6%), added in the beginning of the fermentation stage.	No significant differences between the control and the beers with the addition of seriguela and orange peel.	[58]
Turmeric (*Curcuma longa*), black pepper (*Piper nigrun*) and aromatic hops (*Humulus lupulus*) (1, 1.5, and 5 g/mL, respectively), added during maturation (turmeric and black pepper) or the final boiling stage (aromatic hops) in Red Ale-style beer. Spices incorporated alone and in combination	Increase after adding turmeric and hop (304.17 mg GAE/L), and turmeric, black pepper, and hop (300.00 mg GAE/L) compared to control (298.01 mg GAE/L).	[15]
Umatola (*Parastrephia lucida*) dry leaves (0.1, 0.5, 1 and 5%), added during the maturation stage in Aumauta Porter-style beer.	Significant increase after the addition of umatola (480.16 ± 1.12 to 800.64 ± 4.00 mg GAE/L) compared to control (413.21 ± 2.24 mg GAE/L), $p < 0.05$.	[50]
Spirulina (*Arthrospira platensis*) 0.25%, added after bottling in American Pale Ale.	The addition of spirulina resulted in a non-significant increase in polyphenol content compared to the control.	[16]
Citrus aurantium var. dulcis essential oil at 0.025%, added before bottling in Gruyt-style beer.	After adding *C. aurantium* was equal to 2.2 ± 0.2 mg TAE/mL and in the control, it was 2.2 ± 0.2 mg GAE/L.	[71]

DW: Dry Weight. GAE: Gallic Acid Equivalent. TPC: Total Phenolic Content.

The incorporation of jambu [46] in the bottling stage resulted in a significant increase ($p < 0.05$) in TPC, unlike the addition, in the same stage, of spirulina [16] and *Citrus aurantium* essential oil [71] at the same stage, which showed no differences compared to the control beer. At the beginning of the fermentation stage, the addition of seriguela and orange peel [58] did not prove promising for increasing TPC, while the addition of pitaya [41] significantly decreased the TPC value ($p < 0.05$). The addition of kamchatka berry [42], saskatoon berry [37], and lemongrass [47] at a concentration of 2.5% on the seventh day 7 of fermentation resulted in a significant increase in TPC ($p < 0.05$). However, the addition of lemongrass [47] at concentrations of 1 and 5% resulted in a significant decrease in TPC compared to the control beer ($p < 0.05$). In the remaining studies analyzed, the incorporation of different active ingredients at different stages of the brewing process generally resulted in an increase in TPC. These results support the idea that the impact of adding botanical ingredients depends not only on the ingredient incorporated but also on the concentration incorporated and the stage of incorporation.

Except for the study by Dziedziński et al. [48], which uses chromatographic techniques to determine TPC, the other studies determine TPC using the Folin–Ciocalteu spectrophotometric assay, in which phenolic compounds reduce the reagent, changing its color from yellow to blue (colorimetric assay) [72,73]. Although this is a simple, economical, and rapid method, it is not specific for the determination of phenolic compounds, so the presence of reducing sugars, vitamins, and amino acids in the sample may interfere with the values obtained. In addition to the wide range of composition of the samples to be analyzed, methodological variability also contributes to the variability of results, compromising their reproducibility and comparability [73]. In addition, all included studies incorporate different botanical ingredients into different control beers with different initial TPC values. For example, in the studies by Silva et al. [46], Verdan et al. [45], and Piva et al. [36], jambu, guava, and basil pepper were incorporated into Pilsner-style beer, whose, control beers had TPC values of 203.34 ± 1.52 mg GAE/L, 256.3 ± 2.9 µg GAE/mL and

291.2 ± 4.0 μg GAE/mL, respectively. Thus, the characteristics of the control beer itself may interfere with the extraction and stability of bioactive compounds present in the matrices, conditioning the increase or decrease in TPC values.

Although most studies focus on determining TPC, some studies have evaluated the phenolic profile of beers before and after the addition of bioactive ingredients. In the study performed by Yao et al. [53], the addition of dandelion resulted in an increase in chlorogenic acid (112.50–518.57 mg/L), caffeic acid (8.34–29.25 mg/L), ferulic acid (8.95–15.78 mg/L), and chicoric acid (115.16–581.56 mg/L), in a dose-dependent manner. Similarly, the addition of saskatoon berries increased neochlorogenic acid, chlorogenic acid, sinapic acid, caffeic acid, kaempferol-3-O-glucoside-pentoxide, kaempferol-3-O-rutinose, kaempferol-3-O-rhamnose-7-O-pentoxide, and ferulic acid [37].

The increase in TPC has important technological impacts. On the one hand, it makes beer resistant to the oxidative process resulting from ageing, as demonstrated in the study performed by Dziedziński et al. [48] where beer with pine shoots after one month of storage had a TPC value of 384.71 ± 45.68 μg/g, while the control beer had a TPC value of 82.12 ± 14.81 μg/g. This was also verified in the study by Nunes Filho et al. [15] where beers resulting from the combination of three spices (turmeric, black pepper, and aromatic hops) showed greater resistance to ageing over 196 days (TPC decreased from 288.21–300.00 mg GAE/L to 218.04–223.91 mg GAE/L) compared to the control (TPC decreased from 298.01 mg GAE/L to 217.68 mg GAE/L). Also, in the study by Piva et al. [36], the addition of aqueous extract of basil pepper and *in natura* leaves to beer showed greater stability in terms of TPC after 90 days of storage (TPC decreased from 259.0 ± 2.8–371.9 ± 1.9 μg GAE/mL to 299.0 ± 1.2–350.4 ± 1.9 μg GAE/mL) compared to the control (TPC decreased from 291.2 ± 4.0 μg GAE/mL to 252.6 ± 4.1 μg GAE/mL).

It should be noted that increasing TPC can lead to colloidal instability since phenolic compounds tend to complex with amino acids, increasing beer turbidity [74,75].

Finally, it should be noted that beers with high TPC tend to be of higher quality due to their sensory stability, foam persistence, and longer shelf life [17]. In addition, the potential of phenolic compounds to neutralize reactive oxygen species inherent in cellular metabolism, as well as to mitigate the oxidative damage triggered by the presence of ethanol in beer, represents functional advantages of relevance to health [44].

3.3.2. Antioxidant Activity

The antioxidant activity of an extract is characterized by its ability to neutralize reactive species either by donating hydrogen atoms or electrons or by chelating metal ions [76,77].

Table 4 shows that the antioxidant assays used are the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and oxygen radical absorbance capacity (ORAC) assays, which may be associated with the fact that they are spectrophotometric, sensitive, and rapid assays [54].

The ABTS (color change from blue/greenish to transparent) and DPPH (color change from purple to light yellow) assays allow the antioxidant potential of an extract to be measured by the transfer of electrons or hydrogen atoms, reducing the respective radical [54,78–80]. In turn, the FRAP assay (color change from light purple to dark purple) is based on the ability of an extract to donate electrons, reducing Fe^{3+}, present in the 2,4,6-tripyridyl-s-triazine (TPTZ) complex, to Fe^{2+} [54,80,81]. In contrast, the ORAC assay estimates the antioxidant potential of an extract by its ability to donate hydrogen atoms, neutralizing the peroxyl radical, and resulting in a decrease in fluorescence [79,80].

Table 4. Impact of addition of botanical ingredients to beers in terms of antioxidant activity.

Ingredients/Stage of Incorporation/Beer Style	Antioxidant Activity	Reference
Basil pepper (*Ocimum selloi*): aqueous extract (0.05% and 0.1%) and *in natura* (0.1% and 0.5%), added before and after the fermentation stage in Pilsner-style beer.	DPPH assay: increase in antioxidant potential after adding basil pepper (50.3 ± 0.3 to 83.5 ± 0.4%) compared to control (45.1 ± 0.2%). Greater antioxidant potential in beers with the addition of aqueous basil pepper extract. Greater antioxidant potential in beers where basil pepper was added after the fermentation stage.	[36]
Bergamot (*Citrus bergamia*) juice; olive fruit (*Olea europaea*) extract, variety Carolea, in Blanch-style and Eiss-style beer.	DPPH assay: significant increase in antioxidant potential after incorporation of bergamot (48.75 ± 1.36 mg TE/L) and olive (52.71 ± 1.46 mg TE/L) compared to control (without bergamot, 37.21 ± 1.03 mg TE/L; without olive, 42.44 ± 1.63 mg TE/L), $p < 0.05$. ORAC assay: significant increase in antioxidant potential after incorporation of bergamot (289 ± 2.41 mg TE/L) and olive (296 ± 3.34 mg TE/L) compared to control (without bergamot, 199.12 ± 4.05 mg TE/L; without olive, 247.90 ± 2.37 mg TE/L), $p < 0.05$.	[44]
Coffee bagasse (1, 5, and 10 mg/mL), added during the maturation stage in Stout-style beer.	DPPH assay: significant increase in antioxidant potential after adding coffee bagasse (33.31 ± 0.06 to 110.10 ± 2.44 µMol of TE/g DW) compared to control (1.54 ± 0.14 µMol TE/g DW), $p < 0.05$. FRAP assay: significant increase in antioxidant potential after adding coffee bagasse (55.36 ± 6.03 to 246.71 ± 13.89 µMol of TE/g DW) compared to control (4.36 ± 0.49 µMol TE/g DW), $p < 0.05$.	[9]
Dandelion (*Taraxacum* spp.) (5, 10 and 20 mg/mL), added 45 min after boiling.	ABTS assay: increase in antioxidant potential after adding dandelion (66.73 ± 1.78 to 88.24 ± 2.62%) compared to control (63.12 ± 0.39%). DPPH assay: increase in antioxidant potential after adding dandelion (75.39 ± 2.96 to 95.68 ± 0.21%) compared to control (70.18 ± 1.31%).	[53]
Guava (*Campomanesia adamantium*) fruits and aqueous leaf extract (0.1%), added after the fermentation stage in Pilsner-style beer.	DPPH assay: increase in antioxidant potential with the addition of the fruit and aqueous leaf extract (70.2 ± 0.7 and 54.7 ± 0.3%, respectively) compared to control (39.3 ± 0.1%).	[45]
Huaimi (*Flos Sophorae Immaturus*) varieties: Cyan and Golden (5 g/L), added in the wort saccharification phase, 10 min after the start of wort boiling, 10 min before the end of wort boiling, before the start of primary fermentation	ABTS assay: significant increase in antioxidant potential after incorporation of huaimi (2.85 ± 0.11 to 3.36 ± 0.05 mmol/L TE) compared to control (2.15 ± 0.02 mmol/L TE), $p < 0.05$. DPPH assay: significant increase in antioxidant potential after incorporation of huaimi (1.63 ± 0.04 to 1.92 ± 0.04 mmol/L TE) compared to control (1.59 ± 0.03 mmol/L TE), $p < 0.05$. ORAC assay: significant increase in antioxidant potential after incorporation of huaimi (5.43 ± 0.05 to 6.20 ± 0.13 mmol/L TE) compared to control (4.64 ± 0.02 mmol/L TE), $p < 0.05$.	[39]
Jambu (*Acmella oleracea*): alcoholic extract of flowers 5.0 and 7.5 mL/L), added in the bottling process in Pilsner-style beer.	ABTS assay: significant increase in antioxidant potential after adding 5.0 mL/L (860.30 ± 0.02 µmol TE/L) and 7.5 mL/L of extract (944.8 ± 0.02 µmol TE/L) compared to control (771.40 ± 0.01 µmol TE/mL), $p < 0.05$. DPPH assay: significant increase in antioxidant potential after adding 5.0 mL/L (290.60 ± 0.05 µmol TE/L) and 7.5 mL/L of extract (335.60 ± 0.01 µmol TE/L) compared to control (265.90 ± 0.03 µmol TE/mL) $p < 0.05$. ORAC assay: significant increase in antioxidant potential after adding 5.0 mL/L (5087.21 ± 127.00 µmol TE/L) and 7.5 mL/L of extract (5396.25 ± 141.00 µmol TE/L) compared to control (3810.84 ± 121.84 µmol TE/mL), $p < 0.05$.	[46]

Table 4. *Cont*.

Ingredients/Stage of Incorporation/Beer Style	Antioxidant Activity	Reference
Kamchatka berry (*Lonicera caerulea* var. kamtschatica) 'Duet' and 'Aurora' variety and haskap (*Lonicera caerulea* var. emphyllocalyx) 'Lori' and 'Willa' variety fruit, added after 7 days of fermentation in Wheat-style beer.	ABTS assay: significant increase in antioxidant potential after incorporation of kamchatka berry and haskap fruit (1.27 ± 0.06 to 1.97 ± 0.03 mM TE/L) compared to control (1.01 ± 0.04 mM TE/L), $p < 0.05$. DPPH assay: significant increase in antioxidant potential after incorporation of kamchatka berry and haskap fruit (1.19 ± 0.06 to 2.14 ± 0.06 mM TE/L) compared to control (1.04 ± 0.06 mM TE/L), $p < 0.05$. FRAP assay: significant increase in antioxidant potential after incorporation of kamchatka berry and haskap fruit (2.07 ± 0.03 to 2.71 ± 0.04 mM Fe^{2+}/L) compared to control (0.86 ± 0.04 mM Fe^{2+}/L), $p < 0.05$.	[42]
Lemongrass (*Cymbopogon citratus*) (1, 2.5, and 5%), added on day 7 of fermentation in Wheat-style beer.	ABTS assay: significant decrease of antioxidant potential after adding 2.5 and 5% lemongrass (0.79 ± 0.01 and 0.46 ± 0.06 mM TE/L, respectively) compared to control (0.92 ± 0.10 mM TE/L), $p < 0.05$. The addition of lemongrass 1% significantly increases the antioxidant potential (1.46 ± 0.06 mM TE/L) compared to control. DPPH assay: significant decrease in antioxidant potential after adding lemongrass 2.5 and 5% (1.70 ± 0.05 and 1.08 ± 0.08 mM TE/L, respectively) compared to control (2.38 ± 0.08 mM TE/L), $p < 0.05$. The addition of lemongrass 1% did not significantly change the antioxidant potential compared to control. FRAP assay: significant decrease of antioxidant potential after adding lemongrass (0.92 ± 0.08 to 1.62 ± 0.07 mM Fe^{2+}/L) compared to control (2.42 ± 0.08 mM Fe^{2+}/L), $p < 0.05$.	[47]
Olive (*Olea europaea*): leaf extract (0.5, 1, and 2%), added during the maturation stage in Light-style beer.	ABTS assay: significant increase in antioxidant potential only after incorporation of olive extract at 2% compared to control, $p < 0.05$. DPPH assay: significant increase in antioxidant potential after incorporation of olive extract compared to control, $p < 0.05$. FRAP assay: significant increase in antioxidant potential only after incorporation of olive extract at 2% compared to control, $p < 0.05$.	[40]
Pine (*Pinus sylvestris*) shoot extract (15 g/L) added in the boiling stage in Wheat-style beer.	DPPH assay: no significant differences with the addition of pine shoots after main fermentation and one month of storage compared to control.	[48]
Pitaya (*Hylocereus polyrhizus*): pulp (5, 10 and 20% *v*/*v*), added in the beginning of the fermentation stage in Witbier-style beer.	ABTS assay: non-significant increased antioxidant potential after incorporation of pitaya. DPPH assay: significant increase in antioxidant potential after incorporation of pitaya 20% *v*/*v* (475.26 ± 70.10 mg GAE/L compared to control (311.70 ± 45.19 mg GAE/L), $p < 0.05$. FRAP assay: non-significant increase in antioxidant potential after incorporation of pitaya compared to control.	[41]
Propolis: ethanolic extract (0.05, 0.15, and 0.25 g/L), added during the maturation stage in Golden Ale-style beer.	ABTS assay: Significant increase in antioxidant potential after adding propolis 0.25 g/L (0.808 ± 0.197 mmol TE/L) compared to control beer (0.629 ± 0.038 mmol TE/L), $p < 0.05$. DPPH assay: non-significant change antioxidant potential after adding propolis compared to control. FRAP assay: Significant increase in antioxidant potential after adding propolis 0.15 and 0.25 g/L (1705.0 ± 131.5 and 1892.5 ± 251.0 µmol TE/L, respectively) compared to control (1415.0 ± 241.8 µmol TE/L), $p < 0.05$.	[17]

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Table 4. *Cont.*

Ingredients/Stage of Incorporation/Beer Style	Antioxidant Activity	Reference
Purple grape pomace (1, 5 and 10% *w*/*w*), added before fermentation.	ABTS assay: significant increase in antioxidant potential after incorporating purple grape pomace (3031.81 ± 15.56 to 4294.52 ± 6.60 μM TE/L) compared to control (2468.33 ± 16.67 μM TE/L), $p < 0.05$. DPPH assay: significant increase in antioxidant potential after incorporating purple grape pomace (1258.59 ± 4.91 to 1878.22 ± 6.45 μM TE/L) compared to control (1044.82 ± 4.24 μM TE/L), $p < 0.05$. FRAP assay: significant increase in antioxidant potential after incorporating purple grape pomace (208.64 ± 14.84 to 844.75 ± 18.19 mg of ascorbic acid/L) compared to control (84.75 ± 4.09 mg of ascorbic acid/L), $p < 0.05$.	[43]
Red rice (*Oryza sativa*) from Bahia and Paraíba (3 Kg/35 L), added in the mashing stage.	ABTS assay: decrease in antioxidant potential after incorporating red rice from Bahia (74 ± 4.31 μmol TEAC/L) and Paraíba (73 ± 3.25 μmol TEAC/L) compared to control (77 ± 3.45 μmol TEAC/L). DPPH assay: increase in antioxidant potential after incorporating red rice from Bahia (69 ± 3.84 mg of DPPH/L) and Paraíba (63 ± 3.25 mg of DPPH/L) compared to control (75 ± 3.94 mg DPPH/L). FRAP assay: increase in antioxidant potential after incorporating red rice from Bahia (81 ± 3.88 μmol TEAC/L) compared to control (without red rice, 71 ± 3.96 μmol TEAC/L).	[35]
Freeze-dried cape gooseberry (*Physalis peruviana*) (20, 40, and 60 g/L), added after reaching 75% of fermentation.	ABTS assay: significant increase in antioxidant potential after incorporating gooseberry (1165.33 ± 94.52 to 1869.77 ± 111.82 μmol TE/L) compared to control (896.44 ± 90.76 μmol TE/L), $p < 0.05$. DPPH assay: significant increase in antioxidant potential after incorporating gooseberry (853.22 ± 53.16 to 1202.11 ± 160.05 μmol TE/L) compared to control (762.11 ± 18.36 μmol TE/L), $p < 0.05$.	[49]
Saskatoon berry (*Amelanchier alnifolia*) of the 'Thiessen' and 'Honeywood' species with and without ozonated treatment, added on day 7 of fermentation.	ABTS assay: significant increase in antioxidant potential after adding saskatoon berry (1.96 ± 0.04 to 2.22 ± 0.08 mM TE/L) compared to control (1.81 ± 0.05 mM TE/L), $p < 0.05$ DPPH assay: significant increase in antioxidant potential after adding saskatoon berry (2.34 ± 0.04 to 2.94 ± 0.01 mM TE/L) compared to control (2.27 ± 0.07 mM TE/L), $p < 0.05$. FRAP assay: significant decrease in antioxidant potential after adding saskatoon berry (1.46 ± 0.06 to 1.97 ± 0.03 mM Fe^{2+}/L) compared to control (2.19 ± 0.04 mM Fe^{2+}/L), $p < 0.05$.	[37]
Seriguela (*Spondias purpurea*) (3 and 6%) and orange peel (*Citrus sinensis*) (0.3, and 6%), added in the beginning of the fermentation stage.	DPPH assay: no significant differences in antioxidant potential after incorporation of seriguela and orange peel compared to control. Significant increases in antioxidant potential were found in beer with 6% seriguela (36.02 ± 4.99%) and in beer with 6% seriguela and 0.3% orange peel (26.77 ± 0.98%) compared to control (11.79 ± 1.08%), $p < 0.05$.	[58]
Turmeric (*Curcuma longa*), black pepper (*Piper nigrun*) and aromatic(Curcuma longa), black pepper (Piper nigrun), and aromatic hops (*Humulus lupulus*) (1, 1.5, and 5 g/mL, respectively), added during maturation (turmeric and black pepper) or the final boiling stage (aromatic hops) in Red Ale-style beer. Spices incorporated alone and in combination.	DPPH assay: decrease in antioxidant potential after adding spices (0.58 to 0.73 mmol TE/L) compared to control (0.80 mmol TE/L). FRAP assay: increase in antioxidant potential after adding spices (348.33 to 423.00 mmol TE/L) compared to control (342.64 mmol TE/L).	[15]

Table 4. *Cont.*

Ingredients/Stage of Incorporation/Beer Style	Antioxidant Activity	Reference
Umatola (*Parastrephia lucida*) dry leaves (0.1, 0.5, 1 and 5%), added during the maturation stage in Aumauta Porter-style beer.	ABTS assay: significant increase in antioxidant potential after incorporation of umatola (1.38 ± 0.03 to 3.34 ± 0.11 mmol TE/L) compared to control (1.15 ± 0.10 mmol TE/L), $p < 0.05$. FRAP assay: significant increase in antioxidant potential after incorporation 0.5, 1.0 and 5.0% umatola (2.38 ± 0.04 to 5.46 ± 0.04 mmol TE/L) compared to control (1.88 ± 0.05 mmol TE/L), $p < 0.05$. ORAC assay: significant increase in antioxidant potential after incorporation 1.0 and 5.0% umatola (13.46 ± 0.76 and 30.58 ± 1.20 mmol TE/L, respectively) compared to control (7.86 ± 0.14 mmol TE/L), $p < 0.05$.	[50]
Spirulina (*Arthrospira platensis*) 0.25%, added after bottling in American Pale Ale.	ABTS assay: significant increase in antioxidant potential after incorporating spirulina (IC_{50} = 1.9 µL/mL) compared to control (IC_{50} = 5.4 µL/mL), $p < 0.05$. DPPH assay: no significant differences after adding spirulina.	[16]
Citrus aurantium var. dulcis essential oil at 0.025%, added before bottling in Gruyt-style beer.	ABTS assay: no significant differences in antioxidant potential after addition of *C. aurantium*. DPPH assay: significant increase in antioxidant potential after incorporating *C. aurantium* (IC_{50} = 5.2 µL/mL) compared to control (IC_{50} = 23.9 µL/mL), $p < 0.05$.	[71]

ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid). DPPH: 2,2-diphenyl-1-picrylhydrazyl. DW: Dry Weight. FRAP: Ferric Reducing Antioxidant Power. IC_{50}: Inhibitory Concentration 50%. ORAC: Oxygen Radical Absorbance Capacity. TE: Trolox Equivalent. TEAC: Trolox Equivalent Antioxidant Capacity.

In general, the addition of botanical ingredients to beer resulted in an increase in antioxidant potential compared to the control beer, consistent with the upward trend in TPC.

There were, some exceptions, namely, in the ABTS assay results, where the addition of lemongrass at 2.5 and 5.0% [47], red rice [35], and spirulina [71] decreased the antioxidant potential.

Also, in the DPPH assay alone, the addition of spices (turmeric, black pepper, and aromatic hop) [15] and propolis at 0.05 and 0.15 g/L [17] resulted in a decrease in antioxidant potential.

The FRAP assay was the least used to assess the antioxidant potential of the extracts, and it was also found, in general, there is an increase in antioxidant potential, with the exception of the addition of lemongrass [47] and saskatoon berry [37] which decreased the antioxidant potential.

The ORAC assay was only performed in the studies by Muscolo et al. [44], Zhang et al. [39], Silva et al. [46], and Bustos et al. [50], where the incorporation of bergamot juice and olive fruit extract, jambu, and umatola, respectively, resulted in significant increases in antioxidant activity compared to the control beers ($p < 0.05$).

Although the ABTS and DPPH assays evaluate the same antioxidant mechanism of action, methodological differences reflect variations in the results presented. This emphasizes the need for several complementary assays to be carried out due to the possible interactions between the reagents and the samples themselves [54,82]. This can be observed in the study by Santana et al. [35], where the incorporation of red rice resulted in a significant increase in antioxidant potential in the DPPH assay, while in the ABTS assay, the incorporation of the extract resulted in a significant decrease in antioxidant potential ($p < 0.05$). Therefore, the matrix may be rich in compounds with hydrophobic characteristics [83].

The addition of compounds with antioxidant potential to beer may be advantageous in ensuring/prolonging oxidative stability during its shelf life. Similar to what was observed for

TPC, in the study by Nunes Filho et al. [15], beers resulting from the combination of three spices (turmeric, black pepper, and aromatic hops) showed greater resistance to oxidative ageing after 196 days (DPPH values decreased from 0.64–0.68 mmol TE/L to 0.54–0.57 mmol TE/L, and in the FRAP assay, it decreased from 395.01–423.00 mmol TE/L to 348.67–364.08 mmol TE/L) compared to the control (DPPH value decreased from 0.80 mmol TE/L to 0.54 mmol TE/L, and in the FRAP assay, it decreased from 342.64 mmol TE/L to 309.33 mmol TE/L). Similarly, in the study by Piva et al. [36], the incorporation of aqueous extract of umatola and *in natura* leaves increased resistance to oxidative ageing after 90 days of storage, with a decrease in DPPH values from 50.3 ± 0.5–83.5 ± 0.4% to 34.0 ± 0.2–69.9 ± 0.6%, while in the control beer, DPPH values decreased from 45.1 ± 0.2 to 30.9 ± 0.4%.

The variability of ingredients incorporated and the lack of studies corroborating the findings prevent correlation between botanical ingredients, beer style (e.g., Pilsner-style, Stout-style, Wheat-style, and American Pale Ale) and the incorporation stage (before and/or after fermentation, beginning fermentation, after 7 days of fermentation, maturation, boiling, and bottling) that enhance TPC and antioxidant activity. Thus, it was not possible to identify the ideal conditions with reproducibility that result in beers with functional properties.

In the case of olive, both the addition of leaf extract [40] and extract fruit [44] induced an increase in antioxidant potential compared to the respective control beers ($p < 0.05$), suggesting, in this case, that both parts of the plant have similar effects.

It was also found that the incorporation of some botanical ingredients, although not increasing the TPC, promoted an increase in antioxidant potential. This suggests that although phenolic compounds are characterized by their antioxidant potential, the presence of other compounds, as well as those potentially formed during the brewing process, can increase this potential. Compounds such as melanoidins, sulfites, and vitamins from botanical ingredients may contribute to increased antioxidant potential [39,47,84].

Additionally, it was observed that control beers of the same style have different initial antioxidant activity values, which highlights the methodological variability between studies and beer production, which consequently have a distinct impact on the composition and antioxidant activity of the final beer after the incorporation of active ingredients. For example, Pilsner-style beers in the studies by Piva et al. [36] and Verdan et al. [45] presented distinct antioxidant potential (DPPH assay), 45.1 ± 0.2% and 39.3 ± 0.1%, respectively. Thus, the impact of adding botanical ingredients depends on the matrix added, concentration, composition, timing, and conditions of addition.

It should be noted that although the addition of botanical ingredients may influence the volatile profile and sensory parameters of beer, these parameters were not included in the eligibility criteria for the review, as they fell outside its scope. However, the literature included in the study shows that the addition of these matrices can induce changes in the profile of aldehydes, esters, terpenes, alcohols, ketones, and acids in beer, affecting its sensory acceptability [53].

For example, the addition of dandelion at a concentration of 20 mg/mL reduced the acceptability of beer, potentially due to the presence of sesquiterpene lactones [53]. In addition, the intensification of the aroma of the beer after the addition of bergamot and olive fruit is justified by the presence of esters such as butanal, hexanal, butyl acetate, and ethyl octanoate [44].

The addition of matrices (huaimi and mushrooms) before the fermentation stage did not prove promising, with an unpleasant, aged aroma being described in the beer after the addition of huaimi and an oxidized, aged flavor and aroma after the addition of mushrooms [10,39]. Additionally, the addition of mushrooms at a concentration of 10 g/L after the fermentation stage presented a briny and acetic off flavor and a briny and acidic off taste [10]. In the study by Santana et al. [35], despite the significant increase in TPC ($p < 0.05$) in beer with red rice from Paraíba, consumers did not show any intention to purchase it, unlike beer made with red rice from Bahia.

The sensory evaluation (aroma, taste, foam stability, bitterness, saturation, and overall impression) of the addition of Kamchatka, haskap, and lemongrass to craft beers showed that lemongrass significantly increased taste, foam stability, and overall impression at concentrations of 2.5% m/v and 5% m/v ($p < 0.05$) [42,47]. The addition of Kamchatka and haskap generally contributed to an increase in aroma, taste, and overall impression ($p < 0.05$) [42]. The addition of pine shoots to beer resulted in greater acceptability compared to control beer (no significant differences), with no foreign flavors identified [48]. Finally, the addition of saskatoon improved the flavor of the beer without interfering with the overall evaluation, while the addition of seriguela and orange peel improved the aroma without improving the flavor [37,58].

The impact of TPC on sensory characteristics is unclear, as the higher TPC of red rice from Paraíba resulted in lower acceptability, while the addition of pine shoots, with an increase in TPC compared to the control beer, resulted in increased acceptability compared to the control beer [35,48].

In general, it can be seen that the impact on sensory characteristics and volatile profile depends on the type of matrix added, its concentration, and the timing of its addition. It is worth noting the heterogeneity of the methodologies inherent in sensory evaluation, which makes comparative analysis between matrices difficult.

Therefore, future studies should explore and compile the impact of botanical ingredients on the volatile and sensory profile of beer.

4. Conclusions

The incorporation of botanical ingredients in craft beer has emerged as a promising strategy to add nutritional value and expand its sensory diversity. Thus, the matrix type, the incorporated concentration, the addition timing, the beer style, and the brewing process influence the physicochemical parameters, TPC, and antioxidant activity of the final product.

It should be noted that the studies analyzed are limited to the laboratory scale, and it is essential to evaluate the impact of these matrices on the physicochemical characteristics; TPC; antioxidant activity; and chemical, microbiological, and biological stability throughout the shelf life under industrial conditions. In addition, it is crucial to evaluate the sensory characteristics of beers, as well as their acceptability by consumers.

Therefore, future research should ensure the reproducibility of existing studies, the inclusion of new and innovative matrices, as well as the study of possible variables that target the functional quality of beer. In addition, they could prioritize practical application under real production conditions, ensuring that the functional benefits are effective and long-lasting without compromising the stability and acceptance of craft beer.

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Abbreviations

The following abbreviations are used in this manuscript:

ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
DPPH	2,2-diphenyl-1-picrylhydrazyl
DW	Dry Weight
EBC	European Brewery Convention
FRAP	Ferric Reducing Antioxidant Power
GAE	Gallic Acid Equivalent
°Gl	Degrees Gay-Lussac
IBU	International Bitterness
IC_{50}	Inhibitory concentration 50%
Min	Minutes
N.D.	No Data
NaOH	Sodium hydroxide
ORAC	Oxygen Radical Absorbance Capacity
SRM	Standard Reference Method
TE	Trolox Equivalent
TEAC	Trolox Equivalent Antioxidant Capacity
TPC	Total Phenolic Content

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