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Pomegranate Juice Effect on Physicochemical and Nutraceutical Characteristics of a Craft Fruit Beer

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Abstract: While fruit is a common ingredient in beer, our research takes a unique approach by studying the effects of pomegranate juice (PJ) on the physicochemical and nutraceutical characteristics of craft fruit beer. These properties have been studied in PJ and other beverages using pomegranate; however, there is insufficient information on fruit beer. PJ, known for its health benefits, was obtained by compressing the fruit in a manual press and characterizing it. The base beer, a blonde ale with two hops, Cascade (C) and Saaz (S), was used. PJ was added to the beer during the second and third fermentation steps. Beer quality was analyzed using ASBC methods: phenolic compounds, sugars, and ethanol content by HPLC, and antioxidant capacity by ORAC. PJ presented a pH of 3.8 and 14°Brix. The beer evaluated was the third fermentation beer called 3FC and 3FS; due to the type of hops used, in general, 3FS presented better physicochemical characteristics; the relevant result was alcohol content around 6.0%, but ethanol content by HPLC was 7.36% for 3FS and 7.19% for 3FC. PJ in phenolic compounds provides the beer with 4-hydroxybenzoic acid, epicatechin, and synaptic acid. However, the hop used influenced the phenolic profile of each beer. The antioxidant capacity of 3FC was higher at 19.75 mm ET/L. In conclusion, our study demonstrated that pomegranate juice in a fruit beer style provides good physicochemical and nutraceutical characteristics, offering a unique twist to the craft beer industry.

Keywords: antioxidant activity; beer quality; fermentation; phenolic compounds; pomegranate

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1. Introduction

Beer is one of the most popular alcoholic beverages worldwide. However, in the last few decades, small beer production in local breweries has been a trend, and in the previous years, it has been popular because of the raw material used, flavor, and innovation [1–4]. This way of producing and selling beer brings about a special identification with places and social lifestyles that are very interesting for food science. Compared to conventional/commercial beer produced for the public, craft beer is produced in independent breweries using a place-specific artisanal technique, influencing marketing and sales activities in the product value chain. As a result, the brewing industry is drawing attention to the diverse flavors and unique experiences that craft beer can offer, leading to an increase in consumer preferences and actual consumption. New fermentation processes, raw material quality, and certain products' nutritional and sensory impacts have also led to significant growth in food science research [5,6]. Using unconventional raw materials such as hops, locally grown grains, fruits, and spices brings new challenges and opportunities for food scientists. These elements increase the complexity of craft beer flavor profiles, thus necessitating a deeper understanding of their chemical composition and interactions during brewing. In recent years, as consumers have become increasingly aware of the benefits of strong flavors that generate feelings of interest, enjoyment, and novelty, scientists have increasingly investigated the qualitative background of the craft beer brewing process and the influence of the ingredients on physicochemical and nutraceutical properties [7,8].

Fruit is one of the most common ingredients added to the beer process. There are different studies on berries, oranges, pineapples, apples, apricots, plums, etc., and fruit beer is becoming popular because of its sensory attributes, such as flavor and color [9]. The most common fruit beer, "Lambic," is traditionally produced in Belgium with the whole fruit of sour cherries or raspberries. During this process, there is a second fermentation or maturation of the young beer; during this step, sensorial tributes and bioactive compounds are extracted from the fruit. Some researchers have studied fruit beer as an alcoholic beverage with phenolic content and antioxidant activity [10,11].

However, pomegranate is not a commonly used fruit in brewing; this fruit has been used for wine and other alcoholic and nonalcoholic beverages and is more often consumed for fresh arils and used by the food industry [12,13]. Pomegranate (*Punica granatum* L.) has long been a subject of scientific interest because of its many beneficial effects as a food source and its pharmacological and toxicological properties. It exhibits antioxidant, antibacterial, anti-inflammatory, anticancer, and other health-promoting properties. Moreover, pomegranates have versatile applications in various food products. Recognized as a valuable source of nutrients, its production and consumption continue to increase globally. This can be attributed to the growing consumer awareness of the health benefits of pomegranate consumption, driven by advancements in science and technology [14–16].

Nevertheless, pomegranate fruits with secondary quality, small size, peel fractures, and other defects are often considered as waste. Therefore, this study aimed to investigate the potential of utilizing poor-quality fruit, which is typically unsuitable for direct consumption or high-value markets, as a raw material for juice extraction and its subsequent application in producing fruit-style beer. This study evaluated how the incorporation of fruit juice influences the physicochemical properties of beer, including pH, alcohol content, and color, as well as its phenolic profile, sugar composition, and antioxidant activity. By doing so, this study assessed the feasibility of transforming low-grade fruit into a value-added product while enhancing the nutraceutical and physicochemical qualities of fruit beer.

2. Materials and Methods

2.1. Pomegranate Juice

Pomegranate fruits were harvested from Hidalgo, Mexico, during the ripening stage. Each batch contained 25 Kg of pomegranates. The fruits were washed in a dilution of STARSAN® disinfectant (2 mL per liter of water) and dried, and then the peel was removed with a knife. A manual press obtained the juice from the whole fruit without peel, and the pH and Brix° were measured. The juice was then frozen until further use.

2.2. Craft Fruit Beer Production

2.2.1. Base Beer Production

Mexican base malt (2H), Fermentis S04® yeast, and Cascade and Saaz hops were used for beer production. The base beer was a blond ale style; 4.3 Kg of ground malt was placed in a stainless-steel pot with 22 L of purified water at 65 °C. The temperature was maintained for one hour; then, the malt was washed by adding water at 85 °C, and the original gravity of the wort was measured with a densimeter. Washing was stopped when the wort had a gravity of 1.050. The filtered wort was boiled, and at this point, 17 g of Saaz and 17 g of Cascade hops were added, each of the hops in a different container. There were two formulations, one with Saaz hops (S) and another with Cascade hops (C), and the boiling temperature was maintained for one hour. Then, an ice bath was used to lower the temperature of the wort to 35 °C. The wort was placed in a fermenter with a capacity of 10 L, and 5.5 g of yeast was added and kept at 20 °C ± 3 for ten days (first fermentation), checking that each fermenter had constant CO_2 output and avoiding external contaminants (Figure 1).

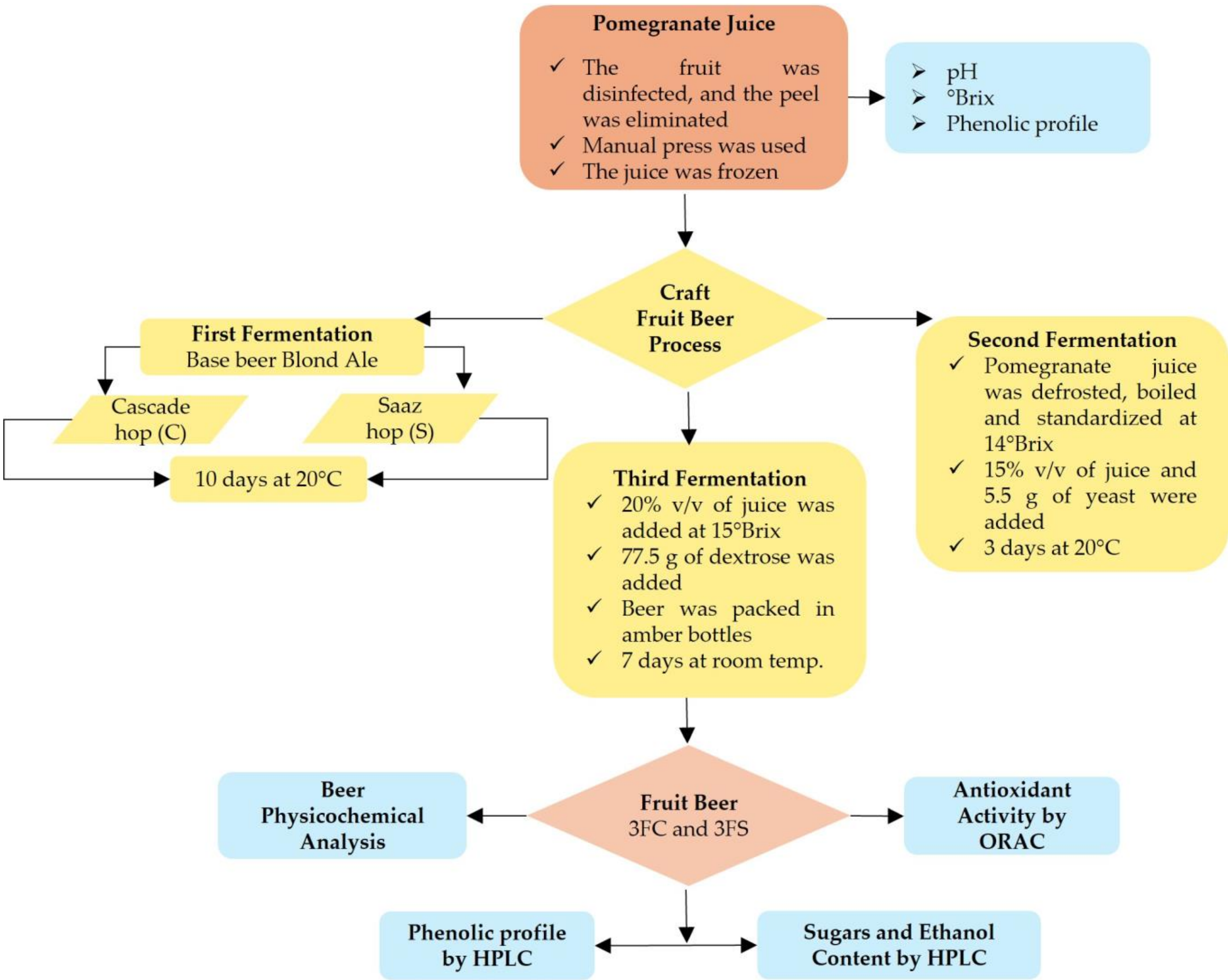

Figure 1. Flow diagram that explains the experimental methodology.

2.2.2. Pomegranate Juice Addition

The pomegranate juice was defrosted at room temperature and boiled to eliminate any microorganisms. Before adding the juice to the base beer, it was at 20 °C. Once the first fermentation time was over, pomegranate juice (15% V/V, 14°Brix) and 5.5 g of yeast were added. Subsequently, the fermenters were hermetically closed and maintained at 20 °C ± 3 for seven days (second fermentation). Subsequently, pomegranate juice concentrate (20% V/V, 15°Brix) and 77.8 g of dextrose powder were added. They were packaged in amber bottles with a capacity of 355 mL and kept at room temperature for seven days until gasification was obtained (third fermentation). They were subsequently refrigerated for ripening to avoid detecting compounds created during the fermentation of the fruit in the beer. The samples were cataloged as 3FC and 3FS, meaning third fermentation with each hop (Figure 1). Three different bottles were used for all analyses.

2.3. Beer Physicochemical Analysis

All parameters were determined using ASBC Methods (2011) [17]: soluble protein content (Beer-11), viscosity (Beer-31), pH (Beer-9), total acidity (Beer-8A), ash (Beer-14), specific gravity using pycnometer (Beer-2A), color (Beer-10A), and alcohol content (Beer-4A).

2.4. Phenolic Profile in Pomegranate Juice and Fruit Beer

Pomegranate juice, pomegranate fruit beer, and two commercial fruit beers were analyzed, and of all samples, three different bottles were used. The method used for separating phenolic compounds by HPLC was described by Ramamurthy et al. [18] with slight modifications. It was performed in reverse phase using a Zorbax (ODS)-C18 column (5 µm particle size, 15 cm × 4.6 mm i.d). The mobile phase ran at 1.5 mL/min and consisted of solvent A (acetic acid/water, 2:98 *V*/*V*) and solvent B (acetic acid/acetonitrile/water, 2:30:68 *V*/*V*). During the analysis, the solvent gradient was programmed from 10 to 100% B in A for 30 min. The diode array detector was programmed at four different wavelengths: 260, 280, 320, and 360 nm. The injection volume used was 20 µL. All solvents used were filtered through 0.45 µm membranes. Phenolic compounds were identified by comparison with the retention time and absorption spectra of commercial phenolic compound standards, and their quantification was performed using calibration curves.

2.5. Sugars and Ethanol Content in Fruit Beer

Sugar and alcohol contents were determined according to the method proposed by the column manufacturer used in the analysis. A column Bio-Rad Aminex XHP-87H 300 × 7.9 mm No. Cat 125-0140 was used. The pomegranate and commercial fruit beers were decarbonated, filtered with a 0.45 µm syringe filter, and placed in a vial for subsequent injection. The samples were run on an Agilent Technologies brand liquid chromatograph equipped with a degasser and quaternary pump. A refractive index detector was used, and a 0.6 mL/min flow rate was programmed using 0.005 N H_2SO_4 as the mobile phase. The column temperature was 50 °C, the injection volume was 20 µL, and the run time was 25 min. For quantification, calibration curves for glucose, fructose, sucrose, maltose, and ethanol were prepared and injected under the same conditions as the samples.

2.6. ORAC Antioxidant Activity

The oxygen radical absorbance capacity assay (ORAC) measures the ability of antioxidant compounds in test materials to inhibit the decline in fluorescein fluorescence induced by a peroxyl radical generator, AAPH [2,2′-azobis (2-amidinopropane). The assay was performed according to the procedure reported by Ou and Prior [19]. A quantity of 1.5 mL of fluorescein working solution (8.185×10^{-5} mM) was placed in a spectrophotometric cell. In the same cell, 0.75 mL of sample (diluted in phosphate buffer) was added and placed in a water bath at 37 °C for 5 min. Then, 0.75 mL of AAPH solution (0.415 g in 10 mL of buffer) was added, and the mixture was stirred with the same pipette. Immediately, the first reading was obtained using a fluorometer (λexcitation = 493 λemission = 515), and the cells were returned to the water bath. The cells were read every minute until the intensity value corresponded to 10% of the initial value. In this procedure, a blank was used along with the sample. The results were calculated using the Trolox standard curve by calculating the net area under the curve. The data are reported as mmol equivalents of Trolox per liter of sample (mmol TE/L).

2.7. Statistical Data Analysis

The data presented in this study are the mean values of three replicates and are expressed as the mean ± standard deviation. The results were statistically analyzed using ANOVA analysis. Tukey's test was used to determine significant differences ($p \leq 0.05$). JMP® statistical software, version 16, was used [20].

3. Results and Discussion

Fresh pomegranate juice presented a pH of 3.8 and 14°Brix. According to the results published by Mena et al. [21], which indicate that the pH can vary between 3 and 4, the juice obtained from pomegranates from the Mexican region is similar to that from fruits obtained from other parts of the world. Previously, it was reported that pomegranate juice may present Brix degrees ranging from 14 to 18 [22,23]. Therefore, the concentration of solids in the pomegranate juice used in this study coincides with the samples analyzed in previous studies.

3.1. Physicochemical Characteristics

The quality parameters of the final fruit beer are shown in Table 1. The soluble protein content of non-fruit and commercial beer is 0.30 to 1.31%, depending on its components and additives [23]. Regarding other beers made with fruits, values range from 0.29% to 0.81% [24]. The fruit beer obtained in this research presented 0.35 to 0.57% soluble protein content, higher in 3FS. The protein content in beer is low compared that found in barley grain, after all the processes by which it is transformed and processed. The amount of protein that passes into the final product is minimal, which is recommended to avoid turbidity problems caused by the formation of protein–tannin complexes. Furthermore, the protein content in pomegranate juice was insignificant and did not contribute to the protein content of beer [25].

Table 1. Results of physicochemical analysis of the beer obtained after the third fermentation with different hops.

Parameter/Sample	3FC	3FS
Soluble Protein (%)	0.35 ± 0.05 b	0.57 ± 0.02 a
Viscosity (cps)	1.16 ± 0.01 b	1.59 ± 0.01 a
pH	3.34 ± 0.02 b	3.50 ± 0.01 a
Total Acidity (%)	4.90 ± 0.03 b	5.08 ± 0.03 a
Ash (%)	0.26 ± 0.01 a	0.14 ± 0.00 b
Specific Gravity	1.0087 ± 0.00 a	1.0074 ± 0.00 b
Color (°SRM)	5.89 ± 0.02 b	7.48 ± 0.06 a
Alcohol (%)	6.06 ± 0.11 b	6.69 ± 0.09 a

Equal letters within the same row indicate no statistically significant differences ($p \leq 0.05$). Mean ± standard deviation; 3FC = third fermentation Cascade hops; 3FS = third fermentation Saaz hops.

The viscosity of beer can be influenced by various factors, including alcohol content and ingredients added during maceration or fermentation. Although there is no established parameter, it is generally desirable for the viscosity to be less than 1.6 cps [26]. When pomegranate juice was added to the beer, its viscosity ranged between 1.16 and 1.59 cps (Table 1), with higher values observed in 3FS. Interestingly, there was a statistically significant difference between the two samples, despite the addition of the same amount of pomegranate juice. This difference could suggest variations in the alpha acids and alcohol contents, affecting the specific gravity of the beer.

The pH of the fruit beer was 3.5 (Table 1). For a light beer, the desired pH is between 4.3 and 4.6 [26]. Adding pomegranate juice at a pH of 3.8 decreased the final pH of the beer. Fruity beers typically have a pH between 3.5 and 4.2, while ale beers have a pH range of 4.3 to 4.8. These beers have a good flavor and are well preserved [27]. Therefore, the beers in this study may taste acidic but have a lower pH, which helps prevent the growth of harmful microorganisms.

Significant statistical differences were observed in total acidity (Table 1), with the 3FS sample showing a higher acidity of 5.08% than the 3FC sample at 4.90%. This difference can be attributed to the use of different hops types. The beer produced exhibited higher total acidity than the base beers or those without added fruit. Typically, total acidity averages between 0.30% and 0.43% in light commercial beers and between 0.23% and 0.40% in dark beers [26].

Table 1 shows that the residual ash content for 3FC was 0.26% and for 3FS was 0.14%. This inorganic material primarily comes from malt, hops, and, in this specific case, pomegranate juice. When comparing these values with the ash content of light beers (1.61%) and dark beers (1.84%) [28], the beers in this study presented less mineral content. The specific gravity (SG) is a parameter that various factors can influence, such as the addition of pomegranate juice. For this reason, this cannot be compared individually with commercial or other craft beers because these parameters are not specified in the regulations for the distribution and consumption of the beer type. Sample 3FC had an SG value of 1.0087, higher than 3FS, indicating that this beer has more soluble solids as part of the malt, pomegranate juice, and hops. In different commercial fruit beers, SG was 0.9971 to 1.0362; most samples had an SG like that of pomegranate fruit beer [29].

Color is an attribute that consumers receive as a sensory stimulus before consuming a product. The 3FS beer had a value of 5.89°SRM, placing it in the light beer category on the beer scale. As a pale ale style, the 3FS beer had a value of 7.48°SRM. Baigts-Allende et al. [29] studied 26 commercial fruit beers; the color varied between 4.16 to 34.81°SRM depending on the fruit and concentration in the process. The addition of pomegranate juice influences the beer's final color, which becomes amber and coppery due to the oxidation of pomegranate anthocyanins during beer aging.

The alcohol content in the beer brewed with pomegranate juice exceeded 6% for both recipes (Table 1), with 3FS having a higher alcohol concentration of 6.69%. The alcohol content influences the SG; more alcohol decreases the SG, and alcohol density is lower than that of water. Therefore, as previously stated, 3FS has a lower SG because it contains more alcohol than 3FC (6.06%). Most commercial beer sold worldwide, whether light or dark, has an alcohol concentration of 4 to 5.5%. Furthermore, the ideal alcohol concentration is estimated to range between 4.3 and 5.8% [26]. In fruit beers from different countries, Baigts-Allende et al. [29] found a range of 2.5 to 8.6% alcohol content; the pomegranate fruit beer is in this range.

3.2. Phenolic Profile in Pomegranate Juice and Beer

Pomegranate juice contained the following phenolic compounds at high concentrations: 4-hydroxybenzoic acid, catechin, chlorogenic acid, syringic acid, epicatechin, and sinapic acid (Table 2). Previous studies have reported that pomegranate juice contains polyphenols such as catechin, epicatechin, quercetin, epigallocatechin 3-gallate, rutin, kaempferol, luteolin, and naringenin. Not all pomegranate varieties contain the same phenolic compounds; their presence depends on several factors, and some of these compounds respond when the plant is subjected to stress. Furthermore, the metabolism of microorganisms can produce or use other phenolic compounds, which can modify the profile of phenolic compounds during fermentation [25].

Table 2. Phenolic compounds by HPLC in pomegranate juice and fruit beer of the third fermentation (results expressed in mg/100 mL).

Phenolic Compound	PJ	3FC	3FS	Delirium	Liefmans
Gallic acid	0.09 ± 0.01 c	0.27 ± 0.01 bc	0.80 ± 0.09 b	3.79 ± 0.19 a	0.77 ± 0.02 b
4-hydroxybenzoic acid	2.37 ± 0.01 a	0.33 ± 0.03 b	0.28 ± 0.01 b	0.19 ± 0.05 c	0.11 ± 0.01 d
Catechin	1.08 ± 0.05 d	2.33 ± 0.01 b	2.58 ± 0.03 a	1.99 ± 0.09 c	0.97 ± 0.15 d
Chlorogenic acid	0.43 ± 0.02 c	0.12 ± 0.00 e	0.17 ± 0.02 d	1.17 ± 0.01 b	1.71 ± 0.02 a
Caffeic acid	0.06 ± 0.00a b	0.05 ± 0.01 ab	0.03 ± 0.02 c	0.05 ± 0.00 bc	0.07 ± 0.01 a
Syringic acid	0.14 ± 0.00 a	0.12 ± 0.01 b	nd	nd	0.08 ± 0.01 c
Epicatechin	0.42 ± 0.06 b	0.57 ± 0.09 a	0.33 ± 0.06 b	nd	nd
p-Coumaric acid	0.03 ± 0.00 a	0.02 ± 0.02 b	0.03 ± 0.00 a	0.03 ± 0.00 a	nd
Ferulic acid	0.03 ± 0.01 c	0.13 ± 0.01 a	0.05 ± 0.01 b	0.01 ± 0.01 d	nd
Sinapic acid	0.41 ± 0.01 b	0.40 ± 0.00 bc	0.46 ± 0.01 a	0.37 ± 0.01 c	nd

Equal letters within the same row indicate no statistically significant differences ($p \leq 0.05$). Mean ± standard deviation; PJ = pomegranate juice; 3FC = third fermentation Cascade hops; 3FS = third fermentation Saaz hops; nd = not detectable.

Currently, the identification of approximately 50 phenolic compounds in beer has been reported, with malt as the main source, accounting for 70–80%, and the remaining 20 to 30% originating from hops [30]. The beer with the addition of pomegranate juice, as well as the commercial red fruit beers, contained gallic acid; the commercial fruit beer Delirium presented the highest concentration at 3.79 mg/100 mL, and 3FS the lowest with 0.80 mg/100 mL (Table 2). Commercial beers are made with more than one red fruit, which increases the content of some phenolic compounds. However, the previous study did not detect gallic acid in Liefmans beer; this study determined it at 0.77 mg/100 mL [29]. The gallic acid in the beer produced in this study cannot be attributed to the addition of pomegranate juice; the presence of this compound in the juice was low at 0.09 mg/100 mL. Gallic acid was previously reported in pomegranate juice in a range from 0.07 to 1.74 mg/100 mL [31].

In the barley malting process, the gallic acid content increases from steeping to drying [32]. The raw materials for beer brewing provided gallic acid for both the malt and hops. Mikyška and Jurková [32] reported that Saaz hops contain a large amount of gallic acid, which is consistent with the results of this study. The 3FS beer brewed with Saaz hops contained a higher concentration of gallic acid than 3FC with Cascade hops. The concentration in beer ranged from 0.01 to 1.04 mg/100 mL [32], and adding fruit increased the gallic acid content, reaching values higher than previously reported.

As mentioned previously, pomegranate juice is rich in 4-hydroxybenzoic acid (4-HBA) and coincides with what was reported by Mena et al. [21]; this acid is present in both sweet and sour pomegranate juice in concentrations ranging from 0.56 to 1.61 mg/100 mL. The addition of pomegranate juice to the beer on two occasions resulted in an increase in 4-HBA in the final product, resulting in higher concentrations than in commercial beers. Furthermore, there were no significant differences between the beers brewed: 3FC (0.33 mg/100 mL) and 3FS (0.28 mg/100 mL) (Table 2). It is inferred that the hops used do not determine the concentration of 4-hydroxybenzoic acid in beer. 4-HBA has been reported in concentrations of 0.04 to 0.90 mg/100 mL in beer [33]; brewed and commercial beers are within these values.

In previous studies, pomegranate juice obtained a catechin content of 5.63 to 41.23 µg/mL in various cultivars from China [31], and cultivars from other regions showed catechin values from 4.19 to 40.3 mg/100 mL [25,34]. In this study, pomegranate juice had a catechin concentration of 1.08 mg/100 mL (Table 2), similar to those reported by Hmid et al. [35]. In contrast, in different cultivars from Morocco, the catechin content ranged from 0.18 to 0.68 mg/100 mL in juice. In the 3FC and 3FS study beers, catechin was one of the phenolic compounds with the highest concentrations, with values of 2.33 and 2.58 mg/100 mL, respectively, with significant differences between the samples. Cascade hops contain 0.09 mg/100 g of catechin, a lower concentration than Saaz hops, with 3.45 mg/100 g [36,37]. Saaz hops provide a more significant amount of catechin to the beer, resulting in 3FS beer having a more substantial presence of catechin.

The source of catechin in beer is mainly barley malt. Barley values of 0.1–10.5 mg/100 g, higher than in other cereals, have been reported [38,39]. During the malting process, this content increases during the drying process after germination [32]. In most cases, catechin values of 1.86 to 3.84 mg/100 mL have been reported [40]. In commercial beers, Delirium showed a similar trend to beers with pomegranate; however, in Liefmans, the catechin concentration was low at 0.97 mg/100 g. Nardini and Garaguso [27] reported that in different fruit beers made with other fruits, those that contained cherry had the highest catechin content, of 2.40 mg/100 mL. In contrast, the commercial beers under study contained cherries in their formulation.

The pomegranate juice provides a small amount of chlorogenic acid (0.43 mg/100 mL), such as previously reported in the juice, which ranged from non-detectable to 0.47 mg/100 mL. This was observed in more than 18 cultivars in Morocco and Turkey [36.42]. Pomegranate's low acid content results in beers with lower values than commercial beers, with 0.12 mg/100 mL in 3FC and 0.17 mg/100 mL in 3FS, compared to 1.17 and 1.71 mg/100 mL in commercial beers, respectively. Liefmans showed the highest concentration (Table 2). However, the chlorogenic acid level is low in beer treated with pomegranate juice; 3FS exhibited a slightly higher content than 3FC, which was statistically significant and could be linked to the chlorogenic acid content in Saaz hops.

Both commercial beers analyzed in this research have cherries in their formulation; this fruit is rich in chlorogenic acid, at 19.3 mg/100 g [41,42], which, unlike pomegranate, provides a more significant amount of this phenolic compound that passes into the beer. For this reason, commercial beers presented a higher content of this phenolic compound. In fruit beers, those that showed higher concentrations of chlorogenic acid of 1.02 and

1.27 mg/100 mL contained cherries and apricots, respectively [27]. Values of chlorogenic acid in beer have been reported to be between 0.01 and 1.04 mg/100 mL [31], comparable to the blonde ale that formed the base beer in this study.

Liefmans beer contains a blend of red fruits such as strawberry, raspberry, cherry, elderberry, and bilberry, which gives it a high caffeic acid content of 0.07 mg/100 mL and high levels of other phenolic compounds; the level in this beer has been reported to be 0.63 mg/100 mL by mass chromatography [29]. Pomegranate juice has a value of 0.06 mg/100 mL, as Hmid et al. [35] reported, with concentrations ranging from 0.02 to 0.16 mg/100 mL for different pomegranate varieties. Pomegranate juice did not add any value in terms of caffeic acid content. The difference between the content of 3FC (0.05 mg/100 mL) and 3FS (0.03 mg/100 mL) can be attributed to the hops used. Cascade hops contain 1.38 mg/100 g of this compound [37]. Values have been reported from 0.01 to 15.8 mg/100 g for various hops and 0 to 0.25 mg/100 mL for beer [33].

Ferulic acid in pomegranate juice from different cultivars has been reported in concentrations from 0.07 to 0.47 mg/100 mL [35]. However, Poyrazoğlu et al. [43] reported that most varieties lacked this acid. In the pomegranate juice used in this study, 0.03 mg/100 mL of ferulic acid is reported (Table 2). In Liefmans beer, it was not detectable, and in Delirium, the concentration was low, at 0.01 mg/100 mL, compared to beers with added pomegranate juice. The 3FC beer contained 0.13 mg/100 mL of ferulic acid, but the 3FS beer contained 0.05 mg/100 mL, indicating that pomegranate juice is not the source of this phenolic compound; the hops contribute. Da Rosa Almeida et al. [37] found ferulic acid in Cascade hops at a concentration of 4.48 mg/100 g, making it one of the phenolic compounds with the highest presence after isoquercitin (4.90 mg/100 g). According to Mikyška and Jurková [36], the ferulic acid level in Saaz hops is 0.13 mg/100 g, resulting in a lower content in 3FS beer compared to 3FC. Ferulic acid levels in beer range from 0.01 to 0.11 mg/100 mL [33], with 3FC beer having a greater concentration.

In beer, syringic acid was detectable in 3FC, at 0.12 mg/100 mL, but not in 3FS. Pomegranate juice does not provide this acid to beer, as it is found in a low concentration in the juice and is not present in beers with pomegranate. The presence of syringic acid in beer is due to Cascade hops containing 0.31 mg/100 g, compared to Saaz hops with 0.04 mg/100 g [36,37]. Liefmans commercial beer exhibited a value of 0.08 mg/100 mL, whereas it was undetectable in Delirium. The first commercial beer has more red fruits than Delirium and does not indicate the hop type used. In beer, 0 to 0.13 mg/100 mL has been found [33], consistent with what has been measured in beer containing pomegranate juice and commercial beers.

One of the phenolic compounds found in pomegranates that has been studied is epicatechin; in the juice, several cultivars from China and Morocco have reported concentrations ranging from 0.10 to 0.48 mg/100 mL [31,35]. The resulting pomegranate juice has an epicatechin concentration of 0.42 mg/100 mL (Table 2), within the highest previously recorded range.

Epicatechin was found in the beer containing pomegranate juice, with 3FC having the most significant amount at 0.57 mg/100 mL and 3FS at 0.33 mg/100 mL; commercial beers did not contain it (Table 2). In addition to hops, the pomegranate gives the beer epicatechin; Cascade exhibited the most significant level of 3.00 mg/100 g, while Saaz showed 0.45 mg/100 g [36,37]. According to Nardini [33], epicatechin levels in beer ranged from 0.002 to 0.455 mg/100 mL; the 3FC sample had the highest epicatechin content found in these investigations. Among other things, epicatechin has been demonstrated to have anti-inflammatory and neuroprotective properties, avoid metabolic irregularities, and have cardioprotective benefits [44].

Table 2 shows that the sinapic acid content of pomegranate juice was 0.41 mg/100 g. Previously, sinapic acid was not found in the juice of sweet pomegranates; nevertheless, 1.80 to 3.87 mg/100 mL was found in the pomegranate acids [34]. This phenolic component is provided by pomegranate juice, which was added to the beer; 3FC obtained 0.40 mg/100 mL and 3FS 0.46 mg/100 mL (Table 2). Notable variations are also seen between the samples, with the 3FS content being higher due to sinapic acid 0.04 mg/100 g in Saaz hops, as opposed to none in Cascade hops [36,37]. Commercial beers showed sinapic acid levels of 0.37 mg/100 mL in Delirium, while Liefmans showed no detectable acid levels.

Various studies on beer have shown that phenolic compounds, mainly from barley and hops, provide health benefits when ingesting low amounts of beer. These benefits have been linked to a cardioprotective effect, a decrease in triglycerides and bad cholesterol (LDL), a neuroprotective effect with prevention of dementia, glucose tolerance, the counteracting of carcinogenesis, reduced risk of osteoporosis, and an antioxidant effect and reduced inflation [45–50]. These health benefits are primarily observable in craft beers [51,52]. Therefore, increasing the phenolic compound content by adding pomegranate juice would enhance these benefits, as previously reported in beers without red fruits. Pomegranate juice improves the nutraceutical properties of the drink, and moderate consumption could result in better health benefits than from industrial beer.

3.3. Sugars and Ethanol Content by HPLC

The yeast used, style, and production conditions of beer influence the final sugar content. Monosaccharides are considered entirely fermented, and the final content of maltose and other oligosaccharides varies according to the type of yeast used in brewing [53]. Table 3 shows that most sugars are present in beer; in this beer style, sugars come from malt and fruit. Maltose is the principal sugar in malt used by yeast during the fermentation process. Pomegranate juice has been detected to be from 0.21 to 0.27 mg/100 mL [54]. Both raw materials are sources of maltose in the pomegranate fruit beer. The fruit beers with the highest maltose content were 3FC and Delirium, and Liefmans presented the lowest content. The 3FC and 3FS beers presented significant differences in maltose; both beers had the same amount of malt and juice, which is possible in 3FS. The yeast metabolized more maltose in this batch than in 3FC. In the different beer styles, maltose was not detectable at 2.61 g/L [53,55].

Table 3. Principal sugars (g/100 mL), ethanol (%V/V) content, and antioxidant activity (mM = mmol ET/L) in fruit beer.

Sample	Maltose	Sucrose	Glucose	Fructose	Ethanol	ORAC
3FC	0.43 ± 0.00 a	0.12 ± 0.00 b	0.80 ± 0.03 b	1.43 ± 0.03 a	7.19 ± 0.29 a	19.75 ± 0.09 a
3FS	0.37 ± 0.00 b	0.12 ± 0.00 b	0.85 ± 0.01 a	1.43 ± 0.01 a	7.36 ± 0.35 a	15.05 ± 0.68 b
Delirium	0.43 ± 0.00 a	0.05 ± 0.00 c	0.84 ± 0.01 ab	1.18 ± 0.00 b	4.95 ± 0.06 b	19.62 ± 0.02 a
Liefmans	0.17 ± 0.00 c	0.15 ± 0.00 a	0.53 ± 0.00 c	1.06 ± 0.00 c	3.21 ± 0.02 c	4.89 ± 0.21 c

Equal letters within the same column indicate no statistically significant differences ($p \leq 0.05$). Mean ± standard deviation; 3FC = third fermentation Cascade hops; 3FS = third fermentation Saaz hops.

Sucrose is not detectable in beer; this sugar is mainly used for refermentation in bottles to produce CO_2 and is used faster by the yeast before five days [56]. The pomegranate and commercial fruit beer contained sucrose, and the fruit used in each beer was a source of this oligosaccharide. Pomegranate juice has sucrose content of 4.8 to 6.6 g/100 mL [57,58]; this remains after fermentation in the beer produced. In the samples, 3FC and 3FS did not show significant differences (Table 3); the lowest content was presented in Delirium and the highest in Liefmans; in the recipe of this last beer, five fruits could bring sucrose into the beer.

Glucose is a monosaccharide that is mainly metabolized by yeast and is not detectable in some beers. Fructose was lower, at 0.07 g/mL [55]. The fruit beer glucose range is 0.53 to 0.85 g/100 mL (Table 3); Liefmans presented the lowest content. The yeast used in this beer is more efficient at metabolizing glucose. Fructose is the sugar most present in beer; for the beer style, it is possible that fructose is present in fruits and is not the principal yeast substrate. The fructose content of pomegranate fruit beer was higher than that of commercial beers. In beer without fruit, fructose content is 0.07 g/100 mL [55], lower than in fruit beers. Glucose and fructose are the major sugars in pomegranate juice, at 11.20 and 6.42 g/100 mL, respectively [53,58].

Regarding the ethanol content, pomegranate fruit beers had a more significant presence, unlike commercial beers of a similar style. Fruit beers from this study contained approximately 7.0% ethanol (Table 3). No significant differences were observed among the samples. The different hops used in each recipe did not affect sugar or alcohol content. For commercial beers, Delirium has 4.95% and Liefmans 3.21%. Depending on the style, beer's ethanol content can range from 3.47 to 7.66% [59].

3.4. Antioxidant Capacity (AC)

Table 3 shows the data on the antioxidant capacity of the pomegranate and commercial fruit beers; for a better illustration of this information, it is shown in Figure 2. Red fruits contain antioxidant compounds such as polyphenolic compounds, anthocyanins, flavonoids, tannins, and vitamins. Pomegranate juice has been shown as the AC by ORAC. Some authors have found that depending on the cultivar and cycle, the antioxidant capacity could range from 11 to 24 mmol ET/L in pomegranate juice [22,60]. Using the ORAC method, pomegranate juice showed more AC than blueberries, grapefruit, and grapes [61]. Therefore, 3FS presented a higher AC, of 19.75 mmol ET/L, which is similar to Delirium, of 19.62 mmol ET/L. Pomegranate fruit beers showed significant differences; using different hops in the recipe affected AC. Rothe et al. [62] described how hop varieties and hopping regimes affect the AC. Liefmans beer, besides using more fruits in the brewing process, presented the lowest AC, of 4.89 mmol ET/L. In beers, the antioxidant capacity ranges from 4.87 to 10.15 mmol ET/L [62,63], and fruit beers presented a higher AC than other beers without fruit, except for Liefmans.

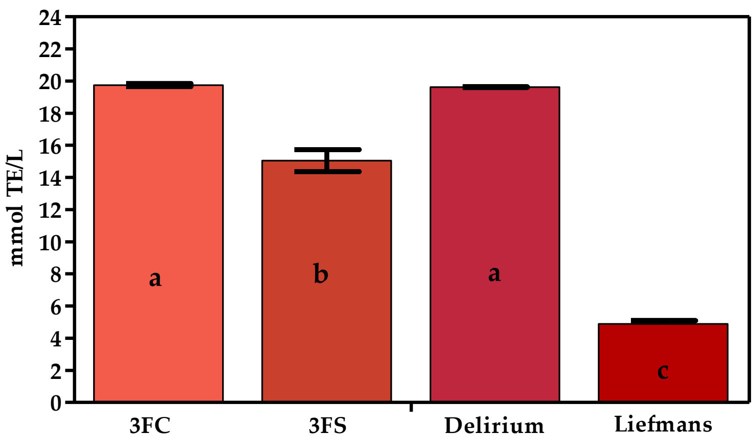

Figure 2. Antioxidant capacity by ORAC assay of pomegranate and commercial fruit beers. Equal letters within the same row indicate no statistically significant differences ($p \leq 0.05$).

4. Conclusions

The pomegranate juice added in the second and third fermentation in the brewing process of a fruit beer affected the physicochemical properties, mainly in terms of pH, total acidity, color, and alcohol content, classifying the product as a specialty fruit beer style. Although Liefmans beer had the lowest amount of identifiable phenolic components, no coumaric acid, ferulic acid, synaptic acid, or epicatechin was found; in Delirium, however, syringic acid and epicatechin were not found. The absence of epicatechin was consistent with that observed in both commercial beers. The fruit beers had different contents primarily because of the hops used: 3FC with Cascade hops is rich in 4-HBA, caffeic, syringic, and epicatechin acids, whereas 3FS with Saaz hops contains more gallic, chlorogenic, coumaric, sinapic, and catechin acids. Overall, beers with pomegranate juice had a better profile of phenolic compounds than commercial beers. This is attributed to the nutraceutical properties of pomegranate juice, malt, and hops, and the processing used in production.

The same trend was observed for ethanol content and AC; pomegranate fruit beer showed better behavior than commercial beer. Sugars remain in fruity beers, and the fruit used and the way yeast metabolizes provides a higher concentration. The different contents of phenolic compounds in beer affected the AC; in this case, using one fruit (pomegranate) is enough to have some or better AC than commercial beer that uses more than one fruit. However, commercial fruit beers do not specify the amount of fruit added or the brewing process, which has an impact on physicochemical and nutraceutical characteristics.

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