

## Original Research Article

### Physicochemical characterization of craft beers produced with passion fruit (*Passiflora edulis* Sims)

#### Abstract

This study aimed to produce and evaluate the physicochemical characteristics of craft beers with the addition of yellow passion fruit pulp and evaluate the acceptance level of these products through sensory analysis. The addition of 2% (w/v) of passion fruit pulp to beers was performed in two stages: A) in the wort, before the primary alcoholic fermentation and B) in the green beer, after primary alcoholic fermentation and before the maturation stage. The pH, titratable acidity, total soluble solids, reducing sugars and total phenolic compounds were determined in wort and beers, and antioxidant capacity and alcohol content were determined in beers. The passion fruit beers were characterized by pH of 3.65-3.66, acidity of 0.33%, phenolic compound values of 67-68 mg/100 mL, antioxidant activities between 2.45-3.04 mM TEAC by ABTS and 0.62-0.70 mM TEAC by DPPH and alcoholic contents of 4.50-4.70°GL. The two formulations of passion fruit beers had good acceptance, however beer B where the addition of passion fruit was carried out in green beer (before the maturation stage) showed higher antioxidant activity and obtained higher scores in flavor and aroma attributes in sensory analysis compared to beer A where the addition of passion fruit was carried out in wort (before primary alcoholic fermentation). The results of the present study suggested that quality characteristics of beer can be enhanced by addition of passion fruit.

*Keywords: fruit beer; polyphenols; antioxidant activity; physicochemical analysis; sensory analysis*

## 1. INTRODUCTION

In recent years, there has been a big increase in the Brazilian market for craft beer production and consumption [1]. And, as a result of the growing market of craft beers, more breweries are exploring the manufacture of varied styles of beer with new flavors, aromas and modified manufacturing processes [2]. The product diversity in the craft beer market exploit the potential of unconventional starch-rich ingredients and the addition of local fruit, herbs, spices, and vegetables [3].

The fruit addition in beer production not only adds new flavors, but also promotes residual sweetness, increasing the aroma to beer. The nutritional and functional values can be further enhanced by incorporating fruits [4]. During fermentation and maturation of fruits beers, flavors, and bioactive compounds, particularly carotenoids and polyphenols, are extracted from fruits. Therefore, fruits addition during the beer production process might contribute to enhance the content of bioactive compounds of beer [5].

The passion fruit (*Passiflora edulis* Sims), also known as yellow passion fruit or sour passion fruit, is native to Brazil. Currently, Brazil is the largest producer and consumer of this fruit, which is mainly used for in natura consumption and industrial juice processing [6]. The passion fruit has potential functional components, such as fibers, vitamins, phenolic compounds, and carotenoids. Due to its pleasant aroma and characteristic flavor, passion fruit is highly appreciated by Brazilian consumers and considered a tropical fruit with an exotic flavor in the world [7].

Considering that craft beer is focused on a consumer market that seeks products with different aromas and flavors and prioritizes a product with a higher sensory quality [1-3], this study aimed to produce and evaluate the physicochemical characteristics of craft beers with the addition of yellow passion fruit pulp and evaluate the acceptance level of these products through sensory analysis.

## 2. MATERIALS AND METHODS

### 2.1 Brewing process

A total of 4.0 kg of ground Pilsner malt (Agraria<sup>®</sup>, Brazil) was mixed with 12 L of mineral water at 50°C. Mash was conducted at 50-55°C for 30 min, 60-65°C for 30 min and 70-75°C for 30 min. After mashing, wort was transferred to kettle and spent grain was washed using 8 L of mineral water at 78°C. Subsequently the liquid part proceeded to the boiling step and the solid part was discarded. Wort was boiled for 60 min and then 30 g of Saaz hop (Bart Hass<sup>®</sup>, Czech Republic) were added. During the wort boiling process, the hop pellets were added at three stages: 15 g after 30 min of boiling and 15 g after 50 min of boiling. The boiled wort was separated from the hot trub and then cooled at 18°C. The passion fruit pulp (2%, w/v) were added in wort, before the primary alcoholic fermentation, which led to the formulation of beer A. Prior to fermentation, the yeast (0.7 g/L) (Lager W 34/70 Fermentis<sup>®</sup>), was rehydrated and added into the wort. Fermentation was carried out at 15°C for 10 days to obtain green beer. The passion fruit pulp (2%, w/v) was added in green beer before the maturation, which led to the formulation of beer B. Then the temperature was reduced to 4°C for 15 days to obtain matured beers. The beers were filled in 600 mL amber glass bottles and for the carbonation process, glucose syrup (1 g/L) and a yeast suspension (0.2 g/L) were added. Bottled beers were sealed and stored at room temperature for 15 days to form carbon dioxide.

### 2.2 Addition of yellow passion fruit to beers

Passion fruits were obtained in a market of local farmers of Distrito Federal (Brazil) in July 2019. In the laboratory, the fruits were sanitized in a solution of sodium hypochlorite (200 ppm) for 15 minutes and washed in running water before use. The fruits were opened

approximately into two equal halves and the pulp were scooped using stainless-steel spoons. The passion fruit pulp was passed through a stainless-steel sieve and thus the seeds were discarded. The addition of 2% (w/v) of passion fruit pulp to beers was performed in two stages: A) in the wort, before the primary alcoholic fermentation and B) in the green beer, after primary alcoholic fermentation and before the maturation stage.

### **2.3 Physicochemical analyses**

The soluble solids were determined through refractometer Shimadzu and expressed in °Brix. The pH was measured using a digital potentiometer Micronal B222 and total acidity was determined by titration with 0.1 N NaOH [8]. Reducing sugars were determined using the 3,5-dinitrosalicylic acid method [9]. Total polyphenols were determined by the Folin-Denis method [10], using gallic acid as reference compound. The evaluation of **the in vitro** antioxidant activity was performed through the evaluation of free radical capture DPPH [11] and ABTS [12]. The results were expressed as Trolox equivalents (mM TEAC) and calculated from a standard curve. The alcoholic content of the beers was determined using a Gay-Lussac alcoholometer placed directly in a volume of 250 mL of distillate at 20°C [13].

### **2.4 Sensory analysis**

The sensory analysis of the beers was performed by a well-trained panel of 12 evaluators of both sexes aged between 21 and 60 years. Each evaluator received 70 mL of each beer sample at approximately 8°C, coded with three random digits. Mineral water and salt and cracker were also served to clean the palate. Flavor, aroma, foam persistency and carbonation, and global impression of beers were evaluated following nine-point hedonic

scale (9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely) [13].

## 2.5 Statistical analysis

Data were expressed as mean values  $\pm$  standard deviation of three independent replicates. Physicochemical and sensory analyzes results were submitted to analysis of variance (ANOVA) and the means were compared by Tukey test (Statistica 10<sup>®</sup>, Statsoft Inc). Results were considered significantly different when p values were  $< 0.05$ .

## 3. RESULTS AND DISCUSSION

### 3.1 Physicochemical characterization of passion fruit and wort

The results of the physicochemical analyzes of the yellow passion fruit pulp and the wort are shown in Table 1. The result of the soluble solids obtained for the yellow passion fruit pulp was 12.0°Brix. Greco et al. [14] reported similar results for the composition of some yellow passion fruit cultivars with soluble solids ranging from 10.9 to 13.8°Brix.

**Table 1. Physicochemical analyses of passion fruit pulp and wort**

Analyses	Passion fruit pulp	Wort
Soluble solids (°Brix)	12.00 $\pm$ 0.01	13.00 $\pm$ 0.01

<b>Reducing sugars (%)</b>	4.11 ± 0.04	7.83 ± 0.12
<b>Total acidity</b>	3.30 ± 0.04	0.10 ± 0.02
<b>pH</b>	2.84 ± 0.14	5.77 ± 0.04
<b>Total phenolic compounds (mg/100 mL)</b>	119.00 ± 4.00	99.00 ± 3.00

Values are the means ± standard deviations of triplicate measurements

In the present study, the acidity of the yellow passion fruit pulp was 3.30% and the pH was 2.84. Santos et. al. [15] reported similar results for yellow passion fruit pulp with values of 3.00-3.60% for acidity and pH from 3.00 to 3.25. Regarding the content of phenolic compounds, in this study, it was obtained the value of 119 mg/100 mL. Silva et al. [16] described similar results from 104 to 108 mg/100 mL of phenolic compounds for yellow passion fruit.

The composition of yellow passion fruit may vary considering factors such as climate, planting region, fruit harvest period and maturation state. The studies show that yellow passion fruit has high acidity, with a moderate content of reducing sugars and a high content of total phenolic compounds. Many studies highlight the functional properties of yellow passion fruit due to the presence of bioactive compounds such as phenolic compounds,  $\beta$ -carotene, and vitamin C [7,16], making it an interesting option to be added in fruit beers.

The result of the soluble solids obtained for the wort was 13°Brix and the reducing sugar content was 7.83%. According to Zhuang et al. [17], the value of 13-15°Brix represents a wort with standard sugar content, while wort with 16-20°Brix have a high sugar concentration and is used to produce beers with a high alcohol content. Zhuang et al. [17]

reported that the wort with 13°Brix had 6% maltose, and 2% glucose, totaling 8% sugars. The sugar profile in the wort mainly depends on the barley malt and wort preparation procedures. Maltose and maltotriose are the most abundant sugars in the wort and are normally consumed by yeasts after depletion of monosaccharides. Sugars are converted by yeasts into ethanol and carbon dioxide and many other compounds with organoleptic properties, including esters and higher alcohols [18].

The wort pH in this study was 5.77 and the acidity was 0.10%. Similar results were reported by Pater et al. [19], where the barley malt wort had a pH of 5.95-5.99. Li and Liu [20] reported that the barley malt wort had a pH of 5.45 and the predominant organic acids were citric acid, malic acid, and lactic acid. In our study, the content of phenolic compounds in the wort was 99 mg/100 mL. Ozcan et al. [21], reported the content of phenolic compounds of 107.78 mg/100 g. According to Bustos et al. [22] beer is a beverage rich in polyphenols, with around 70% originating from malt, and numerous studies have associated the intake of polyphenols derived from fermented beverages with the prevention of cardiovascular diseases and certain types of cancer.

### **3.2 Physicochemical characterization of passion fruit beers**

Physicochemical analyses of passion fruit beers were performed in three stages of production: at the end of primary alcoholic fermentation (green beer), at the end of maturation phase (matured beer), and after bottle refermentation (carbonated beer) (Table 2).

**Table 2. Physicochemical analyses of green, matured, and carbonated beers**

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**Analyses**

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Passion fruit beers	Stages of production	SS (°Brix)	RS (%)	pH	TA (%)	TPC (mg/100 mL)
	Green	6.00±0.00 <sup>a</sup>	1.37±0.03 <sup>a</sup>	3.82±0.01 <sup>a</sup>	0.23±0.00 <sup>a</sup>	91.00±1.00 <sup>a</sup>
Beer A	Matured	6.00±0.00 <sup>a</sup>	1.15±0.04 <sup>b</sup>	3.79±0.02 <sup>a</sup>	0.28±0.01 <sup>a</sup>	83.00±1.00 <sup>b</sup>
	Carbonated	6.00±0.00 <sup>a</sup>	1.03±0.02 <sup>c</sup>	3.66±0.01 <sup>b</sup>	0.33±0.01 <sup>b</sup>	67.00±2.00 <sup>c</sup>
	Green	6.00±0.00 <sup>a</sup>	1.30±0.04 <sup>d</sup>	3.95±0.04 <sup>c</sup>	0.26±0.01 <sup>a</sup>	87.00±2.00 <sup>d</sup>
Beer B	Matured	6.30±0.00 <sup>b</sup>	1.58±0.03 <sup>e</sup>	3.76±0.01 <sup>d</sup>	0.29±0.01 <sup>a</sup>	84.00±0.60 <sup>b</sup>
	Carbonated	6.10±0.00 <sup>a</sup>	1.04±0.01 <sup>c</sup>	3.65±0.02 <sup>b</sup>	0.33±0.02 <sup>b</sup>	68.00±2.00 <sup>c</sup>

Beer A = addition of 2% (w/v) of passion fruit pulp in the wort, before the primary alcoholic fermentation; Beer B) = addition of 2% (w/v) of passion fruit pulp in the green beer, after primary alcoholic fermentation and before the maturation stage. SST = Soluble Solids, RS = Reducing sugars, TA = Total acidity, TPC = Total phenolic compounds. Values in the same column with different letters are statistically different ( $p < 0.05$ ).

The soluble solids content of beer A was 6.00°Brix in the three stages of production (green, matured, and carbonated), and reducing sugars content of beer A decreased from 1.37 to 1.15% in matured beer and even decreased to 1.03% in carbonated beer, showing that the secondary alcoholic fermentation process occurred and reduced the sugar content. And in beer B, the soluble solids content increased from 6.00 to 6.30°Brix and the reducing sugars content increased from 1.30 to 1.58% in the matured beer, as there was the addition of passion fruit pulp in this stage, but the carbonated beer showed 6.10°Brix and a reducing sugars value of 1.04%, showing that there was consumption of the fruit sugars by yeasts in this stage.

Therefore, there was a reduction of 13°Brix and 7.83% of reducing sugars in the wort to 6.00-6.10°Brix and 1.03-1.04% of reducing sugars in the passion fruit beers in this study.

The values obtained in passion fruit beers (6,00-6,10°Brix) were similar to the values observed in beer with quince fruits (5,78°Brix) (23) and in beer with soursop fruits (5,80°Brix) [4]. Kawa-Rygielska et al. [24] obtained 1.05-1.37% of reducing sugars in beers formulated with Cornelian cherry fruits. And Costa et al. [25] obtained 0.80% of reducing sugars in beer formulated with pineapple pulp.

The pH of beer A (3.82) was lower than that of beer B (3.95) in the green stage, because in beer A the passion fruit pulp had already been added. Then, the addition of passion fruit to beer B reduced its pH from 3.95 (green) to 3.76 (matured). And the carbonated beers had a similar pH of 3.65-3.66. The acidity of carbonated beers also had a similar value of 0.33%. In contrast with wort (pH 5.77 and 0.10%), the corresponding beers had lower pH and higher acidity due to the formation of volatile organic acids (acetic and formic acids) and non-volatile organic acids (pyruvic, malic, citric and succinic acids) by the yeasts during beer fermentation [20,26]. The addition of passion fruit pulp, which is a fruit with high acidity, also contributed to lower the pH and increase the acidity of the beers. Such pH values could inhibit certain spoilage microorganisms and increase the biological stability of beer [26]. The final pH and acidity values obtained in the beers (pH 3.65-3.66 and total acidity 0.33%) agreed with similar studies by Kawa-Rygielska et al. [24] (pH 3.42-3.64), Alves et al. [4] (pH 3.90 and total acidity 0.33%) and Costa et al. [25] (3.90 and total acidity 0.26%).

Green beers showed a higher content of phenolic compounds (87-91 mg/100 mL) than matured beers (83-84 mg/100 mL), regardless of the addition of passion fruit pulp in the fermentation or maturation stage. And it can be observed a decline in the phenolic compounds content of carbonated beers (67-68 mg/100 mL) compared to matured beers. According to Siqueira et al. [27], the concentration and different types of hops and malts

used in the beers influence the content of phenolic compounds and during the storage, beers age and show a decline in the content of phenolic compounds. Li et al. [28] evaluated the evolution of the total phenolic compounds during storage of beers. A decrease in phenolic compounds of beers was observed during storage, and at the end of storage (6 months) phenolic compounds decreased by approximately 18.6% than the starting concentration. According to Li et al. [28] this behavior was attributed to the oxidation of phenolic compounds by free radicals and polymerization with proteins.

The phenolic compounds values obtained in passion fruit beers (67-68 mg/100 mL) were similar to the values observed in beer with omija fruits (60.7 mg/100 mL) [29] and in beer with goji berries (62.3 mg/100 mL) [30]. Nardini and Garaguso [5] reported that total polyphenols content (51.0-76.7 mg/100 mL) in cherry, orange, grape, plum, raspberry and peach beers was considerably higher in respect to that of conventional beers (32.1-48.2 mg/100 mL). And Koren et al. [31] reported that beers aged with sour cherry had higher values of total polyphenols content (42.8-103.4 mg/100 mL) compared to conventional beers (11.9-20.0 mg/100 mL).

Table 3 presents the values of antioxidant activity and alcohol content of carbonated beers. The antioxidant activity of beer B (3.04 mM TEAC by ABTS and 0.70 mM TEAC by DPPH) was higher than that of beer A (2.45 mM TEAC by ABTS and 0.62 mM TEAC by DPPH). Kawa-Rygielska et al. [24] showed that the method of beer production in which fruit pulp were added before the stage of secondary fermentation enabled producing beer with a higher concentration of antioxidants than the method in which fruit pulp were added to wort before the beginning of the primary alcoholic fermentation.

**Table 3. Results of antioxidant activity and alcohol content of passion fruit beers**

Analyses	Carbonated passion fruit beers	
	Beer A	Beer B
<b>ABTS (mM TEAC)</b>	2.45 ± 0.39 <sup>a</sup>	3.04 ± 0.22 <sup>b</sup>
<b>DPPH (mM TEAC)</b>	0.62 ± 0.04 <sup>a</sup>	0.70 ± 0.06 <sup>b</sup>
<b>Alcohol content (°GL)</b>	4.50 ± 0.01 <sup>a</sup>	4.70 ± 0.01 <sup>a</sup>

Beer A = addition of 2% (w/v) of passion fruit pulp in the wort, before the primary alcoholic fermentation; Beer B) = addition of 2% (w/v) of passion fruit pulp in the green beer, after primary alcoholic fermentation and before the maturation stage. Values in the same line with different letters are statistically different ( $p < 0.05$ ).

Other studies reported high values of antioxidant activity in fruit beers. Ducruet et al. [30] obtained values of 2.26-3.70 mM TEAC by ABTS method in goji berries beers and according to authors these results clearly indicated that an addition of goji berries allows obtaining a beer with high antioxidant activity. Zapata et al. [23], found antioxidant activity of 0.72-0.73 mM TEAC by the ABTS method in beers with quince fruits. Deng et al. [29] reported values of 0.18-0.76 mM TEAC by the DPPH method in omija fruit beers.

According to Nardini and Garaguso [5] most of the fruit beers showed antioxidant activity markedly higher in respect to conventional beers. Kawa-Rygielska et al. [24] showed that the addition of pulp from all studied cultivars of Cornelian cherries improved the antioxidant properties of the beers. And the strong correlation observed between antioxidant activity and total phenolic acids content suggest a central role of phenolic compounds in the antioxidant properties of beers.

It is reported in the literature that a moderate ingestion of alcoholic beverages, such as beer, is associated with protective cardiovascular function and a reduction in the development of neurodegenerative diseases. Several studies describe the effects of beer and wine on the increase of antioxidant, anti-inflammatory and anticoagulant activities, and a positive effect on plasma lipid levels of humans [32,33].

The alcohol content of passion fruit beers was 4.50-4.70°GL. Similar results were reported by Kawa-Rygielska [24], and Cornelian cherries beers showed an alcohol content of 4.89-5.09°GL. Fanari et al. [34] reported that the cherry beer had an alcohol content of 5.63°GL and the plum beer had 5.51°GL. And Zapata et al. [23] obtained an alcohol content of 5.51-5.62°GL in beer with quince fruits. Alves et al. [4], Costa et al. [25] and Deng et al. [29] obtained an alcohol content of 4.00, 5.24 and 5.10°GL in fruit beers of soursop, pineapple and omija, respectively. According to Sohrabvandi et al. [35], beers can have from 2.5 to 13% (v/v) of ethanol and most beers around the world have an alcohol content of 3 to 6% (v/v).

In the sensory analysis, both passion fruit beers had good acceptance (Table 4). The beer B had higher mean scores of flavor and aroma than beer A, however the global impression of the beers A and B was similar. So, the addition of passion fruit after primary fermentation had positive effects on the flavor and aroma of beer. The average score for foam persistence and carbonation was lower in relation to the score of the other attributes, indicating a need to increase the carbonation of the beers.

**Table 4. Sensory profiles of passion fruit beers**

<b>Attributes</b>	<b>Beer A</b>	<b>Beer B</b>
<b>Flavor</b>	6.40 <sup>a</sup>	7.00 <sup>b</sup>

<b>Aroma</b>	6.90 <sup>a</sup>	7.10 <sup>b</sup>
<b>Foam persistency and carbonation</b>	5.40 <sup>a</sup>	5.60 <sup>a</sup>
<b>Global impression</b>	7.00 <sup>a</sup>	7.10 <sup>a</sup>

Beer A = addition of 2% (w/v) of passion fruit pulp in the wort, before the primary alcoholic fermentation; Beer B) = addition of 2% (w/v) of passion fruit pulp in the green beer, after primary alcoholic fermentation and before the maturation stage. Values in the same line with different letters are statistically different ( $p < 0.05$ ).

In the studies with fruit beer, it was observed the addition of fruits at different stages of beer production. Alves et al. [4], Costa et al. [25] and Zapata et al. [23] added soursop pulp, pineapple pulp and pieces of quince, respectively, after primary alcoholic fermentation, and the maturation stage was continued for 10 days at 4-5°C. Marin et al. [36] and Jahn et al. [37] added red grape juice and Aronia berries, respectively, to wort before primary alcoholic fermentation. Deng et al. [29] studied the addition of dried omija fruits in 3 stages of beer production: 1) to wort at the beginning of boiling, 2) before the primary alcoholic fermentation, and 3) before conditioning beer at bottles. And the addition of omija fruits at the initiation of boiling led to a beer with the best sensorial attributes. Ducret et al. [30] reported that consumers preferred beers which goji berries were added to wort before primary alcoholic fermentation. Thus, the beer production stage where the addition of fruit is carried out depends on each type of fruit and on production tests to obtain the best fruit beer formulation.

#### 4. CONCLUSION

Sensory analysis proved that the passion fruit beers studied had appropriate sensory characteristics that led to good consumer acceptance. The addition of passion fruit resulted in beers with high values of total polyphenol content and antioxidant activity. Thus, adding passion fruit to beer is a good option to improve quality characteristics of beers.

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