Seed oils from different passion fruit species (*Passiflora edulis Sims flavicarpa, Passiflora cincinnata and Passiflora setacea*): extraction and characterization

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Abstract: Passion fruit (*Passiflora edulis* Sims flavicarpa - PF), traditionally cultivated in the state of Bahia, and other species of passion fruit, such as *Passiflora setacea* - PS and *Passiflora cincinnata* - PC, as well as their residues, have been explored as a source of bioactive compounds, in order to enhance Brazilian biodiversity and add value to these representative residues in the processing of the pulp of these fruits. The objective of this work was to extract seed oils from different passion fruit species via Soxhlet and characterize them - fatty acid profile, acid value, peroxide value, refractive index, saponification value, moisture content, relative density, viscosity, and ABTS. The PF oil presented the following characteristics: yield of 22,58%, acidity of 1,03 mg KOH/g, peroxide value of 4,31 meq/kg, refractive index of 1,4720, saponification value of 177,00, moisture content of 6,46%, relative density of 0,87 g/mL, ABTS of 185,11 µmol TE/g, initial viscosity of 22,15 mPa.s, oleic acid 16,10%, linoleic acid 50,47%, palmitic acid 17,02% and stearic acid 16,41%.The PC oil presented the following characteristics: yield of 14,80%, acidity of 2,69 mg KOH/g, peroxide value of 14,91 meq/kg, refractive index of 1,4743, saponification value of 174,78, moisture content of 2,47%, relative density of 0,88 g/mL, ABTS of 298,56 µmol TE/g, initial viscosity of 26,73 mPa.s, oleic acid 13,59%, linoleic acid 72,97%, palmitic acid 8,88% and stearic acid 4,53%.The PS oil presented the following characteristics: yield of 32,45%, acidity of 2,07 mg KOH/g, peroxide value of 29,89 meq/kg, refractive index of 1,4726, saponification value of 173,48, moisture content of 2,31%, relative density of 0,92 g/mL, ABTS of 280,67 µmol TE/g, initial viscosity of 26,81 mPa.s, oleic acid 18,02%, linoleic acid 60,50%, palmitic acid 11,56% and stearic acid 9,92%.The oils presented interesting characteristics and compositions that give them potential for industrial application.

Keywords: Extraction. Oil. Seeds. *Passiflora spp.*

Abbreviations: PF, *Passiflora edulis* Sims flavicarpa. PC, *Passiflora cincinnata*. PS, *Passiflora setacea*.

1. Introduction

Over the years, researchers have sought to overcome the barriers of two major challenges: reducing the amount of agro-industrial waste through reduction or reuse strategies and finding alternative sources of vegetable oils to meet the growing demand for this raw material [1].

Passion fruit has emerged as one of Brazil's most widely produced fruits. Much of this production is directed toward the industrial production of concentrated juice, generating large amounts of waste, consisting of peels, albedo, and seeds. What is known is that this waste, which is largely discarded in landfills, causing environmental pollution, is rich in nutrients and has great potential for industrial use [2].

One potential effective industrial application is the production of oils from passion fruit seeds for cosmetic, pharmaceutical, and food applications. This practice is already common with the famous yellow passion fruit; however, other species in this family may offer similar potential. Thus, the objective of this study was to perform the Soxhlet extraction of oils from three different species of passion fruit: *Passiflora edulis* Sims Flavicarpa, *Passiflora cincinnata*, and *Passiflora setacea*. Furthermore, the objective was to perform the physicochemical characterization and fatty acid profile of each of the matrices.

2. Methodology

2.1. Obtaining raw materials

The fruits were purchased locally (*Passiflora edulis Sims flavicarpa*) and from small producers (*Passiflora cincinnata* and *Passiflora setacea*). The fruits were sanitized, pulped, and the seeds were separated from the pulp using a sieve. The seeds were divided into 20 g portions and stored in a freezer at -10°C.

2.2. Seed preparation

The seeds were thawed at refrigerated temperature. Afterward, were dried in an air-circulating oven at 40°C for two days. After drying, the seeds were manually cleaned to remove any remaining pulp residue. The dried and cleaned seeds were vacuum-packed and stored at refrigerated temperature.

2.3. Oil extraction

The seeds were crushed, and the particle size was adjusted using a 16-mesh stainless steel sieve. Oil extraction was performed via Soxhlet extraction, where 10 g of the crushed seed was weighed and added to a cartridge, which was inserted into a Soxhlet extractor. 150 mL of hexane was used as the extracting solvent. The extraction was completed after 24 complete cycles. To separate the solvent from the oil, rotary evaporation (15 RPM and 50°C) was performed.

2.4. Oil characterization

All analyses were performed in triplicate, unless otherwise stated.

2.4.1. Detemination of oil yield

The samples yield was determined considering the weight of oil obtained in relation to the total initial seed mass.

2.4.2. Determination of fatty acid profile

The fatty acid profile was determined by gas chromatography using a GC-FID 2010 apparatus with a SUPELCOWAX10 column (30 m x 0.25 mm x 0.25 µm). The temperature was initially set at 70 °C for 3 min, then increased to 210 °C at a rate of 24 °C/min, followed by an increase to 230 °C at a rate of 5 °C/min, where it was then held for 8 min. Nitrogen was used as the carrier gas at a rate of 1.7 mL/min. The injector was operated in Split mode (1-20) at a temperature of 230 °C, with an injection volume of 1 µL. Injections were performed in triplicate for all samples. To determine the fatty acid content of the oil samples, the methyl esters present were identified by comparison with the retention times of the external standard FAME. A correlation factor was calculated for all fatty acid methyl esters present in the oil samples [3].

2.4.3. Determination of acidity

In a 125 mL Erlenmeyer flask, 2 g of sample was weighed. 25 mL of ether:alcohol (2:1) solution and two drops of phenolphthalein were added. Titration was performed using 0.1 M sodium hydroxide solution until a persistent pink color appeared [4].

2.4.4. Determination of the peroxide value

In a 250 mL Erlenmeyer flask, 5 g of sample was weighed. 30 mL of acetic acid:chloroform (3:2) solution was added, and the flask was shaken until completely dissolved. After that, 0.5 mL of potassium iodate solution was added, and the flask was left to stand for 1 minute in the dark. After the time elapsed, 30 mL of water was added, and titration was performed with 0.1 M sodium thiosulfate solution until the yellow color had almost disappeared. Then, 0.5 mL starch indicator solution was added, and titration was performed until the blue color disappeared [4].

2.4.5. Determination of refractive index

Initially, the device was calibrated using water. After that, two to three drops of the sample were placed on the lower prism of the device, the prism was closed, locked, held towards the light, and the result was read [4].

2.4.6. Determination of saponification index

A 5 g sample was weighed, and 50 mL of an alcoholic KOH solution was added. The system was connected to a condenser and allowed to boil for approximately 1 hour. Afterward, the system was disconnected from the condenser, 1 mL of phenolphthalein indicator was added, and titration with a 0.5 M hydrochloric acid solution was performed [4].

2.4.7. Moisture determination

A 3 g sample was weighed into a porcelain crucible and dried in an oven at 105°C for 3 hours, then stored in a desiccator for cooling and weighing. The heating and cooling process was repeated until a constant weight was achieved [4].

2.4.8. Determination of relative density

Initially, the empty pycnometer was weighed. After that, the sample was added to the pycnometer, and it was weighed again. The same procedure was performed with distilled water [4].

2.4.9. Determination of viscosity

Viscosity determination was performed in a controlled stress rheometer at room temperature. 5 mL of sample was added to the rheometer measuring vessel, and measurements were taken at 12 different speeds, representing shear rates ranging from 25 to 1000 s^{-1}. The rheological data were fitted to the Oswald de Waele model to obtain apparent viscosity values.

2.4.10. Determination of antioxidant capacity - ABTS

Initially, a Trolox standard curve was prepared. To the extract was first prepared. For this, 1 g of sample was weighed into a 100 mL beaker, 40 mL of 50% methanol solution was added, homogenized and left to stand for 60 minutes at room temperature.

After the time was up, centrifugation was performed at 15,000 rpm for 15 minutes and the supernatant was transferred to a 100 mL volumetric flask. 40 mL of 70% acetone solution was added to the residue from the first extraction, homogenized, and left to rest for 60 minutes at room temperature. Centrifugation was performed again at 15,000 rpm for 15 minutes; the supernatant was transferred to a volumetric flask containing the first supernatant and the flask was filled with distilled water.

In a dark environment, a 30 µL aliquot of the extract was transferred to test tubes containing 3.0 mL of the ABTS radical and homogenized by vortexing. After 6 minutes, the absorbance was read at a wavelength of 734 nm. It is important to note that ethyl alcohol was used to calibrate the spectrophotometer. To obtain the data, the values found were substituted into the equation of the straight line of the trolox standard curve.

3.Results

The results of yields and physicochemical characterization of the sample were available in Table 1 below. Table 2 described the kinetic viscosity results of the sample. Table 3 described the fatty acid profiles of the sample analyzed.

Table 1. Physical and chemical characterization of oils *Passiflora edulis Sims flavicarpa* (PF), *Passiflora cincinnata* (PC), *Passiflora setacea* (PS)

	Oil samples		
Parameter	PF	PC	PS
Yield (%)	22.58 ± 0.74	14.80 ± 0.91	32.45 ± 0.32
Acidity (mg KOH/g)	1.03 ± 0.00	2.69 ± 0.30	2.07 ± 0.01
Peroxide value (meq/kg)	4.31 ± 0.53	14.91 ± 0.03	29.89 ± 9.93
Refractive index (40 °C)	1.4720 ± 0.00	1.4743 ± 0.00	1.4726 ± 0.00
Saponification index (mg KOH/g)	177.00 ± 2.00	174.78 ± 0.52	173.48 ± 3.14
Moisture (%)	6.46 ± 1.07	2.47 ± 0.25	2.31 ± 0.27
Relative density (g/mL)	0.87 ± 0.00	0.88 ± 0.00	0.92 ± 0.00
ABTS (µmol TE/g)	185.11	298.56	280.67

Table 2. Kinetic viscosity of oils *Passiflora edulis Sims flavicarpa* (PF), *Passiflora cincinnata* (PC), *Passiflora setacea* (PS)

	Oil samples		
Shear rate (s^{-1})	PF (mPa.s)	PC (mPa.s)	PS (mPa.s)
25	22.15	26.73	26.81
50	17.13	20.77	20.40
100	13.24	16.14	15.53
200	10.23	12.54	11.81
400	7.91	9.75	8.99
600	6.80	8.41	7.66
800	6.11	7.58	6.84
1000	5.63	6.98	6.27

Table 3. Fatty acid profile of oils *Passiflora edulis Sims flavicarpa* (PF), *Passiflora cincinnata* (PC), *Passiflora setacea* (PS)

	Oil samples		
Fatty acid	PF	PC	PS
Oleic acid (%)	16.10 ± 0.13	13.59 ± 0.01	18.02 ± 1.94
Linoleic acid (%)	50.47 ± 0.77	72.97 ± 0.34	60.50 ± 1.65
Palmitic acid (%)	17.02 ± 0.02	8.88 ± 0.04	11.56 ± 2.30
Stearic acid (%)	16.41 ± 0.44	4.53 ± 1.71	9.92 ± 1.68
Saturated fatty acids (%)	33.43	13.41	21.48
Unsaturated fatty acids (%)	66.57	86.56	78.52

4.Discussions

4.1. Oil yield

According to what was presented in Table 1, the PS presented the highest amount of oil extracted per g of sample, with an extraction yield of 32.45%. Meanwhile, PC presented the lowest amount of oil extracted per g of sample, with an extraction yield of 14.80%. The results found were similar to those found in another studies - PF 21,34% and PS 34,07 %. However, a difference was observed in the PC yield, and this difference can be explained due to agroeconomic variations and variations in process conditions [5,6].

4.2. Acidity

Acidity is a fundamentally important parameter, as it indicates the oil's conservation status. Oil stored under inadequate conditions or for a long period of time will experience increased lipase activity, thus increasing the amount of free fatty acids present [5, 7]. According to IN 87, virgin vegetable oil must have a maximum acidity value of 10 mg / KOH g. Thus, the samples presented an acidity index within the established parameter [8]. The acidity indices for PF and PS oils found here were similar to those found in the study by Pereira - PF 1,5 mg KOH / g and PS 2,55 mg KOH / g [10].

4.3. Peroxide value

The peroxide index is also a parameter that indicates the conservation status of the oil, being related to its degree of oxidation [5]. The maximum value of the peroxide index for vegetable oils and fats is 15 meq/kg. The PF and PC oils were within the standard, however the PS oil had a peroxide index much higher than expected. High peroxide levels were expected since the use of high temperatures can result in thermal oxidation [8,9].

4.4. Refractive index

The refractive index can also be used to identify the oxidation state of an oil. Furthermore, this parameter also indicates the oil's degree of purity, allowing the identification of adulteration [10]. Silva, Santos and Melo Filho determined the refractive index of Passiflora edulis Sims flavicarpa to be 1.471, a result similar to that found in this study [5]. No data was found in the literature for PC and PS oils.

4.5. Saponification index

Determining the saponification index is considered important because it is related to the length of the fatty acid carbon chain. This is an inverse relationship, where the higher the saponification index, the shorter the carbon chain. Therefore, knowing this index allows us to understand and direct the oil toward the best industrial application, since, in general, edible oils have a saponification index of 168 to 196 mg KOH /g, and above that, they are used for soap production [11,12].

Silva, Santos and Melo Filho in a study carried out with Passiflora edulis Sims flavicarpa seed oil, determined a saponification index of 179.06 mg KOH/g [5]. Souza et al determined a saponification index for Passiflora cincinnata seed oil equal to 179.35 mg KOH / g and for Passiflora setacea seed oil equal to 191.91 mg KOH / g. Thus, the samples analyzed here presented saponification indexes similar to those found in previous studies and have potential to be used as edible oils [6].

4.6. Moisture

Among the species studied, PF oil had the highest moisture content, and PC and PS oils had statistically similar moisture contents. No data for comparison were found in the literature.

4.7. Relative density

The density of an oil is directly related to the length of its carbon chain. In other words, the longer the carbon chain of fatty acid, the higher the oil's density. According to Table 1, PS oil presented the highest density and PF oil presented the lowest density. Therefore, it is correct to state that the fatty acids present in PS oil have longer carbon chains than the fatty acids in PC oil. This same observation was found when analyzing the saponification indices of the oils. [13]

Furthermore, studies carried out by other authors found similar density values for the three oils, namely: PF - 0.9 g mL; PC - 1.09 g mL; PS - 1.14 g mL [5,6].

4.8. Viscosity

Regarding viscosity, PS oil presented the most viscous and PF oil presented the least viscous. It was observed that the three oils present similar behavior when the shear rate was changed: as the rate increased, the viscosity decreased, thus characterizing the oils as pseudoplastic fluids. This same behavior was observed in other oils already trained, such as palm oil, sunflower oil and olive oil [14].

4.9. Fatty acid profile

Table 3 showed that, passion fruit seed oils are rich in linoleic acid and oleic acid, with PC oil having a higher amount of linoleic acid and PS oil having a higher amount of oleic acid. In a study carried out by Tome-Rodriguez et al, the authors analyzed the fatty acid profile of olive oil and quantified a maximum linoleic acid content of 26.86% [15]. The fatty acid profiles of sunflower and corn oils were also studied. These oils contained 56.29% and 54.73% linoleic acid, respectively [16]. Considering the studies mentioned above, it is correct to state that PF, PC and PS oils can be used as a replacement and effectively replace commonly used oils, considering their linoleic acid content.

4.10. ABTS

The main function of ABTS analysis is to determine the antioxidant activity present through the sequestration of the ABTS radical [17]. Among the species studied, PC oil showed the highest antioxidant activity and PF oil showed the lowest antioxidant activity. The ABTS values found here were lower than those found in another study carried out with olive oil, where the values varied between 311. 66 and 465.91 µmol TE/g [18].

5. Conclusion

It was possible to extract passion fruit seed oils via Soxhlet and characterize each of them, demonstrating the richness of their composition and their potential for industrial application in the food, cosmetics, and pharmaceutical sectors.

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