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# Functional characteristics and antimicrobial activity of supercritical $CO_2$ extracts from passion fruit (*Passiflora edulis*) seeds

## Características funcionales y actividad antimicrobiana de extractos de $CO_2$ supercrítico de semillas de maracuyá (*Passiflora edulis*)

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## ABSTRACT

The passion fruit seed is an important byproduct of the agroindustry, as it accounts for 15% of this fruit, so it is imperative to add value to these seeds. In this context, the present study aimed to evaluate the functional characteristics and antimicrobial activity of passion fruit seeds. Oil extraction was carried out using supercritical $CO_2$ (SC-$CO_2$) and supercritical $CO_2$ with ethanol cosolvent (SC-$CO_2$+et); both oils showed a similar fatty acid profile with a high content of polyunsaturated fatty acids (71.62 and 71.80%, respectively). The study of the functional quality of the oils showed low atherogenicity (AI) and thrombogenicity (TI) indices. The seed flour oil and the defatted passion fruit seed flour extract presented antimicrobial activity against *Klebsiella oxytoca*, *Staphylococcus aureus* and *Proteus vulgaris*. The ethanolic extract of flour defatted with $CO_2$+ethanol (FDCE) obtained higher values of total phenolic compounds and antioxidant capacity by ABTS and FRAP. The present research provides the characterization of the functional properties of the passion fruit seed oils and the defatted seed, being data of interest for future applications of the passion fruit seed.

**Keywords:**
Antioxidant capacity
By-product
Fatty acid profile
Functional quality indices
RapidOxy

## RESUMEN

La semilla de maracuyá es un residuo agroindustrial importante, ya que representa el 15% de esta fruta, por lo que es imperativo añadir valor a estas semillas. En este contexto, el objetivo del presente estudio fue evaluar las características funcionales y actividad antimicrobiana de la semilla de maracuyá. La extracción de aceite se llevó a cabo utilizando $CO_2$ supercrítico (SC-$CO_2$) y $CO_2$ supercrítico con cosolvente etanol (SC-$CO_2$+et), ambos aceites mostraron un perfil de ácidos grasos similar con elevado contenido de ácidos grasos poliinsaturados (71,62 y 71,80%, respectivamente). El estudio de la calidad funcional de los aceites presentó índices de aterogenicidad (IA), trombogenicidad (IT) bajos. El aceite de la harina de semilla y el extracto de harina de semilla de maracuyá desgrasada presentaron actividad antimicrobiana frente a *Klebsiella oxytoca, Staphylococcus aureus* y *Proteus vulgaris*. El extracto etanólico de harina desgrasada con $CO_2$+etanol obtuvo mayores valores de compuestos fenólicos totales y capacidad antioxidante por ABTS y FRAP. La presente investigación proporciona la caracterización de las propiedades funcionales tanto de los aceites de la semilla de maracuyá como la semilla desgrasada, siendo datos de interés para futuras aplicaciones de la semilla de maracuyá.

**Palabras clave:**
Capacidad antioxidante
Subproducto
Perfil de ácidos grasos
Índices de calidad funcional
RapidOxy

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The Passifloraceae family has 18 genera and around 630 species distributed in tropical and subtropical areas worldwide; in America, most species are found in Central and South America (Deginani 2001). It is estimated that the agro-industrial waste from the passion fruit juice industry reaches 40% of the amount of processed fruit, and around 90% of the waste is composed of peels and seeds (Malacrida and Jorge 2012). In this context, it is important to use these wastes and study their bioactive compounds to offer alternative uses for these byproducts.

Studies on *Passiflora edulis* show its use as an anti-inflammatory, antimicrobial, lipid-lowering, antioxidant, anxiolytic and antitumor; various types of preparations, extracts and individual compounds derived from this species possess a wide spectrum of pharmacological effects on various organs, as well as on different biochemical processes and physiological functions (Taïwe and Kuete 2017).

Barrales et al. (2015) evaluated the combined effect of seed oil extraction with ultrasound and SC-$CO_2$, managing to increase overall yield. In relation to the use of passion fruit seed oil, Arturo-Perdomo et al. (2021) and Pantoja-Chamorro et al. (2017) studied the physicochemical composition of passion fruit seed oils obtained by SC-$CO_2$, highlighting its linoleic acid (67.53%) and sterol content. Santos et al. (2021) and Malacrida and Jorge (2012) reported a potential source of polyunsaturated fatty acids such as linoleic acid in passion fruit seed oil, as well as a source of polyphenols and tocopherol. Dos Santos et al. (2021) evaluated passion fruit oil obtained with supercritical $CO_2$, reporting high values of linoleic acid in the fatty acid profile, as well as tocotrienol, squalene, and carotenoids. Pereira et al. (2018) evaluated the effect of extraction methods (subcritical propane, Soxhlet and ultrasound) on the composition of passion fruit seed oil, reaching a maximum yield of 26.12%; and also reported antibacterial activity against *Escherichia coli, Salmonella enteritidis, Staphylococcus aureus* and *Bacillus cereus*.

Considering the information presented in studies related to passion fruit seed oil, these have been focused on evaluating the phytochemical profile, extraction methods and their effect on yield; however, no studies were found on functional quality, antioxidant capacity and antimicrobial properties of the oils obtained with two extraction techniques with $CO_2$ and $CO_2$+et. In this sense, the present study provides information on the functional characteristics and antimicrobial activity of passion fruit seeds.

## MATERIALS AND METHODS

### Sample preparation and reagents

The passion fruit (*Passiflora edulis*) seeds, a byproduct of juice production in Pucallpa (Ucayali, Peru), were frozen and transported to the Technological Institute of Production (Callao, Peru). Upon arrival, they were thawed, dried in an oven (Venticell, Ecocell, Switzerland) at 50 °C for 22 hours until reaching 4% moisture, then placed in vacuum bags and stored at -20 °C. For oil extraction, the seeds were ground in an analytical mill (A 11 Basic, IKA, USA), resulting in passion fruit seed flour (PF).

The following reagents were used in this study: Ethanol 99.5% (Scharlau, Spain), hexane ACS (Fermont, Mexico), Fatty Acid Methyl Ester Mix $C_4$-$C_{24}$ standard mix 37 FAME (Supelco, Germany), methanol HPLC grade (Merck, Germany), Folin 2N (Sigma-Aldrich, Germany), sodium carbonate ACS (Supelco, Canada), Gallic acid monohydrate ≥98.5% (Sigma-Aldrich, United States), ABTS (2 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) diammonium salt >=98% (Bio Basic, Canada), Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) ≥96% (Sigma Aldrich, China), acetic acid ≥99.7% (Merck, Germany), TPTZ (2,4,6-tri(2-pyridyl)-1,3,5-triazine) 98% (Alfa Aesar, England), Iron (III) chloride hexahydrate ACS (Merck, Germany), carbon dioxide 99.5% v/v liquefied gas (Linde, Peru), nitrogen atmosphere Ultrapure (Linde, Peru), oxygen Ultrapure (Praxair, Peru). For the microbiological assays, all strains were obtained in Kwik Stik format (Microbiologics, United States). The following materials were also used: McFarland standard tube No. 0.5 (Liofilchem, Italy), Mueller-Hinton agar (Condalab, Spain), dimethyl sulfoxide (BioBasic, Canada), and Penicillin disc (OXOID, United Kingdom).

### Passion fruit seed oil extraction

#### Supercritical $CO_2$ (SC-$CO_2$)

The extraction of PF oil with SC-$CO_2$ was carried out in duplicate using the multisolvent equipment (Top industries, 2802 0000) described by Barriga-Sánchez et al. (2022), a cell reducer was used in the reactor (87 $cm^3$, dimensions: 2.8 cm internal diameter and 14.1 cm inner height). A

40.88±3.39 g of PF was used, and the process parameters were 220 bar, 50 °C, $CO_2$ flow of 40 g $min^{-1}$ and time of 240 min. The resulting flour will be referred to as $CO_2$-defatted flour (PFDC), which was stored at -20 °C for subsequent analysis.

**Supercritical $CO_2$ and ethanol cosolvent**

A 50.23±0.5 g of PF was weighed in the extraction cell of the multi-solvent equipment, and the extraction was carried out as described by Barriga-Sánchez et al. (2022). The pressure, temperature and $CO_2$ flow were 220 bar, 50 °C and $CO_2$ flow of 40 g $min^{-1}$, respectively. The sample: ethanol ratio was 1: 16 and extraction time was 180 min. The ethanolic extract was concentrated in a rotary evaporator (Buchi, R-300, Switzerland) until dry, then nitrogen was added to eliminate traces of the solvent. Oil extraction was performed in duplicate. The flour defatted with $CO_2$+ethanol (PFDCE) was stored at -20 °C for subsequent analysis.

**Solid-liquid extraction by Soxhlet apparatus**

The extraction was carried out using the Soxhlet apparatus (FatExtractor E-500, Buchi, Switzerland). Three grams of PF was weighed and hexane was used as a solvent, the extraction time was 3 h and two replicates were carried out.

**Extraction of bioactive compounds from defatted passion fruit flour**

Extraction of bioactive compounds from PFDC and PFDCE was performed according to the recommendations of Reis et al. (2020). 70% ethanol was used in a 1: 5 ratio (flour: solvent, w: v) in a thermostatic bath (MEMMERT, WNB 7 - 45, Germany) at 45 °C for 1 h. Subsequently, it was placed on a rotary shaker at 70 RPM for 2 h (MX-RL-Pro, Dragon Lab, USA), centrifuged (Centrifuge 5804 R, Eppendorf, Brazil) at 4 °C for 10 min at 3,200 g, and the ethanolic phase containing the bioactive compounds was recovered and stored at -20 °C for subsequent analysis.

**Extraction yield and oil recovery**

The extraction oil yield, expressed as a percentage, was determined using Equation 1.

$$\text{Extraction oil yield}(\%) = \frac{W_1}{W_2} x\ 100\% \qquad (1)$$

Where: $W_1$ is the mass of the oil (g) obtained after extraction and $W_2$ is the mass of the passion fruit seed (g). The oil recovery, expressed as a percentage (%), was calculated using Equation 2.

$$\text{Oil recovery}(\%) = \frac{R_1}{R_2} x 100\% \qquad (2)$$

Where: $R_1$ is the extraction oil yield obtained by supercritical extraction and $R_2$ is the extraction oil yield obtained by Soxhlet using hexane.

**Fatty acid profile**

The methodology described by Barriga-Sánchez et al. (2021) was followed. A chromatograph with an FID detector (Autosystem XL, Perkin Elmer, USA) was used; the oil sample was saponified and methylated prior to analysis. The hot methylation process was carried out using sodium methoxide, followed by acidification with sulfuric acid in methanol and subsequent heating. Fatty acid peaks were identified by comparison with the retention times of the mixture of C4-C24 fatty acid methyl esters. Peak area was calculated using TotalChrom Navigator software and the percentage of each fatty acid was calculated by comparing the individual area of each peak to the total fatty acid area. The analysis for each oil were performed in duplicate.

**Functional oil quality**

The functional quality of the oil was determined with the data of the fatty acid profile, which were the atherogenicity index (AI) according to Equation 3 (Ratusz et al. 2018), the thrombogenicity index (TI) according to Equation 4 (Ratusz et al. 2018) and the hypocholesterolemic/ hypercholesterolemic ratio (H/h) according to Equation 5 (Santos-Silva et al. 2002).

$$AI = \frac{(C12:0 + 4(C14:0) + (C16:0)}{(\sum MUFA) + (\sum \omega - 6) + (\sum \omega - 3)} \qquad (3)$$

$$TI = \frac{(C14:0) + (C16:0) + (C18:0)}{0.5(\sum MUFA) + 0.5(\sum \omega - 6) + (\sum \omega - 3) + (\frac{\sum \omega - 3}{\sum \omega - 6})} \qquad (4)$$

$$\frac{H}{h} = \frac{(C18:1\omega-9)+(C18:2\omega-6)+(C20:4\omega-6)+(C18:3\omega-3)+(C20:5\omega-3)+(C22:5\omega-3)+(C22:6\omega-3)}{(C14:0)+(C16:0)} \quad (5)$$

Where: C12:0 (lauric acid); C14:0 (myristic acid); C16:0 (palmitic acid); C18:0 (stearic acid); C18:1 ω-9 (oleic acid); C18:2 ω-6 (linoleic acid); C18:3 ω-3 (linolenic acid); C20:4 ω-6 (arachidonic acid); C20:5 ω-3 EPA (eicosapentaenoic acid); C22:5 ω-3 DPA (docosapentaenoic acid); C22:6 ω-3 DHA (docosahexaenoic acid); MUFA (Monounsaturated Fatty Acids).

**Induction period**

The induction period of the oils was measured in a RapidOxy reactor (Anton Paar, Blankenfelde-Mahlow, Germany), 4 g of sample was weighted onto the plate, which was placed in the equipment, the safety cover closed automatically, and the process started. The parameters used were a temperature of 140 °C and a pressure of 700 kPa (Rodríguez et al. 2021). The induction time was calculated with the OXISoft™ software when a 10% decrease in $O_2$ pressure was reached according to the equipment indication. Each oil was analyzed in duplicate.

**Total phenolic compounds (TPC)**

To determine the total polyphenol content in oil, the methodology described by Varas et al. (2020) was used, 0.5 g of oil was weighed in a test tube, then 1.5 mL of 90% methanol was added, vortexed for 4 min, then centrifuged at 3,000 rpm for 5 min, then the supernatant was recovered. The process was carried out twice more and then the extract was evaporated to dryness.

Ethanolic extracts PFDC and PFDCE were used to quantify the TPC as described by Barriga-Sánchez et al. (2021) by performing a 5-point gallic acid calibration curve between 50 to 400 ppm ($y = 2.18X + 0.02$, $R^2 = 0.9980$), the Folin reagent was added and allowed to rest for 8 min, then 6% sodium carbonate and water were added, leaving it to rest for 1 h for its reading at 750 nm. Analyzes were performed in triplicate and expressed as mg gallic acid equivalent (GAE) 100 $g^{-1}$ sample.

**Antioxidant capacity**

The antioxidant capacity of the oils obtained by SC-$CO_2$ and SC-$CO_2$+et, and the ethanolic extracts PFDC and PFDCE were determined in duplicate and by two methods:

**ABTS**

Measurement of ABTS (2-2′-Azino-bis(3-ethyl benzothiazoline-6-sulfonic acid)) radical cation scavenging capacity was performed according to Prior et al. (2005). For the test, the ABTS radical cationic solution was prepared in ethanol, reaching the absorbance of 0.70±0.02 at 734 nm. The absorbance of the mixture was measured in a spectrophotometer (Genesys 180, Thermo Scientific, USA) after 30 min, using ethanol as a blank. The reference curve was constructed with 5 concentrations between 0.1 to 2.0 mM of Trolox (Sigma-Aldrich, China) in ethanol ($y = 0.31X + 0.01$, $R^2 = 0.9975$). The results were expressed as µmol of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent (TE) $g^{-1}$ oil and µmol TE $g^{-1}$ extract (db).

**FRAP**

The methodology of Benzie and Strain (1996) was followed. The FRAP (Ferric Reducing Antioxidant Power) reagent was prepared by mixing acetate buffer solution: TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) solution: Fe solution in the ratio 25: 2.5: 2.5. Trolox was used as a standard for 5 points of the calibration curve between concentrations of 50 to 600 µM ($y = 1.04x + 0.16$, $R^2 = 0.9980$), adding distilled water and FRAP reagent, letting it rest for 30 min, reading at 595 nm. Before reading, the sample was filtered with the 0.2 µm PTFE syringe filter. The results were expressed as µmol TE $g^{-1}$ oil and µmol TE $g^{-1}$ extract (db).

**Evaluation of antimicrobial activity**

The antimicrobial activity of oils and PFDC and PFDCE dry extracts was carried out with the disk diffusion technique in Mueller Hinton agar according to the methodologies of Gómez et al. (2015) and Cecchini et al. (2018).

The strains studied (*E. coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Klebsiella oxytoca* ATCC 700324, *Enterococcus faecalis* ATCC 29212, *Salmonella enterica* subsp*. enterica serovar typhimurium* ATCC 14028, *Staphylococcus aureus* subsp*. Aureus* ATTC 25923, *Staphylococos epidermidis* ATCC 12228, *Proteus vulgaris* ATCC 8427, *Shiguella flexeri* ATCC 12022) were incubated in brain heart infusion for 18 h at 37 °C; then these cultures were inoculated in 5 mL of 0.85% w/v saline solution. The concentrations were adjusted with the McFarland standard tube No. 0.5 (1.5 x $10^8$ CFU $mL^{-1}$). Subsequently, 100 µL of each culture was inoculated in Petri dishes with Mueller-Hinton agar, distributing it evenly with a Drigalsky spatula. In each plate, 3 wells of 6 mm diameter were made by perforating the agar with a sterile punch. 50 µL of each sample dissolved in dimethyl sulfoxide (DMSO) at a concentration of 30 mg of dry extract $mL^{-1}$ was placed in each well. DMSO was used as a negative control and Penicillin disc (10 µg) was used as a positive control. The plates were incubated at 37 °C for 24 h. Tests were performed in triplicate for each strain.

## RESULTS AND DISCUSSION

### Extraction yield and oil recovery of PF

The overall oil yield was 21.75±0.22% and 22.80±0.15%, with the SC-$CO_2$ and SC-$CO_2$+et techniques, respectively, with a significant difference ($P$<0.05) between both extraction techniques. The difference in yields can be attributed to the different solubilities of the solvents. The solubility of SC-$CO_2$ is comparable to that of organic solvents, which significantly enhances its solvation capacity; this phenomenon is due to the variations in the density of the fluid during the extraction of non-polar compounds (Abbas et al. 2008). While, when using the SC-$CO_2$+et technique, $CO_2$ is used and adding ethanol as a cosolvent increases the solubility of the compounds, resulting in greater efficiency in the extraction process, allowing not only the obtaining of non-polar products but also of polar compounds; this is attributed to the hydrogen bonding interactions between ethanol and polar solutes, which significantly improves the extraction yield (Chai et al. 2020). Furthermore, ethanol has been shown to be the most effective solvent for the extraction of polar compounds in supercritical $CO_2$ systems (Asep et al. 2013).

Pantoja-Chamorro et al. (2017) found a yield of 22.23% in dry passion fruit seed after 450 min of extraction with SC-$CO_2$ (275 bar and 50 °C), close to what was obtained in the present study for 240 min. Dos Santos et al. (2021) reported lower overall yield contents between 14.36 to 17.22% in passion fruit seed oil with SC-$CO_2$. The lower yield could be due to the origin of the sample studied (Dos Santos et al. 2021), among other factors.

The PF oil recovery was 90.98±0.93% and 95.37±0.63% with the SC-$CO_2$ and SC-$CO_2$+et techniques, respectively, based on the overall oil yield obtained with hexane (23.91±0.05%). Other authors also obtained recovery percentages lower than 100%. Antoniassi et al. (2022) reported a 28% yield of PF oil and lower recovery values of passion fruit seed oil (81.4 to 89.0%) obtained from different companies that process passion fruit juice in Brazil. Likewise, Reis et al. (2023) performed the extraction of 15% oil from *Passiflora cincinnata* seeds by pressing using petroleum ether and showed a lower recovery (79%) than in this study. On the other hand, Pereira et al. (2017) reported a higher overall yield in Brazilian passion fruit seeds obtained with hexane (28.33% db). This difference could be due to the usage of enzymes for complete removal in the separation of seeds from passion fruit pulp. In this study, an industrial byproduct of seeds was used after the complete extraction of the juice, so there was the presence of passion fruit pulp which could reduce the overall yield of oil extraction since the pulp does not contain oil in its composition.

### Fatty acid profile

The results show 86.58 and 86.51% unsaturated fatty acids, and 13.42 and 13.49% saturated fatty acids in oils obtained with SC-$CO_2$ and SC-$CO_2$+et, respectively (Table 1), which are higher in unsaturated and lower in saturated fatty acids compared to the values reported by Pantoja-Chamorro et al. (2017) for passion fruit seeds from Colombia (84% unsaturated and 15.45% saturated fatty acids). Reis et al. (2023) reported a higher palmitic (12.14±1.00%) and linoleic (78.34±2.22%), and lower stearic (1.09±0.26%) and oleic (8.43±1.32%) contents in *Passiflora cincinnata* seed oil (extracted in a continuous press) compared to this study. The difference in fatty acid contents could be attributed to the passion fruit variety and its growing area.

**Table 1.** Fatty acid content in (%), functional quality indices and induction period.

| Component | SC-$CO_2$ | SC-$CO_2$+et |
|---|---|---|
| C 14:0 (Myristic) | 0.06±0.01[a] | 0.06±0.0[a] |
| C 16:0 (Palmitic) | 10.23±0.01[a] | 10.36±0.06[a] |
| C 16:1 (Palmitoleic) | 0.14±0.0[a] | 0.14±0.01[a] |
| C 17:0 (Heptadecaenoic) | 0.07±0.01[a] | 0.07±0.0[a] |
| C 18:0 (Stearic) | 2.82±0.01[a] | 2.78±0.03[a] |
| C 18:1 w-9 (Oleic) | 13.99±0.01[a] | 13.8±0.21[a] |
| C 18:1 w-7 (Vaccenico) | 0.58±0.01[a] | 0.52±0.07[a] |
| C 18:2 w-6 (Linoleic) | 71.06±0.01[a] | 71.27±0.33[a] |
| C 18:3 w-3 ($\alpha$-Linolenic) | 0.47±0.01[a] | 0.46±0.01[a] |
| C 20:0 (Arachidic) | 0.18±0.0[a] | 0.17±0.0[b] |
| C 20:1 w-9 (Eicosaenoic) | 0.13±0.01[a] | 0.12±0.0[a] |
| C 24:0 (Lignoceric) | 0.06±0.0[a] | 0.05±0.01[a] |
| C 22:5 w-3 (Docosapentaenoic) | 0.09±0.01[a] | 0.07±0.0[a] |
| C 24:1 w-9 (Nervonic) | 0.12±0.0[b] | 0.13±0.0[a] |
| Saturated | 13.42±0.02[a] | 13.49±0.08[a] |
| Monounsaturated | 14.96±0.00[a] | 14.71±0.28[a] |
| Polyunsaturated | 71.62±0.01[a] | 71.80±0.34[a] |
| Unsaturated/saturated | 6.46±0.01[a] | 6.41±0.05[a] |
| AI | 0.121±0.00[a] | 0.123±0.00[a] |
| TI | 0.293±0.00[a] | 0.296±0.00[a] |
| H/h | 8.326±0.00[a] | 8.214± 0.00[a] |
| Induction period (min) | 40.53±0.63[a] | 89.05±0.42[b] |

Values are expressed as the mean ± standard deviation (n=2). Different letters within the same column indicate a significant difference ($P$<0.05) according to the t-test.

**Functional oil quality**

Passion fruit seed oils extracted with supercritical fluids showed AI and TI values, close to zero, being considered favorable in the prevention of coronary heart diseases (Pinto et al. 2020). Pham-Huy et al. (2008) state that the fatty acids present in passion fruit oil are essential for the prevention of cardiovascular diseases, the control of hypertension and the strengthening of the immune system. In fact, the consumption of foods with lower AI is associated with a reduction in total and LDL cholesterol levels in human blood plasma; and the consumption of foods with a lower TI is beneficial for cardiovascular health (Chen and Liu 2020).

There are few reports of studies on functional oil quality indices, one of them is from Barriga-Sánchez et al. (2021), who reported AI (0.20) and TI (0.23) values in *Vitis labrusca* grape seed oil; and Santos et al. (2021), who reported AI (0.16) and TI (0.40) values in passion fruit oil extracted by Soxhlet. The observed differences in values compared to the present study can be attributed to the type of extraction method used. Conventional techniques like Soxhlet employ organic solvents, which, while effective, can degrade the oil during solvent removal. This degradation can impact the fatty acid composition and, consequently, the quality indices of the oil. Therefore, it is crucial to use appropriate technologies for oil extraction and avoid its degradation.

On the other hand, low values of the H/h ratio are considered unfavorable and can induce an increase in cholesterolemia (Santos-Silva et al. 2002). The H/h ratio values of passion fruit seed oil obtained with both methods presented lower values than camelina oil (11.2 to 15.0) reported by Ratusz et al. (2018), but higher than

in passion fruit seed oil reported by Santos et al. (2021) (H/h=6.03); this difference is due to the extraction technique used. It is important to determine the quality indices since they are related to growth, development, maintenance of various functions of human metabolism and the promotion of good health (Santos et al. 2021).

**Induction time**

The induction time of the oils was measured to evaluate their oxidative stability and compare the stability of both oil samples. Despite having a similar fatty acid profile, the oil extracted with SC-$CO_2$+et presented a higher induction time value (Table 1). The shorter induction time of SC-$CO_2$ oil (40.53 min) suggests it may be more susceptible to oxidation compared to the SC-$CO_2$+ethanol oil (89.05 min). Consequently, the oil extracted with SC-$CO_2$+ethanol can be considered more stable, likely due to the presence of polar compounds such as phenols, which contribute antioxidant properties and help extend the oil's shelf life.

Other studies, such as Lau et al. (2006) reported a similar trend in palm-pressed fiber oils where the oil obtained with SC-$CO_2$+et presented greater oxidative stability measured by Rancimat than the oil extracted with SC-$CO_2$. Furthermore, Reis et al. (2020) found longer induction times at 110 °C for *Passiflora alata* (3.52 h), *Passiflora setacea* (7.32 h) and *Passiflora tenuifila* (6.87 h). This could be attributed to the differences in the variety studied, as the fatty acid content differs depending on the plant variety. It is known that the double bonds of linoleic acid are 40 times more unstable than those of oleic acid, which has a single bond (Damodaran and Parkin 2017).

**TPC and Antioxidant capacity of oil and extracts**

The oil extracted with SC-$CO_2$+et showed higher TPC content and antioxidant capacity, as measured by both the ABTS and FRAP assays (Table 2), compared to the oil obtained using SC-$CO_2$ in this study. These results were also higher than those reported by Ribeiro et al. (2020), who extracted oil using pressurized ethanol, obtaining 8.54 mg GAE $g^{-1}$ and an ABTS antioxidant capacity of 18.2 µmol TE $g^{-1}$. Furthermore, it was also higher than the ABTS antioxidant capacity of 0.73 µmol TE $g^{-1}$ obtained through Soxhlet extraction with diethyl ether in Brazilian *Passiflora* seed oils (de Santana et al. 2015). This difference can be attributed to the use of ethanol as a cosolvent in the extraction with supercritical $CO_2$, which improves the solubility of polyphenols (Rahal et al. 2015). Likewise, when a higher TPC content is present, it allows greater electron donation and greater synergistic antioxidant capacity on ABTS free radicals (Purohit et al. 2021) and the ability of the antioxidants present in the oil to reduce the ferric ion to its ferrous form (Pereira et al. 2017).

In general, fruit seeds, often considered byproducts of juice processing, contain oil rich in bioactive compounds such as tocopherols, carotenoids, flavonoids, phenolic acids, and phytosterols (Kaseke et al. 2020). These compounds are responsible for the antioxidant properties of the oil.

**Table 2.** TPC and antioxidant capacity of passion fruit seed oil and defatted passion fruit seed extract with $CO_2$ (PFDC) and $CO_2$+ethanol (PFDCE).

| Sample | TPC (mg GAE 100 $g^{-1}$) | ABTS (µmol TE $g^{-1}$) | FRAP (µmol TE $g^{-1}$) |
|---|---|---|---|
| Oil obtained by SC-$CO_2$ | 65.92±1.78$^{a}$ | 16.39±0.02$^{a}$ | 41.41±0.15$^{a}$ |
| Oil obtained by SC-$CO_2$+et | 251.78±1.07$^{b}$ | 21.83±0.06$^{b}$ | 63.43±1.54$^{b}$ |
| PFDC extract | 1049.66±28.92$^{A}$ | 80.47±1.19$^{A}$ | 75.14±4.07$^{A}$ |
| PFDCE extract | 1243.80±62.45$^{B}$ | 117.79±5.65$^{B}$ | 111.40±3.53$^{B}$ |

Values are expressed as the mean ± standard deviation (n=2). Different lowercase letters within the same column indicate a significant difference ($P$<0.05) according to the Tukey test. Different capital letters within the same column indicate a significant difference ($P$<0.05) according to the Tukey test.

PFDCE extract presents higher values of TPC and antioxidant capacity (Table 2) than PFDC extract ($P$<0.05). Malik et al. (2023) reported a value of 19,360 mg EAG 100 $g^{-1}$ TPC of defatted sample in acetone extract and found improved solubility in contrast to the ethanol reported in this study. Da Costa et al. (2023) evaluated the extract (50% acetone) of purple passion fruit seed and reported 758.43±7.79 mg EAG 100 $g^{-1}$ and 125.207 µmol $g^{-1}$ for TPC

and ABTS, respectively. Reis et al. (2023), after optimizing ethanol concentration and solid-liquid ratio for extracting antioxidant compounds from defatted *Passiflora cincinnata* seeds, reported higher values of 2,868 mg GAE 100 $g^{-1}$ and ABTS antioxidant capacity (195 µmol TE $g^{-1}$) than those found in the present study. The higher ABTS and FRAP values in both SC-$CO_2$+et oil and PFDCE extract may be attributed to the increased total phenolic content. In a previous study, Reis et al. (2020) also reported lower TPC values in ethanolic extracts from *Passiflora alata* and *Passiflora tenuifila* seeds but higher ABTS values.

**Antimicrobial activity**

The antimicrobial activity of the passion fruit seed oil and defatted passion fruit seed dry extract samples against gram (+) and gram (-) bacterial strains is shown in Table 3. The results show that the passion fruit seed oil extracted with SC-$CO_2$ and SC-$CO_2$+et presented antimicrobial activity against the bacteria *Escherichia coli, Klebsiella oxytoca, Salmonella enterica, Staphylococcus aureus* and *Proteus vulgaris* with larger halo sizes for the oil obtained with SC-$CO_2$+et, this positive effect would be due to the composition of fatty acids, antioxidants and flavonoids of passion fruit oils (Purohit et al. 2021).

Pereira et al. (2018) evaluated the antibacterial effect of passion fruit seed oil extracted by Soxhlet against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella enteritidis*. Their results demonstrated the oil's antimicrobial effect on these bacteria, which is consistent with the findings of the present study. It is important to mention that the oils did not register an effect on the microorganisms *Enterococcus faecalis* and *Staphylococcus epidermidis*, possibly due to the resistance of the cell walls of these bacteria, which prevented the penetration of the oil (Fathi-Achachlouei et al. 2020). PFDC and PFDCE dry extracts showed greater antimicrobial activity against the bacteria *Klebsiella oxytoca, Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis* and *Proteus vulgaris*. The greatest halo of inhibition was formed by PFDC dry extract.

Noguera-Machado et al. (2017) evaluated the antibacterial potential of ethanolic extracts of *Passiflora edulis* seeds, showing the greatest antibacterial potential on strains of *S. aureus* and *E. coli*, with a bactericidal effect at concentrations of 11.7 and 9.4 mg $mL^{-1}$, lower concentrations than those analyzed in this study. PFDC and PFDCE dry extracts at a concentration of 30 mg $mL^{-1}$ showed antimicrobial activity against *Staphylococcus aureus* with a halo size of 17 and 15 mm, respectively, which is consistent with that reported by Nugraha et al. (2018), who evaluated the antibacterial effect of the ethyl fraction of the peel of *Passiflora edulis* against S*taphylococcus aureus*, finding inhibition zones of 14.23, 19.53 and 20.43 mm for concentrations of 100, 400 and 500 mg $mL^{-1}$, respectively. The antibacterial activity of the defatted passion fruit extracts could be attributed to the effect of phenolic compounds and the effectiveness of the solvent used to recover the highest amount of polyphenols (Ramaiya et al. 2014).

**Table 3.** Antimicrobial activity in passion fruit seed oils and defatted passion fruit seed extracts.

| Bacteria | Halo of inhibition (mm) | | | |
|---|---|---|---|---|
| | Oil | | Extract* | |
| | SC-$CO_2$ | SC-$CO_2$+et | PFDC | PFDCE |
| *Escherichia coli* | 7 | 9 | 0 | 0 |
| *Klebsiella oxytoca* | 7 | 10 | 17 | 12 |
| *Enterococos faecalis* | 0 | 0 | 17 | 10 |
| *Salmonella enterica* | 9 | 11 | 0 | 0 |
| *Staphylococcus aureus* | 8 | 10 | 17 | 15 |
| *Staphylococcus epidermidis* | 0 | 0 | 18 | 16 |
| *Proteus vulgaris* | 7 | 9 | 16 | 14 |

PFDC: Defatted passion fruit seed extract with $CO_2$.
PFDCE: Defatted passion fruit seed extract with $CO_2$ ethanol.
*: 30 mg of dry extract $mL^{-1}$ DMSO.

## CONCLUSION

The present study showed that the fatty acid profile of the oils obtained with SC-$CO_2$ and SC-$CO_2$+et did not present a significant difference and that the functional quality indices (AI, TI and H/h) of the passion fruit seed oils obtained by the two techniques are notable. However, the oil obtained with SC-$CO_2$+et, as well as the ethanolic extract of defatted passion fruit seed, presented a higher content of phenolic compounds and antioxidant activity. Furthermore, the oil extracted using SC-$CO_2$+et exhibited greater oxidative stability compared to the oil obtained through SC-$CO_2$ alone. The antimicrobial activity of the oil against pathogens of interest to the food industry was demonstrated, as well as that of the dry extracts obtained from defatted flours. This research provides valuable insights for the food industry, particularly for those seeking to repurpose waste materials into new products with functional properties.

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## CONFLICT OF INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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**Functional characteristics and antimicrobial activity of supercritical $CO_2$ extracts from passion fruit (Passiflora edulis) seeds**

**Características funcionales y actividad antimicrobiana de extractos de $CO_2$ supercrítico de semillas de maracuyá (Passiflora edulis)**

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