

## Physical and Chemical Properties of oils of three varieties seeds of passion fruit: *Passiflora alata* Curtis, *Passiflora edulis* f. *flavicarpa* and *Passiflora quadrangularis*

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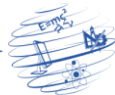
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**Abstract.** This paper reports the physicochemical characteristics of the seed oils from different varieties of passion fruit (*Passiflora alata* Curtis, *Passiflora edulis* f. *flavicarpa* and *Passiflora quadrangularis*) cultivated in Brazil, Roraima. The oil from passion fruit, within the range of 19.29±0.02; 21.34±0.22 e 14.24±0.16%, respectively. The physicochemical characteristics of the extracted oils were: free fatty acid contents (0.84±0.01 - 2.73±0.05 % mg KOH g<sup>-1</sup> as oleic acid), iodine value (101.63±0.18 - 125.96±0.13 g of I<sub>2</sub> 100 g<sup>-1</sup> of oil), and saponification index (90.56±0.32 - 179.06±0.19 mg KOH g<sup>-1</sup> of oil). The oils revealed a reasonable oxidative parameter range as depicted by the determinations of index peroxide value (1.92±0.09 - 3.05±0.03 meq O<sub>2</sub> kg<sup>-1</sup> of oil). Linoleic acid was the major fatty acid found in all the seed oils with contributions of 55.75-63.42% of the total fatty acids (FA). Other fatty acids detected were known to be oleic acid (19.3-20.1%), palmitic acid (10.8-12.8%) and stearic acid (3.25-4.25%). Through the DPPH test we observed the presence of antioxidants in the three oil samples. The results of the present study indicate that the seeds of the tested passion fruit varieties from Roraima are a potential source of high-linoleic oil and thus can be explored for commercial use and value addition.

**Resumo.** O presente manuscrito apresenta as características físico-químicas dos óleos das sementes de três espécies de maracujá cultivadas no estado de Roraima, Brasil: *Passiflora alata* Curtis, *Passiflora edulis* f. *flavicarpa* e *Passiflora quadrangularis*. Os rendimentos obtidos para a extração dos óleos das três espécies de maracujá foram de 19,29±0,02, 21,34±0,22 e 14,24±0,16%, respectivamente. Os óleos extraídos apresentaram um índice de acidez em ácido oléico variando de 0,84±0,01 a 2,73±0,05 %, índice de iodo na faixa de 101,63±0,18 a 125,96±0,13g de I<sub>2</sub> para cada 100 g de óleo e



*índice de saponificação no intervalo de  $90,56 \pm 0,32$  a  $179,06 \pm 0,19$  mg de KOH por grama de óleo. Os óleos apresentaram uma capacidade oxidativa razoável descrita pelos valores do índice de peróxido ( $1,92 \pm 0,09$  -  $3,05 \pm 0,03$  meq  $O_2$  por kg de óleo). O ácido linoléico foi a espécie majoritária encontrada em todos os óleos das sementes cujas concentrações variam de 55,75 a 63,42 %. Outros ácidos como o oléico (19,3 – 20,1%), palmítico (10,8 – 12,8%) e esteárico (3,25 – 4,25%) também foram detectados. A análise de DPPH revelou a presença de altos teores de antioxidantes em todas as amostras. Os resultados apresentados nesse estudo indicam que as três espécies de maracujá cultivadas no Estado de Roraima podem ser consideradas como fontes potenciais em ácido linoléico além de apresentarem um significativo valor comercial agregado.*

## 1. Introduction

In Brazil, the production of concentrated passion fruit juice almost triplicated in recent years [1]. Passion fruit is the popular name for many different species from *Passiflora* genus, a very common fruit in the tropical and subtropical regions of the globe [2]. There are over 500 species worldwide of *Passiflora* in the *Passifloraceae* family [3]. Around 120 species of *Passiflora* are native to Brazil, sixty of which produce fruits that could be direct or indirectly used as food sources [4]. The most cultivated varieties is the yellow passionfruit (*P. edulis*) due to higher industrial productivity and fruit quality, but the *P. alata* and *P. quadrangularis* varieties have potential for the production of juices [5].

The worldwide consumption of natural products such as fruit juice has contributed to increased fruit production in Brazil. Seeds are subproducts in addition to peels (raw materials for passion fruit juice). The amount of waste discarded to the environment is becoming an increasing environmental problem [6]. The passion seeds are usually discarded as a byproduct after extraction of the juice from the fruit. However, some conducted studies suggest that the passion fruit seeds are rich in oil. The passion fruit seed oil contains a great quantity of the unsaturated fatty acids, such as oleic acid (omega 3) and linoleic acid (omega 6) [7]. Omega 6 and Omega 3 fatty acids are essential, wherein both humans and other mammals they are unable to synthesize, and must obtain them from diet [8]. They are essential precursors for fatty acids metabolic and structural functions for human.

The quantity and composition of fatty acids varies significantly within oils from different plant sources, predominantly depending on the variety, but also on the state of ripeness, the area in which the plants are grown, climate conditions, etc [9]. Therefore, the passion fruit seeds would be novel sources of premium-grade edible oils. The objectives of this study were to employ soxhlet technique to extract oil from passion fruit seed, to determinate physical and chemical properties, to determinate the fatty acids composition of the extracted oil using gas chromatographic (GC), and to evaluate the antioxidant activity of the extracted oils by 2,2-diiphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay.

## 2. Material and Methods

### 2.1 Sample preparation

The samples of seeds of three or varieties of passion fruit, from the state of Roraima, Brazil, were dehydrated in a tray drier with the air flow temperature fixed at 55 °C for 48 h. The dehydrated residues were ground in a knife mill equipped with a 20 Mesh sieve, and subsequently analyzed chemically and submitted to oil extraction. The residues consisted mainly of the seeds. The Figure 1 presents the passion fruits species employed.



Figure 1 – Passion fruits species: (A) *P. quadrangularis*, (B) *P. alata* and (C) *P. edulis*.

### 2.2 Extraction of the oil from three varieties seeds passion fruit

The Soxhlet method was selected as conventional extraction technique, using hexane as solvent, which is adequate for oil extraction due to its non-polar characteristic. Reflux was kept for 6 h, then the solvent was evaporated under vacuum and the recovered extract was weighed and stored under freezing until further analyses. The Soxhlet extractions were performed in triplicates [10]. Equation 1 was used to calculate the oil yield.

$$\text{Oil (\%)} = \frac{\text{oil weight (g)}}{\text{sample weight (g)}} \times 100 \quad (1)$$

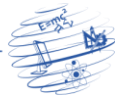
Samples of the oils were stored in amber bottle under atmosphere of nitrogen gas and stored in a freezer at - 4 °C for further analysis.

### 2.3 Characterization of the oil extracted from three varieties seeds passion fruit

#### 2.3.1 Chemical and physical parameters

The oil extracted from three varieties the passion fruit was characterized how much acid index (mg KOH/g), saponification index (mg KOH/g), iodine index (g I<sub>2</sub>/100 g), peroxide index (meq of O<sub>2</sub>/kg), relative density, and refractive index (40 °C) according to AOCS methodology [11].

The acid index indicates the rate oil conservation. The analysis was performed by adding 25 ml of ether-alcohol solution (2: 1) in 2 g of sample. The mixture was stirred



and titrated with KOH 0.1 M to pinkish using phenolphthalein indicator. The acid index was calculated from equation 2.

$$\text{Acid index (as oleic acid)}(\%) = \frac{V \times C \times 282}{m} \times 100 \quad (2)$$

where: V (L) is the KOH 0.1 M spent in the titration, C - KOH concentration, 282 oleic acid molecular mass ( $\text{g mol}^{-1}$ ) and m (g) is the sample weight.

The saponification index indicates the presence of low molecular weight oils that comprise the fatty acids. The analysis was performed by adding 25 ml of KOH alcoholic 4 % m/v in 2 g of sample. The mixture was stirred and heated in a suitable condenser system for 30 min and then titrated with HCl 0.5 M solution using phenolphthalein indicator. The saponification index was calculated from equation 3.

$$\text{Saponification index} = \frac{(B - A) \times C \times (5.61 \times 10^4)}{m} \quad (3)$$

where: A (L) HCl 0.5 M dispense volume, B (L) HCl 0.5 M dispense volume in the white titration, C is the HCl concentration,  $5.61 \times 10^4$  KOH molecular mass ( $\text{mg mol}^{-1}$ ) and m (g) is the sample weight.

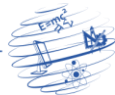
Iodine index is a measure of the unsaturation oils and fats. This parameter was determinate using iodine and mercury chloride solutions [11] and the calculations were made using the equation 4.

$$\text{Iodine index} = \frac{(Vb - Va) \times C \times 126.9 \times 100}{m} \quad (4)$$

where: Va (L) -  $\text{Na}_2\text{S}_2\text{O}_3$  0.1 M solution expense volume for white titration, Vb (L) -  $\text{Na}_2\text{S}_2\text{O}_3$  0.1 M solution expense volume for sample titration, C -  $\text{Na}_2\text{S}_2\text{O}_3$  concentration, m (g) - sample weight and 126.9 is the iodine molecular mass ( $\text{g mol}^{-1}$ ).

Peroxide index indicates oxidation oils degree. High peroxide values indicate that, somehow, the oil was exposed to oxidative process either during the preparation of the raw material, extraction or oil storage<sup>12</sup>. 30 mL of acetic acid - chloroform (3: 2) solution was added to 5 g of the sample. It was stirred until sample complete dissolution and then was added 0.5 mL potassium iodide a saturated solution. The sample was titrated with thiosulphate sodium 0.01 M using starch as indicator. The peroxide index was calculated from equation 5.

$$\text{Peroxide index} = \frac{(A - B) \times C}{m} \quad (5)$$



where: A (L) - Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> 0.01 M solution expense volume for sample titration, V<sub>b</sub> (L) - Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> 0.01 M solution expense volume for white titration, C - Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> concentration and m (g) is the sample weight.

The relative density was determined by pycnometer method at 25 °C using distilled water as a reference liquid. The relative density was calculated from the equation 6.

$$\text{Density at 25 °C} = \frac{A}{B} \times D_{H_2O} \quad (6)$$

where: A (g) - sample weight, B (g) – water mass and D H<sub>2</sub>O is the water density at 25 °C: 0.997071 g cm<sup>-3</sup>.

The refractive index is characteristic for each type of oil to be directly related to the bond saturation degree. For this determination we used the Analytik Jena Abbé refractometer system using water circulation at 40 °C. The reading was done at 40 °C.

### 2.3.2 Determination of fatty acid (FA) composition by gas chromatography (GC)

FA composition of the extracts was determined by GC. Prior to chromatographic analysis, the oils were prepared in the form of fatty acid methyl esters (FAME) according to the method of Hartman and Lago [12]. The chromatographic analyses were performed using HP5890 gas chromatograph equipped with flame ionization detector. Econocap used a carbowax column (Alltech) 30m x 0.32 mm with temperature gradient: 120 °C, 1min, 7 °C/min to 240 °C; gun (split of 1/50) to 250 °C and 250 °C detector. Hydrogen as a carrier gas (2 mL min<sup>-1</sup>) and injection volume 1 µL. Samples were diluted in methanol at a concentration of 1%. The fatty acid methyl esters were identified by comparison with external standard (Supelco 37). Quantification was performed by internal normalization.

### 2.3.3 Antioxidant activity

This measure indicates the total antioxidant capacity which will help understand the samples functional properties (Marxen et al., 2007). The *in vitro* antioxidant activity of the oils was determined by the DPPH (2,2-Diphenyl-1-picryl-hidrazil) method. For this determination were prepared solutions containing the sample at concentrations of 10, 20, 40 and 90 mg per mL which contained in 1 mL of sodium acetate buffer 0.1M pH 5.5, 1 mL of ethanol, 0.5 mL DPPH alcoholic solution, with manual stirring for 30 s. The mixtures were let reacting in the dark at room temperature (25 °C) for 30 min as a control was used 1 mL of buffer solution and 1 mL of ethanol and 0.5 mL of DPPH. Then, the absorbance of the mixture was measured at a wavelength of 517 nm in a UV–VIS spectrophotometer (Hach, DR/4000U), using 1 mL of buffer solution and 1.5 mL of ethanol as blank. Using the values of the absorbance standard curve, the antioxidant activity of the oils was obtained.

The Figure 2 summarizes the analysis performed with passion fruits.

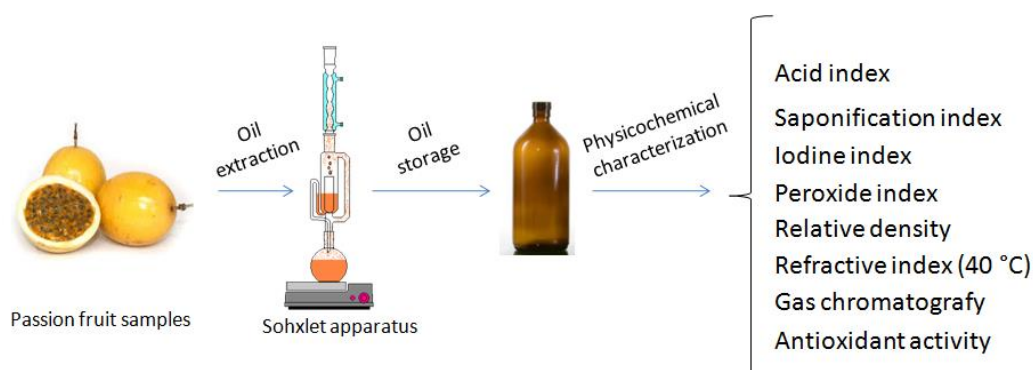


Figure 2 - Summary of the characterization of passion fruits.

### 3. Results and discussion

The three species fruits studied presented different mass ratio, peel, seeds and juices (Table 1).

Table 1 – Fruit composition (%)

Samples	Shell ratio (%)	Seed ratio (%)	Juice ratio (%)	Complete fruit (g)
<i>P. edulis</i>	60.72±0.12	5.43±0.26	33.85±0.09	168.42
<i>P. quadrangularis</i>	91.37±0.43	1.67±0.10	6.96±0.13	918.33
<i>P. alata</i>	66.86±0.20	6.76±0.30	26.38±0.23	346.67

The *P. quadrangularis* fruits were those with the highest mass and shells proportion, however, when compared to the *P. alata* and *P. edulis* there was a lower seeds and juices proportion. The fresh shell is comestible and has sweet slightly acidic flavor. The color of the passion fruit seed oil extracted by Soxhlet was yellow (Figure 3).

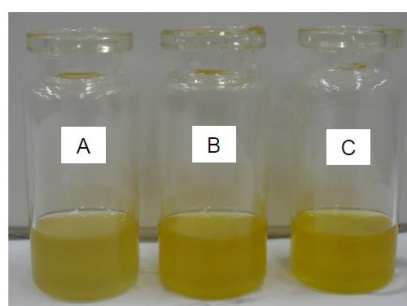
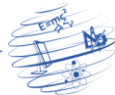


Figure 3 – Passion fruit oils: A - *P. alata*, B - *P. edulis* and C - *P. quadrangularis*.

The state of oil was liquid at room temperature. In the present study, a yield of  $21.34 \pm 0.02$  %,  $19.29 \pm 0.22$ % and  $14.24 \pm 0.16$ % for *P. edulis*, *P. alata* and *P. quadrangularis*,





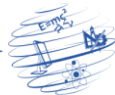
respectively, was obtained for the extraction of oil from passion fruit seeds. Those values they are higher which the reported in the literature that mention a maximum of 25.7 % [13]. This yield may be associated with the soil and climatic conditions of the cultivated areas. Table 2 shows the mean values obtained for the chemical and physical parameters of the oils of seeds passion fruit.

Table 2 - Values for the chemical and physical parameters of the oils extracted from seeds three varieties of the passion fruit.

Parameters	<i>P. edulis</i>	<i>P. quadrangularis</i>	<i>P. alata</i>
Acidity index (mg KOH/g)	0.84±0.01	2.53±0.05	1.57±0.02
Saponification index (mg KOH/g)	179.06±0.19	90.56±0.32	135.48±0.23
Peroxide index meq of O <sub>2</sub> /Kg	3.05±0.03	1.92±0.09	2.41±0.20
Iodine index gI/100g	125.96±0.13	101.63±0.18	105.28±0.03
Density (25°C)	0.90±0.01	0.91±0.02	0.91±0.02
Refractive index (40°C)	1.471±0.04	1.470±0.01	1.471±0.02

The determination of acidity index is an important data on the condition of the oil conservation and the peroxide value is used as an indicator of lipid oxidation. The Codex Alimentarius Commission determines the maximum values of the acidity index of 4.0 mg KOH g<sup>-1</sup> and between 10 and 15 meq O<sub>2</sub> kg<sup>-1</sup> for peroxide index as quality parameters [14]. The values shown for the acidity and peroxide index of the oils from passion fruit seeds in Table 2 is much lower than the maximum limit of 4.0 mg KOH/g and 10 meq of O<sub>2</sub>/Kg, for cold pressed crude oils by recommended Codex Alimentarius Commission indicating that the studied oils presented good conservation and can be exposed to oxidative process during the preparation of the raw material, extraction or oil storage, since they present low oxidative capacity [5].

The iodine index is related to the amount of double bonds present in the oils (unsaturation degree) [15]. It is used to control the oils hydrogenation which reflects the susceptibility of the oil to oxidation [15]. The iodine index of 125.96±0.13, 101.63±0.18 and 105.28±0.03 I<sub>2</sub>/100 g for *P. edulis*, *P. alata* and *P. quadrangularis*, respectively, found for the passion fruit seeds oils in the present study (Table 2) was similar to that obtained by Ferreira et al. to study the physical and chemical parameters of passion fruit oil (Soxhlet extraction and Cold pressed) [16]. Oils with iodine index above 100, as the species of this work, can be considered as semi-drying and indicates the presence of high percentage of unsaturated fatty acids in the seed [15]. Thus, they are samples that exhibit low susceptibility to oxidative rancidity and can be used as industrial raw material (in the production of ice-cream, e.g.) [15, 17].



The saponification index also indicates deterioration of the oils [15]. The values found for the saponification index found for the seeds passion fruit oils in the present study (varying between  $90.56 \pm 0.32$  to  $179.06 \pm 0.19$  mg KOH/g) were similar to reported by Kobori and Jorge (174.97 mg KOH/g) and Silva et al. ( $179.06 \pm 0.19$  mg KOH/g) [15,5]. For *P. edulis* while *P. alata* and *P. quadrangularis* varieties had lower values, however all values were consistent with those found for the conventional oils in accordance with the Codex Alimentarius Commission.

The Refractive index found for the three varieties seeds passion fruit oils in the present study, varying between 1.470 to 1.471, were similar to that reported by Malacrida and Jorge (1.4682) [19]. These values are acceptable according to Codex Alimentarius Commission.

In general, the parameters presented in Table 2 suggest that the oils obtained from the passion fruit residues shows the potential for use as a comestible ingredient, for cleaning and cosmetic products, such as soaps and shampoos, since they have good quality parameters.

### 3.2 Fatty acids composition

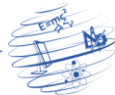
The quantity of free fatty acid in oil may also be an indicator of overall quality [15]. Table 3 presents results obtained fatty acid composition for the three varieties oils of passion fruit seeds by gas chromatography–mass spectrometry (GC–MS).

Table 3 - Fatty acids composition for the three varieties oils of passion fruit seeds extracted by gas chromatography - mass spectrometry (GC–MS).

Fatty acid (%)	<i>P. alata</i>	<i>P. edulis</i>	<i>P. quadrangularis</i>
Palmitic	10.77	11.08	12.81
Stearic	3.25	4.25	4.12
Arachidonic	0.29	0.15	0.30
Palmitoleic	0.68	0.36	1.44
Oleic	20.11	19.32	24.61
Linoleic	63.42	60.62	55.75
Linolenic	0.55	3.46	0.56
Others	0.94	0.55	0.42
Saturated	14.31	15.7	17.3
Unsaturated	85.70	84.31	82.78

The most abundant fatty acids were linoleic (18:2), from 55 to 63%, oleic (18:1), from 19 to 24%, palmitic (C16:0), about 11% and stearic (18:0), from 3 to 4%. The total amount of unsaturated fatty acids was 85.70% having the linoleic acid as the main





component. Regarding Soxhlet extraction, the main components were oleic and linoleic acids with average percentages of around 24.0% and 63.0%, respectively (Table 3). The qualities of edible vegetable oils are determined by the amount and composition of unsaturated fatty acids. The presence of linoleic acid in adequate amounts is essential, since it is an essential fatty acid. The greater the amount of linoleic acid compared to oleic acid, the better the quality of the vegetable oil to prevent the formation of bad cholesterol (LDL) [20]. These values do not differ significantly from the amounts obtained by Ferrari et al. [13] and Piombo et al. [21] to study the chemical composition of passion fruit seed oil *P. edulis* varieties. Accordance with Aremu et al. [15], industrial applications are based on the properties of fatty-acid components of oils. For example, the presences of high levels of free fatty acids especially linoleic acids, such as the case of the samples of this study, are undesirable in finished oils because they can cause off-favours and shorten the shelf life of oils.

### 3.3 Antioxidant activity

DPPH is a free radical compound and has been used to test the free radical-scavenging ability of various samples. It is a stable free radical with a characteristic absorption at 517 nm and was used to study the radical-scavenging effects of oils. Passion fruit seeds oils using both methods showed concentration-dependent scavenging of the DPPH free radical with concentrations ranging from  $1.83 \pm 0.06$  mg/mL  $3.08 \pm 0.13$  mg/mL and  $4.17 \pm 0.21$  mg/mL for *P. edulis*, *P. alata* and *P. quadrangularis*, respectively. These values confirm the antioxidant capacity of the fruits and it is important to reduce the incidence of disease when the fruit is consumed.

## 4 Conclusions

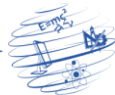
The total yield of passion fruit seed oils by Soxhlet extraction was 14 a 21%. As to the fatty acid profile in the three varieties passion fruit seeds oils, the main components in the all extracts were oleic and linoleic acid. The seeds passion fruit oils presented antioxidant activity. The inhibition concentration varied from 1.83 to 4.17%. Therefore, in view of the results, passion fruit is an important source of both unsaturated fatty acids and natural antioxidants, confirming its role as an important oleaginous crop for human nutrition. Moreover, passion fruit seeds and their oil are potential raw material industry, as they have physicochemical favorable characteristics.

## Acknowledgement

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