JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 25(12), 146–156
https://doi.org/10.12911/22998993/194130
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Received: 2024.09.13
Accepted: 2024.10.20
Published: 2024.11.01

Performance of Passion Fruit (*Passiflora edulis*) Seed Extracts under Solvent Percolation Technique

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ABSTRACT

Passion fruit (*Passiflora edulis*) seed extract is a valuable source of antioxidants with anti-aging potential, but extraction efficiency varies according to the method used. This study focused on determining the optimum yield of passion fruit seed extracts by percolation with n-hexane and ethanol, following regulations such as NTE INEN and Codex Alimentarius. Standardized methods were applied to analyze parameters such as moisture (UNE-EN 14774-3, 2010), ash (Sluiter et al., 2005); free acidity (ac. oleic acidity)/acidity index (NTE INEN 38, 1973), relative density (NTE INEN 35, 1973), loss on heating (NTE INEN 39, 1973), iodine index (NTE INEN 37, 1973), peroxide index (NTE INEN 277,1978), extract yield, fatty acid profile (AOAC 996.06, 2005). Ethanol showed superior performance in most of the parameters, meeting the established standards, except for relative density and heating loss, where n-hexane was more efficient. In conclusion, the use of ethanol is more beneficial for the extraction of passion fruit seed extracts by percolation, based on the yield obtained.

Keywords: extract; percolation; passion fruit; yield; standards.

INTRODUCTION

According to Carvajal de Pabón et al. (2011), the genus Passiflora, comprises about 450 species; these are distributed in temperate and tropical regions of the New World, they are much rarer in Asia, Australia and tropical Africa. Research by Arias-Suárez et al. (2014), mentions that passion fruit (*Passiflora edulis f. flavicarpa Degener*) is the main species of the genus, due to the fact that its fruits are traded in national and international markets as fresh and processed fruit. It is important to highlight the study by Franco et al. (2014), where it is described that passionflower species receive different uses; in a review of the genus related to morphology, microscopy, traditional uses, phytoconstituents, pharmacology, applications in medicine, and toxicology, was found in passion fruit (*Passiflora edulis Sims*), the presence of glycosides, phenols, alkaloids, carotenoids, L-ascorbic acid, anthocyanins, lactones, aromas, essential oils, amino acids, carbohydrates, minerals, enzymes and triterpenes. As well as the research of Sabogal-Palma et al. (2016), it is considered that based on the fact that these compounds in passionflowers have been found to have multiple biological functionalities, this genus possesses antibacterial, insecticidal, sedative, anti-aging and antispasmodic properties. A significant amount of vitamin C, carbohydrates and reducing sugars were observed in the barks, while the total protein content predominated in the seeds (Purohit et al., 2021). Passion fruit (*Passiflora edulis*) is one of more than 500 species in the genus Passiflora and the family Passifloraceae. Passion fruit is a woody vine that grows perennially and produces round or ovoid fruits (Gupta et al., 2022). As noted by Kawakami et al. (2021), numerous studies have explored the physiological properties of *Passiflora edulis* seeds, which are high in polyphenols and possess significant antioxidant activity. For skincare, extracts from *P. edulis* seeds offer protective benefits, such as enhancing collagen production and boosting intracellular antioxidant levels. Additionally, human trials have demonstrated that consuming the seed extract improves skin hydration and elasticity. Similarly, Rodriguez et al. (2021) have reported. The research highlights the potential of Passifloras as a source of extracts with antihypertensive capacity, having as possible mechanisms of action the inhibition or antagonism of angiotensin-II. According to Burgos et al. (2023), poor environmental management of solid waste is currently a serious problem, which not only affects the environment, but also the health of the community, including ineffective management policies, which include generation, inadequate treatment, inadequate isolation and lack of technology for the treatment and final disposal of waste. It is estimated that passion fruit processing residues reach between 61–86% of the amount of fruit processed, which can be used to obtain products of interest in the industry, generating added value and mitigating environmental pollution (Pantoja et al., 2017). According to Burgos et al (2023a) extraction is considered an important step in the isolation of bioactive compounds from plant material, so to obtain good results it is vital to choose a suitable extraction method. While, for Proaño et al. (2020), they indicate that passion fruit seeds contain an appreciable amount of oil that varies between 16.7-33.5 % depending on the extraction methodology, the type of solvent used, the operating conditions and the geographical area where the fruit is grown. According to Casas-Cardoso et al. (2021), due to current environmental concerns, environmentally friendly extraction and fractionation processes should be applied with the least possible use of auxiliary chemicals, so ethanol was chosen among the solvents used. Based on the above, the objective of this research is to compare passion fruit (Passiflora edulis) seed extracts using the solvent percolation technique to establish the best extract yield.

MATERIAL AND METHODS

Sample selection

Passion fruit seeds for the experiment were extracted from fruits from the Danzarín site (-0.9286764072501543, -80.35111690644501) in the Rocafuerte canton of the Province of Manabí, Republic of Ecuador.

Preparation of extracts

The present study consisted of providing a sustainable alternative and an added value on the use of passion fruit seeds as part of their residues, which stands out in obtaining extracts by applying the percolation method with the use of solvents, specifically ethanol and n-hexane. This allowed the improvement of the extraction process through the efficiency of the yields of passion fruit seed extracts, specifically through the comparison of these solvents in terms of their parameters and quantity obtained, visualizing the best performance among them. Based on the research of Intriago and Burgos (2021), where the operating conditions of the extracts within the operation process implied taking into consideration the boiling temperatures of the solvents involved, given that when using two solvents such as ethanol and n-hexane, in which their boiling points are different, being for n-hexane, an operating temperature between 69 °C and 75 °C, while for ethanol the temperature should be equal to or higher than 78 °C. When referring to the volume of solvent used inside the flat-bottomed balloons, regardless of the type of solvent used, it is 200 ml, which represents ¾ of the total volume of the balloon. The operation time with the use of both solvents was 4 hours, which was necessary for the extraction of the extracts. As shown in Figure 1, the flowchart of the passion fruit seed extraction process outlines the steps for obtaining extracts from fresh fruit. Initially, the seeds are separated from the pulp, peel, and other residues. Subsequently, the seeds undergo a drying process, where the moisture content must be reduced to below 10% to optimize the extraction. Table 1 shows the parameters used to characterize the seed of the raw material under study, where the method applied in each of them is also mentioned.

Statistical analysis

Each sample was taken in triplicate to obtain better results. Data processing was performed in the statistical software Jamovi version 2.4.14. An ANOVA was applied using Tukey's test with a confidence interval of $p < 0.05$.

RESULTS AND DISCUSSION

Moisture and ashes

Table 2 shows the percentages of moisture and ash of fresh passion fruit seeds (wet basis), which is three times higher than that obtained after drying (dry basis), being favorable for the extraction process from which the greater amount of water present in the matrix has been released. The percentage of ash on a wet basis is higher with

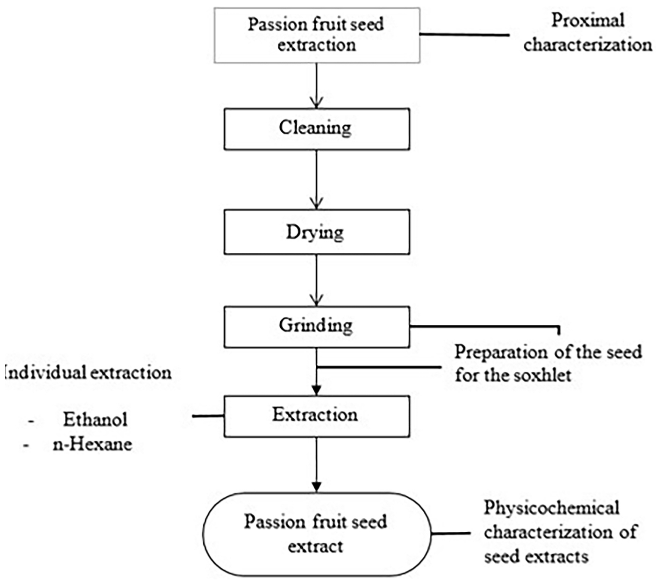

Figure 1. Flow diagram of the seed extraction process

Table 1. Parameter analysis (characterization of passion fruit seeds)

Parameter analysis	Method
Moisture	UNE-EN 14774-3, (2010)
Ashes	Sluiter et al. (2005)
Free acidity (oleic ac.)/acidity index	NTE INEN 38, (1973)
Relative density	NTE INEN 35, (1973)
Loss on heating	NTE INEN 39, (1973)
Iodine index	NTE INEN 37, (1973)
Peroxide value:	NTE INEN 277, (1978)
Extract yield	Chamorrro et al. (2017)
Fatty acid profile	AOAC method 996.06 (2005)

Note: Standardized methods used for the characterization and analysis of passion fruit seeds.

Table 2. Moisture and ash analysis of passion fruit seed on dry and wet basis.

Sample	% H BS	% H BH	% Ash BS	% Ash BH
1	6,70	18,52	1,94	2.83
2	6,25	18,18	1,94	1.96
3	6,14	14.28	1,94	0.95

Note: Moisture (%H) and ash content of passion fruit seeds on a dry basis (BS) and wet basis (BH).

an average of 2.10%, with a variation of 0.86%, which is double that obtained on a dry basis, being lower than the maximum allowed by the standard of 2.7% ash.

Humidity (*BS*)

Mean: 6.36%. Standard deviation: 0.30%. These values indicate that the moisture content on dry basis is quite homogeneous among the samples, with a slight variation of only 0.30%. The three moisture values on a dry basis remain in a very narrow range, suggesting a good stability of the drying process.

Humidity (*BH*)

Mean: 16.99%. Standard deviation: 2.36%. The values show a greater variation. The difference between the maximum and minimum value is approximately 4.24%, which is significant and reflects differences in the condition of the fresh seed. This suggests that the moisture content before drying is much more variable between samples.

Ash (*BS*)

Mean: 1.94%. Standard deviation: Very low (almost constant). Ash values on dry basis are constant in all samples, reflecting uniformity in the mineral composition of the seed after the drying process.

Ash (*BH*)

Mean: 1.91%. Standard deviation: 0.94%. The variation is much greater on a wet basis, with a significant difference between the minimum and maximum. This result shows how ash content is affected by the amount of water present in the fresh seed, being much more variable before drying.

In Figure 2, it is clearly shown that the moisture content is much higher in the wet basis (fresh seed) compared to the dry basis

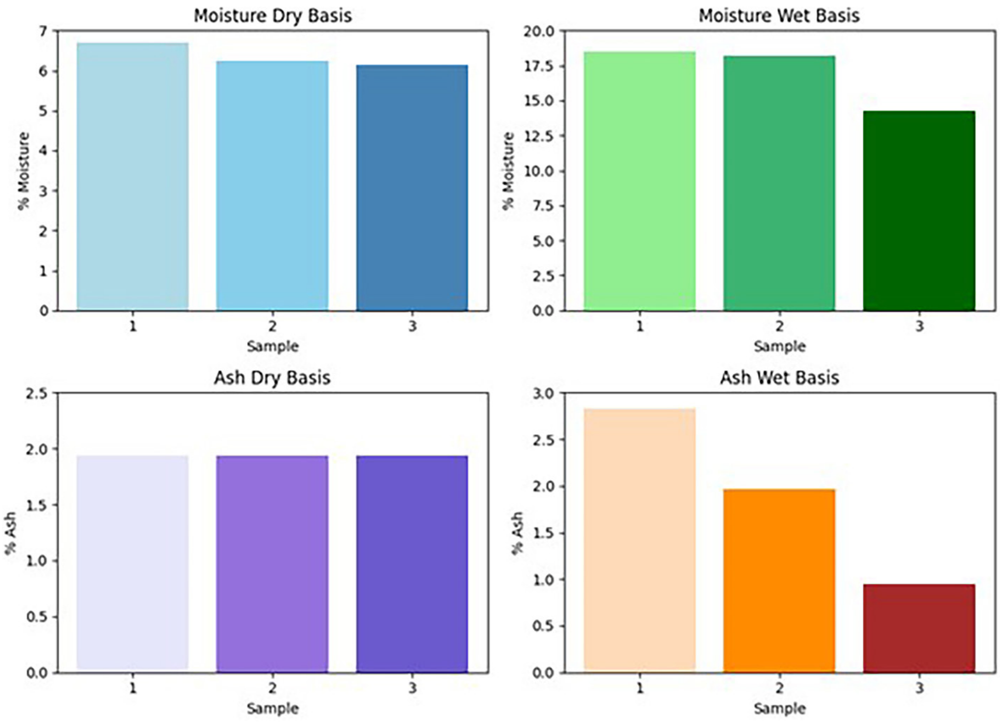

Figure 2. Moisture and ash analysis

(processed seed), which was expected. While ash content on a dry basis is stable in all samples, on a wet basis it has a wider spread, probably due to variability in residual moisture. A clear picture is provided of how drying affects both moisture and ash content. Table 3 shows the results for free acidity, acid number and yield with the two solvents used. Observing that the yield between both solvents is similar, ethanol has a result of almost double compared to n-hexane, which is higher than the % yield of 18.5 obtained (Cardoso de Oliveira et al., 2013), from passion fruit seed extraction with supercritical fluids. Also higher than the maximum % of 25.88 presented by Moia et al. (2019), in their research where they used ethanol as solvent in a PLE type extraction.

Table 4 shows the results of the calculation of the relative density, heating losses, iodine value and peroxide values with ethanol with the two types of solvents used in the extractions.

Figure 3 shows the variability in the results of the different variables measured for the ethanol and n-hexane extractions. Tukey's Post Hoc tests for the three main variables indicate:

Relative density

The difference in relative density between the two solvents is small, but the Post Hoc test indicates that it is not statistically significant (p value is greater than 0.05). This indicates that, although the average value of relative density with ethanol is slightly higher, the differences between the two solvents are not large enough to be considered important. Both solvents provide similar relative densities.

Loss due to heating

The results indicate that extractions with ethanol show a much higher loss than with n-hexane. The ethanol extraction has significantly higher losses compared to n-hexane, which is supported by the Post Hoc test, showing a very small p-value ($p < 0.01$). There are highly significant differences between ethanol and n-hexane extractions in terms of heating loss. Ethanol causes higher losses compared to n-hexane, which could be an important aspect to consider depending on the objective of the extraction process.

Iodine value

The differences in the iodine value between the two solvents are minimal and do not reach a level of statistical significance (p value > 0.05). For the iodine index, the extractions with ethanol and n-hexane do not show significant differences. Both solvents generate similar results in this measure.

Table 5 shows a comparison between the extract obtained with NTE INEN 26 (2012) and Codex Alimentarius 19, (2021), in which it was

Table 3. Free acidity (%Ac), acid number (index (mg NaOH/g)), yield (%R), by extraction with ethanol and n- Hexane.

Sample	Ethanol			n-Hexane		
	% Ac.	Acid number (mg NaOH/g)	% Yields	% Ac.	Acid number (mg NaOH/g)	% Yields
1	0,127	0,25	14.99	0,167	0,33	18.88
2	0,129	0,26	48,96	0,121	0,24	12.73
3	0,204	0,41	32,17	0,200	0,40	15.99

Note: Shows the free acidity, acid number, and yield for passion fruit seed extractions using ethanol and n-hexane as solvents.

Table 4. Relative density extraction at 25 °C (d25), loss on heating (% per. cal), iodine value (i) and peroxide values with ethanol (I) with ethanol and n-hexane.

Sample	Ethanol				n-Hexane			
	d_{25}	% Loss. heat	*i*	*I*	d_{25}	% Loss. heat	*i*	*I*
1	1,16	13,75	128,98	10,72	1,13	1,22	124,91	9,82
2	1,14	14,26	128,50	8.95	1,10	1,23	126,90	7,84
3	1,15	14,02	128,78	9,63	1,11	1,21	125,90	8,85

Note: Presents the relative density, heating loss, iodine value, and peroxide values for passion fruit seed extracts using ethanol and n-hexane as solvents.

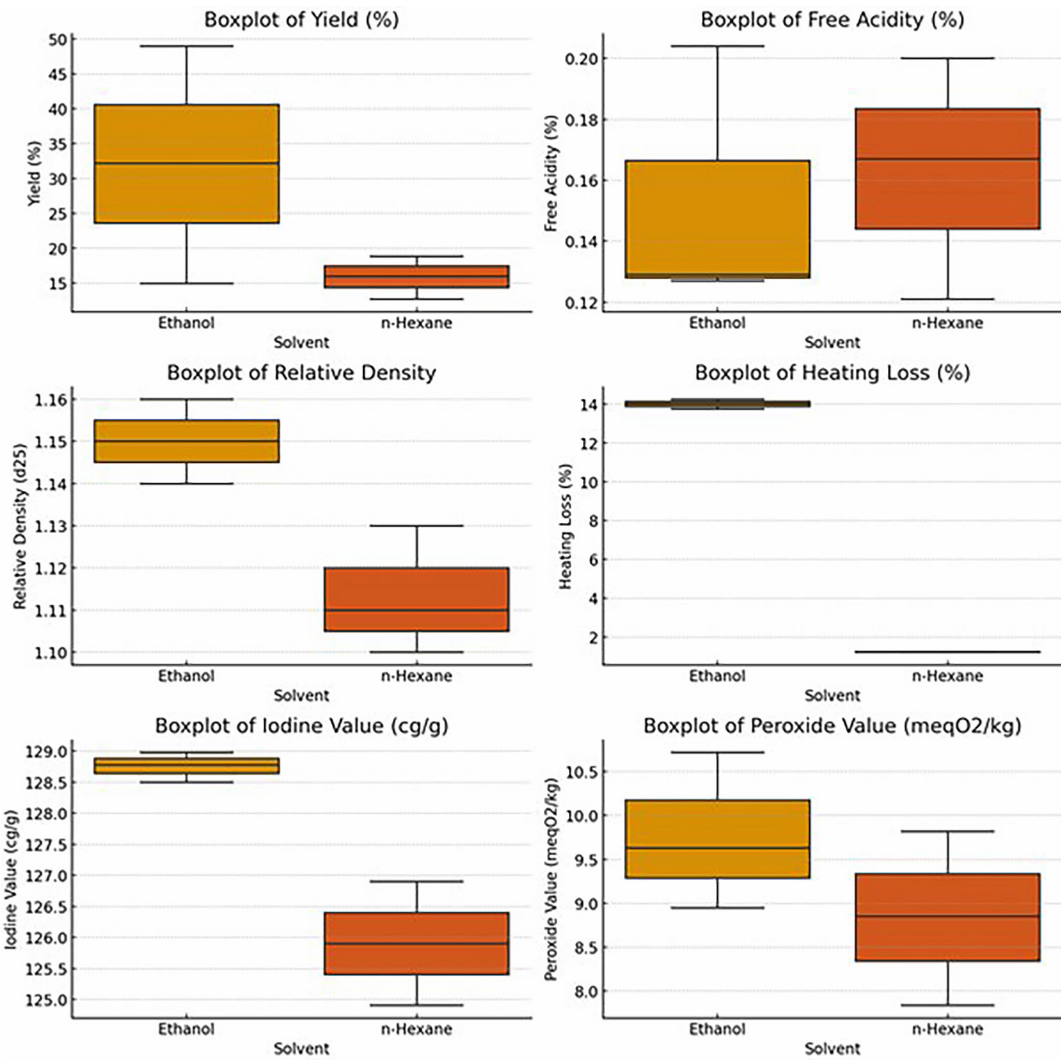

Figure 3. Boxplot of the different variables measured.

Table 5. Compliance of extracts with NTE INEN 26, (2012) and Codex Alimentarius.

Analysis	Extract (Ethanol)	NTE INEN 26:2012	CODEX STAN-19:2015	Extract (n-Hexane)	NTE INEN 26:2012	CODEX STAN-19:2015
Yield (%)	32.04	–	–	15.86	–	–
Heating losses (%)	14.01±0.36	NO	–	1.23±0.01	NO	–
Relative density 25/25°C	1.15±0.02	NO	–	1.11±0.02	NO	–
Free acidity (oleic acid) (%)	0.15±0.04	YES	–	0.16±0.04	YES	–
Acid number (mg NaOH/g)	0.31±0.09	–	YES	0.32±0.08	–	YES
Iodine value (cg/g)	128.74±0.34	YES	–	125.91±1.4	YES	–
Peroxide value (meqO_2/kg)	9.83	YES	YES	8.83±1.40	YES	YES

Note: Comparison of the results of solvent extractions using ethanol and n-hexane with the standards established by NTE INEN 26:2012 and CODEX STAN-19:2015.

sought to analyze whether or not they comply with the parameters established with the regulations. Although there is no specific standard for yield in these regulations, the extraction yield with ethanol is almost twice that with n-hexane. This indicates that ethanol may be a more efficient solvent in terms of extraction, in line with previous studies suggesting high yields with ethanol. For the heating losses parameter, the values obtained are significantly higher than those allowed by the NTE INEN standard, especially for ethanol extraction, which has a very high value compared to what is required.

For relative density 25/25 °C, the NTE INEN 26:2012 standard and other studies (Paucar-Menacho et al., 2015) establish a reference value of approximately 0.92. It does not comply with the standard in either case. Both values exceed the acceptable range of relative density in the standard. The density obtained using n-hexane is closer to the standard value than with ethanol.

Regarding free acidity (% oleic acid). NTE INEN 26:2012 establishes a maximum value of 0.6%. It does comply in both solvents with the regulations. The values obtained are within the permitted limits. This indicates that the extracted oils have an acceptable quality in terms of free acidity.

The acidity numbers indicate that the use of both solvents provides an oil with acidity levels within allowable limits, making it suitable for various industrial and cosmetic applications. Although n-hexane and ethanol have similar efficacy, ethanol may be preferred due to its lower environmental impact. Codex Alimentarius STAN 19:2015 establishes a maximum value of 2 mg NaOH/g. Both solvents comply with the standard. The acid number is significantly below the maximum allowed value, which is a positive indicator of the quality of the extracted oils.

For the iodine index (cg/g). NTE INEN 26:2012 establishes a minimum value of 120 cg/g. Complies for both solvents comply with the standard. The values obtained for the iodine index indicate that the oils are unsaturated, which is favorable from a nutritional and quality point of view.

When referring to the peroxide value (meq O_2/kg). NTE INEN 26:2012 and Codex Alimentarius STAN 19:2015 establish a maximum value of 10 meq O_2/kg. Both solvents comply with the standard. Both peroxide indices are below the permitted limit, indicating that the oils are not highly oxidized and show good stability against rancidity.

It is important to mention that parameters such as loss on heating and relative density 25/25 °C do not conform to any of the standards used for the analogy of the same, however, the closest values for the first mentioned parameter are close when n-hexane was used as solvent, since it is closer to the 0.05% mentioned in the INEN standard. With respect to the relative density 25/25 °C the best solvent was also n- Hexane as it is closer to 0.92 expressed by both Paucar-Menacho et al. (2015), and Hernandez et al. (2016), and by the NTE INEN 26:2012 standard. The results of the research become highly relevant, given the importance that the study of passion fruit has taken, as indicated by the research of (Corrêa et al., 2016), when mentioning that currently, the manufacture of concentrated juices is the activity with the greatest impact involving *Passiflora spp*. as its market is expanding worldwide. Regarding the seeds, Maruki-Uchida et al. (2013) identified piceatannol and scirpusin B, a dimer of piceatannol, as potent antioxidants found in passion fruit (*Passiflora edulis*). As well as corroborated by Kitada et al. (2017) indicating that piceatannol obtained from the seed of (*Passiflora edulis*) can have a strong anti-aging effect. According to dos Santos et al. (2022) passion fruit seeds, by-products of the juice industry, have potential to be used as a low-cost antioxidant and bioactive source for the development of nutraceuticals and dietary supplements to control blood glucose levels and, consequently, reduce the progression of complications of type 2 diabetes mellitus. Table 6 shows the results of the fatty acid profile of the seed samples analyzed with both ethanol and n-hexane.

Linoleic acid is the major component in both extracts. This fatty acid is essential in the human diet, as the body cannot synthesize it. The high linoleic acid content suggests that passion fruit seed oil has a good nutritional profile and may offer benefits for cardiovascular health. This oil could be suitable for the formulation of functional foods or supplements.

Followed by oleic acid which is known for its heart health benefits, as it is the main fatty acid in olive oil. A moderate oleic acid content improves the oxidative stability of the oil and increases its nutritional value by reducing the risk of cardiovascular disease. Both solvents extract similar amounts of this fatty acid, indicating that either method may be useful depending on other processing factors.

Third, palmitic acid is the most common saturated fatty acid in the human diet. Although its consumption should be moderate, it is part of the natural lipids. The content in both extracts is relatively low, which is positive from a health point of view, as excess saturated fatty acids can increase the risk of cardiovascular disease.

Fourth, stearic acid, although saturated, has a lower impact on cholesterol compared to other

Table 6. Fatty acid profile

Fatty acid (%)	Extraction with ethanol	Extraction with n-hexane
Palmitic acid	10.04	9.15
Palmitoleic acid	0.19	0.19
Heptadecanoic acid	–	0.80
Stearic acid	2.41	2.40
Oleic acid	14.46	15.19
Linoleic acid	71.75	72.25
Arachidic acid	0.11	0.14
Cis-11-Eicosenoic Acid	0.14	0.14
Linolenic acid	0.49	0.47
Nervonic acid	0.40	–

Note: Shows the fatty acid profile of passion fruit seed extracts using two different solvents (ethanol and n-hexane).

saturated fatty acids. Low levels of this acid are positive for the stability of the oil and do not represent a significant risk to cardiovascular health.

Fifth, the linolenic acid content is low in both extracts, the presence of this fatty acid is beneficial, as it is essential and has anti-inflammatory properties. In general, the content of omega-3 fatty acids is low, but their presence improves the nutritional value of the oil.

Minor fatty acids such as arachidic acid (C20:0) in both solvents showed low values (0.11% for ethanol and 0.14% for n-hexane), which is typical for vegetable oils. The concentration of Cis-11-eicosenoic acid (C20:1) is low (0.14% in both cases), suggesting that these fatty acids are not a significant part of the passion fruit oil profile. Nervonic acid (C24:1) was only detected in the ethanol extraction (0.40%), which is interesting as this acid is rare in vegetable oils and has potential neuroprotective benefits.

Heptadecanoic acid (C17:0), only appears in the extraction with n-hexane (0.80%), indicating that n-hexane could be extracting some fatty acids that are not obtained with ethanol. However, their presence is not relevant enough to have a significant impact on the nutritional profile.

The Codex Alimentarius and NTE INEN do not establish specific limits for the fatty acid profile, but studies and industry standards tend to value oils with a high content of unsaturated fatty acids (such as oleic and linoleic) for their stability and health benefits. Passion fruit seed oil extracted with both solvents meets the quality criteria for healthy oils, having a high content of unsaturated fatty acids (particularly linoleic and oleic acid) and low levels of saturated fatty acids. When comparing this information with other authors, it is observed that the oleic acid parameters obtained between 15 and 16% exceed those obtained when fresh passion fruit seed was used applying techniques such as cold pressing and solvent extraction, while in parameters such as linoleic acid, % were obtained between 71 and 72 and were also higher when the same techniques mentioned previously were used using fresh seed and waste seed of the species under study (Hoyos Zagaceta et al., 2019).

Results were found for palmitic acid with 14% obtained for the same species of the present study from extraction with supercritical fluids, which were higher than those obtained in the present investigation and which flowed between 9 and 10%. As for palmitoleic acid, the same technology yielded results of 0.23%, slightly higher than the 0.19% found in the present investigation. For stearic acid, the results of the present investigation which hovered around 2.41 % approximately were higher than the 1.29% presented under supercritical fluid technology (Pantoja Chamorrro et al., 2017).

As for arachidic acid, the results of between 0.11 and 0.14% obtained in the present investigation were similar to the 0.15% obtained for passion fruit seed oil by extraction for milling (Santacruz et al., 2020).

Extracted passion fruit seed oil has a favorable fatty acid profile, with high levels of essential (linoleic) and beneficial (oleic) fatty acids, making it an excellent choice for both nutritional and cosmetic applications.

Although the use of ethanol as a solvent presents some problems in terms of density and heating losses and in terms of fatty acid profile, it offers promising results for oil yield and quality, especially in terms of sustainability as it is a more environmentally friendly solvent than n-hexane.

CONCLUSIONS

For the process of obtaining passion fruit seed extracts, the preliminary stages are of great importance, in which the seed must be free of impurities, within which a crucial stage is drying, followed by crushing, selection of the solvent and setting up the equipment to start the extraction process, which gave the best performance with the use of ethanol as solvent.

The fatty acid profile of the extracted oils, especially their high linoleic acid content, suggests that this oil may be beneficial for cardiovascular health and suitable for applications in the food industry, as a functional oil or ingredient in supplements.

Both solvents (ethanol and n-hexane) provide similar fatty acid profiles, although ethanol, being a more environmentally friendly and safer solvent for consumption, could be the preferred option, especially since it also offers a higher extraction yield.

Due to its composition rich in unsaturated fatty acids, this oil has potential for use in cosmetic products, nutraceuticals and health foods.

Acknowledgements

We thank all collaborators for their contributions to this research. Special appreciation to INIAP, Portoviejo Experimental Station, and Universidad Técnica de Manabí (UTM) for their logistical and institutional support, which was crucial for the successful completion of this project.

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