LE STUDIUM
Loire Valley
Institute for Advanced Studies

LE STUDIUM Multidisciplinary Journal
ISSN 2669-7807
www.lestudium-ias.com

FELLOWSHIP FINAL REPORT

Green processes to obtain natural extracts for food, cosmetics and pharmacy - Composition analysis

Alessandra Lopes de Oliveira [1,3], Emilie Destandau[2], Eric Lesellier[2], Caroline West[2]

[1]Food Engineering Department, Faculty of Animal Science and Food Engineering (FZEA), University of São Paulo (USP), Pirassununga, Brasil
[2]Institut de Chimie Organique et Analytique (ICOA), UMR 6005, Université d'Orléans, 45067 Orléans, France
[3] LE STUDIUM Institute for Advanced Studies, 45000 Orléans, France

REPORT INFO

Fellow: Alessandra Lopes de Oliveira
From University of São Paulo, High pressure technology and natural products laboratory, Faculty of Animal Science and Food Engineering
Host laboratory in region Centre-Val de Loire: Institut de Chimie Organique et Analytique (ICOA), Université d'Orléans
Host scientist: **Professor Caroline West**
Period of residence in region Centre-Val de Loire: Aug 2023 – Dec 2023

Keywords:
Green extraction, supercritical fluid extraction, pressurized liquid extraction, Hermetia *illucens*, avocado extracts

ABSTRACT

Brazil contains a large part of the world's biodiversity (around 15 to 20%) and medicinal plants fall into this percentage. In addition to the fact that the country is an important food producer, in this way, cultivated plants or those naturally present in Brazilian flora is responsible for the production of raw materials for the development of foods, cosmetics, medicinal plants and other medicines.
This biodiversity, combined with the rich ethnic and cultural diversity, holds the traditional knowledge associated with the use of these plants. This is why the High Pressure Technology and Natural Products Laboratory (LTAPPN) of the University of São Paulo is developing research aimed at developing green technologies, optimizing processes and increasing the scale of extract production rich in active compounds and free from toxic residues of organic solvent origin.
Although the rich biodiversity and traditional knowledge are a necessary potential to develop research with technologies, the study of the composition of these extracts is fundamental for their applications. In this way, the research interaction with the ICOA (Institute of Organic and Analytical Chemistry) of the University of Orleans emerges with a strong desire to develop common projects, with great attraction for internationalization, in and allow consolidation of existing search links. The aim of the research development period at ICOA is to identify active compounds in vegetable oils and insect oils with strong appeal to the cosmetic industry.

1- Introduction

The development of this research addressed two crucial themes for the food, pharmaceutical, and cosmetics industries. Throughout the international internship, extracts obtained at LTAPPN at the University of São Paulo were and continue to be analyzed at ICOA at the University of Orleans. These extracts include oil and extract from insect flour (black soldier fly produced in Brazil) and oil and extract from six distinct species of avocados grown in Brazil. In anticipation of a global population of around 10 billion by 2050 and a potential shortage of protein foods, insects are emerging as a viable alternative in both animal and human nutrition. The practice of entomophagy, or insect consumption, is gaining traction, especially in regions where it is not traditionally practiced. Insects offer nutritional benefits and serve as an ideal substitute for traditional animal proteins like beef, pork, poultry, and fish. Efforts, notably by the FAO (Food and Agriculture Organization of the United Nations), promote insect consumption due to its nutritional value, functional benefits, and environmentally friendly aspects. Large-scale insect production is highlighted for its efficiency, requiring less cultivation space and water resources compared to traditional protein sources like beef. The

European Union has approved the consumption of *Tenebrio molitor*, *Locusta migratoria*, and *Acheta domesticus* for humans, either in whole form or via their byproducts. Additionally, a list of other insects, including *Gryllodes sigillatus*, *Alphitobius diaperinus*, and *Hermetia illucens* L., is currently awaiting approval for human consumption by the European scientific committee (EFSA Scientific Committee, 2021). Black soldier fly larvae meals are rich in proteins, amino acids, fibers, and lipids, making them a potential inclusion in human nutrition. The larvae meal can have up to 40% lipid content, the second-largest macronutrient in its composition (Cantero-Bahillo et al., 2022). The oil extracted from these larvae exhibits varying levels of monounsaturated and polyunsaturated fatty acids, along with saturated fatty acids (Cruz et al., 2023). Insects in pupal or larval stages have higher lipid concentrations, serving as valuable sources of energy and essential fatty acids. These characteristics suggest their potential use in addressing child malnutrition (Tzompa-Sosa et al., 2014). The use of insect fats in human nutrition remains underexplored, with limited studies and applications. Research has primarily investigated various techniques for extracting insect oil, with a focus on supercritical fluid extraction (SFE) using carbon dioxide (CO_2). Supercritical carbon dioxide (sc-CO_2) serves as an advantageous solvent, providing low toxicity and high solubilization power, leading to elevated mass transfer rates. This method ensures the use of solvents generally recognized as safe (GRAS) in the process, as highlighted in studies (De Oliveira et al., 2019). Pressurized liquid extraction (PLE) utilizing ethanol as a solvent is an unconventional technique for oil extraction. Efficient oil extraction with ethanol requires high pressures. Ethanol, widely used due to its Generally Recognized as Safe (GRAS) status and sustainability (Molino et al., 2018), is a polar solvent leading to oil rich in phospholipids and other minor compounds (Capellini et al., 2017). PLE, a solid-liquid extraction process, is commonly employed in intermittent processes at temperatures above the solvent's boiling point and under high pressures (10 MPa) (Cornelio-Santiago et al., 2019, 2022; Toda et al., 2021; Colivet et al., 2022). This enhances extraction efficiency by facilitating solvent entry into the matrix. The high temperatures involved reduce viscosity, increase solvent diffusivity, and consequently, enhance mass transfer rates and solubility of matrix compounds (Richter et al., 1996; Cornelio-Santiago et al., 2019). Compared to traditional methods, these characteristics result in shorter extraction times and reduced solvent usage in intermittent processes.

When used in an intermittent process, PLE does not require high concentrations of solvent and can be performed in less time, compared to conventional methods (Da Cunha Rodrigues et al., 2023). Both SFE and PLE are innovative methods for obtaining vegetable and animal oils, being considered green and sustainable processes. SFE, in particular, is regarded as a clean technology because it does not leave any organic solvent residues in the extracts. This is because, under ambient or low-pressure conditions, CO_2 is a gas and releases the extracts in pure form. In this context, the aim of this study was to assess the composition of black soldier fly (*Hermetia illucens* L.) larvae oil obtained under different extraction conditions using sc-CO_2, both with and without the incorporation of ethanol as a cosolvent. Insect meal, defatted via SFE, served as the raw material for PLE to obtain an ethanolic extract for composition analysis. Following the extractions, the protein concentrate from the defatted black soldier fly larvae meal was prepared for use in food formulation. The integration of these processes aligns with the concept of microbiorefinery, a bioactive production system that utilizes green and sustainable solvents, which, by way of technology intensification or integration, generate clean products (extracts and co-products free of toxic organic solvent residues) that can be potentially applied in the production of foods, pharmaceuticals, and cosmetics.

Avocados are rich in phenolics, carotenoids, minerals, vitamins, and fatty acids, providing various health benefits (Araújo et al., 2018). Research indicates that avocados play a role in combating hypertension, diabetes, and obesity, demonstrating antiatherosclerotic and cardioprotective effects (Lima et al., 2012; Márquez-Ramírez et al., 2018; Prasad et al., 2017). Additionally, studies suggest that avocados may be used in therapeutic efforts to prevent gastrointestinal and degenerative disorders, as well as the formation of cancers (Abubakar et al., 2017).

Avocado peel and seed extracts inhibits enzymes like acetylcholinesterase, hyaluronidase, and xanthine oxidase (Rojas-García et al., 2022). The antioxidant properties of avocados make them a potential natural food preservative, increasing shelf life and serving as an anti-aging nutraceutical (Figueroa et al., 2021). As interest in "clean label" products grows, the demand for natural preservatives and antioxidants extracted through environmentally friendly processing technologies is increasing in the pharmaceutical and cosmetics sectors. Developing industrial-scale production techniques for avocados is becoming essential to meet these demands (Bastante et al., 2022).

Conventional techniques like maceration or soxhlet for extracting bioactive substances from fruit and avocado by-products have drawbacks, including the use of harmful solvents, prolonged extraction periods, high temperatures leading to potential damage to compounds, and incomplete extraction (Fuente-Ballesteros et al., 2023). There is a recognized need for refining stages in utilizing these substances in the food and pharmaceutical industries. Future research should focus on developing new technologies. Fortunately, modern and environmentally friendly sustainable extraction processes are now available, offering quick, reproducible, and low-solvent methods that minimize environmental impact, increase extract purity, and reduce energy consumption (Chemat et al., 2019). These processes ensure the production of safe, high-quality extracts (Tremocoldi et al., 2018).

Compressed fluid extraction methods like supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) offer sustainable and efficient alternatives for recovering various bioactives from avocados, including oil, carotenoids, phenolics, and phytosterols (Abaide et al., 2017; Barros et al., 2017; Grisales-Mejía et al., 2022; Mostert et al., 2007). Studies have highlighted the effectiveness of these techniques, but variables such as temperature, pressure, cosolvent usage, humidity, particle size, and flow rate influence their efficiency. Limited comprehensive reviews on avocado extraction methods exist, with a notable one by de Satriana et al. (2019) focusing on supercritical carbon dioxide (SC-CO_2) and PLE. Similarly, Afzal et al. (2022) reviewed oil extraction, encompassing avocado seeds, but offered relatively general information on factors affecting bioactive compound extraction and avocado oil.

Research on compressed fluids, including Pressurized Liquid Extraction (PLE) and Supercritical Fluid Extraction (SFE), along with the corresponding process parameters that significantly impact the extraction performance of bioactive compounds from black soldier fly larvae meals and various parts of avocados (pulp, peel, and seed), as well as the examination of the advantages and disadvantages of these techniques, is currently underway in Brazil. The composition of these extracts is concurrently being analyzed in France at ICOA, and partial results will be presented in this report.

2- Experimental details

During the period at ICOA at the University of Orleans, only a limited number of analyses were conducted on the alcoholic extract of soldier fly larvae flour that was defatted via Supercritical Fluid Extraction (SFE). These findings have been incorporated into the submitted paper titled: "Intensification of SFE using ethanol as a cosolvent and integration of the SFE process with sc-CO_2 followed by Pressurized Liquid Extraction (PLE) using pressurized ethanol of black soldier fly (*Hermetia illucens* L.) larvae meal – Extract yields and characterization". The methods employed are described below.

2.1. Preliminary determination of the classes of compounds present in the ethanolic extract of defatted black soldier fly larvae meal obtained via PLE

High performance thin-layer chromatography (HPTLC) was used for preliminary analyzes of the ethanolic extract composition obtained from defatted soldier fly larvae flour by PLE. HPTLC Silica gel 60 plates with fluorescent indicator F_{254}, (Supelco, Ontario, Canada) were used as the stationary phase. Sample deposition was carried out using CAMAG automatic TLC Sampler 4, sample elution in a CAMAG ADC2 automatic developing chamber, and photography were carried out using CAMAG TLC visualizer 2. The data were processed using vision CATs CAMAG software. When plate derivatization was necessary, the CAMAG Derivatizer was used with specific recommendations for each reagent.

In the analysis of the presence of sugars and amino acids, acetonitrile/water (75:25) was used as the mobile phase. For analyze the presence of phenolic compounds, ethyl acetate/water/formic acid/acetic acid (100:27:11:11) was used as the mobile phase as well as the mobile phase normally used for the analysis of organic acids, ethanol/ammonium hydroxide/water (75.5:12.5:12), and in the analysis of condensed tannins the mobile phase was composed of ethyl acetate/methanol/ water (79:11:10). After eluting the samples, the developers were prepared (Wagner & Blat, 2009) and sprayed on the silica plates (CAMAG Derivatizer), specific for each analysis. Ninhydrin (100 mg ninhydrin dissolved in 100 mL ethanol spraying followed by drying at 105 ºC/3 min) was used to identify the presence of amino acids and Molish (2 g α-naphtol solubilized in 100 mL ethanol and sulfuric acid at 5% in ethanol spraying and drying at 120 ºC/5 min) to identify the presence of sugars. To identify phenolic compounds, Neu (1 g of diphenyl boric acid ethylamino ether in 100 mL of MeOH) developer was applied followed by PEG (polyethylene glycol (PEG) 4000 at 5%) in EtOH with reading taken at 366 nm. To identify condensed tannins, the developer Vanillin-HCL was used (spraying and drying at 100ºC/10 min).

UHPLC was also used in the preliminary analysis of the family of compounds present in the hydroalcoholic extract. UHPLC analysis were performed with the Thermo Scientific Ultimate 3000 RSLC analytic system and were analyzed with the Chromeleon 7 software. The column used was the Luna Omega C18 (150 mm × 2.1 mm, 1.6 µm) column. The mobile phase used was water acidified with 0.1% formic acid (A) and acetonitrile acidified with 0.1% formic acid (B) with the following gradient: 0-2 min: 5% of B; 2-22 min: 5-100% of B; 22-31 min: 100% of B; 31-31.5 min: 5% of B; 31.5-41 min: 5% of B. The flow rate was fixed at 0.4 mL/min. Samples were prepared at a concentration of 16 mg/mL in EtOH and standards were prepared at a concentration of 1 mg/mL in EtOH for benzoic acid and water for gallic acid and pyrocatechol. 2 µL of sample and standards were injected and the column was heated to 40°C. Detection was performed with a DAD visualized at 280 mn and ELSD detector.

2.2. Analysis of avocado extracts composition in progress

The oil from six different types of Brazilian avocados, obtained at the LTAPPN at FZEA/USP, is currently under analysis at ICOA. Supercritical fluid chromatography coupled with mass spectrometry is one of the instruments in use. Additionally, high-performance liquid chromatography and high-resolution mass spectrometry may also be employed for analysis. The characterization of these oils commenced during my tenure at the University of Orleans and is persisting as part of the master's program of student Joanna Cios, under the supervision of Professor Carolina West.

Tests on cosmetics can also be conducted under the guidance of Professor Emilie Destandau.

3- Partial results and discussion

This report presents partial results of what has already been released. Data generated and not completed will not be presented.

3.1. *Preliminary determination of the class of compounds present in the ethanolic extract of defatted black soldier fly larvae meal obtained via PLE*

HPTLC analyzes indicated families of compounds that constitute the hydroalcoholic extract of defatted soldier fly larvae flour. It was possible to previously identify the presence of amino acids, by revealing the Ninhydrin reagent (Figure 1A), and the presence of sugars, after revealing with the Molish reagent, (Figure 1B). The standard sugar solution used it was a mixture of glucose (Rf ≈ 0.51), maltose (Rf ≈ 0.40), maltopentaose (Rf ≈ 0.17) and maltoheptaose (Rf ≈ 0.08). The highest concentration of sugar present in the ethanolic extract of soldier fly larvae flour has a Rf ≈ 0.35, a value lower than a disaccharide (maltose) and higher than a pentose, probably a sugar with 3 or 4 monosaccharides (trioses or tetroses).

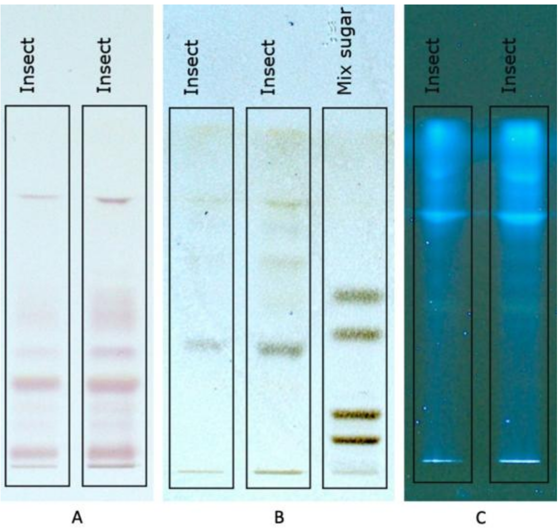

Figure 1. HPTLC plates; (A) indicates the presence of amino acids with Ninhydrin as a developer; (B) indicates the presence of sugars with development using Molish reagent; (C) indicates the presence of compounds with fluorescence at 366 nm with mobile phase ethanol/ammoniumhydroxide/water (75.5:12.5:12).

The identification of compounds that respond to the wavelength of 366 nm, which may be phenolic compounds (Figure 1C), showed distinct bands also with different intensities. Phenolic compounds and carotenoid contents were quantified by colorimetric methods. And in the HPTLC analysis to identify condensed tannins, it was not possible to detect them.

High performance liquid chromatography (HPLC) analysis indicated the presence of compounds with peaks, retention times and maximum absorption spectra similar to those of organic acids. A detection, performed with a DAD at 280 nm indicated, in the ethanolic extract obtained by PLE of defatted black soldier fly larva flour, some peaks with maximum absorption of 215.45, 216, 220.34, 225 and 225.22 nm (Figure 2) and, the same HPLC measurement made with gallic and benzoic acids standards, shows maximum absorption spectra of 217 and 221.8 nm, respectively (Figure 3). Insects are rich in organic acids (Levenbook, Hollis Jr., 1961; Beldean et al., 2022) and the composition varies between species.

Although we are working with preliminary analyses, the presence of organic acids in these extracts can be confirmed by their high acidity.

4- Partial Conclusion

During my four-month tenure as a guest researcher at ICOA at the University of Orleans, several projects were initiated. These projects included the characterization of extracts from soldier fly larvae and the analysis of oils from six distinct varieties of Brazilian avocados.

Given the relatively brief duration of four months for project completion, it was possible to make partial progress, particularly in the preliminary characterization of the ethanolic extract of soldier fly larva meal. This extract was obtained using pressurized liquid extraction in an intermittent process from soldier fly larvae that had been degreased through supercritical fluid extraction. Experiments carried out in Brazil.

Analyzes are currently being carried out:

1) Determining the oil composition of soldier fly larvae flour, extracted through supercritical fluid extraction.
2) Analyzing oils extracted from six different species of avocados.
3) Investigating the aqueous fraction of avocado extract, derived from conventional oil extraction methods.

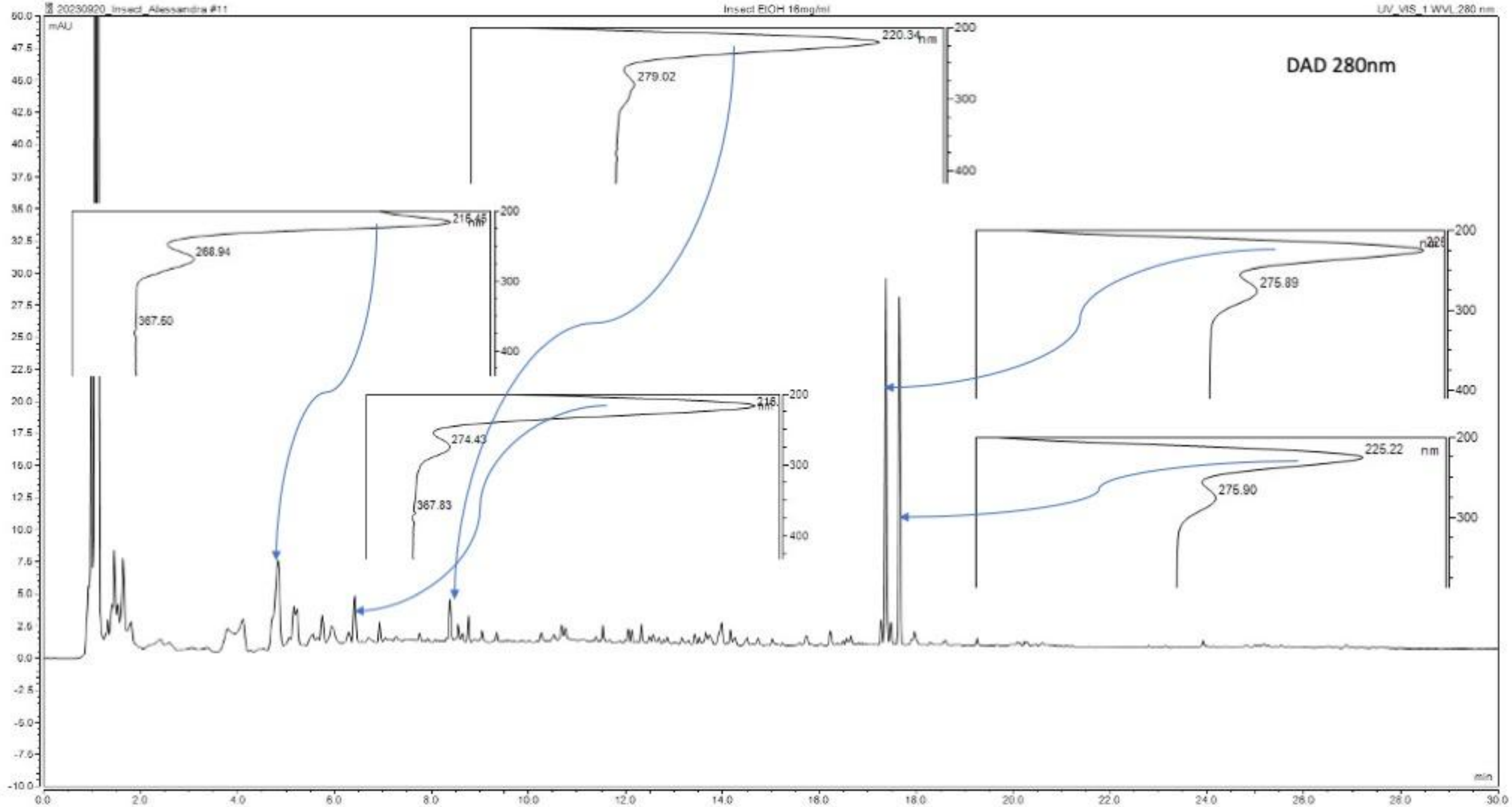

Figure 2. Chromatogram of the ethanolic extract of defatted flour from black soldier fly larvae with some maximum absorption spectra reading at 280 nm.

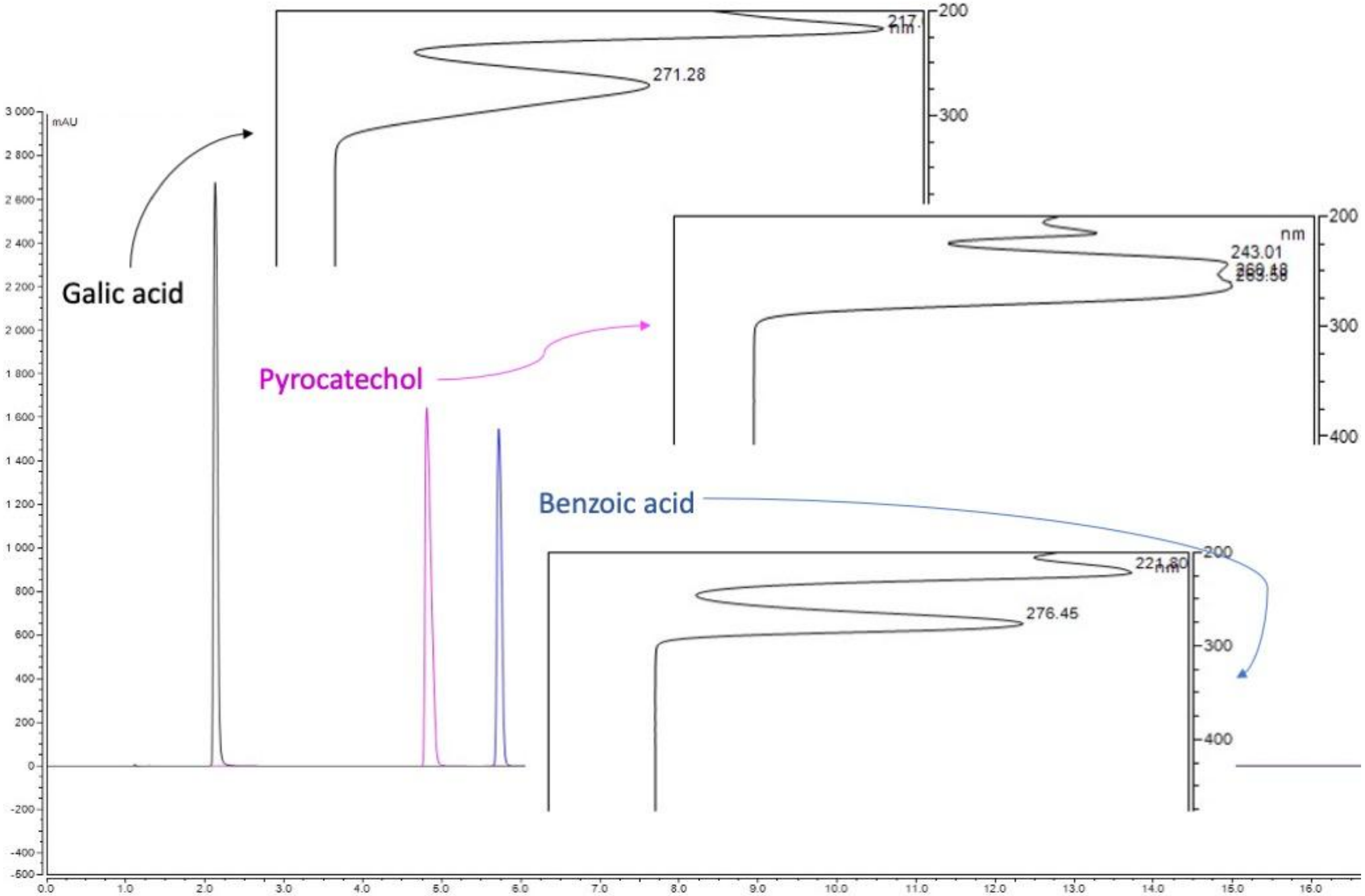

Figure 3. Chromatogram of standard compounds (galic and benzoic acids, pyrocatechol) with maximum absorption spectra reading at 280 nm.

4) Depending on the composition, potential activity tests may be conducted.

During this period, we developed a thematic project entitled: “Microbiorefineries as a bioactive production system through the intensification or integration of high-pressure processes with green and sustainable solvents aiming at zero waste in the industrialization of different avocado species”. This project was drawn up in a proposal between FAPESP (Fundação de Amparo a Pesquisa do Estado de São Paulo) and ANR (Agence Nationale de la Recherche): called “Generic Call for Proposals 2024”. To complete the submission of the project, there is need for an eligibility letter from both funding agencies; FAPESP has already provided us with the eligibility letter. We are awaiting the letter from ANR scheduled for February 2024.

During this period, we developed a thematic project entitled: “Microbiorefineries as a bioactive production system through the intensification or integration of high-pressure processes with green and sustainable solvents aiming at zero waste in the industrialization of different avocado species”. This project was drawn up in a proposal between FAPESP (Fundação de Amparo a Pesquisa do Estado de São Paulo) and ANR (Agence Nationale de la Recherche) called: “Generic Call for Proposals 2024”. To complete the submission of the project, there is need for an eligibility letter from both funding agencies; FAPESP has already provided us with the eligibility letter. We are awaiting the letter from ANR scheduled for February 2024.

5- Perspectives of future collaborations with the host laboratory

Collaboration with ICOA at the University of Orleans has been ongoing for several years, starting in 2008. At present, we are engaged in projects aimed at characterizing extracts from soldier fly larvae, exploring their potential applications. Additionally, ICOA is conducting research on oils derived from various avocado species. This initiative encompasses investigations into the composition and potential uses of extracts derived from the skins and pits of six distinct types of Brazilian avocados.

Should the FAPESP-ANR thematic project receive approval, it will span a duration of five years. Moreover, our collaborations with ICOA entail the exchange of master's and doctoral students between Brazil and France.

6- Acknowledgements

Alessandra Lopes de Oliveira expresses gratitude to LE STUDIUM Loire Valley Institute for Advanced Studies for providing the scholarship and the opportunity to serve as a guest researcher at the University of Orleans. The Cosmétosciences program facilitated this support for research development, emphasizing the practical application of extracts in the cosmetics industry.

Special thanks are extended to Professor Caroline West for the warm welcome at ICOA, and through her, gratitude is conveyed to the entire analytical team.

Acknowledgment is also extended to the University of Orleans for their hospitality and to the University of São Paulo for granting permission for the leave of absence.

Furthermore, appreciation is expressed to FAPESP for enabling the experiments conducted in Brazil.

7- References

Abaide, E. R., Zabot, G. L., Tres, M. V., Martins, R. F., Fagundez, J. L., Nunes, L. F., Druzian, S., Soares, J. F., Dal Prá, V., Silva, J. R. F., Kuhn, R. C., & Mazutti, M. A. (2017). Yield, composition, and antioxidant activity of avocado pulp oil extracted by pressurized fluids. *Food and Bioproducts Processing*, *102*, 289–298. https://doi.org/10.1016/J.FBP.2017.01.008

Abubakar, A. N. F., Achmadi, S. S., & Suparto, I. H. (2017). Triterpenoid of avocado (Persea americana) seed and its cytotoxic activity toward breast MCF-7 and liver HepG2 cancer cells. *Asian Pacific Journal of Tropical*

Biomedicine, *7*(5), 397–400. https://doi.org/10.1016/J.APJTB.2017.01.010

Afzal, M. F., Khalid, W., Armghan Khalid, M., Zubair, M., Akram, S., Kauser, S., Noreen, S., Jamal, A., Kamran Khan, M., & Al-Farga, A. (2022). Recent industrials extraction of plants seeds oil used in the development of functional food products: A Review. *International Journal of Food Properties*, *25*(1), 2530–2550. https://doi.org/10.1080/10942912.2022.2144882

Araújo, R. G., Rodriguez-Jasso, R. M., Ruiz, H. A., Pintado, M. M. E., & Aguilar, C. N. (2018). Avocado by-products: Nutritional and functional properties. *Trends in Food Science & Technology*, *80*, 51–60. https://doi.org/10.1016/J.TIFS.2018.07.027

Barros, H. D. F. Q., Grimaldi, R., & Cabral, F. A. (2017). Lycopene-rich avocado oil obtained by simultaneous supercritical extraction from avocado pulp and tomato pomace. *The Journal of Supercritical Fluids*, *120*, 1–6. https://doi.org/10.1016/J.SUPFLU.2016.09.021

Bastante, C. C., Vafaei, N., Rempel, C. B., Scanlon, M. G., Jones, P. J. H., & Eskin, M. N. A. (2022). Application of Supercritical Fluid Extraction (SFE) of Tocopherols and Carotenoids (Hydrophobic Antioxidants) Compared to Non-SFE Methods. *AppliedChem 2022, Vol. 2, Pages 68-92*, *2*(2), 68–92. https://doi.org/10.3390/APPLIEDCHEM2020005

Cantero-Bahillo, E., Del Hierro, J. N., Hernández, D. M., Fernández-Felipe, M. T., Fornari, T., & Martin, D. (2022). Supercritical-CO2 for defatting and production of bioactive extracts from black soldier fly (Hermetia illucens) larvae. *Journal of Insects as Food and Feed*, *8*(12), 1441–1453. https://doi.org/10.3920/JIFF2021.0210

Capellini, M. C., Giacomini, V., Cuevas, M. S., & Rodrigues, C. E. C. (2017). Rice bran oil extraction using alcoholic solvents: Physicochemical characterization of oil and protein fraction functionality. *Industrial Crops and Products*, *104*, 133–143. https://doi.org/10.1016/j.indcrop.2017.04.017

Chemat, F., Abert-Vian, M., Fabiano-Tixier, A. S., Strube, J., Uhlenbrock, L., Gunjevic, V., & Cravotto, G. (2019). Green extraction of natural products. Origins, current status, and future challenges. *TrAC Trends in Analytical Chemistry*, *118*, 248–263. https://doi.org/10.1016/J.TRAC.2019.05.037

Colivet, J., Garcia, V. A. D. S., Lourenço, R. V., Yoshida, C. M. P., Oliveira, A. L. D., Vanin, F. M., & Carvalho, R. A. D. (2022). Characterization of Films Produced with Cross-Linked Cassava Starch and Emulsions of Watermelon Seed Oils. *Foods*, *11*(23), 3803. https://doi.org/10.3390/foods11233803

Cornelio-Santiago, H. P., Mazalli, M. R., Rodrigues, C. E. C., & de Oliveira, A. L. (2019). Extraction of Brazil nut kernel oil using green solvents: Effects of the process variables in the oil yield and composition. *Journal of Food Process Engineering*, *42*(7), e13271. https://doi.org/10.1111/jfpe.13271

Cruz, V. A., Ferreira, N. J., Cornelio-Santiago, H. P., Santos, G. M. T., & Oliveira, A. L. (2023). Oil extraction from black soldier fly (*Hermetia illucens* L.) larvae meal by dynamic and intermittent processes of supercritical CO2--Global yield, oil characterization, and solvent consumption. *The Journal of Supercritical Fluids*, *195*, 105861. https://doi.org/10.1016/j.supflu.2023.105861

Da Cunha Rodrigues, L., Bodini, R. B., Caneppele, F. D. L., Dacanal, G. C., Crevelin, E. J., de Moraes, L. A. B., & de Oliveira, A. L. (2023). Pressurized Liquid Extraction (PLE) in an Intermittent Process as an Alternative for Obtaining Passion Fruit (Passiflora edulis) Leaf Hydroalcoholic Extract (Tincture). *Processes*, *11*(8), 2308. https://doi.org/10.3390/pr11082308

De Oliveira, N. A., Mazzali, M. R., Fukumasu, H., Goncalves, C. B., & de Oliveira, A. L. (2019). Composition and physical properties of babassu seed (*Orbignya phalerata*) oil obtained by supercritical CO2 extraction. *The*

Journal of Supercritical Fluids, *150*, 21–29. https://doi.org/10.1016/j.supflu.2019.04.009

Figueroa, J. G., Borrás-Linares, I., Lozano-Sánchez, J., & Segura-Carretero, A. (2018). Comprehensive characterization of phenolic and other polar compounds in the seed and seed coat of avocado by HPLC-DAD-ESI-QTOF-MS. *Food Research International*, *105*, 752–763. https://doi.org/10.1016/J.FOODRES.2017.11.082

Fuente-Ballesteros, A., Priovolos, I., Ares, A. M., Samanidou, V., & Bernal, J. (2023). Green sample preparation methods for the analysis of bioactive compounds in bee products: A review. *Advances in Sample Preparation*, *6*, 100060. https://doi.org/10.1016/J.SAMPRE.2023.100060

Grisales-Mejía, J. F., Torres-Castañeda, H., Andrade-Mahecha, M. M., & Martínez-Correa, H. A. (2022). *Green Extraction Methods for Recovery of Antioxidant Compounds from Epicarp, Seed, and Seed Tegument of Avocado var. Hass (Persea americana Mill.)*. https://doi.org/10.1155/2022/1965757

Levenbook, L., Hollis Jr., V.W. (1961) Organic acid in insects—I. Citric acid. *Journal of Insect Physiology*, *6*(1), 52-61. https://doi.org/10.1016/0022-1910(61)90091-9

Lima, C. R., Vasconcelos, C. F. B., Costa-Silva, J. H., Maranhão, C. A., Costa, J., Batista, T. M., Carneiro, E. M., Soares, L. A. L., Ferreira, F., & Wanderley, A. G. (2012). Anti-diabetic activity of extract from Persea americana Mill. leaf via the activation of protein kinase B (PKB/Akt) in streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology*, *141*(1), 517–525. https://doi.org/10.1016/J.JEP.2012.03.026

Márquez-Ramírez, C. A., Hernández de la Paz, J. L., Ortiz-Avila, O., Raya-Farias, A., González-Hernández, J. C., Rodríguez-Orozco, A. R., Salgado-Garciglia, R., Saavedra-Molina, A., Godínez-Hernández, D., & Cortés-Rojo, C. (2018). Comparative effects of avocado oil and losartan on blood pressure, renal vascular function, and mitochondrial oxidative stress in hypertensive rats. *Nutrition*, *54*, 60–67. https://doi.org/10.1016/J.NUT.2018.02.024

Molino, A., Mehariya, S., Iovine, A., Larocca, V., Di Sanzo, G., Martino, M., … Musmarra, D. (2018). Extraction of astaxanthin and lutein from microalga Haematococcus pluvialis in the red phase using CO2 supercritical fluid extraction technology with ethanol as co-solvent. *Marine Drugs*, *16*(11), 432. https://doi.org/10.3390/md16110432

Mostert, M. E., Botha, B. M., Du Plessis, L. M., & Duodu, K. G. (2007). Effect of fruit ripeness and method of fruit drying on the extractability of avocado oil with hexane and supercritical carbon dioxide. *Journal of the Science of Food and Agriculture*, *87*(15), 2880–2885. https://doi.org/10.1002/JSFA.3051

Prasad, S., Gupta, S. C., & Tyagi, A. K. (2017). Reactive oxygen species (ROS) and cancer: Role of antioxidative nutraceuticals. *Cancer Letters*, *387*, 95–105. https://doi.org/10.1016/J.CANLET.2016.03.042

Richter, B. E., Jones, B. A., Ezzell, J. L., Porter, N. L., Avdalovic, N., & Pohl, C. (1996). Accelerated solvent extraction: a technique for sample preparation. *Analytical Chemistry*, *68*(6), 1033–1039.

Rojas-García, A., Fuentes, E., Cádiz-Gurrea, M. de la L., Rodriguez, L., Villegas-Aguilar, M. D. C., Palomo, I., Arráez-Román, D., & Segura-Carretero, A. (2022). Biological Evaluation of Avocado Residues as a Potential Source of Bioactive Compounds. *Antioxidants*, *11*(6), 1049. https://doi.org/10.3390/ANTIOX11061049/S1

Satriana, S., Supardan, M. D., Arpi, N., & Wan Mustapha, W. A. (2019). Development of Methods Used in the Extraction of Avocado Oil. *European Journal of Lipid Science and Technology*, *121*(1), 1800210. https://doi.org/10.1002/EJLT.201800210

Toda, T. A., Visioli, P. D. C. F., de Oliveira, A. L., & da Costa Rodrigues, C. E. (2021).

Conventional and pressurized ethanolic extraction of oil from spent coffee grounds: Kinetics study and evaluation of lipid and defatted solid fractions. *The Journal of Supercritical Fluids*, *177*, 105332. https://doi.org/10.1016/j.supflu.2021.105332

Tremocoldi, M. A., Rosalen, P. L., Franchin, M., Massarioli, A. P., Denny, C., Daiuto, É. R., Paschoal, J. A. R., Melo, P. S., & De Alencar, S. M. (2018). Exploration of avocado by-products as natural sources of bioactive compounds. *PLOS ONE*, *13*(2), e0192577. https://doi.org/10.1371/JOURNAL.PONE.0192577

Tzompa-Sosa, D. A., Yi, L., van Valenberg, H. J. F., van Boekel, M. A. J. S., & Lakemond, C. M. M. (2014). Insect lipid profile: aqueous versus organic solvent-based extraction methods. *Food Research International*, *62*, 1087–1094. https://doi.org/10.1016/j.foodres.2014.05.052

Wagner, H.; Bladt, S. **Plant drug analysis – A thin layer chromatography atlas**. Springer, London, 384p., 2009.