GC×GC-TOFMS of bio-oils from pyrolysis of açaí seeds (*Euterpe oleracea* Marth)

Nathalia M. Conrado,[a] Anaí L. dos Santos,[d] Rafael O. Farrapeira,[a] Yasmine B. Andrade,[b] Allan S. Polidoro,[d] Laiza C. Krause[b,d], Lisiane dos S. Freitas,[c] Elina B. Caramão [a, b, d]

[a] INCT Energia & Ambiente, UFBA, Salvador, BA, Brazil.
[b] Programa de pós-graduação em biotecnologia industrial, Universidade Tiradentes, Aracaju, SE, Brazil.
[c] Universidade Federal de Sergipe, Aracaju, SE, Brazil
[d] Instituto de Tecnologia e Pesquisa - ITP, Universidade Tiradentes, Aracaju, SE, Brazil

Abstract: The açaí processing industry generates a significant amount of waste, predominantly seeds, which constitute about 80% of its weight. The aim of this study is to evaluate the chemical composition of the bio-oil from açaí seeds using GC×GC-TOFMS. The açaí seeds underwent a preliminary treatment involving the extraction of the lipid fraction utilizing an EDGE™ extractor (sequential extraction - conducted 20 times with 20 mL of petroleum ether at 70°C, held for 10 minutes). Following this, the solid residue underwent pyrolysis in a mini fixed-bed reactor (with a heating rate of 100 °C min^{-1}, a nitrogen flow rate of 2 mL min^{-1} and maintained at 600 °C for a one-minute residence time). Analysis revealed a bio-oil yield of 21.44%, devoid of water content. This analysis, carried out through GC×GC-TOFMS, demonstrated that phenols were the primary category of identified compounds, comprising 59.4%, followed by other compounds typically found in bio-oils from seed biomass. This indicates the potential to add value to a residual biomass that previously had no commercial use.

Keywords: GC×GC-TOFMS, açai, pyrolysis, EDGE

GC×GC-TOFMS de bio-óleos de pirólise de sementes de açaí (*Euterpe oleracea* Marth)

Resumo: A indústria de processamento de açaí gera uma quantidade significativa de resíduos, predominantemente sementes, que constituem cerca de 80% do seu peso. O objetivo deste estudo é avaliar a composição química do bio-óleo de sementes de açaí usando GC×GC-TOFMS. As sementes de açaí passaram por um tratamento preliminar que envolveu a extração da fração lipídica utilizando um extrator EDGE™ (extração sequencial - realizada 20 vezes com 20 mL de éter de petróleo a 70°C, mantida por 10 minutos). Após isso, o resíduo sólido passou por pirólise em um reator de leito fixo mini (com uma taxa de aquecimento de 100 °C min-1, uma taxa de fluxo de nitrogênio de 2 mL min^{-1} e mantido a 600 °C por um minuto de tempo de residência). A análise revelou um rendimento de bio-óleo de 21,44%, isento de água. Esta análise, realizada através de GC×GC-TOFMS, demonstrou que os fenóis foram a principal categoria de compostos identificados, compreendendo 59,4%, seguidos por outros compostos tipicamente encontrados em bio-óleos de biomassa de semente. Isso indica o potencial de agregar valor a uma biomassa residual que anteriormente não tinha uso comercial.

Palavras-chave: GC×GC-TOFMS, açaí, pirólise, EDGE

1. INTRODUCTION

Euterpe oleracea Marth., commonly known as açaí, is a fruit with a high concentration of bioactive compounds and substantial economic value in Brazil. In 2020, around 1.4 million tons of açaí were produced in Brazil.[1,2] The industrial processing of the fruit generates a large volume of waste, primarily consisting of seeds, which make up 80% of the fruit's total weight.[3]

Pyrolysis, defined as the thermal decomposition of biomass in an oxygen-free environment, is a straightforward and cost-effective method for converting biomass into high-value by-products. When conducted at temperatures exceeding 500 °C, this process seeks to break down biopolymers, with bio-oil being a favored outcome.[4-6]

Bio-oil, obtained from pyrolysis, is a complex mixture encompassing various organic compounds. The Comprehensive Two-Dimensional Gas Chromatography (GC×GC) technique is aptly suited for exploring its intricate chemical characteristics.[4,5] Within the GC×GC framework, the modulator emerges as a vital component. Predominantly, commercially available modulators for GC×GC operate based on cryogenic or flow modulation principles. Notably, flow modulators are valued for their simplicity, affordability, and efficacy in analyzing a range of samples. Such modulators collect and concentrate the effluent from the ^{1}D column into a collection channel before swiftly releasing it into the ^{2}D column.[7]

Although research exists on the pyrolysis of comparable biomass sources like mango.[8] peach,[9] Mangaba,[10] grape seeds,[11] and sugarcane bagasse,[12] studies focusing exclusively on açaí seed pyrolysis are relatively scarce.

Bio-oils derived from the pyrolysis of triglyceride-based biomasses, like seeds, exhibit distinct characteristics compared to other lignocellulosic biomasses. While triglyceride-based vegetable oils hold promises as potential fuel or hydrocarbon sources under appropriate processing conditions, [13] these are generally extracted from seeds through methods other than pyrolysis. Therefore, the presence of fatty acids is not deemed favorable in pyrolytic bio-oils. One alternative is to pre-extract these fatty substances utilizing solvents such as hexane or petroleum ether.

Recently, the Energized Dispersive Guided Extraction (EDGE) method emerged as an innovative approach for plant materials, combining the strengths of pressurized liquid extraction (PLE) and solid-phase extraction (SPE).[14,15] Claimed to surpass the Soxhlet method in speed, automation than QuEChERS, and simplicity than other solvent extraction systems, EDGE® presents numerous advantages including reduced solvent usage, shorter operation durations, and enhanced yield and extract quality [16,14] This method employs a two-piece open sample vessel named the "Q-Cup" to contain the sample. During the process, the solvent is introduced to the sample housed within the Q-Cup, followed by pressurization and heating to predefined temperature and duration settings. Upon completion, the extract undergoes filtration and is collected in a designated vial.[14,15] The remaining solid material is then suitable for bio-oil production through pyrolysis.

This study aims to analyze the chemical composition of bio-oil from açaí seeds employing GC×GC-TOFMS with a forward fill/flush modulator and the bio-oil samples, with and without pre-treatment using the EDGE method for lipid extraction, were obtained through pyrolysis in a mini pyrolizer fixed-bed reactor at 600 ºC.

2. METHODOLOGY

2.1. Samples

Seeds of *E. oleracea* were supplied by Isa Foods (Ipiaú, BA, Brazil) and subsequently oven-dried at 40 °C for a duration of 24 hours. Post-drying, the seeds were ground and sieved to a particle size between 8-16 mesh, then stored in dark containers until further analysis.

2.2 Extraction of Oil Content (EDGE™ procedure)

Following the optimization process,[15] the seeds were directly weighed into a Q-Cup, which was placed in the EDGE removable rack with a 40 mL amber glass collection vial. The sequential extraction process was carried out 20 times, wherein 20 mL of petroleum ether was added to the top of the Q-Cup, subsequently heated at 70 °C and maintained at this temperature for 10 minutes. After this, the extracts were gathered, and the solvent was evaporated using a rotary evaporator. The yield of the extraction was determined by calculating the percentage ratio of the weight of the produced oil to the weight of the initial dried material.

To synthesize fatty acid methyl esters (FAMEs), the obtained vegetable oil was subjected to esterification. Prior to analysis, the sample was dissolved in hexane. A standard solution of FAMEs (C12:0, C16:0, C16:1, C18:0, C18:1, C18:2) was used for compound identification, with margaric acid methyl ester as the internal standard. The composition of açai seed oil was analyzed through GC/qMS. All experiments were performed in triplicate. The dried seeds were subjected to the pyrolysis process.

2.3 Pyrolysis conditions

Pyrolysis experiments were conducted using a mini fixed-bed reactor, as depicted in **Figure 1**. Two types of samples were assessed: treated and untreated açaí seeds. A 1.0 g portion of each sample was firmly packed within the reactor, cushioned by glass wool at both ends to minimize displacement. The oven was heated to 600 °C before the introduction of the reactor. Prior to inserting the reactor, the oven was preheated to 600 °C. The reactor, laden with biomass and already connected to a nitrogen flow at a rate of 2 mL min^{-1}, was then introduced into the preheated oven.

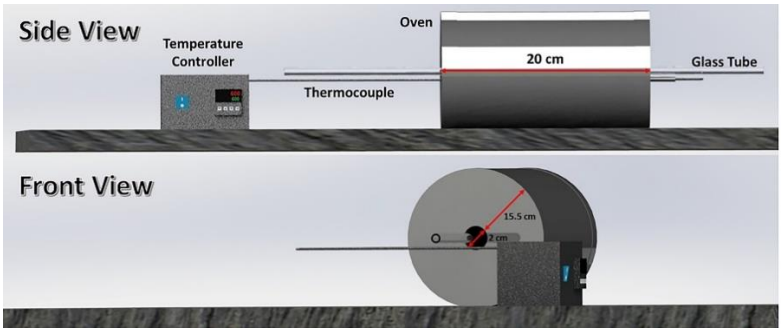

Figure 1: Pyrolysis system

Following the process, the reactor was removed and allowed to cool. Subsequently, the bio-oil was extracted using acetone, passed through a filter containing anhydrous sodium sulfate, and then left to permit the solvent to evaporate. Both the bio-oil and the biochar (the solid residue) were then weighed, with their

respective yields being calculated as percentages of the initial biomass weight. Gas yields and potential losses, including coke formation, were assessed based on weight differences. This experiment was replicated thrice. The composition of the resulting bio-oil was then analyzed using GC×GC-TOFMS.

2.4 Chromatographic analysis

2.4.1. GC-qMS analysis of the oil

The analysis of the FAME (fatty acid methyl esters) composition in açai seed oil was carried out using a Shimadzu system, which included a GC2010 gas chromatograph integrated with a QP2010-ultra quadrupole mass spectrometer. Operating at 70 eV, the mass spectrometer scanned a range from 40 to 450 m/z. Separations were achieved on a ZB-5 analytical column (5% phenyl – 95% dimethylpolysiloxane, 50 m × 0.25 mm × 0.25 µm). The injector, interface, and ion source temperatures were maintained at 300 °C. Samples were injected in splitless mode at a concentration of 2500 mg L^{-1}, with a carrier gas flow rate of 1 mL min^{-1} using He. The oven temperature program started at 120 °C (hold 5 min) and increased at a rate of 3 °C min^{-1} until reaching 285 °C. Data processing was performed using GC-qMS solution 2.6.1 software. FAMEs standards, including lauric, palmitoleic, palmitic, linoleic, oleic, and stearic acid methyl esters, were used for identification and quantification, with margaric acid methyl ester as an IS. The FAMEs in the açaí seed oil samples were quantified utilizing their respective response factors. Results were expressed in mg g^{-1} of oil, and the experiment was conducted in triplicate.

2.4.2. GC×GC-TOFMS analysis of the bio-oil

The bio-oils from treated and untreated açai seeds were analyzed using a gas chromatograph 8890 Agilent with an RFF flow modulator coupled with a 7250 Q-TOFMS system. The ionization energy for electron impact (EI) was set at 70 eV, and the mass acquisition was conducted within the range of 50 to 500 Da at 50 Hz. The ion source, transfer line, and injector were maintained at a temperature of 300 °C. Samples were introduced into the GC×GC inlet system through an autosampler PAL RTC 120 in splitless mode, with helium as the carrier gas. A conventional column set was employed: DB-5 (5% phenyl–95% dimethylpolysiloxane) with 20 m length, 0.18 mm of internal diameter, and 0.18 µm of film thickness in the first dimension and a DB-17 ms (50% phenyl–50% dimethylpolysiloxane) with 3,8 m length, 0.18 mm of internal diameter and 0.18 µm of film thickness. The temperature program started at 50 °C for 5 minutes, followed by a heating rate of 5 °C min^{-1} until reaching 300 °C, which was held for 5 minutes.

The RFF flow modulator used in this study has three ports connecting the ^{1}D and ^{2}D columns, as well as a restrictor capillary. The RFF's collection channel was etched in the plate, and connected via two metal branches to a three-way micro solenoid valve. The solenoid valve received a controlled gas supply from an auxiliary electronic pressure control (EPC) module, allowing gas flow into the plate. The effluent flow rate from the ^{1}D column entered the modulator plate's center port, filling the fixed-size collection channel connected to a restrictor capillary port (in this study, a 5 m × 100 µm silanized fused silica capillary was used). This process occurred during the sampling time of 4.5 seconds, with a constant flow rate of 0.5 mL/min in the ^{1}D column.

The restrictor capillary enabled the carrier gas to pass through the accumulation capillary during the filling cycle and reversed the flow direction during the flush cycle. The effluent exiting the capillary restrictor was monitored by a flame ionization detector (FID), which allowed for the monitoring of analyte breakthrough. After the loading of the collection channel, the three-way solenoid microvalve switched the flow from the EPC module, and the channel was flushed for 0.500 seconds in the reverse direction of the fill flow into the ^{2}D column. The ^{2}D column was connected to the Q-TOFMS and maintained a constant flow rate of 7 mL min^{-1}. During the modulation time of 5 seconds, the components present in this band were separated.

The analysis of both GC and GC×GC utilized linear-temperature-programmed retention indices (LTPRI) for peak detection. The software automatically calculated the indices after injecting a mixture of linear saturated hydrocarbons ranging from 7 to 33 carbon atoms, using the same analytical conditions as the samples. Peaks were considered tentatively identified if they exhibited an identity spectrum match factor equal to or above 80% when compared to the library. Besides the spectral analysis, a qualitative manual verification of the mass spectra of each compound was performed, comparing them with those from the NIST library.

3. RESULTS AND DISCUSSION

3.1. Pre-treatment of seeds

The oil yield from the seeds was 3.88 ± 0.19%. The EDGE system used in this study achieved higher oil yields from açai seeds compared to conventional Soxhlet extraction methods mentioned in the literature.[19,20] The EDGE, with its fully automated nature for lipid recovery from seeds, offered advantages such as reduced solvent usage, shorter processing time, and high reproducibility, as indicated by a lower relative standard deviation, besides to the high mass yield.

Table 1 shows that oleic and linoleic acids account for over 58% of the fatty acid methyl esters (FAMEs) in açai seed oil, making it a valuable source of unsaturated fatty acids. The myristic acid content is 45.90 mg g^{-1}, while the remaining fraction includes lauric (35.46 mg g^{-1}) and palmitic (33.20 mg g^{-1}) acids, among others. This FAMEs profile aligns with previous reports on açai seed oil.[19,20]

Table 1: FAMEs composition of the açai seed oil.

Saturates	Conc.(*)	Unsaturated	Conc.(*)
C 12:0 - Lauric acid	35.46 ± 0.33	C 18:1- Oleic acid (ω-9)	75.30 ± 0.77
C 14:0 - Myristic acid	45.90 ± 0.63		
C 16:0 - Palmitic acid	33.20 ± 0.26	C 18:2 - Linoleic acid (ω-6)	91.97 ± 0.86
C 18:0 - Stearic acid	4.56 ± 0.09		
∑ ***saturated***	119.12 ± 0.025	∑ ***unsaturated***	167.27 ± 0.025

Conc. (*) = concentration in mg g^{-1} of oil

3.2. Pyrolysis of the seeds

The pyrolysis yield (%w/w) of bio-oil and biochar from treated and untreated açai seeds were as follows: untreated seeds yielded **16.02 ± 0.65%** for bio-oil and **26.97 ± 2.05%** for biochar, while treated seeds yielded **21.44 ± 1.01%** for bio-oil and **12.56 ± 1.51%** for biochar. The results demonstrated that the extraction of lipids led to an increase in bio-oil yield but a decrease in biochar production. The procedure for

extracting the lipids might have unobstructed the biomass pores, improving its structure and forming semi-volatile compounds. At operation conditions (600 °C, 1 minute, and 2 mL min^{-1} N_2 flow), the mini-fixed-bed reactor yield was similar/substantially higher compared to the literature data of other systems [21,22]

3.3. Chromatographic analysis of bio-oils

Figure 2 illustrates the color diagram of the GC×GC-TOFMS analysis for the bio-oils obtained from (a) untreated and (b) treated açai seeds. The untreated seed bio-oil (**Fig. 2a**) exhibited dominant peaks of fatty acids, including lauric, myristic, palmitic, and oleic acids. In contrast, the treated seed bio-oil (**Fig. 2b**) displayed a greater number of peaks in the chromatographic region from 10 to 45 minutes, indicating the presence of semi-volatile compounds commonly found in bio-oils [21,22]. The pre-treatment effectively extracted fatty acids and enhanced the bio-oil's quality. This extracted oil can be utilized in the food and cosmetic industries, ensuring the use of açai seed residues for both purposes without creating competition between food and biofuel production.

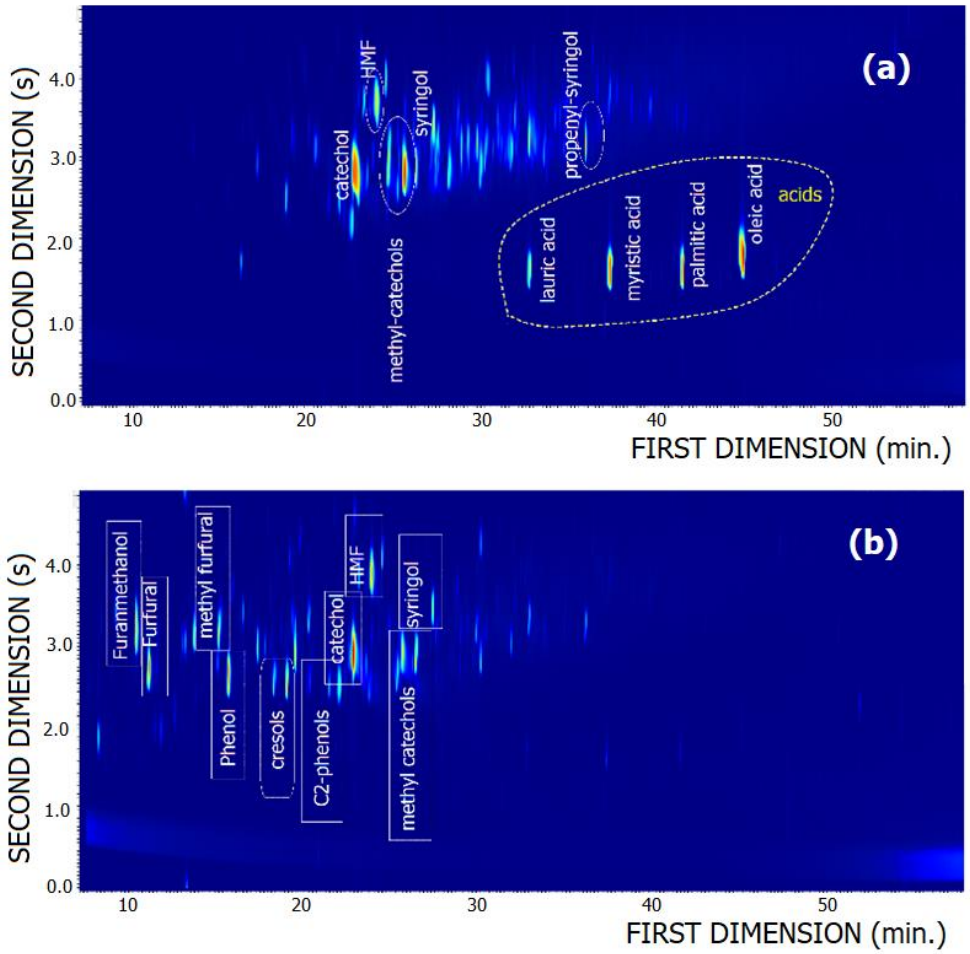

Figure 2: GC×GC-TOFMS results for the bio-oils from (a) untreated and (b) treated açai seeds.

Semi-quantification was performed by analyzing the relative volume of the identified peaks (**Figure 3**). The bio-oil from treated seeds contained a variety of

compounds, including phenols, aldehydes, ketones, hydrocarbons, sugars, and some fatty acids that were not extracted during the seed treatment.

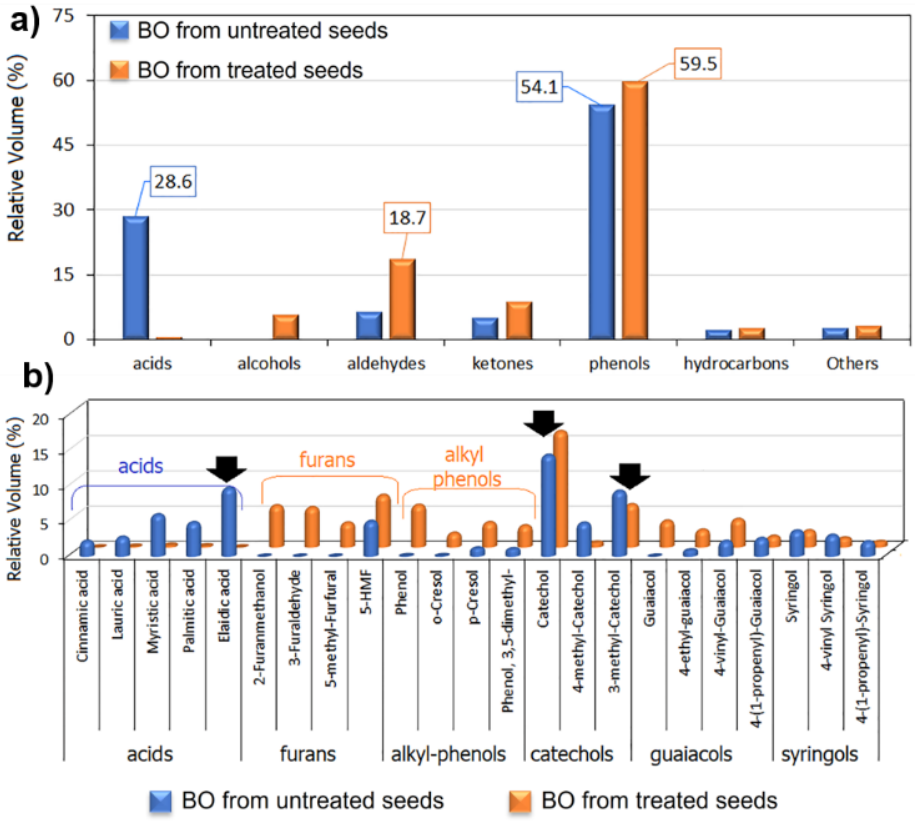

Figure 3: (a) bio-oil chemical class composition and (b) main compounds (with a volume % higher than 2.0%).

Due to the lower fatty acids relative content, seeds treated produced a bio-oil with higher relative levels of phenols, aldehydes, and ketones. Besides that, both samples exhibited a predominance of benzenediols (derived from catechol). On the other hand, alkyl phenols were barely detected in the bio-oil from untreated seeds. Similarly, aldehydes derived from furfural were only observed in the bio-oil from untreated biomass, with hydroxy methyl furfural (HMF) being the detected compound.

4. CONCLUSION

In conclusion, the biomass pre-treatment process facilitated enhancements in both the yield and quality of bio-oil, albeit at the expense of a reduced biochar yield. Notably, the mini pyrolyzer reactor exhibited yields akin to those attained by larger-scale fixed-bed reactors, thereby positing it as a viable candidate for future pyrolysis investigations. Moreover, the bio-oil extracted from açai seeds—a notable byproduct of the fruit processing sector—showcased a high concentration of valuable oxygenated organic compounds, encompassing benzenediols, alkyl-phenols, and furans. This underscores its potential to serve as a lucrative resource within the chemical industry.

Acknowledgments

The authors thank FAPITEC, CAPES, CNPq, INCT E&A, and Petrobras for the financial support to this study.

5. REFERENCES

[1] Benchimol, M. et al. Losing our palms: The influence of landscape-scale deforestation on Arecaceae diversity in the Atlantic forest. Forest Eco. Manag. 384, 314–322, 2017.
[2] IBGE, Produção Agrícola Municipal, IBGE, Brazil, 2020. https://www.ibge.gov.br/estatisticas/economicas/agricultura-e-pecuaria/9117-producao-agricola-municipal-culturas-temporarias-e- permanentes.html?edicao=31675&t=destaques (accessed July 8, 2022)
[3] Coelho, G.M. et al. Genetic structure among morphotypes of the endangered Brazilian palm *Euterpe edulis* Mart (Arecaceae). **Eco. Evol.** 10, 6039–6048, 2020.
[4] Tripathi, M. et al. Effect of process parameters on production of biochar from biomass waste through pyrolysis: A review. **Renew. Sust. Energy Rev.** 55, 467–481, 2016.
[5] Mohan, D. et al. Pyrolysis of Wood/Biomass for Bio-oil: A Critical Review, **Energy & Fuels**. 20, 848–889, 2006.
[6] Mohammed, I.Y. et al. Valorization of Bambara groundnut shell via intermediate pyrolysis: Products distribution and characterization, **J. Clean. Prod.** 139, 717–728, 2016.
[7] Krupčík, J. et al. Comparison of the performance of forward fill/flush and reverse fill/flush flow modulation in comprehensive two-dimensional gas chromatography, **J. Chromatogr. A.** 1466, 113–128, 2016.
[8] Lazzari, E. et al. Production and chromatographic characterization of bio-oil from the pyrolysis of mango seed waste, **Ind. Crops Prod**. 83, 529–536, 2016.
[9] Migliorini, M.V. et al, Caracterização de fenóis no bio-óleo da pirólise de caroço de pêssego por GC-MS e GC×GC-TOFMS, **Scientia Chromatographica**. 5, 47–65, 2013.
[10] Santos, R.M. et al. Pyrolysis of mangaba seed: Production and characterization of bio-oil, **Biores. Technol.** 196, 43–48, 2015.
[11] Sanahuja-Parejo, et al. Catalytic co-pyrolysis of grape seeds and waste tyres for the production of drop-in biofuels, **Energy Conv. Manag.** 171, 1202–1212, 2018.
[12] Barros, J.A.S. et al. Chromatographic characterization of bio-oils from fast pyrolysis of sugar cane residues (straw and bagasse) from four genotypes of the Saccharum Complex, **Microchem. J.** 137 30–36, 2018.
[13] Maher K.D et al. Pyrolysis of triglyceride materials for the production of renewable fuels and chemicals, **Biores. Technol.** 98, 2351–2368, 2007.
[14] CEM Corporation, A Comparison of Potency Extraction Methods on the EDGE, (2022). Evalable in https://cem.com/en/a-comparison-of-cannabis-potency-extraction-methods, accessed June/2023).
[15] Dos Santos., Paulo. Natan et al. Optimization of Energized Dispersive Guided Extraction (EDGE) of antioxidants from Eugenia uniflora L. (Pitanga) leaves using Response Surface Methodology, **Microchem. J.** 187, 108411, 2023.
[16] Kumar, J.H.A.A. et al. Extraction of bioactive compounds from plant materials using combination of various novel methods: A review, **Trends Food Sci. Technol.** 119, 579–591, 2022.
[17] Kinross, A.D. et al. Comparison of Accelerated Solvent Extraction (ASE) and Energized Dispersive Guided Extraction (EDGE) for the analysis of pesticides in leaves, **J. Chromatogr. A.** 1627, 461414, 2020.
[18] Freitas, L.S. et al. Effect of experimental parameters in the pressurized liquid extraction of Brazilian grape seed oil, **Sep. Pur. Technol.** 116, 313–318, 2013.
[19] Okada, Y. et al. A Study on Fatty Acids in Seeds of Euterpe oleracea Mart Seeds, **J. Oleo Sci**. 60, 463–467, 2011.
[20] Melo, P.S., et al. Açaí seeds: An unexplored agroindustrial residue as a potential source of lipids, fibers, and antioxidant phenolic compounds, **Ind. Crops Prod**. 161, 113204, 2021.
[21] Farrapeira R. O et al. Characterization by Fast-GC × GC/TOFMS of the Acidic/Basic/Neutral Fractions of Bio-Oils from Fast Pyrolysis of Green Coconut Fibers, **Ind. Eng. Chem. Res**. 61, 9567–9574, 2022.
[22] Andrade, Y.B, et al. Chromatographic analysis of N-compounds from the pyrolysis of spent coffee grounds, **Sep. Sci. Plus**, 6(1), 2200057, 2023.