High pressure extraction techniques for the recovery of carotenoid-rich extracts from pressed palm fiber: A case of study

Fiorella P. Cárdenas-Toro, PhD[1], G. Keiko Nakama-Hokamura, Mg[1], Janclei P. Coutinho, PhD[2], Helena Texeira Godoy, PhD[3] and M. Angela A. Meireles, PhD[4]
[1]Pontifical Catholic University of Peru, Department of Engineering, Section of Industrial Engineering, Peru, fcardenas@pucp.pe, nakama.gk@pucp.edu.pe
[2]State University of Santa Cruz, Department of Exact and Technological Sciences, Brazil, jpcoutinho@uesc.br
[3]University of Campinas, School of Food Engineering, Lab Análise/DCA/FEA/UNICAMP, Brazil, helenatg@unicamp.br
[4]University of Campinas, School of Food Engineering, LASEFI/DETEA/FEA/UNICAMP, Brazil, maameireles@lasefi.com

Abstract– ***In this work, a comparison of two high extraction techniques: pressurized liquid extraction (PLE) and supercritical fluid extraction (SFE) for the recovery of carotenoid-rich extracts from pressed palm fiber (PPF) was carried out in terms of yield and carotenoid profile to evaluate their industrial applicability. The PLE experiment was performed at 35°C, 4 MPa, 2.4 g/min and S/F ratio of 1, 2, 4, 8, 12, 16 and 20 with ethanol as extraction solvent. The SFE was performed at 45°C, 15 MPa, 3.4 g/min and S/F ratio of 2, 4, 6, 8, 12, 16, 20, 30 and 40 with carbon dioxide as supercritical solvent. The results indicated that the extraction yield and the carotenoid recovery obtained at S/F of 20 for PLE was of 42.6 ± 0.2 mg extract/ g PPF d.b., and 48 ± 3 μg α-carotene/g PPF d.b. and 117 ± 4 μg β-carotene/g PPF d.b. (1136 ± 54 μg α-carotene/g extract and 2740 ± 110 μg β-carotene/g extract). For SFE, the extraction yield and the carotenoid recovery obtained at S/F of 20 was of 58 ± 3 mg extract/g PPF d.b., and 26 ± 11 μg α-carotene/g PPF d.b. and 66 ± 22 μg β-carotene/g PPF d.b. (461 ± 211 μg α-carotene/g extract and 1144 ± 439 μg β-carotene/g extract).***

***Keywords*-- Carotenoid, pressed palm fiber, pressurized liquid extraction, supercritical fluid extraction**

Digital Object Identifier (DOI):
http://dx.doi.org/10.18687/LACCEI2022.1.1.634
ISBN: 978-628-95207-0-5 **ISSN:** 2414-6390

I. INTRODUCTION

Pressed palm fiber (PPF) is a byproduct of the palm oil extraction industry which is composed mainly of a mixture of mesocarp fiber and crushed husk [1]. In South America, palm oil tree is cultivated mainly in Peru and Brazil [2]. PPF is a source of carotenoids, especially of α- and β-carotene, which are recognized for its antioxidant properties and provitamin A activity [3,4]. The process of recovery of these bioactives compounds from this residue as part of the palm industrialization process can reduce the environmental impact generated by these wastes. In addition, an economical evaluation of this process is required for its industrial application. There are several technologies that can be applied to the recovery of carotenoid rich extracts from vegetal matrix. These include low pressure extraction techniques such as Soxhlet (LPSE-SOX), percolation (LPSE-PE), and high pressure extraction techniques such as pressurized liquid extraction (PLE) and supercritical fluid extraction (SFE) [5-7].

A previous work shows that the extracts obtained using PLE process resulted in the best results in terms of carotenoid concentration in extracts and manufacturing cost of extracts showing that high-pressure techniques using clean solvents have potential for industrial scale facility [8]. Furthermore, carotenoid recovery from PPF using SFE with carbon dioxide as solvent have the advantage of solvent separation after separator due to the change in solvent properties when compared with PLE [1,9].

A comparison of these two techniques in terms of highest carotenoid recovery in extract can give us an insight of business opportunities using high pressure techniques. In this work, the kinetic study of PLE and SFE techniques using ethanol and carbon dioxide as solvents was carried out at the optimal operational conditions found in previous studies [8,10]. PLE extraction was performed at temperature of 35°C, pressure of 4 MPa, and flow rate of 2.4 g/min. SFE extraction was performed at temperature of 45°C, 15 MPa and flow rate

of 3.4 g/min. The results were evaluated in terms of extraction yield and carotenoid concentration of overall extraction curve.

II. POTENTIAL APPLICATIONS IN SOUTH AMERICA

Obtaining industrial and biotechnological products and by-products from the palm fruit is diverse. Figure 1 shows the different industrial and biotechnological products that can be obtained from palm fruit as a bio refinery.

.

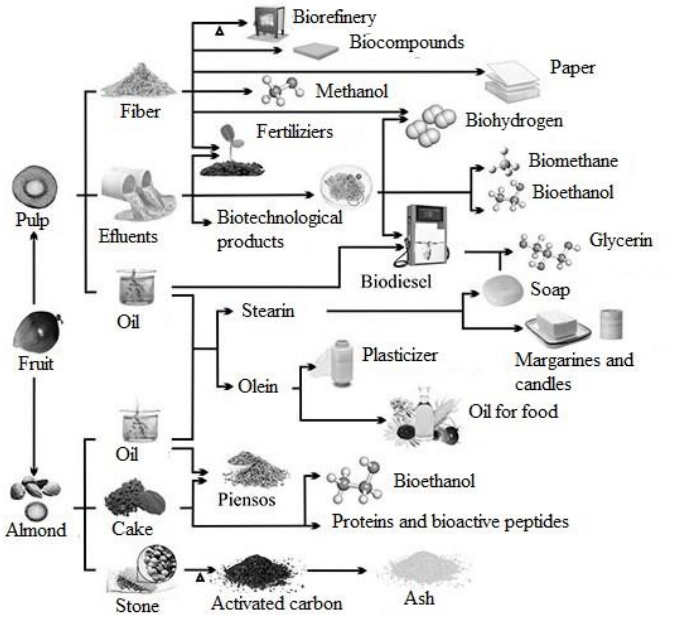

Figure 1. Alternatives for comprehensive use of industrial and biotechnological products and byproducts, that can be produced from oil palm fruit [11]

From Figure 1, we noticed that residues from oil palm industry can be used in different industries, generating opportunities for the development of by-products. Figure 2 shows that countries in South America (Ecuador, Colombia and Peru) can develop its economic potential from palm oil residues. Since they are among the 10 countries with the highest growth rate of palm oil production.

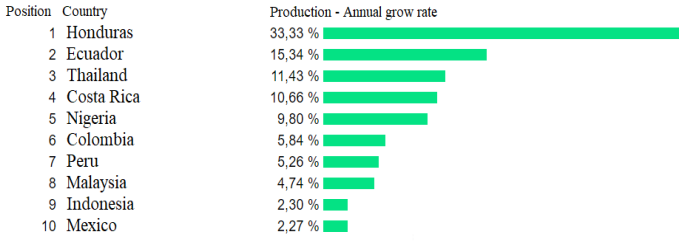

Figure 2. Production – Annual growth rate 2021 [12]

An important characteristic of red palm oil is that it is the richest vegetable source of carotenoids in terms of Provitamin A, with antioxidant qualities that can protect different diseases [13]. The WHO includes within the Guidelines for the fortification of foods with micronutrients [14], it mentions vitamin A compounds for use in the fortification of specific foods as shown in Table 1.

Table 1. Vitamin A compounds and their suitability for specific food vehicles [14]

Food vehicle	Compound of Vitamin A	Stability
Cereal flours	Retinyl palmitate or acetate (stabilized dry forms)	Adequate
Fats and oils	β-carotene and retinyl palmitate or acetate (soluble in oil)	Good
Sugar	Retinyl palmitate (water dispersible forms)	Adequate
Milk powder	Retinyl palmitate or acetate (water dispersible dry forms)	Good
Liquid milk	Retinyl acetate or palmitate (oily form, emulsified)	Good/adequate depending of packing
Infant formulas	Retinyl palmitate (Water dispersible microspheres)	Good
Fat spreads	Retinyl acetate or palmitate (oily form)	Good

In the last decade of South American countries, such as Colombia, there are strategies by the government for the control and reduction of micronutrients [15] through the consumption of vitamin A, likewise in Peru the same strategies are applied for children in extreme poverty or poverty with anemia problems [16]

III. HIGH PRESSURE TECHNOLOGY FOR RECOVERY OF BY-PRODUCTS FROM PRESSED PALM FIBER

A. MATERIAL AND METHODS

A.1. Chemical and reagents.

Ethanol (99.5%) was obtained from Chemco Ltda. (São Paulo, Brazil). Carbon dioxide (99.9%) was obtained from White Martins Inc. (São Paulo, Brazil). The analytical reagents used in carotenoid analysis, namely petroleum ether (≥ 99.5%), ethylic ether (≥ 99.5%), acetone (≥ 99.5%), methanol (≥ 98%) and potassium hydroxide (> 90%), were obtained from Synth (São Paulo, Brazil). Acetonitrile (≥ 99.9%, HPLC grade) was obtained from JT Baker (New Jersey, USA). Methanol (≥ 99.9%, HPLC grade) was obtained from Merck (Darmstadt, Germany). Ultrapure water (18.2 mΩ) was obtained using a Direct-Q 3 UV ultrapure water system (Millipore Corporation, France). Magnesium oxide (97%) and Celite® Hyflo Supercel were obtained from Merck (Darmstadt, Germany).

A.2. Raw material preparation and characterization

The sample of PPF was dried at 30°C for 24 hours in a forced air circulation drying oven (Marconi, model MA-35, São Paulo, Brazil). Then, the material was comminuted using

a knife mill (Marconi, model A340/ 0204244, São Paulo, Brazil) to homogenize the sample. Particles smaller than 80 mesh were separated using sieves (Series Tyler, W.S. Tyler, Wheeling, USA) in a vertical vibratory shaker (Bertel Metallurgic Ind. Ltda., São Paulo, Brazil) to prevent clogging problems during the extraction process.

A.3. Extraction experiments

A.3.1 Pressurized liquid extraction (PLE)

PLE was performed in a homemade unit [17], which was composed of an HPLC pump (Thermoseparation Products, Model Constametric 3200 P/F, Fremoni, USA) to pump the solvent, a 6.57 cm^3 extraction cell of 2.09 cm x 2.0 cm i.d.) with sintered metal filters at the bottom and top parts (Thar Designs, Pittsburg, USA), an electrical heating jacket to heat the extraction medium , stop valves (Autoclave Engineers, Model 10V2071, Philadelphia, USA) and a back pressure valve (Tescom, Model 261700, Seimsdorf, Germany) to maintain constant pressure during the extraction as shown in Figure 3. An apparent density of 294.0 ± 0.2 kg/m^3 was considered for all experiments. Two grams of PPF was packed inside the extraction cell, connected to the preheated system at the temperature of 35°C and held for a period of 5 minutes to ensure thermal equilibrium prior to system pressurization. Then, the system was pressurized with ethanol to 4 MPa by closing the solvent outlet with the stop valve. The pressure was kept constant for the static extraction time of 5 minutes until reaching system stabilization. The dynamic extraction was started by pumping ethanol, which percolated through the vegetal matrix, extracting ethanol soluble-compounds at a specific flow rate of 2.4 g/min at the set temperature and pressure controlled by the back pressure valve. Samples were collected in a flask collector at solvent/feed ratio (S/F) of 1, 2, 4, 8, 12, 16 and 20. The solvent was evaporated using a vacuum evaporator. The dried extracts were weighed in analytical balance (Sartorius Analytic, A200S, GMBH Gottingen, Germany) for yield calculation and stored at -5°C for carotenoid analysis. The experiments were performed in duplicate.

A.3.2. Supercritical fluid extraction (SFE)

The extraction experiments were performed in a homemade SFE unit equipped with a cooling bath (Marconi, Model MA-126, São Paulo, Brazil), a booster pump (M111, Maximator, Niedersachen, Germany), a heating bath (Marconi, Model MA-126, São Paulo, Brazil), extraction vessel of 54.4 cm^3 with a jacket (Autic, São Paulo, Brazil), a compressor (Shulz S/A, Model MS 6V, São Paulo, Brazil), and a flow totalizer (Itrón Inc., Model ACDG1.0, Argentin) as shown in Figure 4. Supercritical CO_2 was used as the extracting solvent. An apparent density of 294.33 ± 0.05 kg/m^3 was considered for all experiments. The extraction vessel was assembled and placed inside the jacket at the temperature of 45°C and CO_2 was pumped into the system until reaching the pressure of 15 MPa. A static period of 10 min was used before the dynamic extraction step. The total CO_2 mass was measured by means of the flow totalizer and it was not recirculated. The extract was collected inside a sealed 100 cm^3 amber glass flask immersed on an ice bath. The samples were collected for S/F ratio of 2, 4, 6, 8, 12, 16, 20, 30 and 40. The extract mass was measured in analytical balance (Sartorius Analytic, A200S, GMBH Gottingen, Germany) and stored under freezing (−4°C) in the absence of light for further analyses.

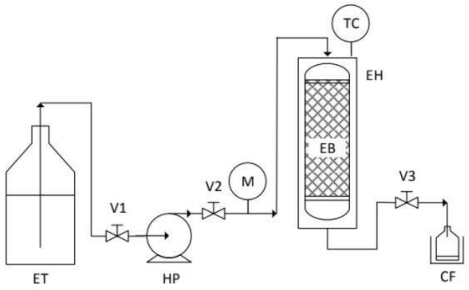

Figure 3. Schematic diagram of PLE unit ET: ethanol tank; HP: high-pressure pump; FC: flow controller; TC: temperature controller; M: manometer; EH: electric heater; V1, V2 and V3: valves; EB: extraction bed; CF: collection flask

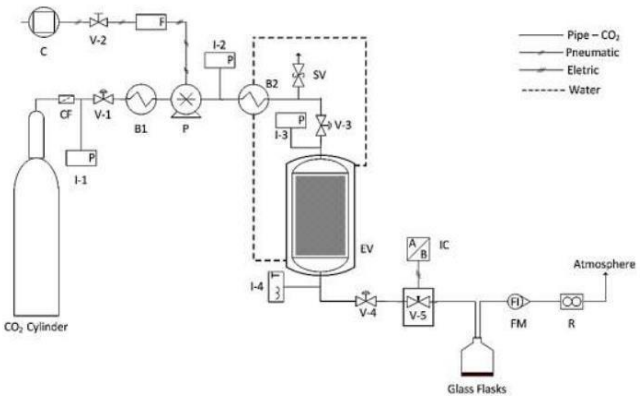

Figure 4. Schematic diagram of SFE unit: V-1, V-2, V-3, V-4: control valves; V-5: micrometer valve; SV: safety valve; C: compressor; F: compressed air filter; CF–CO_2 filter; B1: cooling bath; P: booster pump; B2: heating bath; I-1, I-2 and I-3: pressure indicators; I-4: temperature indicator; IC: temperature indicator and controller of the micrometer valve; R: flow totalizer; FM: flow meter; EV: jacketed extraction vessel

A.4. Extract characterization

A.4.1. Global yield

The global yield X_0 was calculated as the ratio of the total mass of soluble material extracted at each S/F ratio ($m_{extract}$) to the initial mass of PPF (m_{PPF}) on a dry basis.

A.4.2. Analysis of α- and β-carotene

Sample preparation was performed for carotenoid analysis with some modifications [18]. A known amount of extract (0.003 – 0.2 g) was dissolved in 20 cm^3 of petroleum ether and mixed with 20 cm^3 of potassium hydroxide in

methanol (10% w/v). This mixture was stored in the dark at room temperature for a period of 14 hours. After that, the mixture was placed in a separatory funnel containing 20 cm^3 of petroleum ether. Two hundred cubic centimeters of distilled water was added, yielding two phases: the upper phase, containing petroleum ether and carotenoids; and the lower phase, containing methanol, water, salts of fatty acids and water-soluble secondary metabolites. The aqueous fraction was discarded, and the ethereal fraction containing carotenoids was collected in a rotary evaporator flask. The distilled-water partitioning step was repeated three times. The ethereal extract was concentrated on a rotary evaporator at 35°C under vacuum (Fisatom, São Paulo, Brazil). Finally, the extracts were dissolved in 1 or 2 cm^3 of HPLC grade ethanol for UHPLC analysis.

Chromatographic separation was performed on an Acquity UPLC system (Waters, United Kingdom) equipped with a binary pump, degasser, autosampler, photodiode detector array (PDA) and a Hypersil Gold C18 chromatographic column (100 x 2.1 mm, 1.9 µm, Thermo Scientific, Waltham, MA, USA). The PDA was set at 450 nm, and the column was maintained at 40°C. The mobile phase consisted of a mixture of ultrapure water (solvent A) and acetonitrile grade HPLC (solvent B), with an elution flow rate of 0.6 cm^3/min and the following gradient: 0 min: 80% B, 0.5 min: 100% B, 5.5 min: 80% B, 6.5 min: 80% B (equilibration time). The injection volume was 10 µL. The software used for equipment control and data acquisition was Empower Pro. The identification of carotenoids was performed by comparing retention times and absorption spectra (UV-Vis) with standards of α- and β-carotene isolated from carrots. The results were expressed as µg of compound per g of raw material on a dry basis.

A.4.3. Modelling of the overall extraction curve

The experimental data was fitted with a spline of 3 straight lines, using procedures LIN PROC and NLIN PROC of the software SAS ® (SAS Institute Inc., version 9.4 Cary, USA). Each straight line represents the constant extraction rate where mass transfer is dominated by convection (CER), falling extraction rate (FER) which represents convection and diffusion in solid matrix, and diffusion-controlled rate period (DC). The parameters obtained from the spline model were used in the equation described by Meireles [19] to calculate the extraction yield on a given S/F ratio: $m_{ext} = (b_0 - C_1b_1 - C_2b_2) + (b_1 + b_2 + b_3)*S/F$. According with this equation, the linear coefficients (b_1, b_2 and b_3) represents the slope of CER, FER and DC adjusted lines; and intercepts (C_1 and C_2) represents the period S/F_{CER} and S/F_{FER}. Also, the S/F ratio which gives the best balance between constant extraction time and completion time is located between the CER and FER period and can be calculated by the intersection of CER and DC straight lines.

B. RESULTS AND DISCUSSION

B.1. Kinetic Experiments.

The overall extraction curve (OEC) of the selected conditions for PLE (temperature of 35°C, pressure of 4 MPa, ethanol flow rate of 2.4 g/min) and SFE (temperature of 45°C, pressure of 15 MPa, CO_2 flow rate of 3.4 g/min) were performed in duplicate. Figure 5 and 6 show the extract yield, carotenoid recovery and carotenoid content in extracts as a function of S/F ratio for each process. The extraction kinetic was adjusted to a spline of three straight lines (Figure 5a and Figure 6a) which described the constant extraction period (CER), falling extraction period (FER) and diffusion-controlled period (DC). The extraction yield corresponding to S/F of 5.5 and 9 for PLE and SFE, respectively, after the CER period was about 70% of the total recovered extract at the diffusion-controlled region. In addition, the accumulated extraction curve for carotenoid content and recovery presented in Figure 5b, 5c and 6b, 6c clearly shows that the carotene concentration increased with time and the dominant mass transfer resistance is located primarily in the extract mixture. The triglycerides are preferably solubilized as compared to carotene during the CER period. A similar trend was observed by other authors [20,21]. The extraction yield, the carotenoid recovery and the carotenoid content obtained at S/F of 20 for PLE was of 42.6 ± 0.2 mg extract/ g PPF d.b.; 48 ± 3 µg α-carotene/g PPF d.b. and 117 ± 4 µg β-carotene/g PPF d.b.; 1136 ± 54 µg α-carotene/g extract and 2740 ± 110 µg β-carotene/g extract. For SFE, the extraction yield, the carotenoid recovery and the carotenoid content obtained at S/F of 20 was of 58 ± 3 mg extract/g PPF d.b.; 26 ± 11 µg α-carotene/g PPF d.b. and 66 ± 22 µg β-carotene/g PPF d.b.; 461 ± 211 µg α-carotene/g extract and 1144 ± 439 µg β-carotene/g extract. From these results, it was observed that the extract yield for SFE process was higher than the yield obtained by PLE process at the end of the studied S/F ratio. One reason could be that the diffusion controlled period for PLE process did not finished at S/F ratio of 20 due to the ethanol flow rate of 2.4 g/min, and it was lower than the carbon dioxide flow rate of 3.4 g/min. In addition, similar values of carotenoid recovery were obtained for PLE and SFE; however, the carotenoid content in extract for PLE process was about 1.5 times higher than SFE. The use of pressure in this process could promote the separation of carotenoids along with polar compounds related to ethanol. In the SFE process, the oil present in the pressed palm fiber acted as a co-solvent, which promoted the extraction of apolar compounds of low molecular weight such as carotenoids [6].

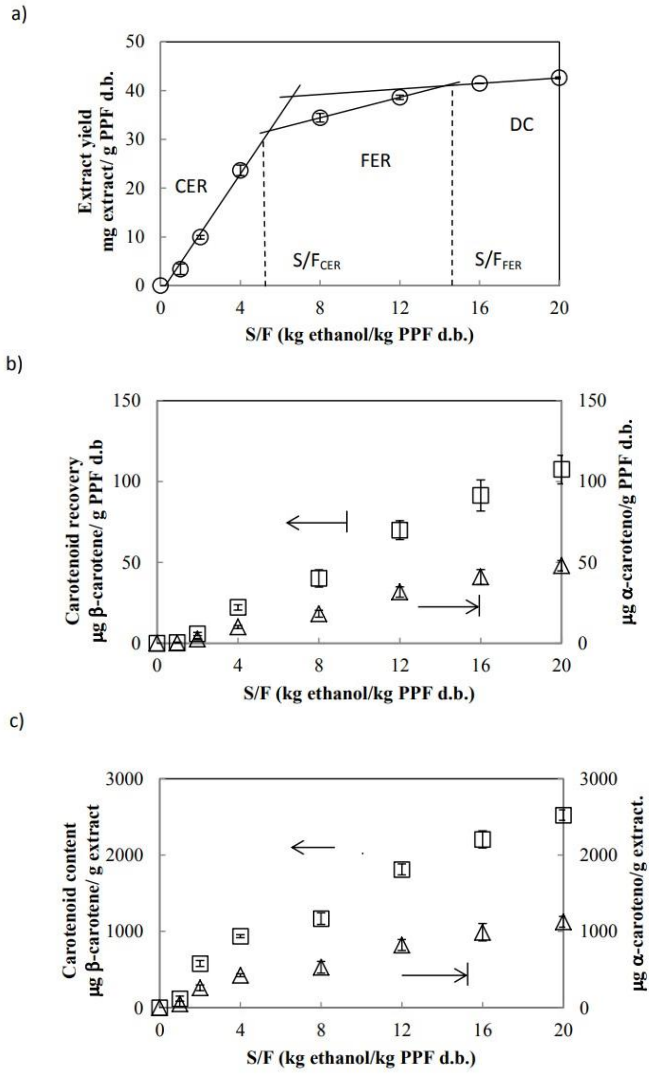

Figure 5. Overall extraction curve (OEC) of a) Extract yield, b) Carotenoid recovery and c) Carotenoid content obtained by PLE at 35°C, 4 MPa and ethanol flow rate of 2.4 g/min.

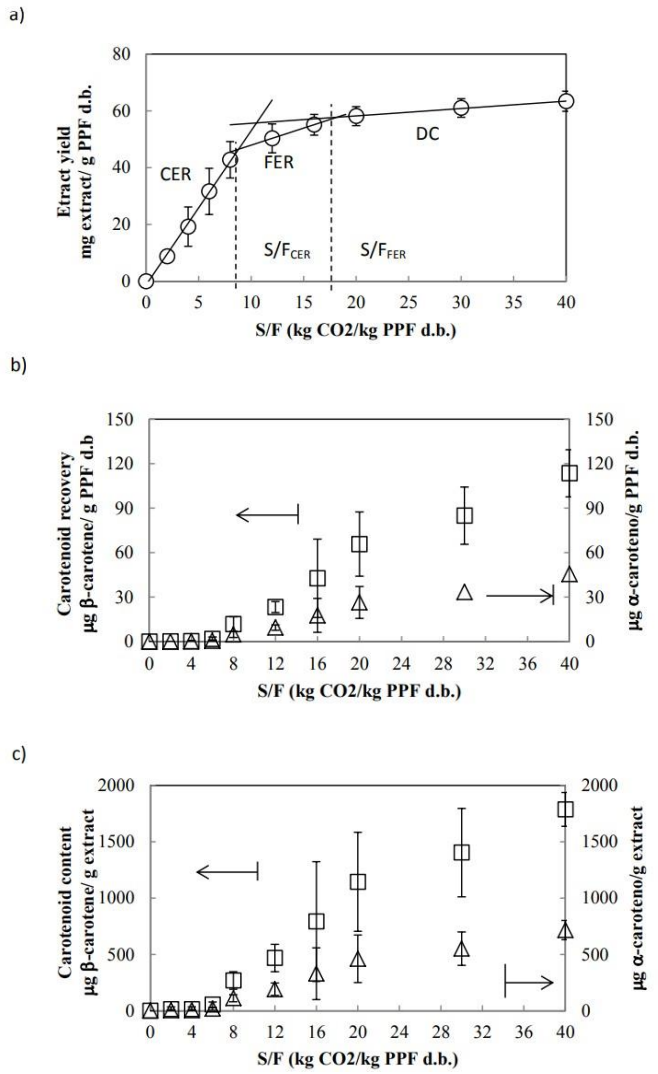

Figure 6. Overall extraction curve (OEC) of a) Extract yield, b) Carotenoid recovery and c) Carotenoid content obtained by SFE at 45°C, 4 MPa and carbon dioxide flow rate of 3.4 g/min

IV. CONCLUSION

In this work, two high pressure extraction techniques were studied. A kinetic study of the pressurized liquid extraction and supercritical fluid extraction for the recovery of carotenoid rich extracts from pressed palm fiber was performed. The PLE and SFE processes presented a similar behavior in terms of extract yield, carotenoid recovery and carotenoid content. However, the highest extract yields were observed for SFE process, while highest carotenoid recovery and content in extract were obtained for PLE process. This result indicates that PLE process is a promising technology for the obtaining of carotenoid rich extracts from pressed palm fiber. In addition, it is possible to obtain high value-added products using high pressure technology from pressed palm fiber.

ACKNOWLEDGMENT

F.P. Cárdenas-Toro is grateful to CAPES-PEC-PG-UNICAMP (5945100) for the Ph.D. assistantship and PUCP for the financial support; and M. A. A. Meireles is grateful to CNPq for the productivity grant (301301/2010-7).

REFERENCES

[1] LAU, H.L.N., CHOO, Y.M., MA, A.N., CHUAH, C.H. Quality of residual oil from palm- pressed mesocarp fiber (Elaeis guineensis) using supercritical CO_2 with and without ethanol. Journal of the American Oil Chemist´s Society. v.83, p.893-898, 2006.

[2] BRAZILIO, M.; BISTACHIO, N.; PERINA, V.; NASCIMENTO, D. Revisão: O Dendeizero (Elaeis guineensis Jacq.). Bioenergia em revista: dialogos, v.2, p. 27-45, 2012.

[3] MINGUEZ-MOSQUERA, M.I.; HOMERO-Mendez, D.; PEREZ-GALVEZ, A. Carotenoids and Provitamin A in Functional Foods. In: HURST, J.W. (Ed.). Methods of Analysis for Functional Foods and Nutraceuticals. EUA: CRC Press, 2008. p. 277-336.
[4] MEINKE, M.C.; FRIEDRICH, A.; TSCHERCH, K.; HAAG, S.F.; DARVIN, M.E.; VOLLERT, H.; GROTH, N.; LADERMANN, J.; ROHN, S. Influence of dietary carotenoids on radical scavenging capacity of the skin and skin lipids. European Jorunal of Pharmaceutics and Biopharmaceutics. v. 84, p.365-373, 2013.
[5] LUTHRIA, D.; VINJAMOORI, D.; NOEL, K.; EZZELL, J. Accelerated Solvent Extraction. In: Oil Extraction and Analysis: Critical Issues and Comparative Studies. EUA: AOCS Publishing, 2004. p. 25-38.
[6] SALDAÑA, M.D.A.; TEMELLI, F.; GUIGARD, S.E.; TOMBERLI, B.; GRAY, C.G. Apparent solubility of lycopene and β-carotene in supercritical CO_2, CO_2 + ethanol and CO_2 + canola oil using dynamic extraction of tomatoes. Journal of Food Engineering. v.99, p.1-8, 2010.
[7] LLOYD, P.J.; WYK, J.V. Introduction to Extraction in Food Processing. In: LEBOVKA, N.; VOROBIEV, E.; CHEMAT, F. (Eds). Enhancing Extraction Processes in the Food Industry. USA: CRC Press, 2011. p. 1-24.
[8] CARDENAS-TORO, F.P.; ALCAZAR-ALAY, S.C.; COUTINHO, J.P.; GODOY, H.T.; FORSTER-CARNEIRO, T.; MEIRELES, M.A.A. Pressurized liquid extraction and low pressure solvent extraction of carotenoids from pressed palm fiber: experimental and economical evaluation. Food and Bioproducts processing, 2014.
[9] MUSTAFA, A.; TURNER, C. Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review. Analytica Chimica Acta, v. 703, n. 1, p. 8-18, 2011.
[10] CARDENAS-TORO, F.P.; FORSTER-CARNEIRO, T.; ROSTAGNO, M.A.; PETENATE, A.J.; MAUGERI-FILHO, F.; MEIRELES, M.A.A. Integrated supercritical fluid extraction and subcritical water hydrolysis for the recovery of bioactive compounds from pressed palm fiber. Journal of Supercritical Fluids. v.93, p.42-48, 2014.
[11] SIERRA, J; SIERRA,L;OLIVERO,J. Economic potential of the oil palm. Agronomía Mesoamericana, vol. 28, núm. 2, pp. 523-534, 2017
[12] INDEX MUNDI, Tasa de crecimiento anual de aceite de palma por país, 2021.
[13] CENIPALMA. Guía del aceite de palma y aplicaciones. 2013.
[14] WHO. Guías para la fortificación de alimentos con micronutrientes. 2017.
[15] MINSALUD. Estrategia Nacional de Prevención y Control de Micronutrientes 2014-2021, 2015. p 13-14.
[16] MINSA. Plan Nacional y Control de la Anemia 2017-2021, 2018.p 27, 31.
[17] SANTOS, D.T., VEGGI, P.C., MEIRELES, M.A.A. Optimization and economic evaluation of pressurized liquid extraction of phenolic compounds from jabuticaba skins. Journal of Food Engineering. v.108, p.444-452, 2012.
[18] RODRIGUEZ-AMAYA, D. A guide to carotenoid analysis in foods. Washington D.C., USA: ILSI press, 2001. 71 p.
[19] MEIRELES, M. A. A. Extraction of bioactive compounds from Latin American plants. In: MARTINEZ, J.L. (Ed.). Supercritical Fluid Extraction of Nutraceuticals and Bioactive Compounds. EUA: CRC Press, 2008. p.243–274.
[20] ALBUQUERQUE, C.L.C.; MEIRELES, M.A.A. Defatting of annatto seeds using supercritical carbon dioxide as a pretreatment for the production of bixin: Experimental, modeling and economic evaluation of the process. Journal of Supercritical Fluids, v.66, p.86-95, 2012.
[21] RODRIGUES, V.M.; SOUSA, E.M.B.D.; MONTEIRO, A.R.; CHIAVONE-FILHO, O.; MARQUES, M.O.M.; MEIRELES, M.A.A. Determination of the solubility of extracts from vegetable raw material in pressurized CO_2: a pseudo-ternary mixture formed by cellulosic structure+solute+solvent. Journal of Supercritical Fluids. v.22, p.21–36, 2002.