

DEVELOPMENT AND ASSEMBLY OF A HOME-MADE PRESSURIZED LIQUID EXTRACTION (PLE) UNIT

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Abstract. Pressurized liquid extraction (PLE) is an efficient extraction technique that combines high pressures and temperatures with liquid solvents to achieve rapid and effective extraction of compounds of interest from solid matrices. PLE is widely accepted for the analysis of different pesticides, PCBs, PAHs in sludge and in the extraction of bioactive compounds as capsaicinoids from *Capsicum* peppers. The objective of the present study is the development, montage and validation of a home-made pressurized liquid extraction unit (PLE) to the extraction of capsaicinoids from dedo-de-moça peppers (*Capsicum annuum*) with 100% methanol as solvent. The objective of this work was to development and assembly of a home-made pressurized liquid extraction (PLE) unit. Dedo-de-moça pepper extracts were obtained by using the home-made PLE unit and analyzed from the global yield and total phenolics. The PLE equipment is composed of a HPLC pump, which operates with flow rates from 0.001 to 10.0 mL/min, manometers and stainless steel vessels. The equipment has three different vessel volumes, 5, 50 and 100 mL. The vessel heating system is composed of an electric heating jacket and the system pressure is controlled by a back pressure valve. The equipment can operate at pressures from 0.5 to 40 MPa and temperatures from 30 to 150 °C. The home-made pressurized liquid extraction proved to be suitable for use in the extraction of vegetable matrices. The results follow the same behavior studies in the literature. Twenty minutes of extraction for global yield tests was sufficient to extract compounds from the vegetable matrix. Only the temperature factor was significant at the 5% level ($p < 0.05$). The best condition for the extraction with pressurized methanol from dedo-de-moça pepper was 20 MPa at 80 °C.

Keywords: Pepper; PLE; home-made equipments.

1. Introduction

The extraction of active compounds from vegetable raw materials is a promising area in the food industry. Classical extraction methods, such as Soxhlet extraction (SE) and heat-reflux extraction, are both time and solvent consuming. Therefore, it is of considerable interest to find a better extraction method for the natural product industry. The new extraction techniques emerged that will certainly overcome traditional extraction technologies in the future. These include supercritical fluid extraction, pressurized liquid extraction (PLE), ultrasound-assisted extraction and microwave-assisted extraction [1, 2].

PLE is a technique in which pressure is applied during the extraction, allowing the use of temperatures above the boiling point of solvents. The use of higher temperatures increases mass transfer and extraction rates and PLE generally involves shorter extraction times and a lower consumption of organic solvents than conventional techniques. In the last decades, many applications of PLE have been reported, such as the extraction of saponins from *Lysimachia clethroides*. [3], fatty acids from the *Piper* species [4] and capsaicinoids from red peppers [5].

Red peppers (*Capsicum* sp.) are vegetables rich in capsaicinoids, substances responsible for the pungency of the fruits, among which capsaicin is the most representative [6]. Currently, capsaicin is used in the development of new drugs because it has many beneficial properties, such as antioxidant, antimicrobial, anti-inflammatory and antitumor activities, and contributes to the control of diabetes and pain relief [7]. Taking into account the beneficial power of capsaicin, there is great interest in developing new technologies to obtain concentrated extracts.

The main objective of this work was to develop and assemble a home-made pressurized liquid extraction (PLE) unit. Dedo-de-moça pepper extracts were obtained by using the home-made PLE unit and analyzed in terms of global yield and total phenolics.

2. Materials and methods

The work was conducted in the Laboratory of Supercritical Technology, Extraction, Fractionation e Identification of Vegetal Extracts – LASEFI/DEA-FEA/UNICAMP. The raw material was dedo-de-moça pepper (*Capsicum annuum*) acquired at “Central de Abastecimento de Campinas S.A. (CEASA)”, a local market in Campinas, southeastern Brazil.

2.1 Chemicals

The solvent used in PLE was methanol P.A. (Êxodo Científica - Hortolândia, SP, Brazil). For the total phenolics analysis, the Folin-Ciocalteu reagent was purchased from Dinâmica (Dinâmica, SP, Brazil) and gallic acid from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Sample preparation and characterization

The fruits with good physical integrity were selected, sanitized by immersion in a solution of 100 mL/L of sodium hypochlorite for 20 minutes, washed with running water to remove the excess of hygienic solution and finally, triturated in a common food crusher and conditioned under refrigeration ($\approx 4^{\circ}\text{C}$) for further utilization. Analyses of moisture and ash were performed according to AOAC techniques [8].

The total phenolics content was determined spectrophotometrically using the Folin–Ciocalteu method, according to the methodology proposed by Singleton, Orthofer and Lamuela-Raventos [9] with modifications. Briefly, 2.5 mL of the diluted Folin–Ciocalteu reagent (1:10 v/v) was added to 0.5 mL of solution of extract in methanol. After 5 min, 2.0 mL of sodium carbonate solution (7.5 %) was added. The absorbance was measured at 760 nm after the mixture remains 2 h in the dark. Gallic acid was used as a standard, and results were expressed as milligrams of gallic acid equivalent (GAE) per gram of extract.

2.3 Soxhlet extraction

The Soxhlet method was selected as conventional extraction technique, using hexane as solvent. In each extraction about 5.0 g of prepared sample were packed in filter paper and inserted in the extractor. Methanol (0.20 L) was added and the system was heated until boiling ($\sim 65^{\circ}\text{C}$). The reflux was kept for 6 h, then the solvent was evaporated under vacuum (at 25°C), and the recovered extract was weighed and stored under freezing (-18°C) for further analyses. The Soxhlet extractions were performed in duplicate.

2.4 Pressurized liquid extraction (PLE) assembly

The assembly and testing of home-made pressurized liquid extraction unit was performed in stages: Design, assembly and extraction tests. First, with the aid of designs computer software the unit was designed to use the least amount of material, to occupy as less space as possible on bench experimentation, perform extractions with the lowest experimental error and being easy to operate and maintenance. Later the unit was constructed in about 4 weeks. Finally, extraction assays were performed using dedo-de-moça pepper. Figure 1 shows the steps carried out to assemble the home-made pressurized liquid extraction unit.

The unit was mounted on an aluminum profiled structure of 45x45mm (Jartec ® / SC-Brazil) with 60 cm high, 60 cm long and 30 cm wide (base). In the profile were set at block valve, the cell extract (5, 50, and 100 ml), the back-pressure valve, indicator and controller jacket temperature of the cell and manometer. To interconnect system components stainless steel tubes (316) 1/8" seamless were used. The unit was designed

with the intention to operate with different extraction cells. Figure 2 shows the different cells made for home-made PLE unit.

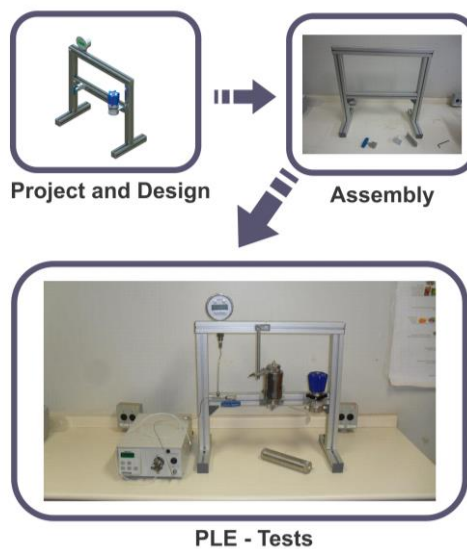


Figure 1. Steps performed for constructing the home-made pressurized liquid extraction unit.

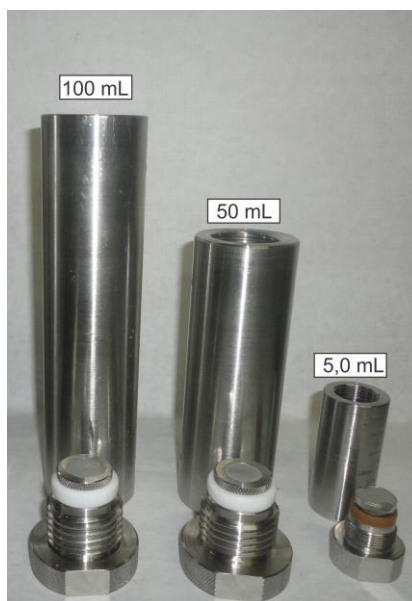


Figure 2. Different cells prepared for the home-made pressurized liquid extraction unit.

All the extraction cells were made with stainless steel (316) and the inlet and outlet filters were installed with the intention of not contaminating the sample and/or preventing the passage of raw materials for the line.

Figure 3 shows the schematic diagram of the home-made PLE unit. The system consists of an HPLC pump (Scientific Systems, Inc., Pennsylvania / USA, volume flow rate of 0.1 to 10.0 ml/min and a maximum pressure of 344 bar), two block valves (Autoclave Engineers, Pennsylvania/USA, maximum pressure of 1034 bar), extraction cells of 5, 10 or 50 mL, vessel heating system (Marconi, São Paulo / Brazil, maximum temperature 200 ° C), a manometer (Zurich, Sao Paulo/Brazil) and back-pressure valve (Tescon Industrial Controls Minnesota / USA, Model: 26-1761-22-161 maximum pressure 689 bar).

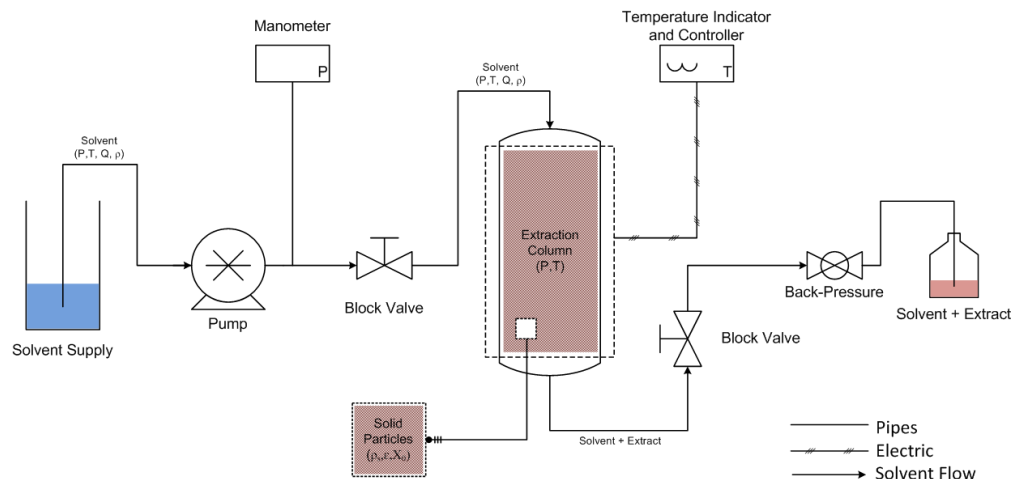


Figure 3. Schematic diagram of the home-made pressurized liquid extraction unit: P – Pressure (MPa); T – Temperature (°C); Q- Flow rate solvent (mL/min); ρ – solvent density (kg/m³); ρ_s – solid particles density (kg/m³); ϵ - particle porosity (ad.); X_0 – extraction global yield.

2.5 Experimental conditions and statistical analysis

The extractions of dedo-de-moça pepper with pressurized liquid were performed in two temperatures (40 and 80 ° C) and two different pressures (10 and 20 MPa), totaling eight tests in duplicate. Each global yield test was performed with approximately 5 g of raw material with the extraction vessel of 5.0 mL, over a period of 20 minutes. The kinetic extractions were performed in the 50 mL cell, for 40 minutes, with about 10 g of raw material. All tests were performed with a temperature static time of 10 minutes and with the same solvent, methanol [5], with solvent flow rate of 10mL/min. Residual solvent was evaporated under vacuum and extract mass was quantified on an analytical balance. All the experimental results obtained were expressed as means \pm amplitude. Statistical analyses of the effects of temperature and pressure were performed using analyses of variance (ANOVA) and the significant differences of the means were analyzed by the Tukey test at level of 5 % ($p < 0.05$), with the aid ‘Statistica’ software (release 7, StatSoft, Tulsa, USA).

3. Results and discussion

The moisture content of fresh dedo-de-moça pepper was 87.0 ± 0.2 (%). Table 1 shows the experimental data of global yield and total phenolics in the extracts obtained in the home-made PLE unit and Soxhlet for dedo-de-moça pepper in the different working conditions.

Table 1. Experimental results of Yields (%) and Total Phenolics content (mg GAE/g extract) in the extracts obtained in experiments with PLE and Soxhlet.

Temperature (°C)	Pressure (MPa)	Yield (%)	Total Phenolics
40	10	7.29 ± 0.70^b	9.60 ± 0.5^{ab}
40	20	7.33 ± 0.31^b	7.80 ± 0.5^{bc}
80	10	9.96 ± 1.75^{ab}	7.60 ± 0.7^{bc}
80	20	10.79 ± 0.29^a	5.26 ± 0.02^c
Soxhlet			
Methanol		12.27 ± 2.24^a	11.2 ± 0.4^a

It is verified in the Table 1 that the values obtained for the global yield in temperature of 40°C at 10 and 20 MPa and at 80 ° C and 10 MPa did not differ significantly among themselves at 5%. The global yield values for the condition of higher temperature and pressure did not differ significantly from the average obtained for the Soxhlet extraction, i.e. the yield of extract obtained by PLE is close to the yield of the conventional technique. It can also be observed in Table 1 that the values for the global yield (%) increased with increasing

temperature and pressure, from $7.29 \pm 0.70\%$ at the lower pressure and temperature to $10.79 \pm 0.29\%$ for the higher pressure and temperature. Such behavior was found by Barbero, Palm and Barroso [5], who studied the extraction of capsaicinoids from the peppers in 5 different temperatures, where the higher temperature was selected.

Concerning total phenolics, it can be observed that the Soxhlet extraction provided the highest concentration (Table 1), followed by the PLE extraction at 40 °C and 10 MPa. This behavior is the opposite of that observed for the extraction global yield. The results indicate that increasing temperature and pressure results in lower total phenolics content of PLE extracts. The PLE conditions of 40 °C/20 MPa and 80 °C/10 MPa showed no significant difference ($p < 0.05$) by the Tukey's test.

According to Mustafa and Turner [1] the critical factor in studies of PLE is the temperature, because it affects the process efficiency and selectivity of the solvent, since the use of high temperatures helps the disruption of analyte-sample matrix interactions caused by van der Waals forces, hydrogen bonding and dipole attraction. Table 2 shows of variance for the yield (%) of extraction of dedo-de-moça pepper.

Table 2. Analysis of variance for the yield (%) of PLE of dedo-de-moça pepper.

Source	DF	SS	MS	F-value	p-value
Temperature (1)	1	17.0480	17.0480	36.64	0.004
Pressure (2)	1	0.6662	0.6662	1.43	0.298
(1)*(2)	1	0.1321	0.1321	0.28	0.622
Error	4	1.8612	0.4653		
Total	7	19.7074			

where: SS - sum of squares; DF- degrees of freedom; MS - mean square.

It is verified in Table 2 that only the temperature factor was significant at the 5% level ($p < 0.05$), because usually the effect of pressure on the recovery of most substances is generally negligible. The main advantage of the application of pressure during extraction at temperatures above the boiling point is that the solvent remains at its liquid state. The same behavior was observed by Deng et al. [10], Choi et al. [11] and Deng et al. [12]. Figure 4 showed the experimental results of extraction yields of dedo-de-moça pepper with home-made PLE unit.

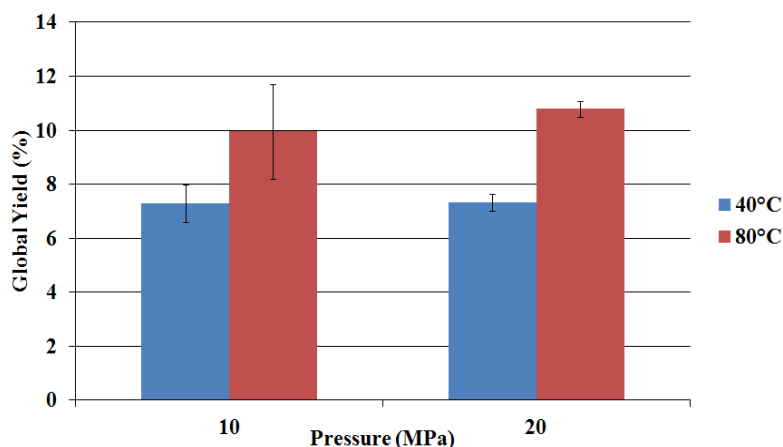


Figure 4. Experimental results of extraction yields of dedo-de-moça pepper with a home-made PLE unit.

Figure 5 shows the kinetics of PLE of dedo-de-moça pepper at a pressure of 20 MPa and temperature of 80 °C.

The extraction process can be explained in two steps. First, the extraction begins controlled by solubility, followed by a diffusion-controlled step. As in supercritical fluid extraction, the increase of global yield in the diffusive period is very low and in this step becomes uneconomical. Such behavior demonstrates that the time to 20 minutes of extraction for global yield tests was sufficient to extract compounds from vegetable matrix, as shown by the kinetics of extraction of dedo-de-moça pepper presented in Figure 5.

The extraction unit with pressurized liquid proved to be adequate for the use in research projects, being efficient with the cells of various volumes, different temperatures, pressures and solvent flow rates. Furthermore, low variations of the experimental data in both global yield and kinetic experiments have shown

the good functioning and reproducibility of the data obtained by home-made PLE unit, according to Figure 3 and 4, respectively.

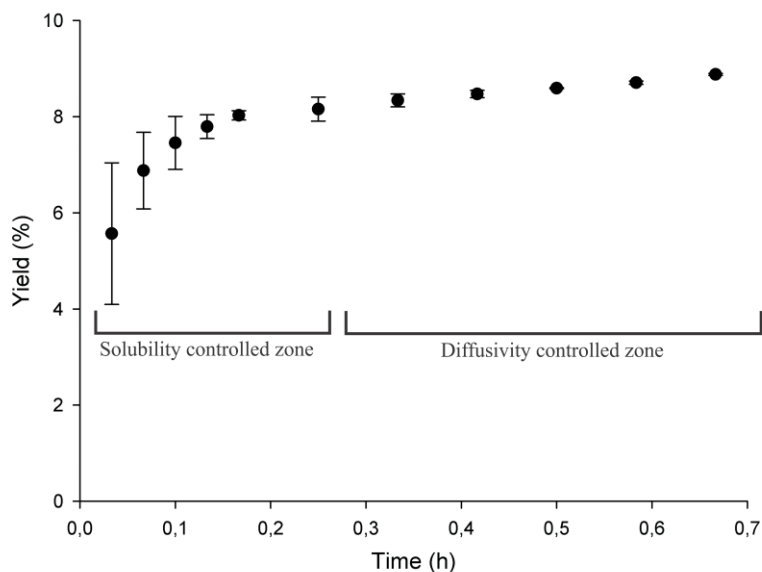


Figure 5. Experimental results of kinetics extraction of dedo-de-moça pepper with a home-made PLE unit.

4. Conclusions

The results obtained indicate that the home-made pressurized liquid extraction proved to be suitable for use in the extraction of vegetable matrices. The results follow the same behavior studies in the literature. The 20 minutes of extraction for global yield tests was sufficient to extract compounds from vegetable matrix. Only the temperature factor was significant at the 5% level ($p < 0.05$). The best condition for extraction with pressurized methanol for PLE from dedo-de-moça pepper was 20MPa at 80 ° C.

Acknowledgments

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References

- [1] Mustafa, A. and 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.
- [2] Li, W.; Zhao, L.-C.; Sun, Y.-S.; Lei, F.-J.; Wang, Z.; Gui, X.-B.; Wang, H. Optimization of Pressurized Liquid Extraction of Three Major Acetophenones from *Cynanchum bungei* Using a Box-Behnken Design. *Int. J. Mol. Sci.* 2012, 13, 14533-14544.
- [3] Jiang, Y. et al. Optimization of pressurized liquid extraction of five major flavanoids from *Lysimachia clethroides*. *Journal of Pharmaceutical and Biomedical Analysis*, v. 43, n. 1, p. 341-345, 2007.
- [4] Péres, V. F. Saffi, J. Melechi, M. I. S. Abad, F. C. Jacques, A. R. Martinez, M. M. Oliveira, E. C. Caramão, E. B. Comparison of soxhlet, ultrasound-assisted and pressurized liquid extraction of terpenes, fatty acids and Vitamin E from *Piper gaudichaudianum* Kunth. *Journal of Chromatography A*, v. 1105, n. 1-2, p. 115-118, 2006.
- [5] Barbero, G.F.; Palma, M. and Barroso, C.G. Pressurized liquid extraction of capsaicinoids from peppers. *J. Agric. Food Chem.* 2006, 54, 3231-3236.
- [6] Damodaran, S.; Parkin, K. L. e Fennema, O. R. *Química de Alimentos de Fennema*. Porto Alegre: Artmed, 2010. 900p.
- [7] Reyes-Escogido, M.; Gonzalez-Mondragon, E. G.; Vazquez-Tzompantzi, E. Chemical and Pharmacological Aspects of Capsaicin. *Molecules*. v. 16, n. 2, p.1253-1270, 2011.

- [8] AOAC, Association of Official Analytical Chemists, Official Methods of Analysis, (16th ed.) AOAC, Arlington, VA (1995).
- [9] Singleton, V. L., Orthofer, R., Lamuela-Raventos, R. M. Analysis of Total Phenols and Others Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. *Methods in Enzymology*, vol. 299, p. 152, 1999.
- [10] Deng, C. JI, J., Wang, X., Zhang, X. Development of pressurized hot water extraction followed by headspace solid-phase microextraction and gas chromatography-mass spectrometry for determination of ligustilides in *Ligusticum chuanxiong* and *Angelica sinensis*. *Journal of Separation Science*, v. 28, n. 11, p. 1237-1243, 2005.
- [11] Choi, M.P.K., Chan, K. K. C., Leung, H.W., Huie, C.W. Pressurized liquid extraction of active ingredients (ginsenosides) from medicinal plants using nonionic surfactant solutions, *Journal of Chromatography A* 983 (1–2) (2003) 153–162.
- [12] Deng, C.H, Li, N., Zhang, X.M. Rapid determination of essential oil in *Acorus tatarinowii* Schott by pressurized hot water extraction followed by solidphas emicroextraction and gas chromatography-mass spectrometry, *Journal of Chromatography A* 1059 (1–2) (2004) 149–155.