Supercritical CO_2 Extraction as Pretreatment On Low-Pressure Extractions: Global Extraction Yield

Hugo Alexander Martínez-Correa[a,b] ; Fernando Antonio Cabral[a,*]; Pedro Melillo Magalhães[c]; Alessandra Lopes de Oliveira[d]

[a] Laboratório ExTrAE**,** Departamento de Engenharia de Alimentos, Universidade Estadual de Campinas-UNICAMP, Campinas, SP, Brasil, P.O Box: 6121, CEP:13083-862
[b] Universidad Nacional de Colombia, Departamento de Ingenieria, Cr 32 Chapinero, Palmira, Colombia.
[c] CPQBA - UNICAMP, Cidade Universitária Zeferino Vaz, Campinas, SP, Brasil, CEP:13083-970.
[d] Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, SP, Brasil, CEP: 13635-900.

* Corresponding author. Tel.:+ 55-19-3521-4030; Fax: 55-19-3521-4027.
E-mail address: cabral@fea.unicamp.br (F.A. Cabral)

ABSTRACT

The present work proposed the study the influence of previous SC-CO_2 extraction as pretreatment on the low pressure extraction employing water or ethanol as solvent on five vegetables matrixes : *1)* alecrim-do-campo leaves (*Baccharis dracunculifolia*), *2)* Pitanga leaves (*Eugenia uniflora L.*), *3)* Curcuma rhyzomes (*Cúrcuma longa L.*), *4)* Artemisia leaves (*Artemisia annua*) and *5)* Bidens roots (*Bidens pilosa l.*) Extraction process was made in order to obtain supercritical and low pressure extracts: SC-CO_2, low-pressure (aqueous and ethanolic) and sequential process: SC-CO_2 extraction + aqueous extraction (SC+A) and SC-CO_2 extraction + ethanolic extraction (SC+E). The results showed that global extraction yield for the different matrixes was a function of the process, which the highest yield was for low-pressure (aqueous > ethanolic). When SC-CO_2 was used as pretreatment for low-pressure extractions, it was obtained: SC+A > SFE+E. It was evident that sequential process (SC+low-pressure) improved cumulative extraction yield.

Key-words:.Supercritical Extration, low-pressure, extraction, global yield.

INTRODUCTION

Extracts from natural sources containing functional and bioactive compounds such phytochemicals, pharmaceuticals, lipids, flavors, fragrances, pigments are widely used in food, pharmaceutical and cosmetical industries. This extracts has traditionally obtained by means of organic solvents (e.g., acetone, hexane, methanol, ethanol, methylene chloride) and by water (e.g., steam distillation , hidrodistillation). Processes employing organic solvents are very time consuming and requires relative large quantity of solvent [1], the process requires to remove this solvent in order to obtain a product (extract) with minimum residuals content. Some disadvantages of this classical extractions techniques are the toxicity of the solvents and his presence in the concentrated extract must be regulated, besides during the extract concentration very volatile compound are lost and thermolabiles are hydrolyzed and oxidized [2].

Since these bioactive compounds are usually present in low concentrations a great effort to seek more effective and selective extractions process. Supercritical fluid extraction using carbon dioxide (SF-CO_2) as solvent has become more important because the employing low temperatures, relatively fast extractions, generally high selectivity and is considered a environmentally friendly process [3]. The main disadvantage of SFE-CO_2 is related low polarity of CO_2 that makes extraction that guides low recoveries of polar components.

In the present study SFE-CO_2 was employed extraction as pretreatment on the low pressure extraction employing water or ethanol as solvent on five vegetables matrixes : *1)* alecrim-do-campo (*Baccharis dracunculifolia*): sample 1 (developed leaves) and 2 (bud leaves), *2)* Pitanga leaves (*Eugenia uniflora L.*), *3)* Curcuma rhyzomes (*Cúrcuma longa L.*), *4)* Artemisia leaves (*Artemisia annua*) and *5)* Bidens roots (*Bidens pilosa l.*)

1. MATERIALS AND METHODS

Plant material

Different vegetables matrixes were collected in the herbary of the "Centro de Pesquisas Químicas, Biológicas e Agrícolas" (CPQBA, Campinas Brazil). All solvents used were of analytical grade. The carbon dioxide, 99.5 % (w/w), was purchased from White Martins Gases industriais (Campinas, Brazil), ethanol, 99.5 % (w/w) was provided by Synth (Brazil), and ultra pure water (Milli Q, Millipore Corporation, USA) was employed.

Sample aconditioning

All samples were dried under forced convection dryer at 40˚C, after that plants were thoroughly ground in knife Mill (Marconi, Brasil). The moisture content were in 10 to 13 wt.% for all crushed matrixes.

Extraction procedures

Single extraction process was made in order to obtain supercritical and low pressure extracts: SC-CO_2, low-pressure aqueous (A) and ethanolic (A). Sequential process: SC-CO_2 extraction + aqueous extraction (SFE+A) and SC-CO_2 extraction + ethanolic extraction (SFE+E).

Supercritical Fluid extractions were performed in experimental unit (Fig. 1). It consisted of a 50 mL equilibrium cell (stainless steel AISI 316, Suprilab, Campinas, Brazil) (5) immersed in a water bath controlled by a heater (Suprilab, Campinas, Brazil) to within ±0.1 ◦C. The CO_2 from the supply tank (1) was cooled to the liquid state (refrigerated bath model 12101-30, Cole Parmer Instrument Company, Vernon Hills, IL, USA) (2) and compressed into the extractor using a high-pressure pump (Model AA100S, Eldex Laboratories Inc., Napa, CA, USA) (3). The other equipments used were: CO_2 cylinder (4), manometers, glass flasks (7) and a peristaltic pump (Model L/S 77910, Cole Parmer Instrument Company) (6) to clean the system. After extraction, all the tubing in the process line was washed with ethanol to recover the extract deposited in it. The global extraction yields were calculated as the ratio of the total mass of extract (extraction + cleaning process) to the initial mass of raw material .For all supercritical extractions the conditions was fixed in 400 MPa , 60 "C and flow rate of SC-CO_2 4.10^{-5} Kg/min. Extractions were finished after 6 hours (540 L of CO_2 at 0.93 bar and 25^{o}C).

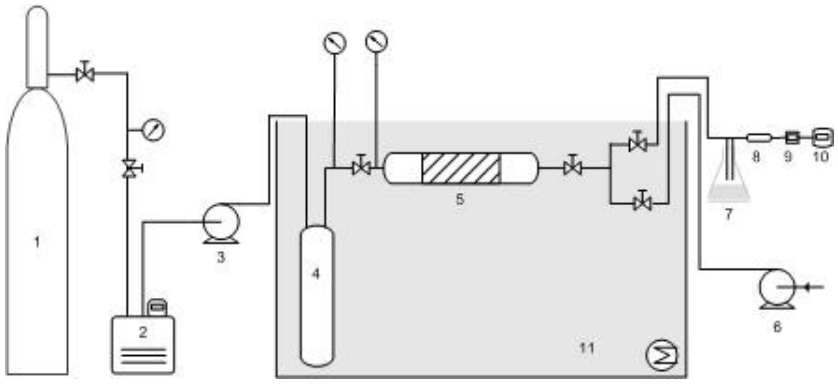


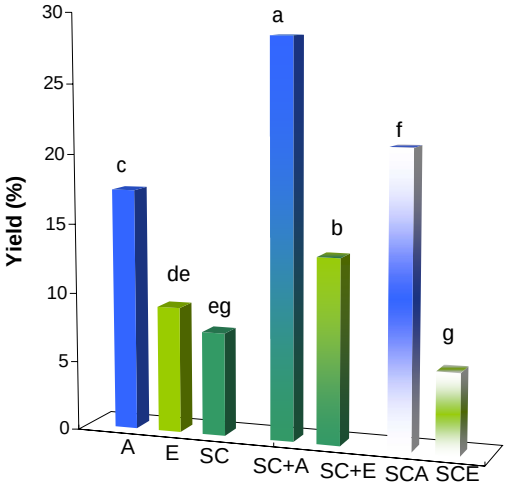
Figure 1. Schematic diagram of the SC-CO_2 extractor unit.

Ethanolic extracts were obtained from 3 g of plant with 20 mL ethanol in agitated bath (Marconi, Brazil) at 25 C for 42 hours. The mixture is filtered and solid waste extracted again with 10 ml ethanol and centrifuged (Jouan, BR4i, France) at 3000 rpm for 5 min.
Aqueous extracts were obtained using modified technique of Cseke *et al*. [4] , employing 3 g of each vegetable matrix and 60 ml of ultra pure water at 60 °C in a water bath (Tecnal, Brazil) for 10 min, after that the mixture was centrifuged for 10 min at 10000 rpm (Jouan, BR4i, France), finally extract was obtained from vacuum filtration system.

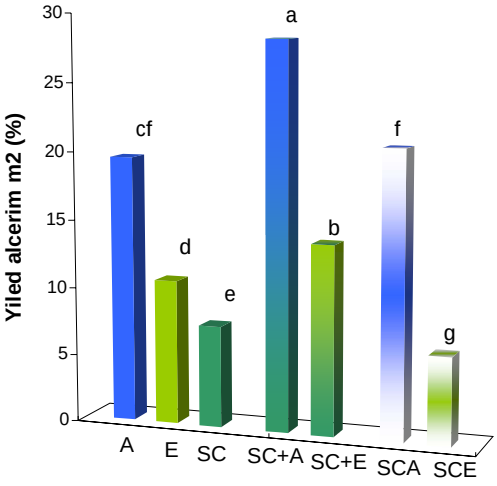
2. RESULTS AND DISCUSSION

In the figure 2 can be seen the results for global yields extraction for five extraction procedures studied for the different vegetable matrixes. There also appear results for second extraction stage: aqueous (SCA) and ethanolic (SCA) when they are preceded for supercritical CO_2 extraction. Different letters means significative difference when Tukey test (STATISTICA 7.0®) was applied on the means.

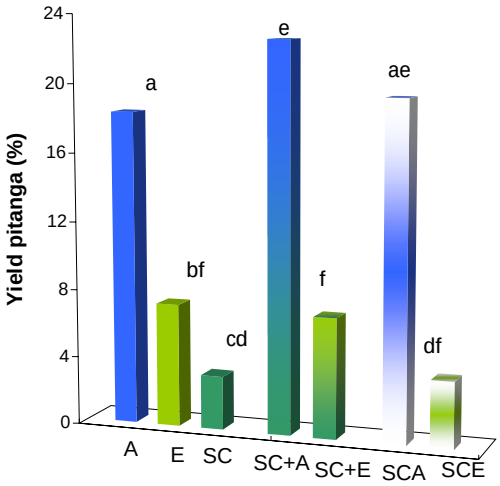
The results for global yield extraction for single extraction process all vegetables matrixes, shows: A>E > SC-CO_2. When SC-CO_2 was used as pretreatment for low-pressure extractions, for alls matrixes was obtained: SC+A > SC+E. Statistically differences were found when the tukey test was applied for comparing means between one-step extraction (SC, A, E) and two-step extraction (SC+A, SC+E), indicating that the pretreatment with SC-CO_2 (previous extraction) improve the accumulative extraction yield in this 2-step extraction.



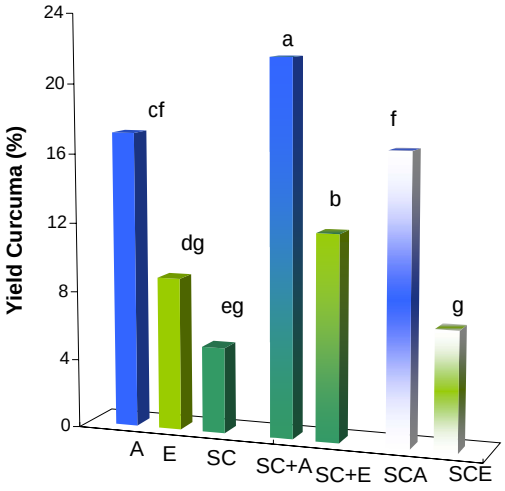
B.dracunculifolia (sample 1)



B.dracunculifolia (sample 2)



E. uniflora



C. longa

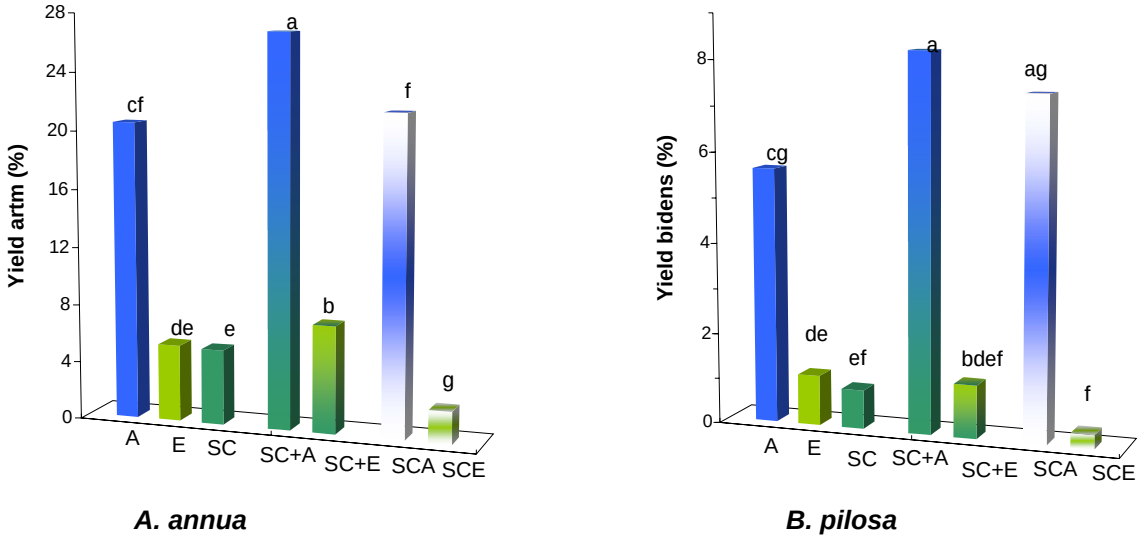


Figure 2. global yields extraction for different extraction process.

The performance of the two-step process was too studied. Then was calculated factor 1, which represent yields ratio: (SCE)/E, (SCA)/A, and the factor 2 which represent yields ratio: (SC+E)/E, (SC+A)/A.

For two-step process in the figure 3 can be seen that the performance were always better for aqueous extractions (SCA) than the ethanolics extractions (SCE), for alls vegetables matrixes. Also can be shown for ethanolic extraction, the yield produced on the SCE stage is lower than in one-step process (E) (Factor 1 < 1).
Two-step process involving aqueous extraction had always factor 2 > 1 (figure 4), indicating the positive effect of previously SC-CO_2 extraction over the global process. For two-step process involving ethanol can be seen that the previously SC-CO_2 extraction improves the global process for *B. dracunculifolía* (samples 1 and 2), *C. longa* and *A. annua*.
.

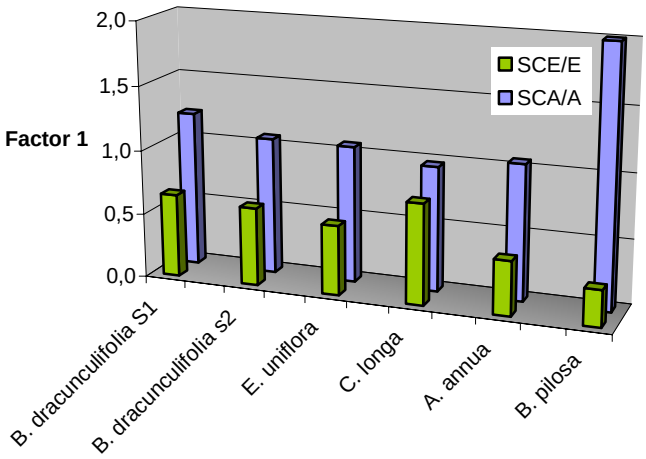


Figure 3. Second extraction stage performance

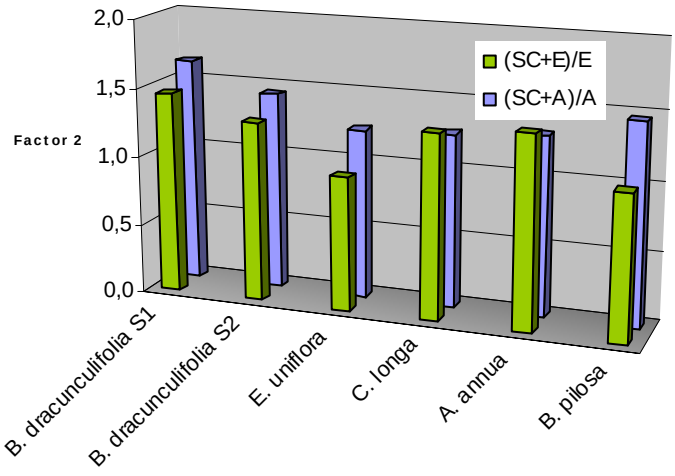


Figure 4. Two-step process performance.

3. CONCLUSIONS

Different extraction process were used to obtain extracts for six vegetable matrixes, The results for global yield extraction for single extraction process all vegetables matrixes, following order : A > E > SC-CO_2 and for two-step process we found: SC+A > SC+E.
The influence of the previous SC-CO_2 extraction results in improving the global yield in two-step extraction process.
Future works will reports chemical analysis of this different extracts related chemical analysis, quantity of phenolic compound and antioxidant activity.

Acknowledgments
The authors are grateful to CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and to Fapesp (Fundação de Amparo à Pesquisa do Estado de São Paulo) for their financial assistance

REFERENCES

[1] WANG, L, WELLER, C. .Recent advances in extraction of nutraceuticals from plants. Trends in Food Science & Technology 17, **2006**, 300–312.
[2] MUKHOPADHYAY, M. Natural extracts using supercritical carbon dioxide. CRC Press, Boca Raton, USA, **2000**.
[3] LANG, Q, WAI, C.M. Chien M..Supercritical fluid extraction in herbal and natural product. studies — a practical review. Talanta 53, **2001**, 771–782.
[4] CSEKE, L. J., KIRAKOSYAN, A., KAUFMAN, P B., WARBER, S. L., DUKE, J, A., BRIELMANN, H. L. Natural Products From Plants. 2nd ed., CRC Press, Boca Raton, USA, **2006**.