Achyrocline Satureioides (Macela) Flowers Extracts Obtained By Supercritical Fluid Extraction

Melina P. Peruchi, Mara E. M. Braga, M. Angela A. Meireles

LAboratory of **S**upercritical Technology: **E**xtraction, **F**ractionation, and **I**dentification of Vegetable Extracts (**LASEFI**). Department of Food Engineering (FEA), State University of Campinas (Unicamp), P.O. Box 6177, 13083-970, Campinas, SP, Brazil.
E-mail: meireles@fea.unicamp.br

"Macela" is the common name of *Achyrocline satureioides*, a native plant from Brazil, Argentine and Uruguay. The extracts of *Achyrocline satureioides* have showed functional properties such as antioxidant, hepatoprotective, anti-inflammatory, antiglycating and hypoglycemic activities. This herb has high concentration of polyphenols and flavonoids that have been associated with its pharmacological properties. Quercetin is the most important flavonoid present in *A. satureioides*. Furthermore, the functional properties of *A. satureioides* suggest its use in formulation of foods with the purpose of treating (or preventing) type-2 diabetes and easing the digestion process. The extractions were conducted in a supercritical fluid extraction (SFE) unity with a fixed bed extractor. The assays were made at 30 ^{o}C and 150, 200 and 250 bar. The antioxidant activity of the extracts was determined using the coupled reaction of beta-carotene and linolenic acid. The concentration of total phenols was measured by the Singleton and Rossi methodology. Thin layer chromatographic (TLC) was used to verify the sample phytochemical profiles. The maximum global yield (2.3 ± 0.2 %) and the greater antioxidant activity (81.6 ± 0.5 %) were achieved at 250 bar. The higher phenols content (85 ± 3 mg of total phenols/g of extract) was obtained at 200 bar, but it was similar to the content at 250 bar. The phytochemical profiles of the extracts were similar for all conditions studied.

INTRODUCTION

The search for natural antioxidants has intensified in the last years, since the toxicity of phenolics synthetic compounds that are used, as antioxidants in fat food, have been questioned and studied. Tert-butylhydroquinone (TBHQ), for example, did not have its use approved in Japan, Europe and Canada [1]. Antioxidants are used in the food industry to preserve the products quality, keeping their nutritional and sensory integrities.

The traditional use of *Achyrocline satureioides* by popular medicine is related to the treatment of some gastrointestinal dysfunctions [2], [3]. Moreover, previous experimental studies established many pharmacological activities attributed to this plant, such as: anti-inflammatory [3], antioxidant [2], [3], [4], antiulcerative, antihepatotoxic, antispamodic attributed to quercetina, hepatoprotective, antiglycating related to achyrofuran [5]; analgesic related to kawapyrone, vasodilatatory, antitumor and immunodulatory [6]. The species *Achyrocline satureioides* is a native aromatic plant known as "macela", "macela do campo", "marcela", from Asteraceae family. Original from South America, *A. satureioides* is found in Brazil, Argentina, Paraguay and Uruguay [2],[7].

Studies on the composition of *A. satureioides* extracts showed the presence of high content of phenolics compounds (related to its therapeutic properties) [8] as well high content

of flavonoids, which are responsible for the antioxidant activity [9]. Quercetin, luteolin and 3-O-methylquercetin are the main flavonoids present in *A. satureioides* aqueous extracts [10]. Gucliucci and Menini [4] studied the extracts of *A. satureioides* obtained by infusion that showed antiglycating effect (avoid the glucose formation from proteins), added to hypoglycemic and antioxidant activities suggest the use of *A. satureioides* in the type-2 diabetes treatment. Thus, the phenolics and flavonoids content that are related to gastrointestinal treatment could justify the use of *A. satureioides* extracts in functional food formulations, especially in some aperitif with the purpose to help the digestive process; as functional and dietary food supplement for glucose blood reduction or, even, like a medicine. The daily intake of quercetin in food is 50 to 500 mg [11].

Lorenzo et al [2] obtained extracts of *A. satureioides* inflorescences using a Likens-Nickerson type apparatus and *n*-hexane; the extract yield was 0.3 to 0.45% (dry base). Rocha et al [12] studied extracts of *A. satureioides* inflorescences obtained by ethanol maceration (80%) at 6 ºC for 8 days with yield of 7.8 %. To use the extracts in foods it is necessary to be clean, in other words, without solvents residues, and then supercritical fluid extraction (SFE) is a feasible option, mostly when carbon dioxide (CO_2) is employed as solvent [13].

The goal of this work was to quantify the SFE yield applied to *A. satureioides* flowers in different pressures and to compare the phytochemical profiles of the extracts.

I. MATERIALS AND METHODS

Raw material characterization - Achyrocline satureioides flowers were cultivated at the Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas (CPQBA) - UNICAMP, SP, Brazil. The material was comminuted in a knife mill (Tecnal, model YOU-631, Piracicaba, Brazil) and it was packed in plastic bags and kept in domestic freezer (Metalfrio, model HC4, São Paulo, Brazil).

The humidity was determined by the microwave method (CEM, model Smart System 5, USA) at the Laboratory of Applied Microwaves, College of Food Engineering, Unicamp, Campinas, Brazil.

Supercritical Fluid Extraction (SFE) - The supercritical unit used have an extractor (length of 37.5×10^{-2} m and internal diameter of 2.74×10^{-2} m), similar to the apparatus described by Braga et al [15]. The used pressures were 150, 200 and 250 bar at 30 ºC. The CO_2 flow rate was approximately of 5×10^{-5} kg/s and the extraction time was 90 minutes. The ethanol used in the cleaning was separated from the extract using a rotavap (Laborota, model 4001, Viertrieb, Germany) with vacuum control (Heidolph Instruments GMBH, model Rotavac control, Viertrieb, Germany). The total extract mass was obtained adding the extract mass collected during the extraction, the depressurization and the cleaning stages. The global yield was calculated as the ratio between the total extract mass to the dried solid mass.

Extract characterization: Thin Layer Chromatography (TLC) – The TLC (silica gel plates $60F_{254}$-Merck, USA) mobile phase was chloroform:ethyl acetate:formic acid (75:16:8.5) (chloroform: 99.4%, Merck; ethyl acetate: 99.8%, Merck; formic acid: 98%, Vetec) [14] or chloroform:ethyl acetate (8:1). The spray reagent was: (i) anisaldehyde solution [14] to observe the volatile oil and (ii) natural products (NP) revelator to observe flavonoids in an UV light chamber (Entela, model UVGL –58, Upland, USA) at 366 nm [14].

Total phenolics content – The Singleton and Rossi's method with modifications proposed by Cheung et al [16] was used for the determination of the total phenolics content. The total phenolics content was expressed as mg of gallic acid equivalent (GAE)/g of extract.

Antioxidant activity - The extracts antioxidant activity was determined using Hammerschmidt and Pratt method [17] based in the coupled reaction of beta-carotene and linolenic acid.

II. RESULTS AND DISCUSSION

The global yields are presented in Table 1. The maximum yield obtained by SFE (250 bar, 30 ^{O}C) was approximately 2.3 %. The low phenolics content found in the SFE extracts can be explained by the moderated to high polarity of these compounds and thus it should have moderate solubility in supercritical CO_2, which is much less polar, presenting similar characteristics to pentane or hexane [18].

Table 1. Global yields and content of total phenolics in the *A. satureioides* flowers extracts obtained by SFE at 30 ºC.

Extraction Pressure (bar)	Yield ± range, %	Total phenolic content ± range, mg GAE/g of extract
150	2.0 ± 0.3	35 ± 3
200	2.1 ± 0.2	85 ± 3
250	2.3 ± 0.2	76 ± 3

However, the high values of oxidation inhibition (Table 2) presented by the extracts, the same ones that presented low phenolics contents (Table 1), can indicate the presence of other compounds in *A. satureioides* extracts that can also act as antioxidant, since the antioxidant activity did not showed dependence on the phenolics concentration (Figure 1).

Table 2. Antioxidant activity of *A. satureioides* extracts obtained by SFE at 30 ºC

Extraction Pressure (bar)	Antioxidant Activity ± range, %		
	t=1h	t=2h	t=3h
150	75 ± 5	70 ± 4	64 ± 4
200	76 ± 2	71 ± 2	66 ± 2
250	83 ± 5	84 ± 8	81 ± 10

The oxidation inhibition remained in the range of 60 to 85 % (Figure 1). Desmachelier et al **[7]** quantified the antioxidant activity of *A. satureioides* aqueous extracts by the hydroperoxide-iniated chemiluminescence method and they obtained 63 % of oxidation inhibition using the concentration of 0.001 g of extract/ml.

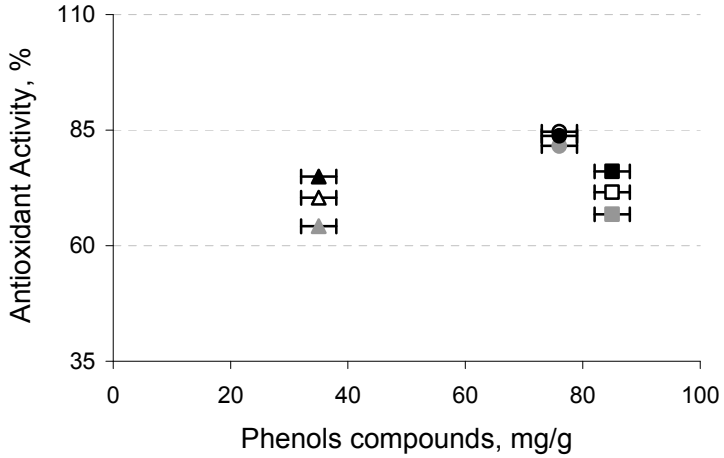

Figure 1. Comparison between antioxidant activity and total phenolics content of *A. satureioides* flowers extracts obtained by SFE ■ 200 bar ● 250 bar▲ 150 bar (■ □ ■ = triplicate data)

The chromatographic plate (Figure 2A) shows that the phytochemical profile of the extracts obtained by SFE at 150, 200 and 250 bar are similar; in the 150 bar extract appears a different red band on the plate bottom (Figure 2A). At the moment in which TLC (Figure 2B) was visualized, bands of yellowish coloration and with low intensity (situated at the same height of quercetin standard band) were detected, indicating the presence of this flavonoid in the extracts.

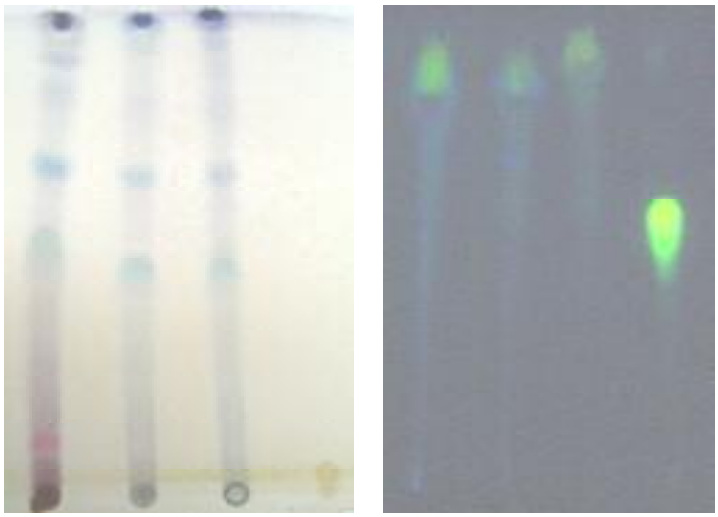

Figure 2. TLC of the *A. satureioides* flowers extracts obtained by SFE: (**1**) 150 bar, (**2**) 200 bar e (**3**) 250 bar e (**4**) quercetin standard. (**A**) Solvent system: chloroform:ethyl acetate (8:1), detection: anisaldehyde solution; (**B**) Solvent system: chloroform:ethyl acetate:formic acid (75:16:8.5), detection: NP solution.

Scalia et al [19] studied chamomile flowers extracts obtained by SFE with CO_2 and verified low flavonoids yields of extraction. This result was attributed to the high polarity of the flavonoids present in the chamomile flowers. Other studies showed that the addition of polar organic co-solvents, for example ethanol or methanol, can promote a significant

increase in the recovered flavonoids by SFE [18], [19], [20]. Chamomile flowers extracts obtained by SFE with CO_2 (200 bar, 45 ºC) using methanol 5% (v/v) as co-solvent resulted in extraction yield increase of 15 and 20% for the flavonoid apigenin-7-glucoside and of 143.3 and 187.7% for apigenin, in relation to the quantities obtained by Soxhlet and maceration, respectively [19].

These results suggest the utilization of polar organic co-solvents in SFE, in order to increase CO_2 polarity and to maximize the total phenolics and flavonoids content recovered in the *A. satureioides* extracts. In this case, ethanol is the polarity modifier more indicated so that the extracts can be used in alimentary formulations.

CONCLUSION

The maximum global yield in the extraction with supercritical carbon dioxide of the *A. satureioides* flowers and the maximum antioxidant activity were obtained at 250 bar and 30 ºC. The maximum amount of phenolics content was recovered at 200 bar and 30 ^{o}C. The antioxidant activity of extracts did not showed dependence of the phenolics concentration, suggesting the presence of other substances (not phenolics) with antioxidant properties in extracts of the *A. satureioides*. It was verified that the phytochemical profile of extracts did not vary significantly for the tested conditions of extraction. The use of co-solvent should be a good option to increase the phenolics fraction in the extract.

REFERENCES

[1] WANASUDARA, U.N., SHAHIDI, F., Food Chemistry, Vol 63, **1998**, p.335

[2] LORENZO, D., ATTI-SERAFINI, L., SANTOS, A.C., FRIZZO, C.D., PAROUL, N., PAZ, D., DELLACASSA, E., MOYNA, P., Planta Medica, Vol 66, **2000**, p. 476

[3] SIMÕES, C.M.O., SCHENKEL, E.P., BAUER, L., LANGELOH, A., Journal of Ethnopharmacology, Vol 22, **1988**, p. 281

[4] GUGLUCCI, A, MENINI, T., Life Science, Vol 71, **2002**, p. 693

[5] CARNEY, J.R., KRENISKY, J.M., WILLIAMSON, R.T., LUO, J., Journal of Natural Products, Vol 65, **2002**, p. 203

[6] SANTOS, A.L.G., RIPOLL, D., NARDI, N., BASSANI, V.L., Phytotherapy Research, Vol 13, **1999**, p. 65

[7] DESMARCHELIER, C., COUSSIO, J., CICCIA, G., Brazilian Jounal of Medical and Biological Research, Vol 31, **1998**, p. 1163

[8] BROUSSALIS, A.M., FERRARO, G.E., GURNI, A., COUSSIO, J. D., Biochemical Systematics and Ecology, Vol 16, **1988**, p. 401

[9] HARBONE, J.B., WILLIANS, Phytochemistry, Vol 55, **2000**, p.481.

[10] ARREDONDO, M.F., BLASINA, F., ECHEVERRY, C., MORQUIO, A., FERREIRA, M., ABIN-CARRIQUIRY, J. A., LAFON, L., DAJAS, F., Journal of Ethnopharmacology, Vol 91, **2004**, p. 13

[11] SHAHIDI, F., NACZK, M., Phenolics in Food and Nutraceuticals, 1ª ed., CRC Press, **2004**.

[12] ROCHA, M.J.A., FULGÊNCIO, S.F., RABETTI, A.C., NICOLAU, M., POLI, A., SIMÕES, C.M.O., RIBEIRO-DO-VALLE, R.M., Jounal of Ethnopharmacology, Vol 43, **1994**, p. 179

[13] PELLERIN, P., Perfumer & Flavorist, Vol 16, **1991**, p. 37

[14] WAGNER, H., BLADT, S., ZGCINSKI. Plant Drug Analysis, 2ª ed, Springer-Verlag, **1983**.
[15] BRAGA, M.E.M., LEAL, P.F., CARVALHO, J.E., MEIRELES, M.A.A. Journal of Agricultural and Food Chemistry, Vol. 51, **2003**, p. 6604
[16] CHEUNG, L.M., CHEUNG, P.C.K., OOI, V.E.C., Food Chemistry, Vol 81, **2003**, p. 249
[17] HAMMERSCHMIDT, P.A., PRATT, D.E., Journal of Food Science, Vol 43, **1978**, p. 556
[18] RIBEIRO, M.A., BERNARDO-GIL, M.G., ESQUÍVEL, M.M., The Journal of Supercritical Fluids, Vol 21, **2001**, p. 51
[19] SCALIA, S., GIUFFREDA, L., PALLADO, P., Journal of Pharmaceutical and Biomedical Analysis, Vol 21, **1999**, p. 549
[20] CATCHPOLE, O. J., FREY, J. B., MITCHELL, J. S. L., Journal of supercritical Fluids, Vol 29, **2004**, p. 97