# PN28

# Supercritical fluid extraction of *Eugenia pyriformis* leaves

**Elissandro Jair Klein [a*], Kátia Andressa Santos [b], Francieli Narok [b], Edson Antônio da Silva [b], Melissa Gurgel Adeodato Vieira [a]**

[a]School of Chemical Engineering, University of Campinas (UNICAMP), Avenida Albert Einstein, 500, 13083-852, Campinas, SP, Brazil.
[b]Center of Engineering and Exact Sciences, Western Paraná State University, Rua da Faculdade, 645, 85903-000, Toledo, PR, Brazil.
[*] elissandro.klein@hotmail.com

**ABSTRACT**
*Eugenia pyriformis* Cambess (popularly known as *Uvaia*) is a subtropical fruit tree that belongs to the Myrtaceae family, native to Brazil. Few reports are found in the literature regarding the extraction process of its bioactive compounds. Technology with supercritical fluids is a promising alternative method to obtain plant extracts, providing satisfactory yields and solvent-free extracts. In this research, supercritical $CO_2$ ($SCCO_2$) extraction was applied and the parameter influences on extraction yield and composition of Uvaia leaf extracts were studied. The extractions were performed according to an experimental design $2^2$ with triplicate at the central point, with pressures of 150, 175, and 200 bar and temperatures of 60, 70, and 80 °C. The chemical profile of the extracts was determined by gas chromatography-mass spectrometry (GC/MS), as well as High Performance Liquid Chromatography (HPLC) for quantification of the major compounds, α-amyrin and β-amyrin. The best yield of uvaia extract, 1.65%, was obtained under the condition of 200 bar and 60 °C. The effects of temperature and pressure were statistically significant on the extraction yield at the 5% level of significance, according to ANOVA (p-value). The main compounds identified by GC/MS were α-amyrin, β-amyrin, vitamin E, and β-sitosterol. Among these, the pentacyclic triterpenes α-amyrin and β-amyrin present therapeutic potentials such as gastroprotective, antidepressant, analgesic, hypolipidemic, antihyperglycemic, antiplatelet drug, and anti-inflammatory, as reported in the literature. The yields of α-amyrin and β-amyrin was in the range of 0.38-2.27 mg $g^{-1}$ of dry leaves for α-amyrin and 1.26-8.22 mg $g^{-1}$ of dry leaves for β-amyrin. The highest yields of those compounds were also obtained at 200 bar and 60 °C.

## INTRODUCTION

The extraction with supercritical fluids offers an interesting alternative to obtain bioactive compounds, overcoming some of the limitations presented by conventional extraction techniques. This technique offers good mass transfer conditions, density control due modifications of experimental conditions and fast extraction rates [1]. Carbon dioxide is the most commonly used solvent in the supercritical fluid extraction process because it is non-toxic, non-flammable, can be used at moderate temperatures, and protects the extracts from oxidation during the extraction process. *Eugenia pyriformis*, popularly known as uvaia, belongs to the Myrtaceae family and is a typical subtropical plant of the South region of Brazil [2]. The essential oils obtained by hydrodistillation from the leaves of uvaia, showed components how β-pinene, limonene, 1,8-cineol, and caryophyllene oxide [3]. The essential oil obtained from fresh leaves of *E. pyriformis*, using hydrodistillation, has the following major compounds: bicyclogermacrene, δ-cadinene, and β-caryophyllene [4]. The previous study showed that the major compounds were α-amyrin and β-amyrin [5].

The compounds α-amyrin and β-amyrin show potential in biological and pharmacological applications, with gastroprotective potential [6], sedative properties, anxiolytic, and antidepressant effects [7]. They also showed potential in relieving induced orofacial pain [8]; reducing VLDL and LDL cholesterol levels and elevating HDL levels [9]; as well as significantly reducing itching [10]. The mixture of α-amyrin and β-amyrin presents therapeutic interest for the treatment of inflammatory bowel disease [11]. The isolated β-amyrin was six times more potent than acetylsalicylic acid in inhibiting platelet aggregation in rabbit's blood [12].

The aim of this study was to evaluate the parameters effect of $SCCO_2$ extraction in the yield and obtention of α/β-amyrin rich extracts.

## MATERIALS AND METHODS

The plant material of *E. pyriformis* was collected in a rural property located in Marechal Cândido Rondon, Paraná – Brazil. The leaves were dried at 40 °C for 24 hours. After drying, the material was milled to an average particle diameter of 0.6 mm. The crushed material moisture, 7.04 wt %, was determined by drying the sample at 105 °C until no mass variation was observed.

The extractions were carried out in an extraction unit with capacity of 48.1 $cm^3$. The extraction cell was filled with approximately 14 g of the crushed leaves. Next, $CO_2$ was pumped into the extractor until the working pressure was reached. The extraction cell was heated to the desired extraction temperature and the sample was maintained in contact of $SCCO_2$ for 15 min (static period). The mass flow of $CO_2$ was maintained constant at 2.0 $\times 10^{-3}$ kg $min^{-1}$ by using the expansion valve.

A $2^2$ full factorial experimental design with triplicate of the center point was used to analyze the effect of the variables pressure and temperature on the extraction yield and α-amyrin and β-amyrin content. The experiments were performed at pressures of 150, 175, and 200 bar and temperatures of 60, 70, and 80 °C.

The chemical profile of the extracts was determined by gas chromatography-mass spectrometry (GC/MS) as described by Klein *et al.* [5]. The compounds were identified by comparing the experimental mass spectra with those of the NIST mass spectra library. The quantification of the major compounds, α-amyrin and β-amyrin was performed using high performance liquid chromatography according to the method described by [13] with a calibration curve of α-amyrin and β-amyrin standard solutions.

## RESULTS

Table 1 depicts the results of the extraction yield at the conditions determined by the experimental design. For the extraction of the *E. pyriformis* leaves, the highest yield (1.65 wt%) was obtained at the highest pressure and lowest temperature, 200 bar and 60 °C, respectively. The lowest yield (0.33 wt%) was obtained in the experiment where lowest pressure and highest temperature values were used, 150 bar and 80 °C, respectively.

From the data presented, it is observed that at 150 bar, the yield decreases from 0.94 to 0.33% with the increase in temperature from 60 to 80 °C. At this condition of lowest pressure, the effect of solvent density appears to be predominant, because with the increase of the temperature, the yield reduced considerably with the decrease of density. A similar behavior was reported by Santos *et al.* [14]. In the extractions carried out at the pressure of 200 bar, the yield reduced from 1.65 to 1.46% with temperature increase, from 60 to 80 °C. For the highest pressure condition, solvent density and vapor pressure influenced the yield. The increase in temperature decreased the yield and this may be due the decrease of the solvent's density, but the extractions presented high yields even with low density.

With the statistical analysis (ANOVA), it was verified that the effect of pressure and temperature are significant. The effect of the pressure is positive, which explains the increase of the yield when larger pressures are used. In an antagonistic way, the effect of the temperature is negative, thus, the yield decrease occurs in conditions with higher temperatures. However, the effect of the interaction of the two factors did not show to be significant in the range of values investigated.

**Table 1**. Experimental conditions and yields obtained with the $SCCO_2$ extraction

| Pressure (bar) | Temperature (°C) | $CO_2$ Density (kg m$^{-3}$) | Yield (wt.%) |
|---|---|---|---|
| 150 | 60 | 604.61 | 0.94 |
| 150 | 80 | 427.52 | 0.33 |
| 200 | 60 | 724.11 | 1.65 |
| 200 | 80 | 594.31 | 1.46 |
| 175 | 70 | 598.56 | 0.94 |
| 175 | 70 | 598.56 | 0.92 |
| 175 | 70 | 598.56 | 0.96 |

Table 2 shows the results of GC/MS analysis of the volatile fraction of the extract at 200 bar and 60 °C, corresponding to the condition of the best extraction yield of the experimental design. The presented compounds are identified with their respective CAS registry numbers and relative area value (%). The major compounds were β-amyrin (68.24%) and α-amyrin (26.65%). The percentage area of the 4 peaks of the volatile compounds accounted for more than 98%. The same compounds (α-amyrin and β-amyrin) were previously identified in extracts obtained of *E. pyriformis* leaves with $SCCO_2$ extraction and extraction with hexane assisted by ultrasound [5].

**Table 2** – Composition of *E. pyriformis* extracts obtained at 200 bar and 60 °C using $SCCO_2$.

| **Compound Name** | **CAS number** | **% Relative area** |
|---|---|---|
| Vitamin E | 059-02-9 | 1.06 |
| β-Sitosterol | 083-46-5 | 2.40 |
| β-amyrin | 559-70-6 | 68.24 |
| α-amyrin | 638-95-9 | 26.65 |
| **Total** | - | **98.35** |

Table 3 shows the results of the quantification of α/β-amyrin for different conditions of the experimental design of the $SCCO_2$ extraction. High levels of α/β amyrin, in the uvaia extracts, were obtained ranging from 50.15 to 64.85%. For the α-amyrin the highest purity (13.74%) was obtained at 200 bar and 60 °C, whereas for β-amyrin the highest content was 50.85% in the condition of 200 bar and 80 °C.

The obtained results in this study, regarding the amount of α-amyrin and β-amyrin, were higher than those found in the literature. Dias *et al* [15] got 3.1 g of α-amyrin and 1.7 g of β-amyrin per kg of the resin of *Protium sp*. Other authors [16] identified 1.2 g of β-amyrin per kg of *Ficus carica* latex. In other study, Manguro *et al*. [17] detected 200 mg of α-amyrin per kg of *Commiphora holtziana* oleo-gum resin.

**Table 3**. α-amyrin and β-amyrin content obtained with the $SCCO_2$ extraction of *E. pyriformis*

| Experimental condition | α-amyrin (g/100 g)[a] | β-amyrin (g/100 g)[a] | Total (g/100 g)[a] | α-amyrin (g/kg)[b] | β-amyrin (g/kg)[b] | Total (g/kg)[b] |
|---|---|---|---|---|---|---|
| 150 bar/60 ºC | 12.94 | 38.92 | 51.86 | 1.21 | 3.65 | 4.86 |
| 150 bar/80 ºC | 11.65 | 38.50 | 50.15 | 0.38 | 1.26 | 1.64 |
| 200 bar/60 ºC | 13.74 | 49.75 | 63.49 | 2.27 | 8.22 | 10.50 |
| 200 bar/80 ºC | 13.50 | 50.85 | 64.85 | 1.97 | 7.42 | 9.39 |
| 175 bar/70 ºC | 12.71±0.45[c] | 40.76±0.75[c] | 53.47±0.32[c] | 1.20±0.07[c] | 3.84±0.02[c] | 5.04±0.09[c] |

[a] g of α/β amyrin per 100 g of extract.
[b] g of α/β amyrin per kg of leaves.
[c] Average value ± standard deviation of three replicates.

## CONCLUSION

The obtained results showed that the extracts of *E. pyriformis* present high amounts of α-amyrin and β-amyrin. Both compounds present several properties with possible applications in foods, medicines, and cosmetics.

The parameters studied in the $SCCO_2$ extraction showed that the process can be selective to obtain α/β-amyrin rich extracts of leaves of uvaia. The highest yield of the extraction and greater amount of α/β-amyrin per mass of leaves was obtained at 200 bar and 60 °C. The highest purity of the α/β-amyrin was obtained at 200 bar and 80 °C.


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