Supercritical Fluid fractionation of mentha suaveolens: concentration of functional properties.

A. M. Mainar[1], C. Giménez-Rota[1], A. González-Coloma[2], J. S. Urieta[1], S. Lorán[3], M. C. Rota[3]

[1] Grupo GATHERS. Instituto de Investigación en Ingeniería de Aragón (I3A) Universidad de Zaragoza, Mariano Esquillor s/n, 50018, Zaragoza, Spain. Tel. +34-976762707, e-mail: gathers@unizar.es

[2] Instituto de Ciencias Agrarias, CSIC, Calle Serrano 115 dpdo, 28006 Madrid

[3] Instituto Agronómico de Aragón (IA2), Universidad de Zaragoza-CITA, Facultad de Veterinaria. Miguel Servet 177, 50013 Zaragoza. crota@unizar.es.

Abstract

Supercritial Antisolvent Fractionation process was optimiced for the concentration of Mentha suaveolens bioactives in two different fractions. Their composition was analysed with HPLC. The antimicrobial activity of these fractions was assayed. The SAF process fractionated M. suaveolens tincture in two enriched fractions in different bioactives concentrating those with antimicrobial activity in one of them.

Introduction

Plant kingdom is an inexhaustible source of bioactive ingredients valuable in the management of many intractable diseases. *Mentha suaveolens*, also known as apple mint, has been traditionally used as treatment in the Mediterranean traditional medicine as analgesic, sedative, digestive and antimicrobial. Supercritical fluids are a green alternative for the extraction and concentration of this natural bioactives. As an attempt to raise the value of the well-recognized aromatic plant *Mentha suaveolens*, the advanced Supercritical Antisolvent Fractionation (SAF) operational contiditions have been optimized to enrich *M. suaveolens* extract in bioactives of interest.

SAF process and composition analysis

The *tincture* of this plant was processed through SAF, pumped in to a precitipation vessel simultaneously with supercritical CO_2, which schematic structure is represented in Figure 1. The compounds insoluble in this new mixture of solvents precipitate generating the *solid fraction*. Those compounds still soluble in the mixture ethanol CO_2 are collected in the downstream vessel as *liquid fraction*. In order to determine the influence of the factors pressure and CO_2 flow rate in compounds concentration, response surface methodology (RSM) based on Central Composite Design (CCD) was employed. This statistical tool set out 13 different experiments (Table 1) to analise the influence of the 2 variables in side a range (*pressure*: 80 - 150, CO_2 flow rate: 10 - 60 g/min).

M. suaveolens tincture and its two fractions were analised with HPLC reverse fase column C18 2,7µm (4,6x150mm), flow 0.8ml/min, phase mobile 27%CH_3CN:20% CH_3COOH 0.1% and temperature 30±5^{o}C. Three compounds were identified and quantified: Rosmarinic acid (RA), luteolin (LUT) and Apigenin (API). The three of them concentrated in the *solid fraction* regarding to the initial *tincture* Table 1. Other three non identified compounds were concentrated in the final *liquid fraction*.

Antimicrobial activity

The antimicrobial activity of the two SAF fraction and the initial tincture was determined through microdilution broth method (CLSI/EUCAST adaptation) in 96 wells plates against 3 gram positive bacteria, 2 gram negative bacteria and 2 fungi strains, named in Table 2. The extract concentrations ranged from 2500 to 2.5 µg/mL, the microbial inocula was 10^5 CFU/mL. The plates were incubated 37ºC 24hours. Minimum inhibitory concentration (MIC) and Minimum microbiocidal concentration (MBB/MFC) were determined. From all extracts studied only liquid fraction showed antimicrobial activity (Table 2) but low if compared with the essential oil assayed in previous works.

Tabla 1. Experiments performed based on a Central Composite design

Exp	Pressure (bar)	CO_2 flow rate (g/min)
1	115	35
2	90	17
3	115	10
4	115	35
5	80	35
6	150	35
7	115	35
8	115	35
9	90	53
10	115	60
11	140	53
12	140	17
13	115	35

Tabla 2. Minimum inhibitory and bactericidal concentrations of the liquid fraction against 7 different microorganisms.

Microorganism	MIC (µg/mL)	MBC (µg/mL)
Enterococcus faecium	1250	-
Staphycoccus aureus	-	-
Listeria monocytogenes	1250	2500
Escherichia coli	-	-
Salmonella Typhimurium	-	-
Aspergillus flavus	1250	-
Aspergillus parasiticus	1250	-

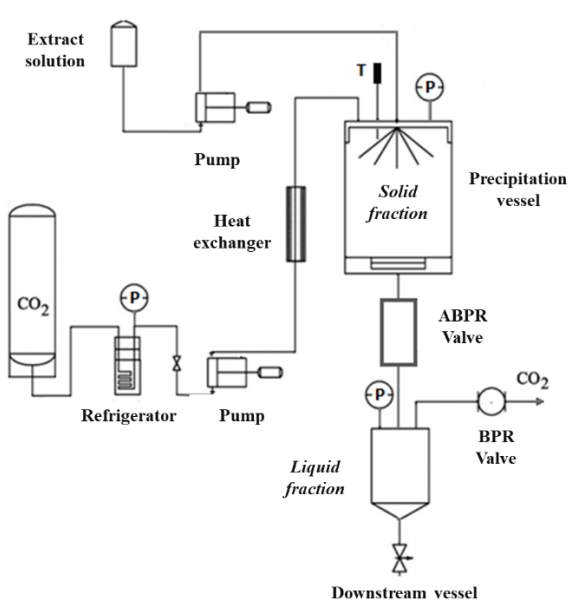

Figure 1. SAF equipment diagram

Conclusiones

Supercritical antisolvent Fractionation concentrated different bioactive compounds in both fractions. Rosmarinic acid, luteolin and apigenin were concentrated in the solid fraction while other non identified compounds were concentrated in the liquid fraction. This last liquid fraction showed antimicrobial activity against two gram positive bacteria and two fungi strains.

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