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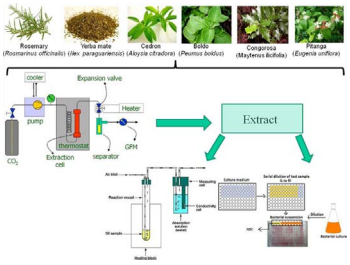
Antioxidant and antibacterial activity of different extracts from herbs obtained by maceration or supercritical technology

I. Vieitez[a,*], L. Maceiras[a], I. Jachmanián[a], S. Alborés[b]

[a] Departamento de Ciencia y Tecnología de los Alimentos, Facultad de Química, Universidad de la República, Montevideo, Uruguay
[b] Departamento de Biociencias, Área de Microbiología, Facultad de Química, Universidad de la República, Montevideo, Uruguay

GRAPHICAL ABSTRACT



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ABSTRACT

Supercritical fluid extraction with carbon dioxide has been demonstrated to be an attractive method to apply to different processes involved in the food industry due to several advantages when it is compared with conventional organic solvents.

Supercritical CO_2 extracts from rosemary (*Rosmarinus officinalis*), boldo (*Peumus boldus*), cedron (*Aloysia citrodora*), congorosa (*Maytenus ilicifolia*), yerba mate (*Ilex paraguariensis*) and pitanga (*Eugenia uniflora*) were obtained at different extraction conditions; their antioxidant effect was studied and compared with that obtained by traditional solvent maceration methods and with that shown by different common synthetic antioxidants. Additionally, antibacterial activity of extracts from the different herbs obtained was determined against different microorganisms.

Supercritical extracts from these herbs were efficient in stabilizing sunflower oil; thus, they could represent a valuable natural alternative to synthetic antioxidants. Herbal extracts prove to be a source of compounds with antibacterial activity, with promising applications in the control of microbial deterioration of food.

1. Introduction

Nutritionally, the basic function of lipids is to provide energy and the essential fatty acid contribution, both being affected after oxidative degradation [1]. Compounds that are generated during oxidation process are not only sensory counterproductive but can be harmful to the organism, with compounds like peroxides being pro-oxidant that generate oxidative stress upon entering the organism. If there are not enough antioxidants in the body to eliminate them, these compounds cause damage to tissues, which can cause DNA mutations or attack any macromolecule, causing, for example, the loss of enzymatic activity or affecting membrane lipids both in structure and function [2]. Antioxidants can interrupt or slow down the oxidation process by stabilizing the radicals or acting to slow down the autoxidation process. Natural antioxidants such as tocopherols, flavonoids and phenols can be found in various sources. However, compounds that naturally act as

* Corresponding author at: (I. Vieitez) Av Gral Flores 2124, C.P. 11800, Montevideo, Uruguay.
E-mail address: ivieitez@fq.edu.uy (I. Vieitez).

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antioxidants are often found in low amounts, have poor activity or are lost during food processing, leading to the need of using more powerful synthetic substances. The synthetic antioxidants that are most commonly used as food additives are butylhydroxyanisole (BHA), butylhydroxytoluene (BHT) and tert-butylhydroxyquinone (TBHQ) [3]. However, in recent years there has been a strong interest in replacing synthetic antioxidants with natural compounds because of possible toxicity and their possible promotion of carcinogenesis [4]. As consumers tend to shift their preferences towards labels with "natural" ingredients, many producers choose to preserve the quality of their products by employing natural antioxidants. Therefore, the utilization of synthetic antioxidants in foods is mostly limited because consumers are increasingly demanding additive-free or natural products. The use of certain amounts of BHA, BHT and TBHQ as additives is allowed in some foods. The limits on fats and oils are 100 mg/kg for BHA, and 200 mg/kg for BHT and TBHQ respectively [5].

Additionally, the control of food deterioration due to the presence of microorganisms and the growth of pathogenic bacteria is mainly achieved by chemical control, but the use of synthetic chemicals is limited by the fact that they are potentially carcinogenic, toxic, teratogenic and because of their slow degradation, which can lead to environmental problems [6]. Consequently, as in antioxidants field, there has been an extensive search for potential natural food additives. It has been found that secondary plant metabolites, such as essential oils and flavonoids, have been studied for their antibacterial, insecticidal and antifungal properties [7]. It is believed that essential oils are produced by plants in response to stress conditions, so crop conditions can affect their composition and content [8,9]. The antimicrobial action of essential oils can be attributed to their ability to penetrate the bacterial membrane and inhibit the functional properties of the cell [10–13]. This ability is due to its hydrophobicity, which allows essential oils to separate the lipids from the cell membrane and cause an increase in cell permeability [14,15]. Phenolic compounds can alter the permeability of the microbial cell, damage the cytoplasmic membrane, interfere with the cellular energy generation system (ATP), and disrupt the proton motive force, leading to cell death [10,14–16]. In general, Gram-positive bacteria are more susceptible to essential oils than Gram-negative bacteria [17]. This is due to the mechanism of action of essential oils; they may interact better with the hydrophobic membrane of the Gram-positive bacteria while Gram-negative ones are more resistant. The cell envelope of Gram-negative bacteria is composed of a cytoplasmic membrane (inner membrane), a thin cell wall of peptidoglycan surrounding the anterior wall, and an outer membrane that lines the cell wall of these bacteria. The composition of this outer membrane is distinct from that of the inner cytoplasmic cell membrane. The outer leaflet includes a complex lipopolysaccharide, with the lipid part in direct contact with the membrane and facing outwards the polysaccharide part. This structure explains the slow flow of hydrophobic compounds [13,18,19–21]. When using essential oils as preservatives in foods, factors of the food matrix such as fat, protein, water activity, pH and enzymes that could reduce the effectiveness of essential oils should be taken into account. Additional methods such as increasing salt content or decreasing storage temperatures may improve the activity [14,15,22]. Many essential oils have a synergistic effect when used in combination with other antimicrobial compounds such as organic acids and trisodium phosphate which are considered GRAS and effective sanitizers [23,24]. Although it has been established that in foods the concentration of bactericides should be higher than that derived from laboratory studies in artificial media, it should also be taken into account that the concentrations of microorganisms in foods are normally lower than in the experimental media, reaching values no higher than 105 cfu/g [25,26]. Many studies have focused on the biological and antimicrobial properties of essential oil derived from rosemary species and their main components [7,27–29]. The use of extracts from herbs as natural food additives is an applicable option, since it is practical, allows some properties to be promoted selectively and because many herbs are very aromatic or pungent for their direct use.

To obtain natural extracts, methods using solvents are the most common, in particular for herbs solid-liquid extraction, with the simplest being maceration. The variables of the method are the solvent to be used, the extraction time and temperature, the grams of sample per solvent volume ratio, the agitation and the final separation method. For example, for the extraction of polyphenols, methanol, ethanol, acetone and water are commonly used as solvents [30,31]. The Soxhlet systems present the disadvantages of high temperature and long time, which can decompose compounds of interest, and low selectivity [32].

Also, extractions with non-polar solvents such as hexane are of interest since the most influential feature of antimicrobial activity is the hydrophobicity of the extracts which allow them to cross bacterial membranes and/or act directly on them [33].

Therefore, research is aimed at obtaining an efficient, economical, high performance method that requires less sample and solvent, which simultaneously extracts a variety of valuable compounds and which results in the least amount of residue possible. In this context, the supecritical fluid extractions (SFE) provide an interesting methodology where solvents are used at temperatures and pressures above their critical point. The most commonly used solvent is Carbon Dioxide (CO_2). In supercritical conditions, the solvents have a high diffusivity, which favors the extraction kinetics of analytes; when optimizing the extraction conditions, extracts with different activity can be obtained [34,35]. Since CO_2 is a non-polar molecule, the supercritical extracts obtained could also have an advantage as antimicrobial agents due to their hydrophobicity.

Therefore, in this study the supercritical extracts of different herbs (rosemary, boldo, cedron, congorosa, yerba mate and pitanga) were obtained at different extraction conditions and their antioxidant and antimicrobial activity against different microorganisms were determined. Results were compared with that obtained with extracts obtained by traditional maceration methods performed using different solvents (75% aqueous ethanol and hexane). Most of these herbs have not been thoroughly studied; except for rosemary which was used as a reference material since it antioxidant power is well known. It is believed that in addition to its antioxidant potential, herbs extracts may have an added value of possessing considerable antimicrobial activity.

2. Materials and methods

2.1. Materials

Dried rosemary (*Rosmarinus officinalis*), boldo (*Peumus boldus*), cedron (*Aloysia citrodora*), congorosa (*Maytenus ilicifolia*), pitanga (*Eugenia uniflora*) and yerba mate (*Ilex paraguariensis*) leaves were obtained from local stores in Uruguay. Refined sunflower oil (SFO, COUSA S.A., Montevideo, Uruguay) was acquired in a local store. Standards and reagents used for the analysis were supplied by Sigma-Aldrich (United States).

2.2. Methods

2.2.1. Extractions by traditional maceration

To perform the macerations, the method described by Dinnies Santos et al. [36] was adapted and scaled. About 2.0 g of the corresponding dried and ground herb were weighed, to which 30 mL of the solvent was added. Two solvents were used: 75% aqueous ethanol (EtOH 75%) and hexane. Subsequently, it was constantly stirred in the dark for 4 h at room temperature. The phases were then separated by centrifugation for 15 min at 4000 rpm and the supernatant was removed. A wash was performed in the herb with 15 mL of solvent, which was stirred for 2 h. Supernatants were pooled together and the solvent was removed in a water bath at 40 °C under nitrogen flow. The extracts were stored in a freezer at −18 °C and protected from light. These determinations were performed at least in duplicate.

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