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Characterization of fennel extracts and quantification of estragole: Optimization and comparison of accelerated solvent extraction and Soxhlet techniques

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ABSTRACT

Fennel (*Foeniculum vulgare* Miller) is an aromatic plant used, among other applications, in the production of traditional herbal liqueurs. In this study, essential oils from fennel were extracted applying two techniques, Soxhlet and accelerated solvent extraction (ASE). The extracts obtained were characterized by GC-MS. Taking into account that estragole is the major constituent of fennel and due to recent studies pointed out its possible carcinogenic properties; this compound was also quantified by GC-FID. The quantification method showed good linearity (r^2 = 0.998) and precision (RSD < 5%) with low values of detection (LOD) and quantification (LOQ) limits. A Box–Behnken design was used to correlate three independent variables (temperature, contact time sample-solvent and number of cycles) with the amount of estragole extracted. Meanwhile, the response surface methodology was applied to optimize the extraction of estragole by ASE. The optimal conditions were 125 °C, 7 min and 3 cycles. On the other hand, the Soxhlet technique was studied step-by-step. Two variables were optimized: time (4 and 8 h) and solvents, according to their polarity. Methanol and 4 h of extraction showed the best results both qualitatively and quantitatively. The Soxhlet technique provided higher performance of extraction and greater amounts of compounds extracted compared to ASE, but similar concentration of estragole. The shorter time of extraction and the lower amount of solvent used justify the ASE technique choice to characterize fennel essential oils.

1. Introduction

Fennel (*Foeniculum vulgare* Miller) is an *Apiaceae* family plant and native from the Mediterranean area. There are two subspecies of the *Foeniculum vulgare*: *piperitum* with bitter seeds and *vulgare* with sweet seeds (He and Huang, 2011). The *vulgare* subspecies is characterized by having as main compounds the phenylpropenes: anethole and estragole, followed by bicyclic oxygenated monoterpene fenchone and the monocyclic monoterpene hydrocarbon limonene (Díaz-Maroto et al., 2006).

Traditionally, fennel has been used to flavor foods, to make liqueurs, in perfumery industry (Gross et al., 2002) and as a home remedy to gastrointestinal and respiratory tract symptoms (Raffo et al., 2011). Due to the known properties as an aromatic and medicinal plant, fennel is traditionally used for making herbal liqueurs by maceration or distillation process with Orujo (the name of grape marc distillate in the North of Spain) (Damjanovic et al., 2005; Piccaglia and Marotti, 2001).

In recent years, estragole has become the subject of several research due to its being the major compound in fennel and also because this volatile compound could possess potential carcinogenic properties (Raffo et al., 2011; Zeller and Rychlik, 2006).

To extract and characterize the essential oils from fennel, which can provide aromatic and medicinal properties to distillates during the liqueur making process, two analytical techniques were applied: Soxhlet and accelerated solvent extraction (ASE). Both techniques are based on solid–liquid extraction. The first is a traditional technique that is time-consuming and uses large volumes of organic solvents (environmentally unfriendly) but it is currently used because of its high recovery. ASE technique is an alternative to the classical extraction method Soxhlet because it uses high pressure (that keeps the solvent below its boiling point) and slightly higher temperatures (that accelerates the extraction kinetics) obtaining similar results to traditional solvent extraction. ASE is

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used in order to reduce the extraction time and volume of solvents and therefore, reduce laboratory time and costs (Heemken et al., 1997; Nakatsu et al., 2000; Raaman, 2006; Romanik et al., 2007).

The optimization of a method can be carried out step-by-step or using an experimental design (Rajaei et al., 2005). Several studies have been carried out on plants using different extraction solvents (with different polarities to evaluate the influence on the extraction yield and on the extract composition) (Almeida et al., 2012; Tsimogiannis et al., 2006). In this study we assayed different solvents and times of extraction to evaluate if this latter variable has an influence on extracting more volatile compounds from fennel seeds.

Furthermore, an experimental design was applied to model and optimize ASE technique for the extraction of volatile compounds from fennel. The modeling tool is known as response surface methodology (RSM) using the Box–Behnken design of experiments. The RSM technique can simulate and optimize complex processes from relatively few experimental combinations of variables (Annadurai and Sheeja, 1998; Zhao et al., 2012). Box–Behnken design method employs a spherical design with excellent predictability within the design space (Nath and Das, 2011). This design has already been used in optimization process of essential oils extraction among others, from tobacco leaves, *Satureja hortensis* and soybean (Jokic et al., 2010; Khajeh, 2011; Zhang et al., 2012).

In this study we focused on the optimization and comparison between Soxhlet and ASE techniques to improve the volatile characterization of fennel with the aim of evaluating the potential volatiles that this aromatic plant gives to the herbal Orujo Liqueurs. Also, optimizing the quantification method to evaluate the quantity of estragole present in the fennel seeds could be useful to improve the making process of herbal liqueurs avoiding a higher concentration of this possibly harmful for health oil.

2. Materials and methods

2.1. Materials

2.1.1. Samples

Dried seeds from fennel (*F. vulgare* Miller) were commercially purchased from a leader phytotherapy company from Spain. According to the information provided by the company, fennel was grown in certified organic area under continental climate conditions. Fennel seeds were air dried at room temperature and vacuum packed in plastic bags (100 g).

Five bags from different lots (500 g) were mixed in order to obtain a homogenous sample previous to the essential oil extraction by ASE and Soxhlet techniques, avoiding possible errors due to the different initial composition in the fennel seeds. Until analysis the whole sample was preserved in a hermetically sealed packaging.

2.1.2. Reagents

The solvents hexane, diethyl ether and methanol and the standard estragole were supplied by Sigma–Aldrich (Steinheim, Germany), ethyl acetate by Panreac (Barcelona, Spain) and ethanol was purchased from Analar Normapur (VWR) (Barcelona, Spain). Alkane standard solution C_8–C_{20} was purchased from Fluka (Steinheim, Germany). Siliceous Earth purified and calcined (USP-NF) RRS-CODEX was supplied by Panreac (Barcelona, Spain).

2.2. Methods

2.2.1. Optimization of fennel seeds extraction

2.2.1.1. Soxhlet extraction. Soxhlet experiments were performed with a Behrotest® Equipment for Soxhlet Extraction (extraction system with six individual extractors (1 sample each) with linear configuration (Düsseldorf, Germany)). A total of 50 g of the previously homogenized fennel seeds was used in the determination of their essential oils by Sohxhlet. In each extraction, 5 g of dried fennel seeds were weighed in cellulose extraction thimbles (33 mm × 94 mm, thickness 1.5 mm purchased from Schleicher & Schuell (Dassel (Germany)), previously homogenized and grinded with coffee grinder (Moulinex (France)). The extraction took place using a solvent volume of 150 mL. Each solvent (hexane, diethyl ether, ethyl acetate, ethanol and methanol) was brought to its corresponding boiling point. The final extract was evaporated in a rotavapor R-215 Buchi (Frankfurt, Germany) at 25 °C and the resulting oleoresin extract was dissolved in 10 mL of the solvent used in each case. All extractions were done in duplicate. The physicochemical characteristics of the five solvents assayed are showed in Table 1.

Table 1
Solvents used during the Soxhlet extraction with their physicochemical characteristics.

		Solvent	Polarity Index	Boiling (°C)
Non polar		Hexane	0	68.85
		Diethyl ether	2.8	34.60
	Aprotic	Ethyl acetate	4.3	77.10
Polar	Protic	Ethanol	5.2	78.40
		Methanol	6.6	64.70

2.2.1.2. Accelerated solvent extraction (ASE). The accelerated solvent extraction of essential oils from fennel seeds was performed with a DIONEX extractor, ASE 350 from Vertex technics (Barcelona, Spain). 160 g of the homogenized sample from fennel seeds was necessary to their essential oil characterization. The extraction was done by weighing 5 grams of sample, previously milled and mixed with diatomaceous earth to remove any moisture that could remain. The resultant sample was placed in a stainless steel cell of 22 mL. According to Heemken et al. (1997) in ASE technique it is recommended to use the same solvent as in Soxhlet technique, so the extraction took place using methanol as solvent because it was found to be the optimal solvent in the Soxhlet technique.

Once the extract obtained by ASE technique was evaporated with a TurboVap LV Caliper LifeSciences (Cardiff, UK) under N_2 at a temperature of 35 °C and a pressure of 12 psi, the resulting oleoresin was redissolved in 10 mL of methanol. All extractions were done in duplicate.

2.2.2. Experimental design and statistical analysis

Temperature, time and cycle number affect aromatic compounds extraction by ASE techniques. An incomplete factorial design with three factors and three levels (Boxt and Behnken, 1960) was used to optimize these parameters. Experiments (15 runs) were carried out in a single base block, of which three were replicates at the center point measuring experimental error. The level of independent variables studied and definition of dimensionless coded of the independent variables are given in Table 2. For statistical calculations, the independent variables were coded as x_1 (coded temperature), x_2 (coded cycle number) and x_3 (coded time). The correspondence between coded and uncoded variables was fitted according to the linear equations showed in Table 2, which were deduced from their respective variations limits. The dependent variable was y_1 (estragole, g/kg). The experimental data were evaluated by response surface methodology using Statistica 5.0 software. Effect of each independent variable to the response was fitted by polynomial quadratic equation, Eq. (1), which includes