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Deodorization by instant controlled pressure drop autovaporization of rosemary leaves prior to solvent extraction of antioxidants

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A B S T R A C T

During the extraction of antioxidants from a natural product, volatile compounds are also extracted and consequently the final extract quality is decreased. Therefore, a deodorization step is necessary. The present study introduces thermo-mechanical treatment of rosemary leaves using the instantaneous controlled pressure drop (DIC) method as both deodorization and expansion process. In addition, DIC-deodorization treatment was compared with the hydrodistillation-deodorization (HD). The extraction of essential oils was achieved on dried rosemary leaves within an optimized time of 3 min by DIC and 4 h by HD. Deodorized leaves were recovered and antioxidants were extracted using solvent extraction (ethanol:water 80:20). With standard protocol extraction, rosmarinic acid of DIC-treated rosemary leaves (12.76 mg/g) was twice as much as untreated leaves (6.74 mg/g). Scanning electron microscopy (SEM) and light microscopy showed that DIC treatment resulted in profound alterations at cytohistological levels which explain the observed effectiveness of DIC process. The specific surface area calculations showed that DIC treatment allows the material to obtain a higher specific surface area. This explains the behaviour of the product towards the extraction. Overall, the results clearly revealed that DIC is an efficient method of deodorization that improved solvent extraction of rosemary antioxidants.
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1. Introduction

Growing consciousness of consumers regarding human health has encouraged agro-industrial firms to substitute synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) by natural antioxidants (Sebranek, Sewalt, Robbins, & Houser, 2005; Yanishlieva, Marinova, & Pokorný, 2006). Antioxidants are compounds capable of scavenging free radicals and consequently delaying or even preventing autooxidation. One of the most important phenomena involved in food deterioration is oxidation (Pérez-Fons, Garzón, & Micol, 2009). Antioxidant molecules must be potent with low quantity taking into account that changes in foodstuff aromas should be minimal (Pokorny, Yanishlieva, & Gordon, 2001). Indeed antioxidant compounds cannot be added to foodstuff when still containing aromas (Ares, Barreiro, Deliza, & Gámbaro, 2009).

Rosemary (*Rosmarinus officinalis*) is known to have great antioxidant activity (Erkan, Ayranci, & Ayranci, 2008). The main substances related to antioxidant activity are phenolic diterpenes (Schwarz & Ternes, 1992a, 1992b; Schwarz, Ternes, & Schmauderer, 1992) such as carnosol, rosmanol, carnosic acid, methyl carnosate, and phenolic acids such as rosmarinic acid (Petersen & Simmonds, 2003). However, rosemary leaves contains between 0.7 and 3% fresh weight material of essential oils depending on the variety, the way of harvesting, their location, etc. (Bousbia et al., 2009; Singh & Guleria, 2013; Sui et al., 2012; Szumny, Figiel, Gutiérrez-Ortíz, & Carbonell-Barrachina, 2010).

As a consequence, processes for obtaining antioxidants should preserve high antioxidant activity, reducing or even completely removing aroma. López-Sebastián et al. (1998) tested at laboratory scale deodorization of rosemary leaves through supercritical fluids. Although this operation was coupled with enzymatic pretreatment, the process itself was quite complicated, making it inconvenient at industrial scale.

DIC (Détente Instantanée Contrôlée) standing for instant controlled pressure drop was defined in 1988 (Allaf, 1988). DIC is

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a thermo-mechanical process generated by subjecting the raw material for a short period of time to high saturated steam pressure followed by an abrupt pressure drop towards a vacuum. It triggers autovaporization of volatile compounds, which results in an expansion of the sample.

An initial study on rosemary leaves showed a relative improvement of DIC treatment compared to other classic volatile compound extraction processes (Rezzoug, Boutekedjiret, & Allaf, 2005). Further studies provided new elements with respect to autovaporization phenomena through instant controlled pressure drop technology. This was observed and explained in terms of fundamental and experimental aspects. Indeed studies were reported for Kananga flowers (Kristiawan, Sobolik, & Allaf, 2008), lavandin (Besombes, Berka-Zougali, & Allaf, 2010), myrtle leaves (Berka-Zougali, Hassani, Besombes, & Allaf, 2010), orange peel (Allaf, Tomao, Ruiz, & Chemat, 2013), etc. providing very relevant results in terms of essential oil extraction. Such results indicated that further studies should be carried out in terms of the feasibility of the instant controlled pressure drop technology as a method of deodorization through autovaporization before solvent extraction of antioxidants. DIC extraction process through autovaporization phenomenon provides essential oil in a stable oil-in-water emulsion.

In this report, a study carried out on the extraction of essential oils of rosemary leaves performed with standard hydrodistillation and DIC technique was described. Scanning electron microscopy and light microscopy were performed in order to understand, at structural scale, the specific effects of HD and DIC treatments on rosemary leaf tissues. In addition specific surface area was calculated to better interpret each matrix behaviour.

2. Materials and methods

2.1. Plant material

In this study 10 kg of dry leaves of Rosemary (*Rosmarinus officinalis* L.) were purchased from the firm Herbier du Diois (Châtillon-en-Diois, France) and have an initial water content of 6.83 ± 0.06% dry basis.

2.2. Protocol treatment

After a first stage of deodorization carried out by hydrodistillation or by DIC-autovaporization, rosemary sample “residue” was treated by ethanol:water (80:20) to extract the antioxidants. Solvent extracts were then analyzed and identified by HPLC. The amount of essential oils was determined and used in order to optimize DIC operating parameters. The performed protocol is shown in Fig. 1.

2.2.1. Hydrodistillation (HD) apparatus and protocol

Hydrodistillation equipment used in the present study was a 6-L modified stainless steel Clevenger apparatus from REUS Company (Contes, France).

200 g of Dried rosemary leaves were immersed in 2-L of distilled water. The extraction of essential oils was achieved during 4 h, from the first drop of distillate until the quantity of extracted essential oils was stabilized. Afterwards the rosemary leaves were recovered and dried at room temperature to be stored for antioxidant extraction and quantification.

2.2.2. DIC apparatus and treatment

DIC reactor used (Fig. 2) was from ABCAR-DIC Process Company (La Rochelle, France). A 7-L processing vessel with a heating jacket was utilized; thermal treatment in this vessel is achieved using saturated steam. A vacuum tank with cooling water jacket is connected with a water ring vacuum pump allowing the vacuum chamber to be maintained at 5 kPa.

DIC cycle can be fractioned in five steps:

1. the sample was put at an initial vacuum;
2. saturated steam was injected in the treatment chamber to reach the selected pressure and kept constant;
3. the pressure was then dropped towards a vacuum instantaneously;
4. the vacuum was maintained for a short moment;
5. the pressure level was then released towards the atmospheric level.

Multi DIC cycles contain several repetitions of stages (2), (3) and (4).

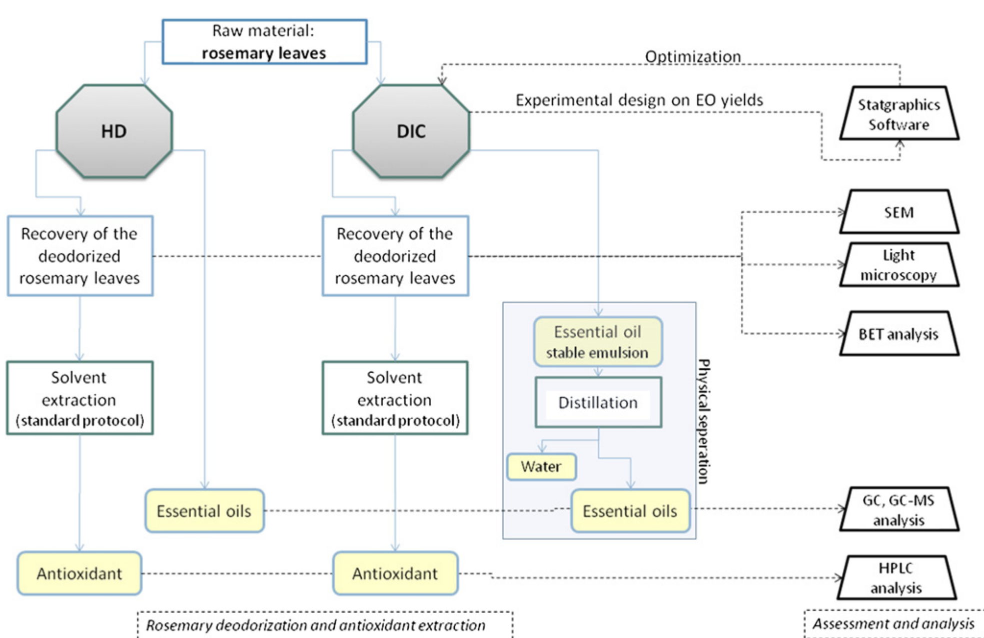

Fig. 1. Protocol treatment of rosemary leaves.

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