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Antioxidant and antimicrobial active paper based on Zataria (*Zataria multiflora*) and two cumin cultivars (*Cuminum cyminum*)

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ABSTRACT

The antioxidant and antimicrobial properties of *Zataria multiflora* and Iranian and European Cumin were evaluated, first as pure extracts and later as active agents incorporated in paper, manufactured at laboratory scale as an active packaging material. Two different procedures were used for quantifying the antioxidant properties and microbiological studies versus *Staphylococcus aureus*, *Listeria innocua, Pseudomonas* sp., *Salmonella enterica* subsp. *enterica and Escherichia coli* were carried out. All bacteria were inhibited when exposed to the atmosphere generated from 4% to 6% (w/w) of Zataria essential oil in the active coating. Zataria showed the best antioxidant properties. The compounds responsible for the antimicrobial and antioxidant properties are analyzed and discussed.

1. Introduction

In recent years, the major driving forces for innovation in food packaging technology have been the increase in consumer demand for minimally processed foods, the change in retail and distribution practices associated to globalization, new consumer product logistics, new distribution trends (such as Internet shopping), automatic handling systems at distribution centers, and stricter requirements regarding consumer health and safety. Active Packaging (AP) technologies are being developed as a result of these driving forces. Active Packaging is an innovative concept that can be regarded as a mode of packaging in which the package contains compounds specifically added to protect the product against deterioration processes, to extend the shelf life or to enhance safety and/or sensory properties, while maintaining the quality of the product (Rodriguez-Lafuente, Batlle, & Nerin, 2007). This concept is very attractive, especially for fresh food, where the addition of protective agents is not allowed for being considered as “fresh food”. Antimicrobial and antioxidant actions are the main target properties searched by active packaging. Active packaging approaches have been studied *in vitro* and *in vivo* by many researchers, although there are many more papers dealing with *in vitro* test than with real foodstuffs.

The antioxidant packaging may be useful in retarding or inhibiting oxidative deterioration of foodstuffs, especially those with high lipid content. The natural antimicrobial agents inhibit or delay the growth of microorganisms, resulting in the prevention of various diseases (Abdoul-Latif et al., 2010). Several plastics (Lopez, Sanchez, Batlle, & Nerin, 2007) as well as paper (Rodriguez-Lafuente et al., 2007), board and biopolymers have been studied and proposed as active packaging materials and a lot of them contain essential oils (EOs) as active agents (Lopez-de-Dicastillo et al., 2011; Lopez-de-Dicastillo et al., 2012; Nerin, 2012; Rodriguez-Lafuente, Nerin, & Batlle, 2010), either as antioxidants or as antimicrobials. This is not surprising, because EOs are rich sources of terpenes and phenols, with strong antioxidant properties and some of them also show antimicrobial properties. Another interesting feature is that these natural compounds do not have any significant toxicity or environmental impact so they constitute efficient alternatives to conventional antimicrobial agents (Gutierrez, Sanchez, Batlle, & Nerin, 2009). The antimicrobial resistances are also much lower than those found for common synthetic antibiotics (Becerril, Gomez-Lus, & Nerin, 2011; Becerril, Nerin, & Gomez-Lus, 2012; Rodriguez-Lafuente, Nerin, & Batlle, 2008), which constitutes an additional advantage. Most of these EOs are traditionally used in food preparation and are included in

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the food additives list, either as flavoring, spices, antioxidants and or antimicrobials. Although this old knowledge about essential oils, their composition and the relationship between the individual components and their properties is unknown in most of the cases.

EOs composition vary with climate, geographic location, collection of plants, drying system and extraction procedure. For this reason, their properties also change with these parameters. Mediterranean countries have a strong tradition of using essential oils and aromatic plants to preserve food, but the specific properties and the real strength as antioxidants and/or antimicrobials are not well known. The main purpose of this research work was to study in depth the antioxidant and antimicrobial behavior of cumin and Zataria EOs, commonly used in Mediterranean countries, as well as the performance of an active paper containing them.

Zataria multiflora is a thyme-like plant that grows wild in central and southern Iran. It is a member of the *Latiatae* family to which mint, rosemary and several other useful medicinal plants also belong. In Iran, *Zataria multiflora* is used in traditional folk remedies for its antiseptic, analgesic (pain-relieving) and carminative (anti-flatulence and intestine-soothing) properties. However, its composition has not been published yet.

Cumin (*Cuminum cyminum*) is one of the most commonly used spice condiment in Asia. Cumin seeds are used as popular aromatic herbs and culinary spices. All the cumin varieties are used in traditional and veterinary medicine as stimulant, carminative, astringent and as a remedy against indigestion, flatulence and diarrhea (Amin, Kalantar, Saeid, & Ahsan, 2010) All these properties make cumin a good candidate for being used as protective agent in food packaging, mainly to protect those foodstuffs that cannot be spiked or produced with additives, such as fresh products.

The present study deals with the chemical composition, antibacterial and antioxidant behavior of *Cuminum cyminum*, *Zataria multiflora* and *European cum*in obtained by steam distillation. Additional tests have been done after incorporating these EOs in active paper packaging. The results obtained are shown and discussed.

2. Materials and methods

2.1. Apparatus

A Clevenger apparatus was used for obtaining the essential oils from the plants. A Varian CP-3400 Saturn 2000 gas chromatograph with a mass spectrometer as detector (GC/MS), a microbial culture media (Scharlau), a UV–VIS Spectrophotometer and an Alliance 2795 Separations Module high performance liquid chromatography (HPLC) with UV detector were used. Active materials were prepared at laboratory scale by coating with a K101 Control Coater RK Print-Coat Instruments Ltd. Chemicals.

2.2. Plant materials

The plants (*Cuminum cyminum* and *Zataria multiflora*) were collected from Kerman and Fars provinces of Iran, respectively. *European cumin* EO was supplied by the company Argolide (Barcelona, Spain).

2.3. Essential oil extraction

The aerial parts of plants (*Cuminum cyminum* and *Zataria multiflora*) were dried in an oven equipped with hot air circulation. They were then ground. The essential oil was obtained by steam distillation of ground material with boiling water up to 2 h utilizing a clevenger-type system. The extracted oils were dried over anhydrous sodium sulfate followed by filtering and they were stored at 4 °C in sealed glass vials for further use.

2.4. Essential oil analysis

The GC/MS analyses were executed on a Varian CP-3400 gas chromatograph equipped with a column BPX5 30 m × 0.25 mm × 0.25 um, SGE (Scientific Instrument Service, NJ, USA) coupled to Saturn 2000 mass spectrometer. The column temperature was programmed at 50 °C as an initial temperature, holding for 2 min, with 2.5 °C increases per minute to 265 °C. Injection port temperature was 250 °C and helium was used as carrier gas at a flow rate of 1 mL/min. Ionization voltage of mass spectrometer in the electron impact mode was equal to 70 eV and ionization trap temperature was 170 °C. The mass spectrometer was scanned from m/z 40 to 250. The individual compounds were identified and confirmed thereafter using Kovats retention indices. Pure standards of the compounds were also used to confirm the identification of the compounds.

2.5. Active paper manufacture

The active paper was manufactured as follows: a US Food and Drug Administration (FDA) quality emulsion of paraffin formula supplied by Repsol (Madrid, Spain) was homogeneously mixed with the appropriate amount of the selected EO. This active coating was then applied to 70 g/m^2 paper provided by Antalis (Zaragoza, Spain) using the coating machine above mentioned. Once coated, the properties of this active paper were evaluated.

2.6. Microbial cultures

The antimicrobial activity of three essential oils were studied using the following bacteria: two gram positive bacteria, namely: *Staphylococcus aureus* (American Type Culture Collection, ATCC 29213), *Listeria innocua* (*Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH,* DSM 20643),three gram negative bacteria, namely *Pseudomonas* sp. (Colección Española de Cultivos Tipo, CECT 4335), *Salmonella enterica* subsp. *enterica* (Colección Española de Cultivos Tipo, CECT 556), *Escherichia coli* (American Type Culture Collection, ATCC 25922).

2.7. Antimicrobial testing

2.7.1. Antimicrobial activity tests

2.7.1.1. Solid diffusion assays. A plastic Petri dish (90 mm diameter) containing the appropriate solidified medium was inoculated with 100 μL of a physiological saline solution containing 10^4 colony-forming units (CFU)/mL of the microorganism under study. Five microliters of the undiluted EO were added to a 5 mm diameter sterile blank filter disk, placed on top of the cultured media. After incubation under optimal conditions (temperature and time), the diameter of inhibition zones was measured. All analyses were carried out in triplicate.

2.7.1.2. Vapor diffusion assays. In each test of the antimicrobial activity of the active packaging materials, a Petri dish with the appropriate solidified agar culture medium was inoculated with 100 uL of a 10^4 colony forming unit (CFU)/mL solution of the microorganism under study. Then, the active paper was placed over the Petri dish and non-hermetically tied using a plastic strip. Controls with paper coated by the paraffin formulation but without active ingredients were also prepared for each set of samples. The inhibition of each microorganism under test was calculated by the rate between the number of viable colonies in the Petri dish