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Antifungal activity of nanoemulsions encapsulating oregano (*Origanum vulgare*) essential oil: *in vitro* study and application in Minas Padrão cheese

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ABSTRACT

The objective of this study was to evaluate the antifungal activity of nanoemulsions encapsulating essential oil of oregano (*Origanum vulgare*), both *in vitro* and after application on Minas Padrão cheese. Nanodispersions were obtained by the phase inversion temperature method. *Cladosporium* sp., *Fusarium* sp., and *Penicillium* sp. genera were isolated from cheese samples and used to evaluate antifungal activity. Minimal inhibitory concentrations of non-encapsulated and encapsulated oregano essential oil were determined, and they were influenced by the encapsulation of the essential oil depending on the type of fungus. The antifungal activity of the nanoencapsulated oregano essential oil in cheese slices showed no evidence of an effect of the MICs, when applied in the matrix. On the other hand, an influence of contact time of the nanoemulsion with the cheese was observed, due to the increase in water activity. It was concluded that nanoencapsulated oregano essential oil presented an inhibitory effect against the three genera of fungi evaluated. If environmental parameters, such as storage temperature and water activity, were controlled, the inhibitory effect of nanoemulsions of oregano oil could possibly be greatly improved, and they could be presented as a potential alternative for the preservation of Minas Padrão cheese against fungal contamination.

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Introduction

Essential oils are aromatic compounds obtained from vegetable materials, characterized by their high volatility and complex composition.[1] Due to their known antimicrobial and antifungal actions, they are considered extensively for use in pharmaceutical, food, agricultural, and cosmetic products.[1,2]

Regarding antifungal activity, several studies have demonstrated the potential of essential oils to inhibit the proliferation of several types of fungi in food products, such as *Aspergillus*, *Microsporum*, *Mucor*, *Penicillium*, *Eurotium*, *Debaryomyces*, *Pichia*, *Zygosaccharomyces*, and *Candida*.[3–6]

Oregano essential oil is among the most investigated essential oils due to its antifungal properties, as it can prevent the growth of a broad spectrum of fungi, such as *Aspergillus* sp., *Fusarium* sp., and *Penicillium* sp.[7–11] Oregano essential oil is mainly composed of carvacrol, thymol, cymene, and terpinene.[12,13] The growing interest in its use in food preservation is mainly due to consumers' demands for a substitution of synthetic preservatives in food by natural substances with a similar effect.[14,15]

From a technological point of view, the application of essential oils as food preservatives presents some disadvantages due to their strong odor and flavor, which could affect the organoleptic properties of the product.[16] Moreover, essential oils are easily degraded by oxidation, volatilization, heating, and exposure to light[17] and difficult to disperse in hydrophilic media.[18] In order to overcome these drawbacks, nanoencapsulation techniques have been proposed, as they increase the dispersibility of essential oils in aqueous formulations, increase chemical stability during storage, minimize organoleptic changes, and in some cases, even improve their antimicrobial action.[19] Due to their very reduced size, nanostructures can lead to an increase in passive cellular absorption mechanisms, thus reducing mass transfer resistance and increasing antimicrobial activity.[20]

Among the nanostructures which can be produced, there are the nanoemulsions, which are emulsions with mean droplet size of 20–200 nm. Nanoemulsions exhibit high kinetic stability and are therefore stable for a long period of storage.[21] Among the production methods used to obtain nanoemulsions with average droplet sizes smaller than 50 nm, there are low-energy methods, such as the phase inversion temperature (PIT) method. Such methods are based on the change in solubility of non-ionic polyethoxylated surfactants with temperature.[22,23].

Minas Padrão is a pressed, semi-hard cured cheese consumed extensively in Brazil.[24] It is produced with pasteurized milk and has characteristic color, odor, and flavor.[25,26] It is a product that requires refrigeration during storage and commercialization, as higher temperatures can decrease its quality.[27] As it is a ripened product, it is susceptible to contamination by pathogenic bacteria and fungi during the maturation process. This may be a cause for serious concern, as the current Brazilian legislation does not establish limits for contamination by fungi.

Therefore, considering the increasing consumer demand for natural ingredients and the possibility of using essential oils in food preservation, the objective of this study was to evaluate the antifungal action of the oregano essential oil encapsulated in nanoemulsions, both *in vitro* and in Minas Padrão cheese.

Materials and methods

Nanoemulsions were produced using essential oil of *Origanum vulgare* (Ferquima, Cotia, SP, Brazil) and sunflower oil (Cargill, Mairinque, SP, Brazil). The surfactants used were PEG-40 hydroxylated castor oil (*Ricinus communis*) (Cremophor RH 40, BASF, Ludwigshafen, Germany), polyoxyethylene 4-lauryl ether (Brij 30, Sigma-Aldrich, St Louis, MO, EUA), and sorbitan monooleate (Span 80, Sigma-Aldrich, St. Louis, MO, USA). Ultrapure water (Direct Q3, Millipore, Billerica, MA, USA) was used throughout the study.

For the isolation and identification of the fungi from the cheese samples, dichloran rose bengal agar chloramphenicol (DRBC) agar (Acumedia, Neogen, São Paulo, SP, Brazil), dextrose potato agar (DPA, Acumedia, Neogen, São Paulo, SP, Brazil), and chloramphenicol (Homeopatia Ouro Preto, Pirassununga, SP, Brazil) were used.

Production of nanoemulsions

Nanoemulsions were produced by the PIT method according to Moraes-Lovison et al.[28]. The components of the nanoemulsions (sunflower oil, surfactants, deionized water, and oregano essential oil) were mixed and magnetically stirred at 1350 rpm, and this mixture was heated to 65 °C. The two formulations of nanoemulsions produced are described in Table 1. Afterwards, this mixture was cooled rapidly by placing the dispersion in jacketed vessels cooled by water at 3 °C, under magnetic stirring, until it reached room temperature (cooling rate: 10 °C/min). The heating/cooling cycles were repeated twice.

Isolation and identification of fungi in Minas Padrão cheese samples

Eighteen samples of fungi-contaminated Minas Padrão cheese were obtained. The fungi were isolated from both the surface and interior of the cheese. A mass of 10 g of each contaminated cheese sample (surface and interior), previously homogenized and diluted, were inoculated on DRBC agar and incubated at 25 °C for 7 days. The colonies of different morphological types were isolated in potato dextrose + chloramphenicol (PDA + C) agar, and after identification by the microculture technique, the fungi were classified by genera.[29]

Evaluation of the in vitro antifungal activity of nanoemulsions

To evaluate the *in vitro* inhibitory activity, non-encapsulated oregano essential oil and nanoemulsions of oregano essential oil were tested. The concentrations of oregano essential oil used are shown in Table 2.

A volume of 0.1 ml of spore suspension of each fungus (*Cladosporium* sp., *Fusarium* sp., or *Penicillium* sp.),

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