RETENTION AND RELEASE OF CARVACROL USED AS ANTIMICROBIAL AGENT INTO SOY PROTEINS MATRIX BASED ACTIVE PACKAGING

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Abstract

In this work, an antimicrobial paper packaging was designed by introducing carvacrol as antimicrobial agent into a soy protein matrix used as coated matrix of paper. The ability of the soy proteins matrix to retain carvacrol added at different ratio (10%, 30%, 60% w/w) was evaluated by measuring the aroma compound losses after process. The losses varied between 12 and 30% depending on the carvacrol load.

The ability of the soy protein to release carvacrol was studied as a function of relative humidity (60, 80 and 100%) for three temperatures 5, 20 and 30°C. Increasing RH and temperature clearly increased carvacrol release. At 30°C and 100% RH, the total amount of carvacrol was released after 2 days of storage. Kinetic release could be modelled by second Fick’s law and diffusivity coefficients evaluated. The control of relative humidity and temperature associated with high retention ability of soy proteins permits to limit the losses of antimicrobial agent during process and storage in adapted conditions and to favour the release of carvacrol in conditions of microbial growth, i.e. high temperature and relative humidity.

Introduction

Nowadays, there is an increasing interest in the possible use of aroma compounds to prevent microbial growth in the food items (1). The use of antimicrobial packaging appears as a promising way preventing microbial growth by the controlled release of an active agent and lowering its concentration in the product (2). Aroma compounds as volatile antimicrobial agents can act through the intra packaging atmosphere without direct contact with food product. In this purpose, an antimicrobial paper packaging was designed by introducing, as antimicrobial agent, an aroma compound into a soy protein solution used as the coated matrix of paper. Carvacrol, the major aroma compound of Oregano essential oil and an efficient inhibitor against a wide spectrum of micro-organisms (3) was selected to be incorporated into the soy proteins matrix based active packaging. The release rate of the volatile agent from the packaging system is an important parameter to control the antimicrobial efficiency. The present work aimed at studying the potentiality of soy proteins matrix to retain and to release carvacrol in controlled conditions of temperature and relative humidity.

Experimental

Preparation of carvacrol/SPI coated paper. Soy Proteins Isolates coated papers were prepared by the procedure previously described (4). SPI (10% w/v) from Seah
International, having 91.8% proteins content, were dissolved in distilled water heated at 50°C by continuously stirring for 30 min at 500 rpm. Carvacrol (Sigma Aldrich) was added at a percentage of 10, 30 or 60% (w/w of SPI). Homogenisation was carried out with an Ultra-Turrax (T-25, IKA Labotechnik, Germany) at 8000 rpm for 10 min. The coating process was performed at 25°C: a support paper (provided by “Ahlstrom Research and Services”) was maintained on a perforated iron plate under partial vacuum and the coating solution was applied by an adjustable micrometer thin layer chromatography applicator. Then, coated papers were dried for 3 hours at 23 ± 2°C and at 50 ± 5% RH.

Kinetic release of carvacrol from SPI coated papers. Pieces of coated papers (3 cm x 3 cm) were put on tray in a controlled temperature incubator at the selected temperature and relative humidity. The incubator was maintained at the following temperatures: 30°C, 20°C, 5°C and for each temperature, the relative humidity (60%, 80% and 100%) were adjusted by humidified air and by the presence of saturated salts solutions. At prescribed time intervals, pieces of coated papers were removed for carvacrol extraction by water and n-pentane mixture (50/50 v/v). After internal standard addition (2-nonanol), the solution was shaken for 16 hours under magnetic agitation (300 min⁻¹). The removed organic phase was analysed by gas chromatography. Quantification of carvacrol was performed using the internal standard method and after extraction yield estimation (87% ± 5%). For each condition, time, temperature and RH, the extraction was done in triplicate from two different coated papers.

Results

Carvacrol retention by SPI matrix after process. Retention of the antimicrobial compound by matrix is one of the most important features of the coating process. It depends on the total compound amount retained in the coating matrices after drying (at ambient temperature) compared to the initial compound quantity introduced in the coating solutions. Carvacrol retention was determined for different ratio of aroma compound/protein (10, 30, 60 % w/w) and losses ranged between 12 and 30% depending on the carvacrol load (Figure 1).

![Figure 1. Effect of Carvacrol/SPI ratio on carvacrol retention by SPI matrix.](image-url)
Weak losses were obtained for the highest carvacrol concentration showing the high ability of SPI matrix to retain carvacrol. The unexpected highest losses observed for the lowest 10% initial carvacrol concentration could be explained by the development of an important air/solution interface on the surface of the thin coating layer due to the foaming properties of soy protein, favouring the migration toward the surface of carvacrol. This amount, independently to the initial load, might easily be eliminated during the drying step. The hydrophobic nature of carvacrol with a \( \log P \) of 3.52 suggested that hydrophobic interactions were preferentially involved. Soy proteins and particularly 11S globulin exhibit high capacity of retention for lipophilic molecules due to its specific quaternary structure characterised by hydrophobic cavity (5).

Carvacrol release from SPI matrix in controlled conditions. Carvacrol release from coated papers as a function of time was determined at 60, 80 and 100% of relative humidity (RH) and for three temperatures 5°C, 20°C and 30°C. Results are reported in Figure 2 (A, B, C).

**Figure 2.** Kinetic of carvacrol release at different relative humidity: (\( \nu \)) 60%RH, (\( \nu \)) 80% RH, (\( \sigma \)) 100 % RH and at A) 5°C, B) 20°C and C) 30°C.
When the SPI coated papers were stored at 5°C, 20°C or 30°C, carvacrol release clearly increased with the RH.

While after 50 days of storage at 5°C and 60% RH, the residual carvacrol in the matrix was about 75% of the quantity present at the beginning of the storage (Figure 2A), at 30°C and 100% RH carvacrol release was total within 1 day (Figure 2C). For a given relative humidity, the increase of temperature acted by fasting the carvacrol release from the coated paper.

Modelling of release kinetic using the Fick’s second law can be realized to characterize the carvacrol transfer by diffusion coefficients. At 5°C and 60% RH, the value of diffusivity was found equal to $0.11 \times 10^{-16}$ m$^2$ s$^{-1}$ while at 30°C and 100% RH, diffusivity was 100 time higher with a value of $138 \times 10^{-16}$ m$^2$ s$^{-1}$. From the results, it can be concluded that the storage of coated papers at 5°C and 60% could be suitable to avoid losses of carvacrol. In contrast, a high release of carvacrol from SPI matrix occurs at the current micro-organism’s growth conditions, i.e. at high temperature and relative humidity.

SPI matrix is promising as antimicrobial delivered support due to its high carvacrol retention ability and to its temperature and relative humidity reactivity permitting the controlled release of carvacrol, an efficient antimicrobial agent.

References