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The use of antimicrobial sachets in the packaging of organic wild rocket: Impact on microorganisms and sensory quality

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ABSTRACT

Natural plant extracts from herbs and spices are regarded as safe products for the control of microorganisms. The aim of this work was to study the effects of natural antimicrobial compounds on the quality of packaged fresh organic wild rocket (*Diplotaxis tenuifolia* L.). The effects of eugenol, carvacrol, *trans*-anethole, *trans*-cinnamaldehyde, and α-pinene were tested *in vitro* against rot and human pathogens. All of the compounds exhibited antimicrobial activity, except for α-pinene. The efficacy of the antimicrobial compounds to control microorganisms, leaf color, spoilage volatiles, such as dimethyl disulfide, and sensory quality was tested in two *in vivo* experiments with sachets: a lab-scale jar experiment and a commercial-scale packaging experiment. In the jar experiment, eugenol showed higher antimicrobial activity than carvacrol against aerobic bacteria. All of the jars contained dimethyl disulfide in the headspace, but the sulfurous rotten odor was only detected in the control and eugenol samples, demonstrating that carvacrol, *trans*-anethole, and *trans*-cinnamaldehyde could mask the unpleasant odor of rotten wild rocket. In the packaging experiment, there was no clear effect of eugenol, carvacrol or *trans*-anethole on the microbial load, but eugenol and *trans*-anethole masked the off-odor ratings of wild rocket. Evaluation of antimicrobial compounds for packaged fresh produce must include *in vitro* and *in vivo* testing because the benefits in real food systems may differ from those obtained in the laboratory.
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1. Introduction

Organic fresh produce may be considered more perishable and more likely to pose a health risk due to higher microbial loads from the use of organic fertilizers and the limited use of agrochemicals in their production (McMahon and Wilson, 2001; Oliveira et al., 2010). These concerns call for the development of new postharvest techniques that are compatible with the rules and regulations of organic production (EC, 2007). Active packaging using plant extracts from herbs and spices has been suggested as a potential technique (Han, 2000) because it is considered 'natural' and agrees with the specific regulations for organic production (EC, 2007).

One of the most important steps in developing active packing solutions for organic fresh produce is the selection of antimicrobial compounds. Several studies have demonstrated a high efficacy of essential oils (EO) and their components as potential antimicrobial and antifungal agents against spoilage pathogens (Çetin et al., 2010; Lopez et al., 2007). EO from the leaves of oregano (*Origanum vulgare* L.) and the buds of clove (*Eugenia caryophyllata* Thumb.) have high antibacterial activity due to their high contents of carvacrol and eugenol (Burt, 2004; Davidson et al., 2013). Lopez et al. (2007) noted a potential antifungal effect of EO from cinnamon (*Cinnamomum zeylanicum* Nees) and its main compound cinnamaldehyde. Anethole from fennel (*Foeniculum vulgare* Mill.) also possess antimicrobial activity and is highly efficient in food preservation (Çetin et al., 2010) and against gram-positive bacteria (Senatore et al., 2013). Gutiérrez et al. (2010) found that film with EO of cinnamon and oregano could inhibit the growth of *Listeria monocytogenes* and *Salmonella choleraesuis in vitro.* However, very few studies have reported the antimicrobial effects of EO and their components in real food systems (*in vivo*) and most studies have been performed with fruits (Montero-Prado et al., 2011; Valero et al., 2006).

EO and their components can be strong odorants and thus impair the organoleptic quality of the packaged produce. Therefore, the use of antimicrobial compounds is a balance

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between sensory acceptability and antimicrobial efficacy (Gutierrez et al., 2009).

Antimicrobial compounds can be applied during the manufacturing of packaging polymers in the melting process, as coatings on material surfaces or to pellets that are inserted into sachets and then used during packaging (Han, 2000; Silva-Weiss et al., 2013). However, the commercial application of antimicrobial compounds in the handling of fresh produce is limited (Kuorwel et al., 2011; Siroli et al., 2015).

Fresh fruit and vegetables vary in their inherited shelf life from a few days to many months, depending on their morphological structure, composition, and general physiology (Kader, 2002). Leafy green vegetables, and baby leaves in particular, are highly perishable products with a short shelf life due to their immature leaves and high respiration rates (Cantwell and Kasmire, 2002). One of the main challenges in the postharvest handling of baby-leaf salads is the rapid oxidation of the green chlorophyll pigments unmasking the underlying yellow carotenoids (Toivonen and Brummell, 2008) and the rapid formation of off-odors when inappropriately handled and stored after harvest (Løkke et al., 2012; Tudela et al., 2013a,b). If conventional and organic baby-leaf salad is sold unwashed or if no sanitizers are applied during the washing process, leaves may be contaminated with rot bacteria (Nielsen et al., 2008) and/or human pathogens (*Escherichia coli*, *Salmonella sp., Yersinia enterocolitica,* and *L. monocytogenes*), which may grow and impair quality during storage (Nielsen et al., 2008).

The aim of the present study was to evaluate eugenol, carvacrol, *trans*-anethole, *trans*-cinnamaldehyde, and α-pinene for their antimicrobial activity *in vitro* and their impact on the sensory quality of organic wild rocket (*Diplotaxis tenuifolia* L.) *in vivo*. The first step was to test the compounds against storage rot (*Pectobacterium carotovorum* and *Pseudomonas fluorescens*) and human (*E. coli* and *Salmonella enteritidis*) pathogens *in vitro* to elucidate their antimicrobial effects. Based on these results, two *in vivo* tests were carried out: a lab-scale experiment and a commercial-scale packaging experiment. In both experiments, biodegradable sachets containing pellets with natural antimicrobial compounds were placed inside jars or packages filled with organic wild rocket, and their effects were evaluated with regards to control of microorganisms, leaf color, spoilage volatile such as dimethyl disulfide, and sensory quality.

2. Materials and methods

2.1. In vitro *testing of antimicrobial compounds*

Active compounds of common plant spices and herbs with high efficacy in food preservation (Burt, 2004; Davidson et al., 2013) were selected for the *in vitro* tests. The compounds were eugenol (99%), carvacrol (99%), *trans*-anethole (99%), *trans*-cinnamaldehyde (≥99%), and α-pinene (98%) (all purchased from Sigma-Aldrich, St. Louis, MO, USA). The antimicrobial activity was tested against bacteria causing storage rot, *P. carotovorum* and *P. fluorescens*, and clinical strains of human pathogens, *E. coli* and *S. enteritidis*, using the disc diffusion method (EUCAST, 2014). Plates containing non-selective Muller-Hinton agar were inoculated with bacteria, and a 6 mm sterile disk (Liofilchem, Roseto degli Abruzzi, TE, Italy) was placed on the plate with sterile tweezers. Dilutions of eugenol, carvacrol, *trans*-anethole, *trans*-cinnamaldehyde, and α-pinene were mixed with dimethyl sulfoxide (Carlo Erba Reagenti SpA, Rodano, Italy) to obtain a concentration of 500 mg L^{-1}. From these solutions, a 10 μL sample was taken and placed on a disk. The plates were sealed with tape to prevent losses of active compounds to the outside atmosphere and incubated at 37 °C for 24 h. The efficacy to inhibit microbial growth was determined using a ruler as the diameter of the inhibition zone. Compounds with an inhibition zone of 0 mm had no antimicrobial activity.

2.2. Development of antimicrobial sachets

Eugenol, carvacrol, *trans*-anethole, and *trans*-cinnamaldehyde were selected for the *in vivo* studies based on the results of the *in vitro* study. The active compounds were applied to pellets for a gradual release of volatiles during storage. The compounds were diluted with sweet almond oil (1:5 w/w; Sigma-Aldrich, St. Louis, MO, USA) to improve their dispersion. This odorless oil was used to ensure that the odor of the oil did not interfere with the odor of the antimicrobial compounds. The pellets were activated by loading 60 mL of oil solution on to 40 g Polylactic Acid (PLA) Accurel XP951B pellets (Membrana GmbH, Obernburg, Germany) and mixing the pellets at 20 rpm (VWR ADV 3500 Shaker, Radnor, PA, USA) for 5 d at room temperature. Sachets (2 × 2 cm) were made of non-woven PLA material (Ahlstrom Chirnside Limited, Manchester, United Kingdom) that was filled with 1 g of pellets containing 10% of the active compound (w/w). Both the pellets and the sachet material were biodegradable according to EN 13432 (2000).

2.3. In vivo *testing of antimicrobial compounds*

Two experiments were carried out: a lab-scale jar experiment and a commercial-scale packaging experiment. In both experiments, biodegradable sachets containing antimicrobial compounds were incubated with fresh produce. Different batches of certified, organic wild rocket (*Diplotaxis tenuifolia* L.) were used as the experiments were carried out during a two-month period. The wild rocket was provided by a commercial company and selected based on the color, brittleness, and odor of the leaves. Only first-class fresh produce was taken. Samples of 100 g were weighed into 1.8 L polyethylene terephthalate trays (185 × 145 × 70 mm), wrapped with micro-perforated polypropylene film, shipped to Aarhus University (Årslev, Denmark) at 2 °C, and stored at 1 °C in a walk-in cold room at the University until beginning of the experiments, 2–4 d later.

Samples of 12.5 g of leaves were carefully selected for the lab-scale jar experiment. This quantity of leaves could fit into the jars without wounding the leaves and allowed for enough O_2 for aerobic respiration during storage (Luca et al., 2016). The leaves were gently placed in 1 L sterile jars with one antimicrobial sachet per jar. The jars were closed with airtight lids, mounted with a septum for gas sampling, and placed in a climate chamber (400 L, −9–99 °C Binder KB400, Binder, Tuttlingen, Germany) at 10 °C for 7 d. In the jar experiment, there were no losses of volatiles during storage, as the jars were airtight (Luca et al., 2016). The gas composition of the jars was measured daily with a gas analyzer (CheckMate 9900 instrument, Dansensor, Ringsted, DK) as described by Seefeldt et al. (2012). Three jars were analyzed per treatment.

For the industrial-scale packaging experiment, organic wild rocket was packaged as described above except that one antimicrobial sachet was inserted into the tray before the leaves were placed. In this experiment, some volatiles were lost to the outside, as perforated packaging material was used to ensure enough O_2 for aerobic respiration during storage (Løkke et al., 2012). The trays were stored at 5 °C for 6 d. The gas composition of the packages at the end of storage was measured as described by Løkke et al. (2012). Only packages with a similar gas composition were selected for quality analyses. Four packages per treatment were used for all of the analyses, except for the sensory analysis. For this analysis, 14 packages in four replicates were prepared per treatment, but only 9 in four replicates were used, corresponding to the number of assessors in the panel.

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