Food Chemistry 259 (2018) 251–260

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Antimicrobial, antioxidant and sensory features of eugenol, carvacrol and *trans*-anethole in active packaging for organic ready-to-eat iceberg lettuce

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ARTICLE INFO

Chemical compounds studied in this article:
Carvacrol (PubChem CID: 10364)
trans-Anethole (PubChem CID: 637563)
Eugenol (PubChem CID: 3314)

Keywords:
Natural compounds
Emitting sachets
Biodegradable packaging
Organic vegetables

ABSTRACT

In this study, bio-based emitting sachets containing eugenol (EUG), carvacrol (CAR) and *trans*-anethole (ANT) were inserted into cellulose (CE) and polypropylene (PP) pillow packages of organic ready-to-eat (RTE) iceberg lettuce to investigate their functional features. EUG, CAR and ANT sachets in CE; and CAR in PP packages showed antimicrobial activities against coliforms (Δlog CFU g^{-1} of −1.38, −0.91, −0.93 and −0.93, respectively). EUG and ANT sachets in both packages reduced discoloration (ΔE of 9.5, 1.8, 9.4 and 5.6, respectively). ANT in both, and EUG only in PP packages induced biosynthesis of caffeoyl derivatives (C_aT_A, $D_iC_aT_A$, $D_iC_aQ_A$), total phenolics and antioxidant activity (FRAP). Also, ANT and EUG in both packages improved overall freshness and odor. Principal component analysis separated ANT and EUG from CAR in both packages. The Pearson correlation confirmed that overall quality improvements were more pronounced by ANT inside the packages in comparison to EUG and CAR.

1. Introduction

Organic vegetable market shares in Europe are increasing in the last years (Willer & Lernoud, 2016), this trend is partially driven by consumer perceptions that organic vegetables are more nutritious and healthier than non-organic one. Organic vegetables are sold in marketplace raw or minimally processed and should be packed in a way to guarantee consumers true origin and produce vital integrity. In the current EU regulation for organic production and processing – EC Reg. No. 834/2007 (EC, 2007), the packaging is not strictly regulated, although some private standards (e.g. IFOAM, Soil Association, Naturland, Bioland, Bio Suisse, Demeter) include packaging. Packaging system for organic produce should satisfy economic, social and environmental requirements related to the production, distribution and consumption. Thus, development of sustainable packaging that is bio-based and biodegradable is needed (Lindh, Olsson, & Williams, 2016). Moreover, the potential adoption of an eco-label on packaging beside organic logo might be an added value, especially if we take in consideration that attitude of organic consumer towards minimally processed vegetables is separated into “health” and “green” perceptions (Sillani & Nassuvera, 2015). The recent trend towards green consumerism is the use of bio-based materials for packaging in combination with essential oils (EOs) (Siracusa, 2016). EOs and their constituents are allowed to be used in organic food processing as additives and flavors because they are generally recognized as safe, but technological solutions for their application in organic food packaging are still lacking.

Up to date, there are different technological solutions for packaging system of conventional produce that are comprised of bio-based polymers and EOs and their constituents. They are divided based on the application as: i) incorporation and ii) addition. Incorporation into bio-based polymers (edible or not) is based on their inclusion either during the melt processing or by coating of the film’s surfaces (García Ibarra, Sendón & Rodríguez-Bernaldo de Quirós, 2016). Both processes can adversely affect the microstructural, physical (tensile, barrier, optical) and bioactive properties of developed films (Silva-Weiss, Ihl, Sobral, Gómez-Guillén, & Bifani, 2013). One of the successful solution, currently in use, is based on addition of EOs or their main constituents into separate carrier, as emitting sachets inside packaging. However, integrated use of bio-based and/or biodegradable materials for carrier, sachets and external packaging materials is still lacking (Otoni, Espitia, Avena-Bustillos, & McHugh, 2016). Hence, there is recent attention on exploring a new generation of bio-based packaging materials with promising applications in organic food packaging, with multifunctional properties and able to improve the intrinsic value of organic food, with minimal adverse impacts on the packaged food and the environment.

In this study, bio-based emitting sachets as carriers of eugenol (EUG), carvacrol (CAR) and *trans*-anethole (ANT) were developed and

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https://doi.org/10.1016/j.foodchem.2018.03.137
Received 3 December 2017; Received in revised form 26 March 2018; Accepted 29 March 2018
Available online 30 March 2018

introduced into natural (cellulose, CE) and synthetic (polypropylene, PP) pillow packaging of organic ready-to-eat (RTE) iceberg lettuce. We hypothesized that EUG, CAR and ANT would have antimicrobial, antioxidant and sensory features on packaged organic RTE vegetables. Therefore, the objective of this study was to examine the potential application of EOs as natural agents for innovative bio-based active packaging. Selection of these compounds was based on previous findings which demonstrated their antimicrobial and antioxidant activities in fresh produce. Among RTE vegetables, lettuce is very popular, but it is very perishable and prone to enzymatic browning and spoiling, especially after it has been fresh-cut (Zhan, Li, Hu, Pang, & Fan, 2012). Thus, organic RTE lettuce was chosen as a model. In particular, the effects were evaluated through microbial, physical (color), biochemical (individual polyphenols, total phenols, vitamin C and antioxidant activities) and sensorial (appearance, odor and texture) properties of RTE lettuce.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals and materials

EUG (99%), CAR (99%), ANT (99%) and sweet almond oil were purchased from Sigma-Aldrich (St. Louis, USA). The nutrient agar, potato dextrose agar and violet red bile agar for microbiology; the reagents Folin-Ciocalteu, DPPH, ABTS, potassium persulphate, sodium acetate, glacial acetic acid, TPTZ, metaphosphoric acid, L-cysteine, formic acid; solvents methanol and acetinitrile; and standards of gallic acid, trolox, C_aT_A, C_aQ_A, $D_iC_aT_A$, $D_iC_aQ_A$ and L (+) ascorbic acid were purchased from Sigma-Aldrich (Steinheim, Germany). All chemicals used were of analytical grade, whereas methanol and acetonitrile was HPLC and LC-MS grade and formic acid was LC-MS grade. Water was purified through a Millipore Q-plus purification system (Millipore Corp., Bedford, MA, USA).

Polylactic acid (PLA) Accurel XP951B pellets (Membrana GmbH, Obernburg, Germany) with loading capacity up to 60%, and non-woven PLA material were purchased from Ahlstrom Chirnside Limited (Duns, United Kingdom). Two packaging films were used: i) micro-perforated polypropylene (PP) film (high OTR, 17.4 pmol s^{-1} kPa^{-1}) from Dansk Klæberulleindustri (Højbjerg, Denmark) and ii) natural cellulose (CE) film (high OTR, 18.1 pmol s^{-1} kPa^{-1}) provided by the NNZ Scandinavia ApS (Odense, Denmark). PLA pellets and non-woven material, and CE are certified to both EN 13432 and ASTM D6400 norms as biodegradable and compostable.

2.1.2. Plant material and growing conditions

Lettuce (*Lactuca sativa* L.) type iceberg, cultivar "Classic" was produced under organic management at the experimental fields of the Mediterranean Agronomic Institute of Bari (CIHEAM Bari) located in Puglia region, Italy (41°.0536 N; 16°.8766 E, and altitude 72 m a.s.L.). Plant nutritional requirements were satisfied by applying compost (N2 plus, Biovegal, Trani, Italy) in pre-cropping phase and organic (plumage, milled meat and manure; Fertil and milled bones and manure; Phoenix, Italpollina, Rivoli Veronese, Italy) and mineral (a crude potassium sulphate salt; Kalisop KALI, Kassel, Germany) fertilizers before transplanting, both approved for the use in organic farming according to regulation (EC889/2008). Four weeks old seedlings at the stage of 3–4 true leaves were transplanted on June 1st 2015 at planting density of 12 plants/m^2 (0.25 m within the row and 0.5 m between the rows). During the growing season plant protection products were not used and weeding was done manually. Lettuce heads were harvested at commercial maturity on July 22nd 2015, and 100 lettuce heads were manually harvested. Immediately after harvest, at early morning hours, heads were trimmed to eliminate outer injured leaves and soil, then transported from the field to the laboratory, where heads were further cleaned to remove external green leaves. In order to keep homogeneity, only heads that weighted 500 ± 50 g were selected for the processing (10 heads for each treatment).

2.2. Methods

2.2.1. Carrier loading and sachets development

Before loading, EUG, CAR and ANT were mixed with sweet almond oil (1:5, w/w) to improve dispersion and loading into PLA pellets. Sachets preparation comprises three steps: loading into the carriers (pellets), carrier addition inside the sachet, and sachet sealing. Loading was done by mixing 40 g of PLA pellets with 60 g of mixtures, or only sweet almond oil in the case of control, at low speed 20 rpm (Reax 2, Heidolph, Torre Boldone, BG, Italy) for 5 days at room temperature in hermetically closed containers. As a result, one gram of activated pellets contained 10% (w:w) of natural compounds – final concentration was chosen based on preliminary *in vitro* study (data not shown). An amount of 1.00*g* of loaded/activated pellets, were added into a 2 × 2 cm^2 sachet made of non-woven PLA materials and then sachet were heat sealed.

2.2.2. Processing, packaging, storage and experimental set-up

Heads were cut horizontally with stainless steel, sharp, sterile knife into 30–40 mm pieces of leaves and midribs and mixed to obtain a homogeneous sample. Before packaging, fresh-cut lettuce pieces were dipped in the cold sterilized deionized water (4 °C) for 10 min and excess of water was removed in salad spinner dryer. Packages were prepared by addition of 200 g of lettuce into 1.2 L pillow packages. Afterward, sachets were attached to the upper layer of packages to avoid direct contact with leaves. Sachets containing eugenol (EUG), carvacrol (CAR) and *trans*-anethole (ANT), and almond oil as control (CON) were inserted into two type of packages, with polypropylene (PP) and cellulose (CE) films. In total, eight systems (CON_CE, EUG_CE, CAR_CE, ANT_CE, CON_PP, EUG_PP, CAR_PP and ANT_PP) were set up, each in 15 replicates. The packages were randomly separated into bunches and were kept in darkness for five days in the storage chamber (Frigoclima, FDM, Gioia del Colle, BA, Italy) at 4 °C and 95% relative air humidity (Fig. S1).

2.2.3. Microbiological analysis

Ten grams of the sample from each package was placed in a sterile 250 mL glass jar with physiological solution (w:v; 1:10) and subjected to shaking for 30 min at 25 °C (HS 260 basic, IKA®-Werke GmbH & Co. KG, Staufen, Germany). From each suspension, appropriate dilutions were made and aliquots of 100 μL were used to inoculate the culture media. After incubation total aerobic bacterial count (AB), yeast and mold (YM) and coliforms (C) were determined using nutrient agar (NA), potato dextrose agar (PDA) and violet red bile agar (VRBA), respectively. In case of NA and PDA by spreading, and by inclusion in case of VRBA. AB, E and YM plates were incubated at 30 °C for 48 h, at 30 °C for 24 h, and at 25 °C for 48 h, respectively.

2.2.4. Color

Measurements were taken on the leaves surface and cut-edges of midribs, initially after cutting and after storage. In each treatment, the reflectance measurement was obtained from the average of ten readings. The color was measured with a spectrophotometer with 3 mm aperture (Minolta CM-700d, Osaka, Japan) and adjusted to the D65 illuminant. The instrument was standardized each time with a black and a white ($L = 91.10$, $a = -1.12$, $b = 1.26$) tile. The color was expressed as L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) values of the color space. Total color differences were calculated as $\Delta E^*[(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}]$, where ΔL^*, Δa^* and Δb^* are the coordinate differences between initial and after storage values.

2.2.5. Chemical analysis

2.2.5.1. Sample extraction for total phenolic contents, antioxidant activities and individual phenolic acids determination. Solvent extraction was