Crop Protection 102 (2017) 94–103

Contents lists available at ScienceDirect

Crop Protection

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

Potential application of spearmint and lavender essential oils for assuring endive quality and safety

Panayiota Xylia, Antonios Chrysargyris, George Botsaris, Nikos Tzortzakis*

Cyprus University of Technology, Department of Agricultural Sciences, Biotechnology & Food Science, 3036, Limassol, Cyprus

ARTICLE INFO

Article history:
Received 23 October 2016
Received in revised form
16 August 2017
Accepted 17 August 2017

Keywords:
Antioxidants
Essential oil
Foodborne pathogens
Leafy vegetables
Quality

ABSTRACT

Many studies have reported the use of essential oils (EOs) as potential sanitizer agents, but there is an increasing need to understand the concentrations not affecting the quality prior investigating the antimicrobial potential. Lavender and spearmint EO sand their mixture were used to retain consumer's acceptability for endive, while the antimicrobial effect for the chosen concentrations were investigated against major foodborne pathogens (*S. aureus*, *L. monocytogenes*, *S.* Enteritidis and *E. coli*) on endive. The results revealed that high concentrations of EOs is not applicable as out of the examined concentrations of EOs, only the 0.001%, 0.01% and 0.1% did not adversely affect the organoleptic characteristics (aroma and color) of endive. Following treatment at these concentrations of spiked endive, antimicrobial activity was observed against all four tested pathogens. Noticeably, lavender EO and its mixture with spearmint EO (50:50% v/v) were more effective against *S. aureus* and *L. monocytogenes*. These findings suggest that EOs can be active at low concentrations not adversely affecting quality, whilst increasing the antioxidants levels of endive and their potential use as chlorine alternatives should not be discarded. Further research is needed to quantify the effect and reveal the mechanisms of their antimicrobial action at low concentrations.

1. Introduction

Endive and other leafy green vegetables, are perishable products, and should be handled with care, to avoid contaminations and quality defects. They provide ideal conditions for microbial growth (low acidity, increased water activity and surface) and any damage may increase the risk of microbial contamination adversely affecting the quality of these products (FAO/WHO, 2008). Factors leading to the contamination of these products include water, soil amendments, animals, birds, the agricultural equipment and workers (FAO/WHO, 2008). More specific, during processing, vegetables come in contact with potential sources of contamination including people, surfaces, water, soil and dust (EFSA, 2011).

Many pathogens have been associated with leafy vegetables, such as enterohaemorrhagic *Escherichia coli*, *Salmonella enterica*, *Campylobacter* spp. *Shigella* spp. *Yersinia pseudotuberculosis*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Vibrio parahaemolyticus* (FAO/WHO, 2008; EFSA, 2013). Viruses and protozoa that have also been linked with leafy vegetables include Hepatitis A virus, Norovirus, *Cyclospora cayetanensis*, *Cryptosporidium parvum* and *Giardia lamblia* (EFSA, 2013).

The main decontamination process applied to minimally processed vegetables is washing, which generally, takes place by dipping the vegetables into a tank, containing water and a sanitizing agent. Sanitizing methods can be divided into three main categories: (i) Application of chemical sanitizers (chlorine, chlorine dioxide, hydrogen peroxide, and ozone), (ii) Physical decontamination methods (irradiation, ultraviolet treatment and electrolyzed water) and (iii) Application of natural antimicrobials (organic acids, protective cultures and plant essential oils-EOs) (Singh et al., 2002; Tzortzakis et al., 2007, 2016; Sánchez-Rubio et al., 2016).

Several fungicides that are active against postharvest pathogens are commercial available and are classified into categories according to the biochemical modes of actions. Therefore, fungicides affect fungal respiration, osmoregulation, microtubule function, biosynthesis of methionine or biosynthesis of sterol (Feliziani and Romanazzi, 2013). The main advantages of using fungicide are the high generally efficacy with a specific spectrum of activity. However, fungicides are suitable for a limited number of crops and possible residues and safety concerns are considered of

* Corresponding author.
E-mail address: nikolaos.tzortzakis@cut.ac.cy (N. Tzortzakis).

http://dx.doi.org/10.1016/j.cropro.2017.08.015
0261-2194/

disadvantage.

The most commonly applied disinfectant agent in the food industry is chlorine -sodium hypochlorite (NaOCl)-with the commercial amount applied usually ranging from 50 to 200 mg/l (Francis et al., 1999). The amount applied depends on the product being decontaminated. Contact time varies from 1 to 10 min (or even longer) at chilling temperatures (Francis et al., 1999). However, it is well established that chlorine can incompletely oxidize organic materials and lead to the production of undesirable byproducts such as chlorophorm, trichalomethanes, haloketones and haloacetic acids and there is accumulating data suggesting adverse effects in the environment and human health (Parish et al., 2003).

Washing in combination with the use of disinfectants may reduce the microbiological load of vegetables (FAO/WHO, 2008). It is noteworthy that the decontamination agents used, often adversely affect the quality of leafy vegetables (flavor, color), thereby affecting consumer's acceptance and preference. There is an increasing need towards the study of naturally occurring agents exhibiting antimicrobial properties, as consumers seek less processed foods (fewer additives) with natural ingredients. New agents should be able to assure the safety of the product, whilst minimizing the nutritional losses of leafy vegetables during processing. Any proposed processing should be consistent with consumer demands for minimally processed and fresh products.

Lavender (*Lavandula angustifolia*) and spearmint (*Mentha spicata*) are two aromatic plants, whose EOs have been studied extensively and found to have many functional effects including antimicrobial (Smith-Palmer et al., 1998; Arrebola et al., 2010; Ma et al., 2016), insecticidal (Da Camara et al., 2015) and antioxidant activity (Chrysargyris et al., 2016). The application of EOs in the disinfection process of vegetables has been reported previously with promising results (De Corato et al., 2010; Stavropoulou et al., 2014; De Medeiros Barbosa et al., 2016). For instance, Singh et al. (2002) reported that the addition of thyme oil in the washing water reduced the population of *E. coli* in lettuce and carrots. Several other studies have shown similar encouraging results, thus the use of EOs of lavender and spearmint, as sanitizing agents of leafy green vegetables and as an alternative way of disinfection must be assessed further.

Endive (*Cichorium endivia* L.) belongs to the genus *Cichorium* of the Asteraceae (Compositae) family. It is an annual plant and has been cultivated for many years in the Mediterranean region. Endive along with lettuce are the most popular leafy vegetables used in salads. The health benefits of the consumption of leafy greens seem to be attributed to the antioxidant compounds they contain as well as their fiber content (Serafini et al., 2002).

The aim of this study was to determine (i) the appropriate treatment concentration of lavender and spearmint essential oils and their combination, which sustains the acceptability and quality of endive and (ii) examine the antimicrobial efficacy of the above treatments against some selected foodborne pathogens.

2. Materials and methods

2.1. Plant materials

Fresh endive (*C. endivia*) was obtained directly from a local retain market in Cyprus and selected for uniformity in appearance and the absence of physical defects or injury and then used for experimental needs. Endive plants were grown for seven weeks in a clay loam soil, frequently irrigated and fertigated according to crop needs, without any pesticide application. Endives were harvested at commercial stage of 320 ± 25 g fresh weight. Additionally, lavender (*L. angustifolia*) and spearmint (*M. spicata*) plants, before flowering stage, were obtained from the experimental farm/greenhouse of Cyprus University of Technology, where they were cultivated in soil for three months and in a hydroponic system (deep flow techniques-DFT) for six weeks, respectively. All chemical reagents used in this study were purchased from Sigma Aldrich (Germany) except if mentioned differently.

2.2. Bacterial strains and inocula preparation

Bacterial strains were obtained from the Agricultural Sciences, Biotechnology and Food Science Department (Lab of Food Microbiology), Cyprus University of Technology. These cultures were stored in −80 °C in 20% glycerol. Fresh cultures were prepared with the addition of 100 μl of pure culture of the bacterium tested (*Escherichia coli* ATCC 11775, *Listeria monocytogenes* NCTC 7973, *Salmonella enterica* subsp. *enterica* serovar Enteritidis NCTC 5188 and *Staphylococcus aureus* ATCC 6538, in 10 ml of Brain Heart Infusion broth (BHI, Biolab, Hungary) and then incubated overnight at 37 °C.

2.3. Essential oil extraction and gas chromatography/mass spectrometry analysis

Lavender and spearmint plant tissue harvested and three biological samples (pooled of three individual plants/sample) for each treatment were air-dried (in oven at 42 °C), chopped and were hydrodistilled for 3 h, using Clevenger apparatus for EO extraction. The EOs were analyzed by Gas chromatography-Mass Spectrometry [Shimadzu GC2010 gas chromatograph interfaced Shimadzu GC/MS QP2010 plus mass spectrometer using an HT280T auto sampler (HTA, Italy), fitted with a ZB-5 column (Zebron, Phenomenex, USA)] and their constituents were determined as described previously (Chrysargyris et al., 2016).

2.4. Phenolic content and antioxidant activity of essential oils

2.4.1. Total phenolic content

Total phenolic content in the EOs was determined using the Folin-Ciocalteu method, as described by Kavoosi and Rowshan (2013). Polyphenols were extracted from three EO samples (each sample extracted by three individual plants) for each treatment. Briefly, 0.1 ml of each EO (100 μg/ml, diluted in methanol) or standard dilutions of gallic acid (0–1 mM) were added to 0.1 ml of Folin-Ciocalteu reagent (Merck, Germany) and 0.9 ml of water. The mixture was incubated at room temperature for 5 min and then 0.3 ml of 10% Na_2CO_3 was added. The reaction was left in the dark, shaking for 1 h and absorbance was read at 755 nm (TECAN, infinite M200PRO, Austria). Results were expressed as milligrams of gallic acid equivalents per gram of EO.

2.4.2. DPPH radical scavenging activity

The bleaching of the purple-colored solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to determine radical scavenging activity according to Oke et al. (2009). Three EO samples (each sample extracted by three individual plants) for each treatment were used. Volume of 0.4 ml of serial EO dilutions (0–100 μg/ml) or of control sample (methanol) was mixed with 0.1 ml of 0.2 mM DPPH solution in methanol. The mixture was shaken and then left in the dark for 30 min. Absorbance was measured at 517 nm. Butylatedhydroxytoluene (BHT) was used as a positive reference. DPPH radical scavenging activity was expressed as the inhibition percentage and was calculated using the equation: $I = 100^*(Ab_{control}\text{-}Ab_{sample})/Ab_{control}$. $Ab_{control}$ is the absorbance of the control and Ab_{sample} is the absorbance of the sample at 30 min. Antiradical activity was expressed as IC_{50} that is the concentration