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Natural antimicrobials to prolong the shelf-life of minimally processed lamb's lettuce

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ABSTRACT

Plant essential oils (EOs) and their components, generally recognized as safe and endowed with a wide antimicrobial activity, have been investigated in this paper as natural sanitizer alternatives to chlorine to control spoilage bacteria and naturally occurring pathogens associated with minimally processed vegetables. From this perspective, the efficacy of oregano and thyme EOs and carvacrol was evaluated in comparison with chlorine for lamb's lettuce decontamination. Their effects were evaluated on mesophilic aerobic bacteria, yeasts, LAB, color parameters and volatile molecule profiles demonstrating the same efficacy of chlorine. A further optimization of the process highlighted that thyme and oregano EOs controlled minimally processed lamb's lettuce spoilage microflora without negatively affecting the quality and sensory properties of the products. These results demonstrate the potential of washing treatments based on natural antimicrobials, as alternatives to chlorine for the sanitization of minimally processed vegetables.

1. Introduction

Fruits and vegetables are well recognized by the consumers for their health benefits and their consumption is strongly promoted for the high levels of nutrients, vitamins, minerals and fibers (Goodburn and Wallace, 2013). Food industry and retail markets offer a broad range of packaged fresh-cut vegetables that merge "health" and "convenience" features as an excellent alternative to raw materials (Allende et al., 2006). However, microbial contamination, spoilage, and in same cases pathogenic species can arise during the different steps from farm-to-consumer (production, harvest, processing, wholesale storage, transportation or retailing and handling in the home) and this contamination can derive from environmental, animal or human sources (WHO/FAO, 2008). In addition, tissue damage and release of nutrients due to cutting, slicing or peeling as well as the packaging of the final product may enhance the microbial growth (Siroli et al., 2014a,b,b). Currently, the shelf-life and safety of minimally processed vegetables are based on tools such as the application of good agricultural practices (GAP) and good manufacturing practices (GMP) and the maintaining of the cold chain (Alegre et al., 2010). However, for minimally processed products, the washing step, performed with sanitizing solution, is the only phase able to reduce the number of spoilage and pathogenic microorganisms (Sao José and Vanetti, 2012) and nowadays, chlorine is the most common decontaminant used at the industrial level (Rico et al., 2007), although its use is prohibited in some European countries such as the Netherlands, Sweden, Germany and Belgium (Gil et al., 2009). However, at the concentration normally employed (50–200 mg L^{-1}) it does not achieve more than a 1–2 log reduction in bacterial populations and it is ineffective in reducing pathogens on vegetables (Oliveira et al., 2012). Moreover, chlorine-based compounds are corrosive, cause skin and respiratory tract irritation and react with the organic matter present in the water leading to the formation of potentially harmful trihalomethanes (López-Gálvez et al., 2009). In addition, some literature reports show that emerging pathogens are more resistant to chlorinated compounds raising further concerns about the effectiveness and the use of chlorine in the minimally processed food industry (Allende et al., 2008).

In recent years the number of foodborne illness outbreaks linked to raw and minimally processed vegetables has increased dramatically (Olaimat and Holley, 2012). Literature shows that *Aeromonas hydrophila*, *Bacillus cereus*, *Clostridium* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella*

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spp., *Shigella* spp., *Vibrio cholerae*, *Campylobacter* spp., and *Yersinia enterocolitica* are frequently associated with illness outbreaks related to consumption of fresh produce and minimally processed fruits (Berger et al., 2010; Van Boxstael et al., 2013). These drawbacks have stimulated research towards non-traditional sanitizers (hydrogen peroxide, peroxyacetic acid and ozone) and other alternative technologies such as physical treatments (UV-C light, ultrasound and gamma rays) (Gil et al., 2009). Plant essential oils (EOs) and their components, which are natural antimicrobials and generally recognized as safe, have been studied as alternative natural sanitizers to control foodborne pathogens and spoilage bacteria associated with minimally processed vegetables, (Gutierrez et al., 2008, 2009). The *in vitro* antimicrobial activity of oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*) EOs and their main components carvacrol and thymol against a variety of Gram-positive, Gram-negative bacteria, yeasts and molds is well documented (Viuda-Martos et al., 2007). In addition, their efficacy has already been experienced in several foods including meat (Skandamis and Nychas, 2001), fish products (Sagdic and Ozturk, 2014) and dairy products (Govaris et al., 2011). However, there are very limited studies that investigate the antimicrobial efficacy of these natural antimicrobials, alone or in combination with other hurdles on fresh produce (Gutierrez et al., 2008, 2009; Scollard et al., 2013).

Thus, the main aim of this work was to evaluate the efficacy of oregano and thyme EOs as well as of carvacrol in comparison with chlorine for lamb's lettuce decontamination, addressing the control of spoilage and pathogenic species and improvement of shelf-life of the minimally processed products. To reach this goal, preliminarily, the two EO were characterized by GC/MS-SPME and the MIC values of the antimicrobials considered against several pathogenic species were determined. The effects of the antimicrobials used as alternative to chlorine in the washing solution of lamb's lettuce were then evaluated on spoilage microorganisms, color parameters and volatile molecule profiles. In addition, in a second experimental phase, the study of the effects of thyme and oregano EOs on the shelf-life of minimally processed lamb's lettuce packaged in synthetic ordinary atmosphere, after some modifications of the washing parameters, was performed and the shelf-life of the products was monitored during storage at 6 °C.

2. Material and methods

2.1. Natural antimicrobials

Thyme and oregano EOs were obtained from Flora s.r.l. (Pisa, Italy). Carvacrol was from Sigma–Aldrich (Milano, Italy). The substances used were selected both for their antimicrobial activity and impact on organoleptic properties.

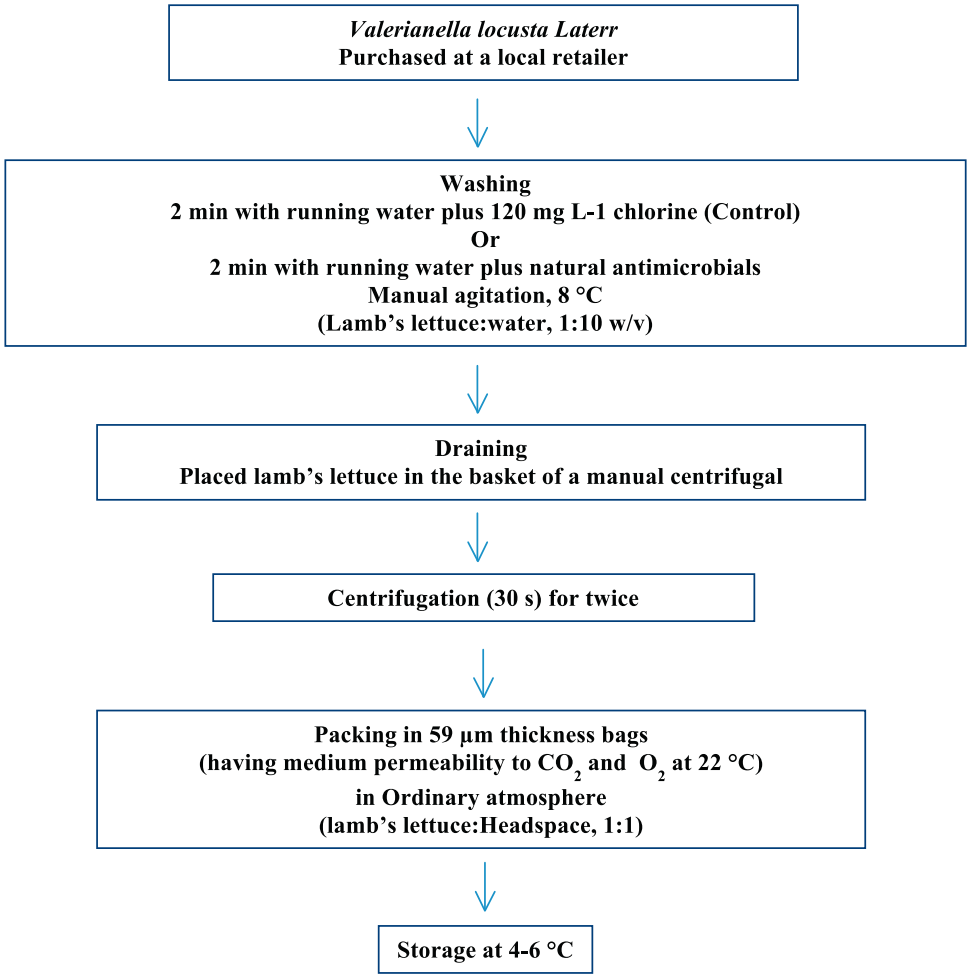

Fig. 1. Working protocol employed to prepare lamb's lettuce; the addition of natural antimicrobials was performed during the washing step, samples washed with chlorine represented the controls.