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Application of carboxymethyl cellulose and chitosan coatings containing *Mentha spicata* essential oil in fresh strawberries

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ABSTRACT

The aim of the present study was to investigate the effects of carboxymethyl cellulose (CMC) and chitosan (CH) coatings containing *Mentha spicata* essential oil (MSO 0.1 and 0.2%) on survival of *Listeria monocytogenes*, and physicochemical (weight loss, titratable acidity and pH), microbial (total viable count, psychrotrophic bacteria as well as yeasts and molds) and sensory (appearance, color, texture and overall acceptability) properties of fresh strawberries during refrigerated storage. The treatments of fruits with CH + MSO 0.2% and CMC + MSO 0.2% resulted in the best microbial, physicochemical and organoleptic properties after 12 days storage. The final population of *L. monocytogenes* in treated samples was decreased by 3.92–3.69 compared to control groups. It can be concluded that CH and CMC coatings enriched with MSO can be used as appropriate active packaging materials to preserve fresh strawberries in the food industry.

1. Introduction

The strawberry fruits are one of the most popular and appreciated summer berries throughout the world, due to their highly desirable flavor, brilliant color and delicious taste [1,2]. They are rich in a variety of bioactive compounds including flavonoids, phenolic acids, tannins, polyphenols, anthocyanins, vitamins and amino acids, the substances related to health benefits [3]. However, fresh strawberries are highly perishable with short postharvest life mainly due to high respiration rate, excessive soft texture, sensitivity to temperature, water loss, microbiological decay, mechanical injury and vibrations, which makes their marketing a challenge [4]. In addition, fresh strawberries and some other berry fruits have been linked to several large food-borne outbreaks caused by contamination with hepatitis A virus, human norovirus, *Cyclospora cayetanensis*, *Salmonella* spp. and *Escherichia coli* [5]. Furthermore, to meet consumer demands for safe and healthy strawberries, some technologies have been developed, including active, modified or controlled atmosphere packaging, addition of chemical preservative substances and irradiation. Most of these treatments have adverse effects on organoleptic properties [6].

At present, food industries use synthetic chemical fungistatics as preservatives to extend the shelf life and subsequently improve the quality of vegetables and fresh fruits. Due to perceived toxic effects, consumers have demanded minimally processed fruits containing no synthetic agents [2,4]. In the last several years, an especial trend to use eco-friendly compounds such as edible films and coatings for food preservation has been occurred [7]. Many studies have shown that edible films and/or coatings derived from protein, polysaccharide and oil-containing materials improve the shelf life and preserve the quality of fish [8], strawberry [9,10], fresh-cut apple [11], cantaloupe [12] and papaya [13]. Among them, sodium carboxymethyl cellulose (CMC) is a promising polysaccharide polymer which consists of β-D-glucose and β-D-glucopyranosyl-2-*O*-(carboxymethyl)-monosodium salt connected via β-(1,4-glycosidic) bonds [14]. It is one of the most suitable polysaccharide polymers that can be used to develop a biodegradable film and/or coating due to its intriguing properties such as excellent film-forming ability, availability, biodegradability and biocompatibility [15]. Previous studies related to CMC showed that it can be potentially used to prolong storage time and control microbial spoilage of many foods such as ground chicken meat [16], soft white cheese [17], fresh-cut apple [11] and citrus fruit [15]. Moreover, chitosan (CH) is a polymer of units of 2-acetamido-2-deoxy-β-D-glucan and 2-amino-2-deoxy-β-D-glucan linked by (1–4) linkages. It is one of the most abundant constituents that can be obtained

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from exoskeleton of crustaceans (e.g. lobsters, crab and shrimp), fungal cell walls and other biological materials [18]. It can be potentially used to improve physic-chemical, textural and organoleptic properties of different types of food due to its capacity to retard microbial growth, reduce dehydration and provide an excellent barrier to moisture and oxygen [19].

The use of natural antimicrobials in edible coatings and/or films can improve the ability of packaging materials for preserving food quality, reduce the use of chemical preservatives and also offer an alternative to satisfy the increasing consumer demand for safe, fresh and minimally processed foods [20]. *Mentha spicata* essential oil (MSO) has been reported to be an effective antibacterial [21,22], larvicidal [23] and antioxidant agent [24]. It can be widely used in the food industries to inhibit growth of spoilage microorganisms and subsequently extend the shelf life of different foods due to its strong antibacterial and antioxidant properties [21,25].

Until recently, there have been some studies that evaluated the effects of the edible coatings on the postharvest quality characteristics of strawberry fruits [3,4,6,26–28]. However, based on our knowledge, there is no scientific literature available regarding the survival of *Listeria monocytogenes* in fresh strawberry fruits coated with CMC and CH containing MSO. Therefore, the aim of the present study was to investigate and compare the effects of antimicrobial active packaging based on CMC and CH coatings containing different concentrations of MSO (0.1 and 0.2%) on (I) survival of *L. monocytogenes*; (Π) physicochemical (weight loss, titratable acidity and pH), microbial (total viable count, psychrotrophic bacteria as well as yeasts and molds) and sensory (appearance, color, texture and overall acceptability) properties; and (Ш) respiration rate of fresh strawberries during storage under refrigerated condition (4 ± 1 °C).

2. Materials and methods

2.1. Raw materials and chemicals

The organic strawberries (*Fragaria ananassa*) were bought from a local farm located in Kermanshah, Iran at the harvest day and immediately transferred to the laboratory under refrigerated condition (4 ± 1 °C) within 30 min. The fruits were gently immersed with a solution of 0.1% sodium hypochlorite for 1 min, rinsed with sterile distilled water, allowed to drip off for 5 min and stored at 4 °C prior to coating. All fruits were selected according to their similarity of color (>75% red surface color), ripeness stage and absence of any surface defects, visual fungal growth and decay. Food grades CH (medium molecular weight: 450 kDa, 75–85% deacetylated CH) and CMC (medium molecular weight: 250 kDa) powders were purchased from Sigma-Aldrich, UK. All media and other chemicals were obtained from Merck, Germany.

2.2. Isolation of Mentha spicata essential oil

M. spicata was obtained from Gilan-e-Gharb, Kermanshah, Iran during May–July 2016. Identification of the plant was conducted by a taxonomist (Dr. Seyed Mohammad Masoumi, Faculty of Agriculture, Razi University, Kermanshah, Iran). The collected plant was washed with tap water, dried in the dark at room temperature for two week, ground using a Lab blender and then used for the isolation of the essential oil by hydro-distillation in a Clevenger-type apparatus for 3 h. The isolated MSO was recovered and stored at 4 °C until gas chromatography–mass spectrometry (GC–MS) analysis.

2.3. Gas chromatography–mass spectrometry (GC–MS) analysis of essential oil

Analytical gas chromatography was conducted on a Thermo Quest Finningan apparatus fitted with HP-5MS 5% phenyl methylsiloxane capillary column (30 m length × 0.25 mm i.d. and 0.25 μm film thickness). Helium (purity: 99.99%; flow rate 1.2 ml/min and split ratio 1:20) was the carrier gas. Column temperature was initially set at 50 °C, then gradually increased to 265 °C at a rate of 2.5 °C/min and finally fixed at 280 °C. MSO analysis was also run on Thermo Quest Finningan coupled to mass spectrometer with the same analytical conditions indicated above. The MS was run in the electron ionization mode, using an ionization energy of 70 eV.

2.4. Preparation of test microorganism

L. monocytogenes (ATCC 19118) was obtained from the culture collection of the Iranian Research Organization for Science and Technology, Tehran, Iran. The bacterial strain was cultured in Brain Heart Infusion (BHI) broth at 37 °C for 24 h and maintained in BHI agar until further use. Before experiment, a single colony of the bacterium was transferred to BHI broth and incubated as described above. The bacterium was diluted to 5 log CFU/ml using 0.1% peptone water for artificial contamination of strawberries.

2.5. Preparation of the coating forming solutions and treatments

CMC solution was prepared by dissolving 1 g of CMC powder in 100 ml distilled water to obtain a 1% coating forming solution with constant agitation for 3 h at room temperature [29]. Moreover, an amount of 1 g CH powder was dissolved in 100 ml of 1% v/v glacial acetic acid and stirred on a hot plate at a controlled temperature of 37 °C for 3.5 h to ensure a complete solubility [7]. Thereafter, glycerol as a plasticizer (0.75 ml/100 ml CH or CMC emulsion) and tween 80 as an emulsifier (0.25 ml/100 ml CH or CMC emulsion) were added into the resulting dispersions, stirred with constant magnetic stirring for half an hour and subsequently homogenized at 12,000 rpm for 1 min [30]. Then, MSO at two concentrations (0.1 and 0.2%) was added to the both solutions. The resulting solutions were used for strawberry coatings.

For each coating forming solution, the fresh strawberries were randomly categorized into two sets (thirty intact fruits per set): set I: without inoculated pathogenic bacterium and set Π: inoculated with *L. monocytogenes*. For inoculation of the pathogenic bacterium, the strawberries were immersed for 5 min at room temperature in the diluted bacterial suspension (5 log CFU/ml) with agitation by a shaker to ensure even distribution of the microorganism. Then, the samples were air dried for 30 min to allow bacterial uniformly attachment onto strawberries. All strawberries were dipped into the solutions of coating materials for 5 min and excess coating materials allowed to drip off for 2 min. The samples were then air-dried at room temperature (25 ± 1 °C) for 30 min. Moreover, uncoated controls have also been handled the same as coated; therefore, it have been dipped into solutions for 5 min, without coatings present. The uncoated samples and coated ones were individually packed in small sterile stomacher bags, immediately stored in the same condition at 4 ± 1 °C for 12 days and sampling was carried out at days 0, 2, 4, 6, 8, 10 and 12 for further analysis.

2.6. Physicochemical, microbial and sensory analysis

The uncoated and coated strawberry fruits without inoculated pathogenic bacterium were mashed, homogenized and used for determination of weight loss (WL), titratable acidity (TA) and pH value according to the previously methods described by Velickova et al. [4]. The thickness of designated coatings was also determined from the obtained micrographs using a stereomicroscope Leica (Heerbrugg, Switzerland) coupled to a computer and a camera. Thickness was randomly measured in different sections. Water vapor resistance (WVR) and respiration rate were determined based on the methods described by Oms-Oliu et al. [31] and Perdones et al. [2], respectively.