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Effect of carboxymethyl cellulose-based coatings incorporated with *Zataria multiflora* Boiss. essential oil and grape seed extract on the shelf life of rainbow trout fillets

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ABSTRACT

To prolong the shelf life of seafood products, lipid oxidation and growth of microorganisms should be retarded. The objective of the current study was evaluating the potential application of carboxymethyl cellulose (CMC) coatings incorporated with *Zataria multiflora* Boiss. essential oil (ZMEO) and grape seed extract (GSE) on chemical (thiobarbituric acid reactive substances (TBARS) and total volatile basic nitrogen (TVB-N)), microbial (total viable count, lactic acid bacteria and *Pseudomonas* spp.) and organoleptic attributes of rainbow trout fillets during refrigerated storage for twenty days. GC–MS analysis showed that ZMEO is rich in monoterpene phenols such as thymol and carvacrol. The following results were obtained after 20 days of storage: The minimum level of TVB-N was measured in the fillets coated with CMC + 2% v/v ZMEO + 0.5% v/v GSE. The minimum number of total viable bacteria, lactic acid bacteria and *Pseudomonas* spp. were determined in the fillets coated with CMC + 2% v/v ZMEO + 1% v/v GSE. The fillets coated with CMC + 1% v/v ZMEO + 1% v/v GSE showed the best organoleptic properties. Our results revealed that CMC-based coatings incorporated with ZMEO and GSE could improve chemical, microbial and sensorial characteristics of rainbow trout fillets during cold storage.

1. Introduction

Fresh fish is one of the most perishable seafood products. It has been reported that the spoilage of fish muscle is a combination of different spoilage mechanisms including lipid oxidation, microbial and endogenous enzymes activities as well as enzymatic browning. These events lead to a decrease in the shelf life of fish meat and other seafood products (Arashisar, Hisara, Kayab, & Yanik, 2004; Mace et al., 2013). In recent years, new techniques have been tried by many researchers to prolong the shelf life of food products. Among different applied methods, application of bio-based films and coatings was the most promising technique (Georgantelis, Ambrosiadis, Katikou, Blekas, & Georgakis, 2007; Jouki, Tabatabaei-Yazdi, Mortazavi, Koocheki, & Khazaei, 2014; Ojagh, Rezaei, Razavi, & Hosseini, 2010).

Edible films and coatings of particular characteristics can be produced from different sources including polysaccharides, proteins and lipids (Sayanjali, Ghanbarzadeh, & Ghiassifar, 2011). Polysaccharides have been frequently used to develop films and coatings because of appropriate film forming properties. CMC (E466) is a cellulose derivative of wide applications in food technology such as thickening, stabilizing and mouthfeel improving. It is composed of linear chains of β (1–4) glucosidic units with methyl and carboxyl substituents (Togrol & Arsalan, 2004). The obtained film from aqueous solutions of CMC has moderate strength; however, has high water vapor permeability because of the inherent hydrophilic nature. In contrast to some biopolymers such as chitosan, CMC does not have any intrinsic antimicrobial properties. A way to improve the moisture barrier properties of CMC together

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with developing antimicrobial characteristics would be the incorporation of hydrophobic compounds such as essential oils. According to Dashipour et al. (2015), *Zataria multiflora* essential oil addition into CMC-based films could improve physical, mechanical and antibacterial properties.

In recent years, there has been an increased interest in the use of natural antimicrobial agents instead of chemical ones. CMC and the other biopolymers can be used as a suitable carrier for natural antimicrobial and antioxidant compounds. Essential oils and their components show promising activities against many food-borne pathogens and spoilage microorganisms. *Z. multiflora* Boiss. (Shirazi thyme) is a member of Labiatae family which grows in some parts of Iran, Pakistan and Afghanistan. *Z. multiflora* Boiss. essential oil (ZMEO) shows strong antimicrobial and antioxidant activities because of having large quantities of phenolic oxygenated monoterpenes. Thymol and carvacrol are the main constituents of this essential oil (Moradi et al., 2012). Grape seed is a byproduct of winery and grape juice industry (Ignea et al., 2013). Grape seed extract (GSE) has different amounts of lipid, protein, carbohydrates and 5–8% polyphenols. Main polyphenol compounds present in GSE include monomeric flavon-3-ols such as catechin, epicatechin and procyanidin dimmers and trimers (Chedea, Braicu, & Socaciu, 2010; Nakamura, Tsuji, & Tonogai, 2003; Peng et al., 2001). In practical applications of biopolymer-based antimicrobial films, higher concentrations of essential oils are needed to exert similar functional effects as those obtained during in vitro assays, which is due to the entrapment of the essential oil components within film matrix. This may result in unfavorable sensory characteristics. Formulating different types of natural preservatives mixtures is thus a new solution to increase the efficacy of essential oils and decrease the unfavorable organoleptic properties by taking the advantage of their synergistic and additive effects. Essential oils containing carvacrol, cinnamaldehyde, cinnamic acid, eugenol and thymol have a synergistic effect (positive interaction) in combination with other polyphenols. The constituents of essential oils may act synergistically by affecting multiple targets and by physicochemical interactions (Bassolé & Juliani, 2012). The efficacy of antimicrobial coatings of different origins has been demonstrated in previous studies (Jouki et al., 2014; Ojagh et al., 2010). Liu, Han, Zhang, Li, and Li (2012) utilized CMC films contained rosemary extract to inhibit microbial and oxidative degradations of fresh beef during cold storage.

To the best of our knowledge the simultaneous incorporation of ZMEO and GSE into CMC-based film and evaluating its potential application in real food systems (such as fish fillets) has not been studied previously. Therefore, the objective of the current study was exploring the antibacterial and antioxidant properties of CMC coatings incorporated with ZMEO and GSE on improving different quality characteristics of rainbow trout fillets during cold storage.

2. Material and methods

2.1. Chemicals and materials

CMC (average MW of 41 kDa) was purchased from Caragum Parsian Co. (Tehran, Iran). Commercial GSE powder was obtained from Mega Natural Inc. (Madera, CA, USA). Stock solution (10% w/v) of GSE powder was prepared by dissolving GSE in distilled water. Glycerol (analytical grade), Standard Plate Count Agar (PCA) and de Man Rogosa Sharpe Agar (MRSA) culture media were purchased from Merck Co. (Darmstadt, Germany). Pseudomonas Isolation Agar (PIA) culture medium was purchased from Oxoid Co. (Cambridge, UK). All other used reagents were of analytical grade.

2.2. Fish sample preparation

Fresh aqua cultured rainbow trout (*Oncorhynchus mykiss*) with average weight of 400–500 g were purchased from a cold water aqua culture farm in Urmia, Iran. The samples were transferred in ice boxes to the laboratory. In the first step, all fishes were eviscerated and filleted and then remained at 4 °C before coating and subsequent analysis.

2.3. Essential oil extraction and its GC/MS analysis

To prepare ZMEO, the plant was purchased from a local grocery and authenticated at the Faculty of Agriculture, Urmia University (Urmia, Iran). Hydrodistillation method using a Clevenger type apparatus was utilized to extract essential oil during a 3 h distillation. The obtained essential oil was dehydrated using sodium sulfate and after filtration, it was stored at 4 °C and darkness. Analysis of ZMEO was performed using an Agilent 6890N instrument equipped with an HP-5MS capillary column (30 × 0.25 mm ID × 0.25 mm film thickness). The carrier gas was helium with a flow rate of 1 ml/min. The column temperature was initially set at 50 °C, and then gradually increased to 120 °C at a 2 °C/min rate, held for 3 min at this temperature, and finally increased to 300 °C. The detection procedure was operated at 70 eV. The compounds were identified by comparing their retention indices with those of authentic samples and mass spectral data available in the library (Wiley 2001 data software).

2.4. Preparation of CMC coating solutions

To prepare coating solution, 1 g carboxymethyl cellulose was dispersed in 100 ml distilled water. Glycerol, as a plasticizer, was added into CMC coating solution at 0.5% v/v. The dispersion was heated at 85 °C for 5 min with subsequent cooling at room temperature (Sayanjali et al., 2011). ZMEO (1% and 2% v/v) and GSE (0.5% and 1% v/v) were incorporated into CMC coating solutions. Fillet coating was performed using immersion method. The coated samples were then allowed to lose excess biopolymer solution before coating development. In this study, nine samples including the blank sample (without any coating) and those coated with CMC contained different amounts of ZMEO, GSE and their possible mixtures were prepared. The samples were stored at 4 °C for 20 days and analyses were carried out during 5 day intervals.

2.5. Chemical analysis

2.5.1. Proximate composition

The AOAC method was used to determine the moisture and the crude ash contents at 103 and 550 °C, respectively (AOAC, 2002). Total crude protein was assessed using Kjeldahl method (AOAC, 2005). The lipid content was measured according to the method described by Bligh and Dyer (1959).

2.5.2. Determination of total volatile basic nitrogen (TVB-N)

Determination of TVB-N values was performed based on the micro-diffusion method described by Pikul, Lesztzynski, and Kummerow (1989). This method was carried out by distillation using a Kjeldahl type apparatus after MgO addition into the homogenized samples. A flask containing aqueous solution of boric acid of 3% concentration as well as indicator (produced by dissolving 0.1 g methyl red and 0.1 g methylene blue in 100 ml absolute ethanol) was utilized to collect the distillate. To determine TVB-N values, the boric acid solution was titrated using 0.05 M sulfuric acid solution. Results have been expressed in milligram of nitrogen per 100 g of sample.