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Potential of antimicrobial active packaging 'containing natamycin, nisin, pomegranate and grape seed extract in chitosan coating' to extend shelf life of fresh strawberry

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ABSTRACT

In this study, chitosan (CH) coatings as carrier of nisin (NS), natamycin (NT), pomegranate (PE) and grape seed extract (GE) were used to maintain quality and extend shelf life of fresh strawberry. The samples were analyzed in terms of gas composition inside packaging, pH, total soluble solid content, water activity, color, texture, FT-NIR and microbial counts for up to 40 days at 4 °C. The experiments showed that all coating materials preserved the quality of fresh strawberry compared to untreated ones. Among the treatments, incorporation of the natamycin in the chitosan coating (CH + NT) reduced the O_2 consumption of the fruit and showed better effects on delaying changes of pH, total soluble solid content, water activity and microbial counts. The incorporation of different antimicrobial agents into chitosan matrix did not reveal any significant effect on lightness (L^*) and redness (a^*) of strawberry. Among the treatments, CH + PE and CH + NT showed significantly higher antimicrobial effect against the mesophilic bacteria and yeast and mold. As a general result, antimicrobial active packaging based on the combination of chitosan coating with antimicrobial agents increased the shelf life of fresh strawberry compared to uncoated fruit.

1. Introduction

Strawberries are one of the most popular fruit in the world due to their unique taste and flavor. However, they have a short shelf-life because of their susceptibility to mechanical damage, physiological deterioration, water loss and fungal infection (Atress et al., 2010) and post-harvest losses can reach as high as 40% during storage period (Satin, 1996). Therefore, manufacturers seek alternative approaches to maintain quality of strawberry. From the consumer point of the view, they demand natural, fresh and healthy foods without using any technologies that have potential risks to human health and the environment (Gol et al., 2013). Thus, natural and environmentally friendly preservation techniques such as antimicrobial agents in edible coatings can be used to fulfill consumers' needs and prevent post-harvest losses of strawberry which result in prolonged periods of marketing.

Edible coatings improve textural properties, enhance product appearance and extend the shelf life of foods by providing a semipermeable barrier to moisture and gases such as carbon dioxide and oxygen (Franssen and Krochta, 2003; Tapia et al., 2008). Chitosan has widely been used in edible coatings because of its non-toxic nature, excellent film-forming characteristic, antioxidant and antimicrobial properties (Rhoades

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and Roller, 2000; Shahidi et al., 1999). Most accepted mechanism of antimicrobial action of chitosan is based on the interaction between positively charged polycationic chitosan molecules and negatively charged microbial cell membranes (Tsai and Su, 1999). Chitosan recognized as safe by the USFDA in 2001 (USFDA, 2001) and European Union permitted that it can be used as a food additive at a daily dose up to 3 g (EU, 2012). Previous works reported that edible coatings had a beneficial effect on strawberry quality. Gol et al. (2013) showed that composite of carboxymethyl cellulose and chitosan coating inhibited the enzyme activity and slowed the changes in weight loss, decay percentage and total soluble solid content. Velickova et al. (2013) found that three-layer and composite chitosan–beeswax coatings slowed down the respiration rate and reduced the senescence of strawberry. Peretto et al. (2014) demonstrated that carvacrol and methyl cinnamate antimicrobial vapors released from pectin film delayed the decay of strawberry and preserved the firmness and brightness.

Incorporation of antimicrobial agents into edible coating which are considered as active packaging (Quintavalla and Vicini, 2002) provides a novel way to maintain quality and extend shelf-life of fruits (Cagri et al., 2004). Antimicrobial agents used in edible coating may contain nisin, natamycin and natural seed extracts (Kallinteri et al., 2013; Kanatt et al., 2007; Moradi et al., 2012; Sivarooban et al., 2008; Song et al., 2012) Nisin is a bacteriocin produced by Lactococcus lactis subsp. Lactis (Delves-Broughton, 1990) and effectively inhibits wide range bacteria when incorporated into hydroxypropylmethylcellulose (Coma et al., 2001), whey protein isolate, soy protein isolate, wheat gluten (Ko et al., 2001) and zein edible films (Janes et al., 2002). Natamycin is another natural antimicrobial agent which inhibits fungal growth by binding to cell membrane sterols which result the cell lyses (Jiang et al., 2013) and widely used on the surface of cheeses with edible coating to prevent mold growth and contamination (Pintado et al., 2010; Ramos et al., 2012; Reps et al., 2002). Pomegranate extract have good antioxidant properties and antibacterial activity against *Staphylococcus aureus* and *Bacillus cereus* when combined with mint extract incorporated into chitosan–polyvinyl alcohol films (Kanatt et al., 2012). Grape seed extract is another compound which show antioxidant and antimicrobial properties alone or in combination with other hurdle technologies (Bañón et al., 2007; Gadang et al., 2008; Theivendran et al., 2006).

Despite this interest no one, to the best of our knowledge, has reported the effectiveness of chitosan coating containing pomegranate and grape seed extract on fresh fruit. Therefore, this paper focused on extending the shelf life of fresh strawberries with antimicrobial active packaging based on the combination of the chitosan coating with nisin, natamycin, pomegranate and grape seed extract. The coated and uncoated fresh strawberries were compared in terms of gas composition inside packages, pH, total soluble solids, water activity, surface color, texture profile, FT-NIR and microbial counts during 40 days of storage period at 4 °C.

2. Material and methods

2.1. Plant material

Fresh strawberries (*Fragaria ananassa cv. Camarosa*) at commercial maturity were harvested from the local farm in Yenice, Canakkale, Turkey. Then fruits were transported at low temperature to the Food Engineering laboratory within 1 h and coatings were applied. Before coating applications, fruits with defects such as decay and mechanical damage were eliminated and fruits of uniform size, shape and color were used in experiment.

2.2. Coating application

Chitosan were obtained from France Chitine (Orange, France) with a deacetylation degree of 89.9% and molecular weight of 1.8×10^5 g/mol. Nisin (<3% moisture content and hydrous potency of 1000 IU/mg) was purchased from Beijing Oriental Roda Biotech (Beijing, China) and natamycin (50% w/w) obtained from DSM Food Specialties Dairy Ingredients (Delft, Netherlands). Pomegranate and grape seed extract were procured from Helvacizade Gida (Konya, Turkey).

Chitosan coating solutions was prepared with some modification of the method described by Aday and Caner (2010) with using 1.5 g (1.5% w/v) chitosan in distilled water while stirring the solution with a magnetic stirrer. Then, acetic acid (1% v/v) was added to dissolve chitosan and polyethylene glycol was used as a plasticizer at a concentration of 0.5% v/v. The solution was stirred and heated at low temperature (40 °C) on a combined hot-plate magnetic-stirrer device for 6 h. The antimicrobial agents such as nisin, natamycin, pomegranate and grape seed extract were added to chitosan solutions at a concentration of 1% w/v in order to obtain optimum films.

Fruits were randomly divided into six groups. Then strawberries were immersed in coating solutions, CH (chitosan 1.5%), CH + NS (chitosan 1.5% + nisin 1%), CH + NT (chitosan 1.5% + natamycin 1%), CH + PE (chitosan 1.5% + pomegranate extract 1%), CH + GE (chitosan 1.5% + grape seed extract 1%), for 1 min and taken out. The sixth group which contained untreated fruit was used as CNT (control). After drying, around 175 g fruits were placed in polylactic acid (PLA) trays supplied by Huhtamaki İstanbul Ambalaj San. A.S. (Istanbul, Turkey). Ninety packages of strawberries were used for per treatment in each replicate and 18 packages were analyzed at per sampling date. Trays were sealed under air atmospheric conditions. Samples were stored at 4 °C and 80–85% RH.

2.3. Composition of atmosphere inside the packages

Concentration of carbon dioxide and oxygen inside the packages were determined using Oxybaby (Hamburg, Germany) gas analyzer. Adhesive silicon septum was glued to sampling point of packages to prevent gas leakage during analysis. Then, needle of gas analyzer was inserted through the septum and results were expressed as O_2 kPa and CO_2 kPa (Park and Kang, 2014). Three packages were used on each test day for per treatment.

2.4. pH and total soluble solids measurement

To determine soluble solids content and pH values of samples, three replicates of strawberry juice was used. Juice was obtained by homogenizing nine fruits in a blender. Measurements were performed by a digital refractometer (Atago, Tokyo, Japan) and pH meter (Sartorius PP-50, Goettingen, Germany) (Aday et al., 2011).