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Antimicrobial efficiency of edible coatings on the preservation of chicken breast fillets

Idoya Fernández-Pan*, Ximena Carrión-Granda, Juan I. Maté

Food Technology Department, Universidad Pública de Navarra, Spain

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ABSTRACT

To improve the quality and extend the shelf-life of chicken breast, Whey Protein Isolate (WPI) edible coatings with oregano or clove essential oils (EOs) incorporated as natural antimicrobials have been developed. These insoluble, homogeneous and continuous WPI coatings formed an imperceptible second skin covering the chicken breast. The antimicrobial effect of the coatings depended on their EO concentration (the higher the better), EO type (oregano EO the most active) and on the microbiological group analysed (*Pseudomonas* spp. the most resistant). Films with 20 g kg^{-1} of oregano EO showed their efficacy by doubling the storage time of chicken breast (from 6 to 13 days), keeping most of the microbiological groups below the recommended limits for distribution and consumption of chicken breast. By comparing the effects of the direct addition of the EOs onto the chicken breast surfaces and the results brought about by the development of edible coatings the usefulness and functionality of the latter as carriers of antimicrobials has been confirmed.

1. Introduction

In the last decades, the wide variety of commercially available products, their relatively low production cost and high nutritional value have given rise to an increase in the consumption of fresh chicken products in many countries. Among the main limitations for the commercialization of fresh chicken products we find their short shelf-life, mainly due to their high content on nutrients and superficial moisture which leads to a fast growth of spoilage and pathogen microorganisms (Aymerich, Picouet, & Monfort, 2008; Patsias, Badeka, Savvaidis, & Kontominas, 2008; Zhou, Xu, & Liu, 2010). The microbial flora that develops primarily on fresh chicken starts off on its surface and it may depend on the initial bacterial counts and storage conditions. It is well established that this bacterial growth and its activity on the surface of the product are the main causes of the changes in flavour, aroma and other organoleptic characteristics of chicken products, which lower their quality and shorten their commercial life with the subsequent industrial economic losses (Mead, 2004; Petrou, Tsiraki, Giatrakou, & Savvaidis, 2012; Samelis, 2006).

Refrigeration as a preservation technique is absolutely necessary to maintain the microbial quality of fresh meat products, but it does not guarantee by itself a long shelf-life, which in the case of chicken breast amounts to a time period of merely 4–5 days. The currently employed consolidated preservation process is based on refrigeration and modified atmosphere packaging, which extends the above mentioned time-length to a minimum shelf-life period of 8 days (Chouliara, Badeka, Savvaidis, & Kontominas, 2007; Chouliara, Karatapanis, Savvaidis, & Kontominas, 2007; Patsias et al., 2008). Nowadays, new preservation techniques are being developed to improve the preservation process and to lengthen the storage time maintaining both the natural appearance and safety of fresh meat and poultry products. To achieve which, not only consumers demands for convenience and easy to prepare natural, nutritive and high quality products, but also long storage periods must be taken take into account (Patsias et al., 2008; Zhou et al., 2010). Thus, the most studied techniques are related with non-thermal microbial inactivation, such as the use of high hydrostatic pressure (Rivas-Cañedo, Fernández-García, & Nuñez, 2009) or ionizing radiation (Zhang, Liu, Li, & Qu, 2010), and also the use of natural preservatives and new packaging systems like vacuum packaging (Belcher, 2006), modified atmosphere (McMillin, 2008) and active packaging (Coma, 2008; Nerín et al., 2006).

Specific objectives related to microbiological quality have been achieved combining the use of different preservation techniques (Aymerich et al., 2008; Zhou et al., 2010). Thus, the addition of biopreservatives and natural antimicrobials combined with

* Corresponding author. Food Technology Department, Universidad Pública de Navarra, Campus Arrosadia s/n, Edificio Los Olivos, 31006 Pamplona, Spain. Tel.: +34 948 16 90 92; fax: +34 948 16 98 93.
E-mail address: idoya.fernandez@unavarra.es (I. Fernández-Pan).

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refrigeration and modified atmosphere packaging (MAP) have lately showed to be highly effective. Different authors as Skandamis, Tsigarida, and Nychas (2002), Chouliara, Karatapanis, et al. (2007) and recently Petrou et al. (2012) studied the effect of the addition of oregano essential oil (EO) as natural antimicrobial in the storage of fresh beef or chicken in combination with MAP and refrigeration, reporting significant increases in the products shelf-life. In fact, certain EOs produce by themselves an important antimicrobial activity against different bacteria related with food spoilage and pathogenicity. However, their direct application requires high EOs concentrations which might modify the organoleptic characteristics of food products (Ntzimani, Giatrakou, & Savvaidis, 2011; Petrou et al., 2012; Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2011). To avoid that, the use of edible films and coatings as carriers of such substances is suggested as an alternative to the direct application of EOs on food products.

Antimicrobial edible films and coatings are presented as an emergent technology capable of increasing the safety and shelf-life of food products upon direct contact (Atarés, De Jesús, Talens, & Chiralt, 2010; Du et al., 2009; Fernández-Pan, Royo, & Maté, 2012; Hosseini, Razavi, Mousavi, Yasaghi, & Hasansaraei, 2008). Their mechanism is based on the controlled release of active agents on the product surface where they are placed, therefore keeping effective concentrations where and when they are needed (Quintavalla & Vicini, 2002).

Many authors have applied antimicrobial edible films and coatings on meat products. Zinoviadou, Koutsoumanis, and Biliaderis (2009, 2010) evaluated the effectiveness of WPI coatings containing different antimicrobial agents on fresh beef under refrigeration. Oregano EO, ε-polylysine or sodium lactate were used as antimicrobial agents and the effect of the films against the development of total viable microorganisms, *Pseudomonas* spp. and lactic acid bacteria (LAB) have been evaluated. The high specific growth rate of total viable microorganisms and *Pseudomonas* spp. were significantly reduced by the use of 1.5% of oregano EO or 0.75% of ε-polylysine. Besides, the development of LAB was completely inhibited. When they used 2% of sodium lactate, both *Pseudomonas* spp., and total viable microorganisms were inhibited, even though the effect on LAB was less intense. Emiroglu, Yemiş, Coşkun, and Candogan (2010) evaluated the effect of soy protein isolate films containing up to 5% of oregano and/or thyme EO in vacuum packaged minced beef burgers for a 12 day period of cold storage (4 °C). Films applied over beef burgers resulted effective against coliforms and *Pseudomonas* spp., although it was no significantly effective against total viable microorganisms, LAB or *Staphylococcus* spp. Some authors have focused on the main active compounds of EOs. Thus, Ravishankar, Zhu, Olsen, McHugh, and Friedman (2009) evaluated the antimicrobial effectiveness of edible films based on apple puree containing 1.5% and 3% of carvacrol or cinnamaldehyde (main active compounds from oregano and cinnamon EOs, respectively) over chicken breast under refrigeration. They found that the films inactivated the autochthonous spoilage microflora of chicken.

Fernández-Pan et al. (2012) and Fernández-Pan, Mendoza, and Maté (2013) developed antimicrobial edible films based on WPI with different EOs. They reported the high effectiveness of these films against both, food industry pathogens and autochthonous spoilage microbiota of fresh chicken breast developed during different refrigerated storage periods by *in vitro* tests. The most effective formulations were the ones containing oregano (high carvacrol content) or clove (high eugenol content) EOs against the following bacteria strains: *Staphylococcus aureus*, *Salmonella enteritidis*, *Listeria innocua,* and *Pseudomona fragi*. Likewise, the same formulations showed effectiveness against total aerobic mesophilic bacteria, *Enterobacteriaceae*, LAB and *Pseudomonas* spp. developed on the surface of chicken breast during 8 days of refrigerated storage.

The objective of the present study is to evaluate both the usefulness and effectiveness of the aforementioned best developed active edible coatings on the microbial quality of chicken breasts and to evaluate the potential extension of their shelf-life. An evaluation of the use of EOs within an edible matrix or directly on the food surface without edible coating is part of this study.

2. Materials and methods

2.1. Raw material

Skinned chicken breast fillets from broilers slaughtered in the same day of the laboratory sample reception were used in this study. Fillets were packed in polypropylene trays upon arrival with no modified atmosphere, and after treatment, but also maintaining an untreated control group, were kept under refrigeration conditions (4 °C) during 13 days.

2.2. Formulation of edible coatings

Film forming solutions (FFS) were prepared using an aqueous solution of 100 g kg^{-1} of WPI (Davisco Food International, USA) and 50 g kg^{-1} of glycerol (Panreac Quimica, Spain) as plasticizer. Following the procedure developed by McHugh and Krochta (1994) the FFS were kept in a thermostatic bath at 90 °C during 30 min under constant agitation in order to denaturalize WPI. After cooling, EOs (Laboratorios Dicana, Spain) of oregano (*Coridothymus capitatus*, with 48.56% carvacrol) or clove (*Eugenia caryophyllata*, with 71.80% eugenol and 19.80% eugenilo acetate) at 10 or 20 g kg^{-1}, were added to the FFS. Homogenization of FFS was done using an Ultra-turrax (T25 basic IKA WERKE, Germany) at 24,000 rpm during 2 min (Fernández-Pan et al., 2012). Finally, the solutions were filtered and degassed, thus obtaining the C-O1, C-O2 (which refer to FFS with oregano EO at 10 or 20 g kg^{-1}), C-Cl1 and C-Cl2 (which refer to FFS with clove EO at 10 or 20 g kg^{-1}). Besides, FFS without EOs were prepared as a coating control (C-C).

2.3. Application of edible coatings on chicken breast fillets

Using a stainless steel punch, homogeneous 50 mm diameter medallions of 5 mm thick chicken breast fillets were prepared and they were coated following three steps: dipping, draining and drying. The three steps were done under sterilized conditions using a laminar flow hood for the initial microbiological population of the samples not to be modified. Edible coatings were formed over the chicken medallions by immersion in 100 ml of FFS during 2 min. FFS excess was drained during 30 s and coatings were formed exposing medallions to a cool air stream during 45 s on each side. The coated samples (C-O1, C-O2, C-Cl1, C-Cl2 and C-C) were packed in polypropylene trays and stored without modified atmosphere at 4 °C during 13 days. Adhesiveness over chicken breast surface, continuity and homogeneity in coatings were visually evaluated. Such evaluation was done at the end of the coating application process, after 24 h of storage and days 3, 6, 8, 10, and 13 of the storage period.

2.4. Direct addition of EOs on chicken breast fillets

In order to verify the effectiveness of the WPI structural matrix, direct addition of oregano and clove EOs on chicken breast fillets was carried out following the same procedure as in the coating application. Then, emulsions of oregano and clove EOs with deionized water at 20 g kg^{-1} were prepared. With the same homogenization conditions as for FFS, the Ultra-turrax was used at