Food Control 80 (2017) 273–280

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Development and performance of whey protein active coatings with *Origanum virens* essential oils in the quality and shelf life improvement of processed meat products

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A R T I C L E I N F O

Article history:
Received 12 December 2016
Received in revised form
19 February 2017
Accepted 19 March 2017
Available online 29 April 2017

Keywords:
Whey protein active coatings
Essential oils
Antimicrobial
Antioxidant
Oregano

A B S T R A C T

Active coating development for prevention and control of microbial contamination is an extremely challenging technology. In this field, the potential of plant-based compounds to be used as a complement or an alternative to the synthetic preservatives have drawn increasing attention from the food industry.

In this study, an active coating containing *Origanum virens* essential oil (EO-WPC) was applied on the surface of two traditional Portuguese sausages (*paínhos* and *alheiras*) during industrial production. For 4 months, both products were regularly monitored for their physicochemical properties and total microbial load. Sensory evaluation was also carried out for each sausage type.

The application of the EO-WPC caused little variations on the moisture content and texture profile. Higher acidity and protection against color fading was observed particularly in *paínhos*, while coated *alheiras* had a significant reduction of the lipid peroxidation. Inhibition of the total microbial load was observed for both coated sausages, resulting in an extension of the shelf life of approximately 20 and 15 days for *paínhos* and *alheiras*, respectively. Sensory analysis revealed an overall positive acceptance. These results support the great potential of *O. virens* EO to be used as food preservative in processed meat products.

1. Introduction

With increasing consumer demand for healthy, safe and convenient minimally processed meat products, and a distribution system requiring adequate shelf life, novel intervention strategies with significant and long-lasting antimicrobial efficacy are being explored (Barros-Velazquez, 2016). In this context, films and coatings made from biodegradable and edible materials, or in combination with synthetic materials, are promising novel systems that can be used as packaging systems for food, because they may protect them from the surrounding environment and act as carriers of antimicrobials. Moreover, a wide range of natural and chemical antimicrobial agents can be incorporated into those systems to inhibit spoilage and pathogenic microorganisms, further contributing to preserve freshness, extend shelf life, and ensure food safety with less severe food treatments (Rawdkuen, Punbusayakul, & Lee, 2016). Still, even though a variety of antimicrobial packaging forms have been reported to effectively inhibit or suppress the microbial growth extending meat shelf life and ensuring food safety, there are only very limited cases of commercialized antimicrobial packages (Rawdkuen et al., 2016).

The added value of bio-based edible coatings, particularly whey protein-based coatings, as opposed to traditional packaging, is firstly due to their edible nature and inherent biodegradability, and also because of their capacity to incorporate functional compounds that offer further protection to foodstuff (Brody, Strupinsky, & Kline, 2001) which can be also valuable due to their intrinsic bioactive properties. The use of whey protein to manufacture films and coatings has received a great deal of attention, since such films

Abbreviations: EO, essential oil; WPC, whey protein concentrate; EO-WPC, whey protein active coating with *O. virens* EO.

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http://dx.doi.org/10.1016/j.foodcont.2017.03.054

possess interesting sensorial, optical and mechanical barrier properties (Gennadios, 2004; Ghanbarzadeh & Oromiehi, 2009; Henriques, Gomes, & Pereira, 2016; Hernandez-Izquierdo & Krochta, 2008; Ramos & Malcata, 2011; Ramos, Fernandes, Silva, Pintado, & Malcata, 2012), and allow the valorization of cheese-making effluents. Research on edible whey protein films has been focused on the use of whey protein isolate (WPI) with a protein content greater than 90%. Nevertheless, several whey protein concentrates (WPCs) ranging from 35 to 80% are available at a considerably lower price, although the presence of other constituents introduces functional changes in WPC-based edible films (Henriques, 2013).

Several factors should be considered when developing antimicrobial films or coatings. The most relevant are the effect of the film-forming solution composition, the presence and amount of plasticizers to reduce brittleness, allowing for the plastic behavior of the film, or even the influence that the antimicrobial agent has on the mechanical and physical properties of the films and coatings. However, other factors are also mentioned, such as the antimicrobial mechanism, migration into the food, toxicological issues, as well as their effect on food product composition (Regalado, Pérez-Pérez, Lara-Cortés, & García-Almendanez, 2006).

The use of aromatic plants and spices in several foodstuffs is an ancient practice based on empirical grounds. More recently, extensive work has been done on plant extracts and EOs in view of the full understanding of their composition, antioxidant and antimicrobial activities (Fernandes et al., 2016; Honório et al., 2015; Roby, Sarhan, Selim, & Khalel, 2013; Shan, Cai, Sun, & Corke, 2005), toxicological aspects and potential health benefits (Atarés & Chiralt, 2016; José Manuel; Lorenzo & Munekata, 2016; Martínez-Graciá, González-Bermúdez, Cabellero-Valcárcel, Santaella-Pascual, & Frontela-Saseta, 2015; Rubió, Motilva, & Romero, 2013). Atarés and Chiralt (2016) reviewed the applications of EOs as additives in biodegradable films and coatings for active food packaging concluding that EOs may provide the films with antioxidant and/or antimicrobial properties and that the oil composition and the specific interactions with the polymer determine its effectiveness as an active ingredient.

Although specific developments and applications of novel antimicrobial films on meat products, poultry, seafood, fruits, vegetables and beverages are studied (Barros-Velazquez, 2016), little information is available about the antimicrobial activity of bioactive agents when incorporated into whey protein films, and the few available studies in this field mainly evaluated this aspect through *in vitro* tests (Cagri, Ustunol, 2001; Min, Harris, & Krochta, 2005; Seydim & Sarikus, 2006). Even less studies have been carried out in real food applications such as cheese, meat or fish products (Dagdelen et al., 2014; Fernández-Pan, Carrión-Granda, & Maté, 2014; Henriques et al., 2013; Zinoviadou, Koutsoumanis, & Biliaderis, 2009).

Therefore, the aim of this study was to develop and evaluate the effect of the application of EO-WPC on the physical, chemical, microbiological and sensorial characteristics of two traditional Portuguese sausages, namely *paínho and alheira*.

2. Material and methods

2.1. Chemicals and materials

Bovine freeze dried WPC obtained by ultrafiltration was produced at Escola Superior Agrária de Coimbra (ESAC) facilities as described by Henriques, Pereira, and Gil (2011). The WPC (47.74 g protein 100 g^{-1}, 43.78 g lactose 100 g^{-1} and 2.00 g fat 100 g^{-1}) was used as the protein source for EO-WPC formulations. The EO from *O. virens* was purchased from Ervitas Catitas Lda. (Pegões, Portugal). The oil is mainly characterized by carvacrol (21.8%), γ-terpinene (20.8%), thymol (12.1%), *p*-cymene (6.1%), β-caryophillene (6.0%), terpinen-4-ol (5.3%), β-bisabolene (4.5%) and 4-carene (3.8%). Glycerol (Gly) of 99% of purity, supplied by JM Vaz Pereira Lda. (Portugal), was used as plasticizer. Sodium hydroxide was supplied by Fisher Scientific (USA).

All the culture media were purchased from Biokar Diagnostics (France). Buffered peptone water (Biokar Diagnostics, France) was used for the sample decimal dilutions. All other chemicals were reagent-grade or better, and used without further purification.

2.2. EO-WPC formulation

In order to obtain a protein concentration of 10% (w/w) in the final forming solution (Henriques, 2013), 24% (w/w) of WPC were weighed and completely dissolved in distilled water at room temperature under continuous agitation. The pH of each solution was adjusted to 7.0 with NaOH (0.1 M) and, distilled water was added until 92 g. This was then heated in a water bath at 90 °C, until an uniform appearance was observed (10 min). After cooling, glycerol was added (at 5% (w/w), on a film-forming solution basis) and homogenized. Finally, 3 g of an 33% EO solution diluted in ethanol was incorporated in the film-forming solution so that the final concentration of EO was 1% (w/w).

2.3. EO-WPC application on meat products

The application of the EO-WPC coating was performed at Irmãos Monteiro S.A. facilities (Gafanha da Encarnação, Portugal) during the industrial production of *paínhos* (i.e., smoked dry-cured sausages made with lean pork and pork back fat, seasoned with garlic, salt and pepper (Elias & Carrascosa, 2014) and hand-cut in pieces of about 100–300 g) and *alheiras* (i.e., smoked fermented sausages made of chopped pork and/or poultry meat, boil with lard, bread and olive oil, seasoned with salt, garlic and spices (Albano et al., 2007)). These sausages have a shelf life period of 3 and 2 months, respectively, if stored at 4 °C under vacuum atmosphere. One mL of the EO-WPC solution was applied to the surface of hand-cut pieces of *paínhos* and intact *alheiras* by brushing the products with a silicone brush at the final stage of the production process, i.e., after hot smoking and dehydration stages and prior to vacuum sealing. Finally, both coated (C) and uncoated (UN) sausages were submitted to packaging by thermal vacuum sealing (30 s at 120 °C) in low density plastic films.

The packed products were stored in the company facilities at 4 °C until further analysis. The effect of the application of EO-WPC coating in *paínhos* and *alheiras* physicochemical properties was assessed at 5 distinct points along the storage period, namely at day 21, 49, 75, 100 and 126 for *paínhos* and day 14, 36, 58, 80 and 106 for *alheiras*. Exception made for the sensory analysis which was evaluated on the 45th and 30th day of storage for *paínhos* and *alheiras*, respectively, corresponding to approximately half of the shelf life of each control product (without coating).

2.3.1. Physicochemical analysis

The physicochemical properties of the sausages were monitored during storage for color, texture profile analysis (TPA), lipid oxidation levels, pH, titratable acidity and moisture content.

The color (ΔE) of *paínhos* and *alheiras* was carried out as described in American Meat Science Associations (AMSA,2015). TPA was determined in a Stable Micro Systems Texture Analyzer, model TA.XT Express Enhanced and data was acquired by the Specific Expression PC Software. A double compression test was performed as described by Herrero et al. (2008). The samples were compressed to 50% of their original height using an aluminum cylinder probe of