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Combined natural antimicrobial treatments (EDTA, lysozyme, rosemary and oregano oil) on semi cooked coated chicken meat stored in vacuum packages at 4 °C: Microbiological and sensory evaluation

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ABSTRACT

The present study examined the effect of natural antimicrobials: Ethylenediaminetetraacetate (EDTA), lysozyme, rosemary and oregano oil and their combinations, on the shelf-life of semi cooked coated chicken fillets stored under vacuum packaging (VP), at 4 ± 0.5 °C for a period of 18 days. The treatments of semi cooked coated chicken fillets examined in the present study were the following: Air-packaged (A, control samples), vacuum-packaged (VP), VP with EDTA–lysozyme solution 1.50% w/w, (VP + EL), VP with rosemary oil 0.20% v/w, (VP + R), VP with oregano oil 0.20% v/w, (VP + O), VP with EDTA–lysozyme solution and rosemary oil (VP + EL + R) and finally VP with EDTA–lysozyme and oregano oil (VP + EL + O). The shelf-life of the samples was determined using both microbiological and sensory analyses. Among the antimicrobial combinations examined in the present study, the treatments VP + EL + R and VP + EL + O were the most effective against the growth of Gram-negative, Gram-positive bacteria, and to a lesser extent on yeasts. Based on both microbiological (TVC data) and sensory (taste attribute) analyses, treatments: VP and VP + O gave a shelf life extension of 6 days, whereas treatments VP + EL + R and VP + EL + O produced a shelf-life extension of 7–8 days, as compared to the control samples.

Industrial Relevance: The present research has highlighted the use of natural antimicrobial treatment combinations, including: EDTA, lysozyme, rosemary and oregano oil and their combinations, in the extension of shelf-life of semi cooked coated chicken fillets stored under vacuum packaging at 4 °C for a period of 18 days. Establishing, the determination of the shelf-life of fresh poultry and products represents a challenge for food companies as poultry meat has a short shelf-life, which causes substantial practical problems for its distribution. Therefore, knowledge of natural preservatives, that can be used as alternatives to chemical additives, that could extend the products' shelf life can have an important economic feedback by reducing losses attributed to spoilage and by allowing the products to reach distant and new markets. This study has shown that combinations of natural antimicrobials can extend the shelf-life of the product.

1. Introduction

Consumer markets are showing an increased demand for a new class of processed foods, the Ready to Eat products. In the production of food it is crucial that proper measures are taken to ensure the safety and stability of the product during its entire shelf-life. Firstly, consumers require better quality, preservative-free, safe but mildly processed foods with extended shelf-life. Secondly, legislation has restricted the use and permitted levels of some currently accepted preservatives in different foods (Brul & Coote, 1999).

The high consumption of poultry, leads to concern that the products marketed should be safe, have a low spoilage rate and show the right composition, packaging, colour, taste and appearance. Products excessively contaminated with microorganisms are undesirable from the standpoint of Public Health, storage quality and general sensory characteristics (Del Río, Panizo-Morán, Prieto, Alonso-Calleja, & Capita, 2007). Despite the work on preservation of fresh poultry meat and/or poultry products using modified atmosphere packaging (MAP) alone (Patsias, Chouliara, Badeka, Savvaidis, & Kontominas, 2006) or in combination with other methods including: treatment with acids (Jimenez, Salsi, Tiburzi, Rafaghelli, & Pirovani, 1997), EDTA–nisin treatment (Cosby, Harrison, & Toledo, 1999), addition of phosphates (Hwang & Beuchat, 1995), essential oils (Chouliara, Badeka, Savvaidis, & Kontominas, 2008), irradiation (Nam and Ahn, 2003; Chouliara, Karatapanis, Savvaidis, & Kontominas, 2007) and recently freeze chilling (Patsias, Badeka, Savvaidis, & Kontominas, 2008) limited or no data are available in the literature on the effect of packaging (MAP/VP) on preservation of pre-cooked chicken products alone or in combination with natural antimicrobial treatments (Patsias et al., 2006). Table 1 summarizes various antimicrobial treatments with hurdle technologies for raw poultry meat and/or poultry products' preservation.

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Table 1
Antimicrobial treatments with hurdle technologies for raw poultry meat and/or poultry products' preservation.

a/a	Poultry	Antimicrobials	Other hurdles	Quality indices	Shelf-life	Reference
1	Fresh chicken wings	Acetic acid	Whirl-pack bags	TVCs, Gram-negative bacterial count	8 days	Kim, Lee, Kim, Moon and Lee (1997)
2	Post-chilled chicken carcasses	Sodium tripolyphosphate		TVCs, *Pseudomonas*, coliforms, *B. thermosphacta*,	10 days	Vareltzis, Soultos, Koidis, Ambrosiadis and Genigeorgis (1997)
3	Post-chilled broiler chicken drummettes with skin	EDTA, Nisin	VP , MAP	TVCs,	18 days	Cosby et al. (1999)
4	Chilled chicken breast portions	Acetic acid	MAP	TVCs	21 days	Jimenez et al. (1997)
5	Refrigerated chicken legs	Trisodium phosphate	–	TVCs	12 days	Kim and Marshall (1999)
6	Raw chicken breast	–	5 °C	TVCs, yeasts and molds	12 days	Ismail et al. (2000)
	Marinated chicken	–	5 °C	TVCs, yeasts and molds	19 days	
7	Refrigerated chicken meat	Nisin, yogurt	–	TVCs, *Pseudomonas*, *Salmonella*	6 days	Göğüş, Bozoglou and Yurdugul (2004)
8	Chicken breasts	Trisodium phosphate, Sodium chloride	Tray-packaged	TVCs, psychrotrophs, *Enterobacteriaceae*	12 days	Sallam and Samejima (2004)
9	Pre-cooked chicken breast fillets	–	MAP	TVCs, LAB, yeasts, molds, *B. thermosphacta*, *Enterobacteriaceae*	20 days	Patsias et al. (2006)
10	Raw chicken breast	Oxalic acid	Oxygen permeable polyethylene bags	TVCs, *Pseudomonas*, *Enterobacteriaceae*	14 days	Anang, Rusul, Radu, Bakar and Beuchat (2006)
11	Fresh chicken breast meat	Oregano oil	MAP	TVCs, *Pseudomonas*, LAB, *B. thermosphacta*, *Enterobacteriaceae*	25 days	Chouliara et al. (2007)
12	Raw chicken fillets	–	Freeze-chilling, MAP	TVCs, *Pseudomonas*, LAB, yeasts, molds, *Enterobacteriaceae*	7 days	Patsias et al. (2008)
13	Fresh chicken meat	Nisin, EDTA	MAP	TVCs, *Pseudomonas*, LAB, *B. thermosphacta*, *Enterobacteriaceae*	14 days	Economou, Pournis, Ntzimani and Savvaidis (2009)
14	Semi-cooked coated chicken fillets	EDTA, lysozyme rosemary, oregano	VP	TVCs, *Pseudomonas*, LAB, yeasts, molds, *B .thermosphacta*	18 days	(our study)

VP = vacuum packaging; MAP = modified atmosphere packaging; TVCs = total viable counts; LAB = lactic acid bacteria.

There has been a great interest within the food industry during the last decade in using enzymes naturally occurring in foods, such as lysozyme (Chumchalová, Josephsen, & Plocková, 1998; Kennedy, O'Rourke, McLay, & Simmonds, 2000; Recio & Visser, 2000). Lysozyme is one of the most frequently used antimicrobial enzymes (Mecitoğlu, Yemenicioğlu, & Arslanoğlu, 2007) and shows antibacterial activity mainly on Gram-positive bacteria by splitting the bonds between *N*-acetylmuramic acid and *N*-acetylglucosamine of the peptidoglycan in cell walls. Because of the protective outer membrane surrounding the peptidoglycan layer of Gram-negative bacteria, lysozyme does not show antibacterial activity against these species (Nattress & Baker, 2003). However, when lysozyme is combined with EDTA the antibacterial spectrum increases significantly and it is effective against Gram-negative organisms (Branen & Davidson, 2004; Padgett, Han, & Dawson, 1998).

Chelators, such as EDTA, can be used as food additives (Russell, 1991). EDTA is known to potentiate the effect of weak acid preservatives against Gram-negative bacteria (Hart, 1984, Shelef & Seiter, 1993). Treatment of Gram-negative bacteria with EDTA makes them sensitive to agents which they normally resist. EDTA will also increase the sensitivity of Gram-negative and Gram positive bacteria to lysozyme (Repaske, 1956). EDTA (21 CFR 172.135) is known as E 385 and its use is permitted in US by the Food and Drug Association (FDA) in contrast to Australia where its use is not allowed. The World Health Organization (WHO) and the FDA have independently assessed the health safety of EDTA and have set the acceptable daily intake of EDTA at 2.5 mg/kg/person/day or roughly 150 mg/day for the calcium disodium salt. Human consumption of EDTA at levels observed in drinking water and taken as part of an average daily food intake, are well within WHO and FDA acceptable guidelines (https://www3.dow.com).

Essential oils (EOs) are known for their antiseptic, i.e. bactericidal, virucidal, fungicidal and medicinal properties and they are used in embalment, preservation of foods and as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and locally anesthesic remedies (Burt, 2004).

Although natural antimicrobial treatments have been used to date on preservation of meat (Gill & Holley, 2000; Mecitoğlu et al., 2007; Nattress & Baker, 2003; Tu & Mustapha, 2002) to our knowledge the combination of hurdles including EDTA, lysozyme, rosemary and oregano EOs for raw poultry and poultry products' preservation has not been reported (Table 1). Thus, the objective of the present study was to investigate the effect of the aforementioned combination in increasing the shelf-life of semi cooked coated chicken fillets stored under VP at 4 °C.

2. Materials and methods

2.1. Preparation of chicken samples and packaging and storage conditions

Semi cooked coated chicken fillets were supplied by a local poultry processing plant (Pindos S.A., Ioannina, Greece) within ca. 8 h after slaughter. Semi cooked coated chicken fillets were prepared using the following procedure: breast fillets, (portions of 100 g) were dipped in a batter, coated with rusk granules and fried at 180 °C for 2 min in a CFC fryer, model GR 3000/40 fryer (CFS, Utrecht, The Netherlands) using corn/sunflower (1:1 v/v) frying oil. Samples were then baked in a CFS oven, model HLT 8000/600 oven (air temperature 150 °C, 5 min) until the core of the product reached an internal temperature of at least 75 °C and immediately cooled at −1 °C, according to the company recipe using standard industrial procedures. Precooked ('Schnitzel' type) poultry products were transported to the laboratory in insulated polystyrene boxes on ice within 1 h of the baking process. The precooked chicken samples (ca. 150 g) were then placed in low-density polyethylene/polyamide/low-density polyethylene (LDPE/PA/LDPE) barrier pouches, 75 mm in thickness having an oxygen permeability of 52.2 ml/m^2 day atm at 60% RH/ 25 °C and water vapour permeability of 2.4 g/m^2 day at 100% RH/25 °C.

The treatments of semi cooked coated chicken fillets examined in the present study were the following: Air-packaged (A, control samples), vacuum-packaged (VP), VP with EDTA–lysozyme solution 1.50% w/w, (VP + EL), VP with rosemary oil 0.20% v/w, (VP + R), VP with oregano oil 0.20% v/w, (VP + O), VP with EDTA–lysozyme solution and rosemary oil (VP + EL + R) and finally VP with EDTA–lysozyme and oregano oil (VP + EL + O).

A PBI-Dansensor model mix 9000 gas mixer (Ringsted, Denmark) was used to achieve the packaging of the samples which was performed in our laboratory. The semi cooked coated chicken fillets