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Application of predictive models to assess the influence of thyme essential oil on *Salmonella* Enteritidis behaviour during shelf life of ready-to-eat turkey products

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ABSTRACT

Consumers' demand for ready-to-eat (RTE) turkey meat is attributed to its convenience and healthy properties. However, as cooked meat product it is subjected to post-process contamination, thus allowing presence and growth of microbial pathogens, such as *Salmonella* spp.. The aim of this study was to include a natural antimicrobial, thyme essential oil (TEO), on RTE turkey products in order to evaluate its effectiveness throughout the shelf life. To do so, the effect of four different formulations of cooked RTE turkey products on *Salmonella* Enteritidis behaviour was investigated. Products' slices were surface inoculated with *S.* Enteritidis (ca. 4 to 5 log cfu/g), subsequently stored at 10 and 25 °C and microbiologically analysed during 18 and 12 days, respectively. Predictive microbiology models fitted to count data were used to evaluate microbial behaviour. Results showed that *S.* Enteritidis behaviour on RTE turkey products slices during storage was strongly dependent on temperature. The pathogen was able to grow on slices at all tested conditions during storage at 25 °C and no statistical differences were detected ($p > 0.05$) between growth parameters. At 10 °C, different behaviour patterns were observed. The application of TEO led to higher *Salmonella* inactivation rates on a product exempt of chemical preservatives. The addition of this novel antimicrobial on meat products or its incorporation on meat active packaging systems as a part of hurdle technology could increase RTE turkey products safety while satisfying the demand of more natural foods.

1. Introduction

Poultry meat consumption has increased worldwide over the last decades (Basu, 2014; Wang et al., 2014). Ready-to-eat (RTE) poultry meats became popular because of their low price, the short preparation time required, besides being characterized by a low fat and salt concentration, which attend the demand of consumers for healthier products (Karadal et al., 2013; Soriano-Santos, 2010).

If hygienic conditions are not accomplished, operations such as packaging, slicing or other RTE meats handling procedures, can lead to cross-contamination in processing facilities or during distribution and storage. In these cases, the risk of foodborne diseases must be considered, since these products are generally not subjected to post-processing treatments that ensure the elimination or reduction of hazardous pathogen levels prior to consumption (Cabedo et al., 2008). Particularly, the slicing operation requires strict hygienic control to prevent cross-contamination as the slicer machines have many components and cavities that may enable bacterial adhesion and biofilm formation (Vorst et al., 2006).

The transfer of *Salmonella* cells from biofilms formed on food-contact surfaces have been demonstrated to be the main cross-contamination source (Wang et al., 2015; Shi and Zhu, 2009). As a consequence, some outbreaks have been linked to the transfer of *Salmonella* biofilm cells from contact-surfaces to food (Srey et al., 2013). Furthermore, *Salmonella* outbreaks linked to the consumption of meat products contaminated during slicing, have been reported (Anonymous, 2007).

Although successful *Salmonella* control programs have contributed to the reduction of human salmonellosis cases associated with poultry meat consumption over the past years (CDC, 2011, 2014, 2015a, 2015b; EFSA, 2015), it is still one of the most important causes of human illnesses worldwide.

It should be noted that at retail points, RTE meat products are sold sliced, and they can be left at abuse conditions, which could allow bacterial growth. In these environments, even when products to be sliced are stored under refrigeration, the variation of temperature between

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slicing events can allow the multiplication of pathogens (Pérez-Rodríguez et al., 2010). At home, on kitchen environments, the sliced products also undergo variation of temperature that could increase the probability of exposure of consumers to microbial risks. The quantitative impact of these handling and storage practices on subsequent pathogen survival and growth has not been yet sufficiently addressed.

Different authors have studied the influence of essential oils (EOs) on the behaviour of pathogens on turkey meat (Ruiz et al., 2009; Sharma et al., 2012). Thanissery and Smith (2014) found out that thyme and orange oils were effective to reduce *S.* Enteritidis and *Campylobacter coli* levels on inoculated broiler breast fillets and whole wings. Nair et al. (2014) evaluated the efficacy of EOs to reduce *Salmonella* levels on turkey breast cutlets. However, few studies have been focused on the influence of plant EOs application as antimicrobials against foodborne pathogens on RTE turkey products.

These edible natural compounds, which have been recognized for their antimicrobial activity, could be added to foods or used in active packaging systems to increase food safety (Bitencourt et al., 2014). Thyme (*Thymus vulgaris* L.) EO (TEO) in vitro activity has been tested against a large range of microorganisms (Burt, 2004; Hyldgaard et al., 2012), nevertheless just a few studies evaluated its antimicrobial activity when applied in meat products or added to active packaging systems (Burt, 2004; Jayasena and Jo, 2013).

Although mathematical models have been used to predict microbial responses to different inactivation treatments, few studies have focused on the inactivation kinetics of EOs on contaminated RTE meats (Zhang et al., 2015). Mathematical models could be tools to help processors to estimate concentrations of these natural antimicrobials on processes to guard RTE products against *Salmonella*.

The aim of this work was to evaluate *S.* Enteritidis behaviour on RTE turkey products, as a function of TEO treatment at different storage temperatures, simulating the contamination occurring during the slicing procedure. Besides, the inhibitory capacity of TEO when added to these products was further modelled and discussed.

2. Material and methods

2.1. Samples

Different products were chosen in order to evaluate how their composition influences *S.* Enteritidis behaviour. Four different formulations of RTE turkey products were chosen and identified as A (low salt and low fat content, presence of sodium nitrite as a preservative), B (preservatives free and low fat content), C (low salt and low fat content) and D (without preservatives) to facilitate comparison. The differences were regarding ingredients concentration, such as salt and fat content (Table 1). The product D was the only one purchased already sliced and modified atmosphere packaged, while the others were available vacuum packaged, in pieces of 0.4 kg each.

The pH values of the products were measured by means of a portable pHmeter (BASIC 20, Crison Instruments, Spain). Water activity (a_w) was measured at 25 °C using the Aqualab equipment (LAB FERRER, Decagon Devices, USA). Measurements were taken at the beginning (day 1) of experiments and can be seen in Table 1.

Table 1
Formulation of the products selected to this study.

Product composition	Product			
	A	B	C	D
Calories (Kcal/100 g)	67.7	62.5	70.9	71.0
Fat (%)	0.5	0.5	0.5	1.0
Salt (NaCl) (%)	1.5	2.1	1.3	2.5
Carbohydrates (%)	1.8	2.5	1.6	1.5
Protein (%)	14.0	12.0	15.0	14.0
a_W	0.97 ± 0.00	0.98 ± 0.02	0.98 ± 0.02	0.98 ± 0.00
pH	6.45 ± 0.02	6.23 ± 0.01	6.34 ± 0.01	6.15 ± 0.03

± represents mean standard deviation.
% Calculated in g/100 g.

2.2. Experimental setup

In an initial set of experiments, the behaviour of *Salmonella* inoculated in products A-D was assessed at 10 and 25 °C without the addition of TEO. Both temperatures were chosen because they could reflect real conditions at distribution points, domestic environments and establishments where products are sliced just before being sold at abuse temperatures (Pérez-Rodríguez et al., 2010).

In a second set of experiments, only products A and B treated with TEO were assessed to study its antibacterial effect. The selection of both RTE turkey products were based on results from the first set of experiments where differences in growth and survival of *S.* Enteritidis were observed among products. In the case of product A, as it was the most permissive to the growth of *S.* Enteritidis in the present study, the addition of TEO was intended to produce a bacteriostatic effect. For product B, as it was the less permissive, TEO addition was mainly performed to evaluate the inhibitory capacity and potential synergistic action with salt present in the composition.

Experiments were conducted three times to capture biological variability and microbiological analysis of slices were replicated twice for each different sample.

2.3. Antimicrobial activity of TEO against Salmonella Enteritidis in vitro

The antimicrobial activity in vitro of TEO against *S.* Enteritidis was studied. Thyme plant oil was chosen based on previously reports of its efficacy against a large range of microorganisms (Burt, 2004; Hyldgaard et al., 2012). The plant was collected on the Collection of Medicinal and Aromatic Plants (CPQBA, Brazil) and its EO was extracted by hydro distillation using a Clevenger-type apparatus (Duarte et al., 2007). Chemical composition was determined by Gas Chromatography-Mass Spectrometry, using a HP6890 (Agilent Technologies, USA) gas chromatograph linked to a HP5975 mass selective detector. The major components of the oil were thymol (60.6%), *p*-cymene (17.0%) and carvacrol (3.8%). Thyme essential oil Minimal Inhibitory Concentration (MIC) against *S.* Enteritidis was determined by means of the colorimetric broth micro dilution technique according to methodology of Salvat et al. (2001).

2.4. Sensory evaluation

A sensory analysis was conducted with the purpose of identifying the maximum concentration of TEO that would be accepted by consumers and could be applied by manufactures on RTE turkey products. The analysis of the products was performed with 50 panellists, potential consumers randomly chosen, with an age range from 18 to 65 years old. The sensory evaluation method was an acceptance test using a 9-point linear hedonic scale, where the answers could vary from "I dislike extremely" (left extreme of the linear scale) and "I like extremely" (right extreme of the linear scale), while the limit of acceptability was 5. Three samples from each product (a total of six) were analysed by each panellist: one sliced sample untreated with EO (control sample) and two sliced samples of the products treated with different TEO concentrations (0.01 and 0.1% v/v), fixed based upon antimicrobial screening conducted in vitro. The products were treated by immersion in different EO concentrations dilutions right before being sliced. Testing was carried out in sensory analysis booths with appropriate lighting conditions, at 25 °C, following Minim (2013) indications.

2.5. Preparation and treatment of products with TEO

A mixture of TEO, Polysorbate 80 (1.0% v/v) (PANREAC, Spain) and sterilized water was stirred up to 2 min on vortex equipment to enable