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The effect of cinnamon, oregano and thyme essential oils in marinade on the microbial shelf life of fish and meat products

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ABSTRACT

Fresh and minimally processed fish and meat are easy targets for microbial spoilage. The demand for natural alternatives to synthetic additives increases. In this study essential oil (EOs) in marinades were used on fish and meat and the effect on the microbial growth during storage was assessed. EOs from *Oreganum compactum* (oregano), *Cinnamomum zeylanicum* (cinnamon), and *Thymus zygis* ct. Thymol (thyme) were chosen. The marinade was composed of water, Na-lactate/lactic acid buffer (2 w/w %), NaCl (10 w/w %), and EO emulsified with Tween 80 and with a pH of 4.5. The necessary Tween 80 to emulsify the EOs in the marinade depended on the EO type and was increased more than tenfold by the NaCl and lactate buffer. The treatment consisted of immersion of meat (pork filet, pork bacon, chicken filets, chicken skin), salmon or scampi for 2 min in marinade solution. The samples were stored at 4 °C in air. Samples were analyzed for microbial counts (dependent on matrix: total coliforms, *Escherichia coli*, lactic acid bacteria, yeasts and molds, total aerobic psychrotrophs). Growth inhibition was achieved with some EO + marinade treatments but marinade itself did not slow down the microbial growth. Most notably, the growth of yeasts and molds was inhibited by immersion of all food matrices in 1 w/w % cinnamon EO. Use of (1 w/w % for all EO) cinnamon EO (+marinade) led to microbial shelf life increase of all matrices (except the chicken matrices as the end of the shelf life was not reached during the experimental duration), oregano EO to shelf life increase of pork filet and salmon, and thyme EO of pork filet and scampi. Sensorial analysis on pork filet and salmon showed that immersion in 3% EO (resulting in 0.09 g EO/100 g pork filet and 0.05 g EO/100 g salmon) resulted in an acceptable odor after 24 h of storage. The results in this study show that the sensorial properties of the meat/fish are inevitably affected when the necessary EO concentrations to extend the microbial shelf life are applied.

1. Introduction

Due to the high water content and availability of important nutrients on the product surface, fresh and minimally processed fish and meat are vulnerable to microbial spoilage (Iturriaga, Olabarrieta, & de Marañón, 2012; Casaburi, Di Martino, Ercolini, Parente, & Villani, 2014). The dominating microbiota on cooled fish products consists of psychrotolerant Gram-negative bacteria (*Pseudomonas* spp., *Shewanella* spp.). When additional stress is created by additional antimicrobial practices (e.g. adding acid, salt, antimicrobial food additives), the harsher environment can lead to a shift in spoilage microorganisms to lactic acid bacteria, yeasts and molds (Gram & Dalgaard, 2002). In meat products, the situation is basically the same although the species of spoilage microorganisms that grow to the highest numbers and dictate the shelf life will differ because the microbial growth rate depends on the nutrient constitution of the food product (Gram et al., 2002).

Marinating is defined as the preincubation of raw meat/fish products with a fluid (Quelhas et al., 2010), aiming to create an additional sensorial value (flavor, tenderness, moistness of the cooked product) and to extend the shelf life (Pathania, McKee, Bilgili, & Singh, 2010). Marinades are water-based solutions that can contain sugar, salt, oil, organic acids, herbs and food additives such as aroma enhancers, antioxidants and antimicrobials (Bjorkroth, 2005). The antimicrobial properties of marinades are

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due to lowering of the pH, lowering of the water activity and addition of certain herbs and antimicrobial food additives (Pathania et al., 2010).

The demand for natural alternatives to synthetic additives increases and the replacement, in foodstuffs, of synthetic antimicrobials such as sorbate and benzoate by essential oils (EOs) is getting considerable attention (Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2014). The active compounds in EOs with antimicrobial properties can be divided as: terpenes, terpenoids, phenylpropenes and others (Hyldgaard, Mygind, & Meyer, 2012). Depending on the active compound in the EO, different microbial targets or processes, especially cellular membranes and cellular energy production, but also less known actions such as inhibition of cell division have been observed or proposed (Hyldgaard et al., 2012). There are indications that the microbial shelf life of certain meat and fish products can be increased by treatment of the foodstuff with certain EOs, and often EO from *Origanum vulgare* or *Thymus vulgaris* has been studied in that context because they contain the antimicrobial compounds thymol and carvacrol (Burt, 2004; Mexis, Chouliara, & Kontominas, 2009; Radha krishnan et al., 2014; Tao, Hill, Peng, & Gomes, 2014). There are precedents that show the potential of EOs for use in marinades. Due to addition of EOs to marinades, both the possibility of reducing pathogens, such as *Salmonella* Enteritidis and *Campylobacter coli* on broiler breast fillet and whole wings (Thanissery & Smith, 2014b), and of inhibiting growth of spoilage microorganisms, such as total mesophilic counts (Thanissery & Smith, 2014a) or *Pseudomonas* spp. and yeasts (Carlos & Harrison, 1999) on broiler breast fillet, have been observed.

Three EOs (from *Origanum compactum*, *Thymus zygis* ct. thymol and *Cinnamomum zeylanicum*) were selected for use in marinades. The effect of the marinades on the spoilage microflora of marinated meat, salmon and scampi was assessed during storage in normal atmospheric conditions at 4 °C.

2. Materials and methods

2.1. Raw materials

Chicken skin, chicken breast fillet, pork (*Longissimus thoracis et lumborum* (LTL)), pork back-fat, salmon (*Salmo salar*) and scampi (*Penaeus monodon*) were acquired from producers and transported (4 °C) to the lab. The used EOs in this study were *C. zeylanicum* (cinnamon EO) from the bark (Biover, Belgium), *Origanum compactum* (oregano EO) from the flowering top (Pranarôm, Belgium) and *T. zygis* ct. thymol (thyme EO) from the flowering plant (Biover, Belgium).

2.2. Marinade solutions

The marinade consisted of 10 w/w % NaCl and 2 w/w % Na-lactate/lactic acid buffer in deionized water with pH 4.5. Tween 80 was added to emulsify the EO (i.e. EO + marinade) in the marinade solution and the appropriate amount of Tween 80 (added as w/w %) was based on the outcome of the stability tests as described in 2.3. Mixing was done at 12,500 rpm for 2 min (T18 digital ultra turrax, IKA, Belgium).

2.3. Stability of essential oil in marinade emulsions

Amounts of Tween 80, EO, NaCl and Na-lactate/lactic acid were varied and the influence on emulsion stability during 24 h of storage at 22 °C was observed. Sunflower oil was added at a concentration of 0–15 w/w %. All emulsions that contained lactic acid were kept at pH 4.5. 10 mL of the emulsions were poured in glass tubes (internal diameter 9 mm) and stored at 22 °C. The stability of emulsions of EO in marinade was assessed by visual observation, i.e. whether a visual (0.5–1 mm layer) creaming layer occurred during the 24 h of storage. At that moment the emulsion was considered unstable. For sensorial and microbial experiments, the optimal settings from the stability experiments (i.e. lowest amount of Tween 80 to emulsify the applied EO concentration and reach a stable emulsion) were applied. The particle size distribution of the emulsions was determined by laser light diffraction (Mastersizer 2000, Malvern, Belgium), with the laser emitting at 633 nm. The Sauter mean diameter for a distribution of discrete entities (d_{32}) was used as this links the area of the dispersed phase to its volume and as such to the mass transfer of the antimicrobial compound (Pacek, Man, & Nienow, 1998):

$$d_{32} = \frac{\sum_{i=1}^{k} n_i d_i^3}{\sum_{i=1}^{k} n_i d_i^2} \quad (1)$$

in which:

n_i is the number of particles with diameter d_i.

The particle size distribution can be represented by its span:

$$span = \frac{d90 - d10}{d50} \quad (2)$$

in which:

dx0 is the diameter corresponding to x0 volume % on a relative cumulative particle size distribution curve.

2.4. Sample preparation and marinating process

For salmon, pork LTL, chicken skin, chicken breast fillet, 10 g of sample was used with a fairly constant surface to volume ratio among samples. The sample was completely immersed in 30 mL of (1 w/w % EO +) marinade for 2 min. The sample was removed from the marinade and left to leak for 5 s. The sample was stored in a sterile stomacher bag (VWR, Belgium) at 4 °C with a small opening to allow gas exchange, i.e. stored in normal atmosphere. For pork back-fat the same was done but with 25 g of sample in 75 mL of (EO+) marinade. The larger sample size was used to assure that the different layers of the pork back-fat (fat layers and meat layers) were represented in each sample.

2.5. Measuring pick-up

The pick-up, i.e. the mass of marinade solution that remains on the sample after marinating, was measured by weighing the sample before and after the immersion and the leaking:

$$pick\ up = \frac{mass_{after} - mass_{before}}{mass_{before}} x\ 100\% \quad (3)$$

in which:

pick up is expressed in g/100 g.
$mass_{after}$ = mass of the sample after immersion in marinade (+EO) solution.
$mass_{before}$ = mass of the sample before immersion in marinade (+EO) solution.