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.

Keywords

Essential oil, microbial shelf life, odor, fish, meat, marinade

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 et al., 2012; Casaburi et al., 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 et al., 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 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 et al., 2014). The active compounds in EOs with antimicrobial properties can be divided as: terpenes, terpenoids, phenylpropenes and others (Hyldgaard et al., 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 et al., 2009; Radha Krishnan et al., 2014; Tao et al., 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 *Cinnamomum zeylanicum* (cinnamon EO) from the bark (Biover, Belgium), *Origanum compactum* (oregano EO) from the flowering top (Pranarôm, Belgium) and *Thymus 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 12500 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 to 15 w/w %. All emulsions that contained lactic acid were kept at pH 4.5. Ten 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 et al., 1998):

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

in which:

n_i is the number of particles with diameter d_i.

The particle size distribution can be represented by its span:

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

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:

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

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.

2.6. Microbial analyses

Ten g of sample was put in a sterile stomacher bag (filter 0.5 mm pore size) (VWR, Belgium) and homogenized during 1 min in 100 mL buffered peptone water (Oxoid, Belgium). Total coliforms and *Escherichia coli* (*E.coli*) were enumerated with Chromocult Coliform-agar (Merck, Germany) using the spreading plate method (incubation at 37 °C, 24 h). Yeasts and molds (Y&M) were enumerated with Rose Bengal Chloramphenicol agar (Oxoid, Belgium) containing 100 mg/L chloramphenicol (incubation at 22 °C, 5 days). Lactic acid bacteria (LAB) were enumerated with MRS (De Man, Rogosa, Sharpe) agar (Oxoid, Belgium), containing 1.4 g/L sorbic acid and with a final pH of 5.7, adjusted with NaOH (1 mol/L), using the pouring plate method with an additional cover layer of agar (incubation at 22°C, 5 days). Total aerobic psychrotrophs (TAP) were enumerated with plate count agar (Oxoid, Belgium) using the pouring plate method (incubation at 22°C, 5 days).

2.7. Sensorial analyses to assess odor acceptability

Sensorial analysis was used to assess whether human subjects could distinguish, based on odor, between samples that were treated with different concentrations of the same EO + marinade (0 to 5 w/w %). For sensorial analyses, triangle tests (ISO 4120:2004) were used in an adjusted form. The subject was asked not only to select the sample that differed from the other two, but also to place the samples on a continuous hedonic scale (0 = very bad, 10 = very good) to assess for the acceptability of the odor of the samples. This value was called the "hedonic value". The samples were prepared as described in section 2.4 and stored for 24 hours in the fridge. After that, samples were assessed by the subjects (raw samples) or baked (baked samples). Baked samples were baked for 1 min at both sides in 1 g butter/ 10 g of meat/fish and subsequently, during baking, turned on the other side every 30 s until the core of the sample reached 72 °C. After baking, these samples were left to cool for 30 min and assessed by the subjects. The control sample consisted of a sample treated with 1 w/w % sunflower oil + marinade and emulsified with 0.1 w/w % Tween 80. The sunflower oil was added in order to avoid visual differentiation by the sensory panel between samples treated with EO + marinade and samples treated with marinade.

2.8. Gas chromatography/mass spectrometry (GC/MS) analysis

GC/MS analysis of the EOs was executed on a 6890 series GC-system (Agilent, Belgium) equipped with a 7683 series injector (Hewlett Packard) and coupled to a 5973 Mass Selective detector (Hewlett Packard, Belgium) in the electron impact ionization mode (70 eV) in the m/z range 40 to 550. The analysis was carried out using a HP-5ms column (methylpolysiloxane, 30 m x 0.25 mm inner diameter, 0.25 µm film thickness, Agilent, Belgium). A time-temperature profile, as described by Espina et al. (2011) was used. The flow of helium, the carrier gas, was kept at 1 mL/min. The EOs were diluted 100 times in n-hexane, and 1 µL was injected in the split mode (ratio 1:100). The analysis was executed three times for each EO. Data acquisition was carried out with GC/MSD ChemStation software (Agilent, United States). Identification was done by matching recorded mass spectra with reference spectra in the computer library (NIST 98 Mass Spectral Library). Carvacrol (Sigma-Aldrich, Belgium), and (E)-cinnamaldehyde (Sigma-Aldrich, Belgium) were also dissolved in n-hexane and injected as described for the EOs in order to use the observed retention times to distinguish between carvacrol and thymol, and between (Z)- and (E)-cinnamaldehyde respectively. For quantification the signal area percentage contribution of each identified compound to the total signal area was used.

2.9. Statistics

To statistically assess the possible presence of growth inhibition due to the treatment solutions the log reduction was used as dependent variable:

$$log\ reduction = log(blank\ as\ cfu/g) - log(treatment\ as\ cfu/g) \quad (3)$$

in which:

blank = stored sample that was not treated (at day x)

treatment = stored sample that was treated with marinade (+EO) (at day x)

Significant growth inhibition compared to the blank or marinade (without EO) samples was assessed with contrast analysis using SPSS Statistics 22 (IBM, United States). As in most cases less importance was given to comparing e.g. 1% cinnamon + marinade with 1% oregano + marinade, contrast analysis was chosen instead of ANOVA or the non-parametric alternatives.

Statistics concerning pick-up and sensorial analyses (hedonic values) were executed with ANOVA, Welch or Kruskal-Wallis (dependent on the presence of normal distributions and/or equal variances between groups) and, if relevant, the respective post-hoc analyses (i.e. Tukey, Games-Howell and Dunn's multiple comparison test). To assess for equal variance among groups Levene's test was used, and for normality Shapiro-Wilk. The probability of a false positive result in the triangle tests was determined via the binomial distribution. The standard deviation was used throughout the manuscript to represent data variation unless otherwise stated.

The microbial shelf life was determined based on microbial shelf life criteria by Uyttendaele et al. (2010). A conservative approach was taken. If, for any measured microbial parameter, the mean log CFU/g food sample, increased with the standard deviation (of the three independent repeats), exceeded the microbial limit for that microbial parameter, the shelf life duration was over. If a treated sample resulted in microbial counts that remained below the microbial limit for a longer duration than the untreated sample, the treatment increased the shelf life. For the meat matrices the following limits were used: 7 log CFU LAB / g, 5 log CFU Y&M /g (and no visible mold growth), 3 log CFU *E. coli* / g. For salmon and scampi the same limits for LAB and Y&M were used, and in addition 7 log CFU TAP /g (Uyttendaele et al., 2010).

3. Results

3.1. Composition of the essential oils

The composition of the *Cinnamomum zeylanicum*, *Origanum compactum* and *Thymus zygis* ct. Thymol used in this research is given in Table 1. Major components (> 5 % abundance) for cinnamon EO were (E)-cinnamaldehyde (66.28 %) and cinnamyl acetate (10.54 %), for oregano EO these were carvacrol (47.80%), thymol (21.41 %), γ-terpinene (13.44%) and p-cymene (8.53 %), and for thyme EO these were thymol (55.91 %), p-cymene (20.61 %), and γ-terpinene (5.59 %).

3.2. Emulsion stability

Cinnamon EO was effectively emulsified in distilled water with a Tween 80:EO ratio of 1:100, whereas a ratio of 1:10 was necessary for oregano and thyme EOs, and for oregano and thyme EO a bimodal particle size distribution was observed at these settings (Table 2), indicating that a small part of the particles had a significantly larger size, and as such indicating a less stable crude emulsion compared to the cinnamon EO-in-water emulsion. More than 10 times the concentration of Tween 80 was required to produce stable EO emulsions in the presence of 10 % NaCl or marinade than in demiwater. The addition of sunflower oil to the EO-in-water emulsions lowered the necessary concentration of Tween 80 for cinnamon and thyme EO but not for oregano EO. The Tween 80:EO ratio and mean particle size of the EO + marinade emulsions that were selected for use in the sensorial and antimicrobial tests are shown in boldface in Table 2, and for each EO the ratio was chosen as the lowest Tween 80:EO ratio that resulted in stable crude emulsions.

3.3. Pick-up

There was a large variability of the pick-up values among food matrices (Table 3), with an order of magnitude difference between the highest (on chicken skin) and lowest pick-up (on scampi). The concentration and type of EO did not influence the pick-up. The pick-up correlated weakly positive with fat (r= 0.453, $p < 5.\ 10^{-4}$), and weakly negative with both protein (r= -0.440; $p < 5.\ 10^{-4}$) and water (r= -0.438; $p < 5.\ 10^{-4}$).

3.4. Influence of essential oils + marinade on the microbial shelf life of fresh meat and fish

Marinade without EO did not reduce the microbial parameters during storage of any researched food matrix except for the reduction of total coliforms on pork back-fat for at least 1 day of storage.

On both chicken matrices, immersion in 1% cinnamon + marinade reduced the counts of some microbial parameters after 6 days of storage (Table 4), i.e. Y&M and LAB in the case of chicken breast fillet and total coliforms, Y&M and LAB in the case of chicken skin. Immersion in 1% oregano + marinade and 1% thyme + marinade were only moderately effective in one case, i.e. a small reduction of Y&M on chicken breast filet was achieved after 6 days. As the microbial shelf life of the chicken matrices was not reached within the duration of the experiment, a potential shelf life increase due to the treatments could not be observed (Table 4).

On pork back-fat, total coliforms were reduced for at least 16 days with 1% cinnamon + marinade and at least 6 days with 1% oregano + marinade and 1% thyme + marinade (Table 5), whereas total coliforms did not grow on pork LTL. *E. coli* did not grow on both the pork matrices. Y&M were reduced during at least 16 days by 1% cinnamon + marinade on both pork matrices and for at least 10 days on pork LTL by 1% oregano + marinade and 1% thyme + marinade. LAB on pork LTL were only reduced after 10 days when treated with 1% oregano + marinade and at least 1 day on pork back-fat with 1% of all three EO + marinade. The microbial shelf life of pork LTL was increased with all three EO + marinade, and that of pork back fat with cinnamon EO + marinade (Table 5).

On salmon, Y&M were reduced for 6 days with 1% cinnamon +marinade, LAB were not reduced, and TAP were reduced for at least 3 days with 1% cinnamon + marinade and 1% oregano + marinade (Table 6). On scampi, there was no growth of Y&M and as such the possible influence of 1% EO + marinade could not be established (Table 6). LAB were reduced for at least 6 days on scampi with 1% oregano + marinade and 1% thyme + marinade and TAP for at least three days for all EO + marinade and at least 6 days for 1% thyme + marinade. The microbial shelf life of salmon was increased with cinnamon and oregano EO, and that of scampi with cinnamon and thyme EO and the marinade treatment (Table 6).

3.5. Sensorial analysis

There is a strong indication that for both the raw and baked pork LTL muscle and salmon a difference in odor was observed between samples treated with 1% sunflower oil + marinade and 1% EO + marinade and between 1% EO + marinade and 5% EO + marinade but not between 1% EO + marinade and 3% EO + marinade (Table 7). For the raw matrices, the samples that were treated with 1 to 5% EO + marinade had a significantly lower hedonic value than those treated with sunflower oil + marinade, except for one instance in the case of salmon (Table 8). For raw salmon, 1% EO + marinade scored higher than 5% EO + marinade. Baking of samples that were treated with EO + marinade increased the acceptability (i.e. hedonic value) of the odor. For the baked matrices the differences in hedonic values between samples treated with EO + marinade and sunflower oil + marinade were mostly insignificant, except for baked pork LTL where oregano EO + marinade scored lower than sunflower oil + marinade. For baked salmon the odor of samples treated with 1% sunflower oil + marinade scored higher than the odor of the samples treated with 5 % EO + marinade. When considering individual treatments (e.g. 1% oregano EO + marinade), some treatments scored lower than 1% sunflower oil + marinade for the raw matrices, but no significant differences were observed for the baked matrices.

4. Discussion

The goal of the EO emulsion stability trials was to create crude EO-in-water emulsions that remained stable during the marinating process, and not to study in detail the influence of the marinade components on the EO emulsion stability. As such, this was not studied nor discussed in depth. However, the detrimental influence of ionic strength on the formation of EO-in-water emulsions is remarkable and an issue that could be relevant for practical application of EOs in certain (food) emulsion systems. The reported used ratios of Tween 80:EO to emulsify EOs are in general between 1:10 to 2:1 (Donsi et al., 2011, 2012; Chang et al., 2012; Terjung et al., 2012; Salvia-Trujillo et al., 2013, 2014; Loeffler et al., 2014; Sugumar et al., 2014; Hashtjin & Abbasi, 2015). Concerning the influence of ionic strength and pH on the stability of EO-in-water however, next to nothing has been published. For non-ionic surfactants such as Tweens, the presence of cations (especially monovalent cations) can be detrimental to the formation of oil-in-water microemulsions due to dehydration of the polar groups which leads to separation of the surfactant from the solution along with the oil (Binks & Dong, 1998; Warisnoicharoen et al. 2000; Hsu & Nacu, 2003). However, in this study the stability of sunflower oil-in-water emulsions was not significantly compromised by the presence of 10 % NaCl. EOs have a relatively low interfacial tension and relatively high polarity. This makes EOs susceptible to Ostwald ripening (i.e. growth of larger droplets at the expense of smaller ones due to diffusion of oil through the aqueous phase) and more susceptible to coalescence (McClements & Rao, 2011). Use of a carrier oil to increase the hydrophobicity of the dispersed phase is a possible strategy for increasing the emulsion stability. Unfortunately, some studies show that, when keeping the absolute concentration of antimicrobial EO (component) constant, a relative increase of carrier oil can decrease the antimicrobial performance of the EO/carrier oil in water emulsion (Chang et al., 2012; Suriyarak & Weiss, 2014). Another strategy would be to apply another surfactant type to prevent coalescence (McClements & Rao, 2011).

The GC/MS results are in line with previous observations that cinnamaldehyde, carvacrol and thymol are the most prevalent compounds in cinnamon EO (Yang et al., 2005; Unlu et al., 2010), oregano EO (Lamiri et al., 2001; Bouchra et al., 2003; Mezzoug et al., 2007), and thyme EO of the thymol type (Bagamboula et al., 2004; Burt, 2004) respectively. Also, p-cymene and γ-terpinene are major compounds of oregano and thyme EOs (Burt et al., 2005), which was also the case in the present study. Most consistent in this study, is the antifungal efficiency of cinnamon EO on all food matrices. In addition to its major abundance in cinnamon EO (> 66 % in this study), cinnamaldehyde is more efficient to inactivate fungi, Gram-negative and Gram-positive bacteria than its structural congeners: cinnamaldehyde > cinnamic acid > cinnamyl alcohol > cinnamyl acetate (Chang et al, 2001; Wang et al., 2005), and as such its contribution to the antimicrobial effect of cinnamon EO is large. Of the compounds found in significant amounts in oregano and thyme EOs, thymol and carvacrol induce the strongest antimicrobial effect as compared to (p-cymene, γ-terpinene etc.) (Bagamboula et al., 2004; Burt et al., 2005; Sokovic et al., 2006). As they are also the compounds with the highest relative abundance in these EOs, the contribution of thymol and carvacrol towards the antimicrobial effect of oregano and thyme EOs is large. Nonetheless, there are some indications that synergy among EO components could occur (Lambert et al., 2002; Periago et al., 2004; Burt et al., 2005), and as such the antimicrobial efficiency of an EO cannot be solely attributed to one or a few of its major compounds without explicit evidence.

Considerable research is published on the use of EOs on meat and fish products in order to extend the microbial shelf life. Chicken breast fillet has been treated with *Oreganum* EOs, mostly *Origanum vulgare* (Chouliara et al., 2007; Khanjari et al., 2013; Fernandez-Pan et al., 2014 ;Radha Krishnan et al., 2014), *Thymus vulgaris* EO (Giatrakou et al., 2010; Thannissery & Smith, 2014a) and *Cinnamomum cassia* (Radha Krishnan et al., 2014). Lean pork meat has been treated with thymol and *Thymus vulgaris* EO (Carramiñana et al., 2008; Tao et al., 2014) and pork back-fat sausages with thymol (Mastromatteo et al., 2011). The published information concerning preservation of salmon (*Salmo salar*) with EOs is limited. However, some research been published on the preservation of the closely related (both belong to the Salmonidae family) rainbow trout (*Onchorynchus mykiss*). Rainbow trout fillet has been treated with *Origanum vulgare* EO (Mexis et al., 2009) and *Cinnamomum zeylanicum* EO (Andevari & Rezaei, 2011). Shrimps (*Palaemon serratus*) have been treated with thymol (Mastromatteo et al., 2010), and precooked peeled shrimps (*Penaeus* spp.) with *Thymus saturoïdes* EO and (E)-cinnamaldehyde (Ouattara et al., 2001). In most of the aforementioned studies, the potential of these EOs to slow the growth of some of the analyzed groups of spoilage microorganisms for a certain period of storage time has been observed, given a sufficient dose of EO. The collective goal of these antimicrobial studies is to gain understanding concerning the dose-response of the EO treatment on the spoilage microorganisms on these foodstuffs. Ultimately the actual EO dose is the pick-up and herein lies the current problem. For virtually all the aforementioned studies, it is unknown how much of the EO actually remained on the food matrix after treatment, which can consist of EO being i) massaged in the food matrix, ii) added to the food matrix, iii) added to the minced food matrix, iv) pipetted on the food matrix, v) the food matrix can be immersed in EO emulsion etc. The results in the current study could be compared with other studies by the pick-up values. In the current study this was done by multiplying the concentration of EO in the marinade with the pick-up values (Table 3). The EO pick-up is a rough estimation because i) not all (EO) components of the marinade are expected to be transferred to the same extent to the food matrix, ii) variance in the pick-up due to transfer of some solid matter from the tissue to the EO + marinade emulsion during the marinating process,

iii) variance in the pick-up due to transfer of water from the tissue to the marinade emulsion because of the high salt content in the marinade emulsion (osmotic effects). These issues were reflected in the relatively high standard deviation in pick-up values for each food matrix. A more accurate method would consist of actually determining the quantity of the adsorbed EO components, through e.g. GC-MS analysis. In order to gain understanding concerning the use of EOs on foods in order to extend the shelf life it is of paramount importance that a method to measure the pick-up is developed and adopted by researchers, because at the moment very little quantitative conclusions can be drawn from the ample collection of generated antimicrobial data.

When EOs are applied in food formulations, the sensorial impact of these EOs is a limitation towards the quantity of EO that can be applied. In this study, baking improved the perception of the odor coming from the baked meat and fish, probably in part due to volatilization of EO compounds during the baking process as well as the mix of the EO odor with generated odorous compounds from the baked matrices. The results suggest that the antimicrobial treatment with 1% EO + marinade could be increased to 3% EO + marinade without compromising the odor of the food matrices. An increase to 5% EO + marinade seems to result in less well perceived odors on baked salmon, as does the use of oregano on baked pork LTL. In this study, only the odor after 1 day of storage was assessed, mainly to detect possible detrimental influences on the fish/meat as this is critical information for valorization of this EO application. As such, the possible beneficial influence of the EOs on the sensorial quality of the meat/fish during storage was not assessed explicitly, only indirectly through microbial enumerations. With an estimated sensorial acceptable concentration in the range between 3 and 5 % EO + marinade immersion treatments, an acceptable pick-up concentration between 0.09 and 0.15 w/w % on pork LTL and between 0.05 and 0.09 w/w % on salmon can be expected. The acceptable EO concentrations are quite diverse when comparing studies. When applying *Origanum vulgare*

EO on meat, the added concentrations that resulted in acceptable odor and taste were in the range of 0.1 to 1 (w/w or v/w) % (Sánchez-Escalante et al., 2003; Skandamis & Nychas, 2001; Chouliara et al., 2007; Govaris et al., 2010; Karabagias et al., 2011; Petrou et al., 2012), while unacceptable added concentrations were in the range 0.2 to 1% (Chouliara et al., 2007; Ntzimani et al., 2010; Karabagias et al., 2011). When applied on fish, acceptable concentrations were in the range 0.1 to 0.4 % (Giatrakou et al., 2008; Mexis et al., 2009; Frangos et al., 2010), while 0.4 % was considered unacceptable on rainbow trout fillet (Frangos et al., 2010). Use of *Thymus vulgaris* EO on meat was acceptable concerning odor and taste in the range of 0.2 to 0.6 % (Solomakos et al., 2008; Giatrakou et al., 2010) but unacceptable at 0.9 % on minced beef (Solomakos et al., 2008). For fish, acceptability was in the range 0.1 to 0.4 % (Kostaki et al., 2009; Kykkidou et al., 2009; Abdollahzadeh et al., 2014) but unacceptable at 0.8 % on minced silver carp (Abdollahzadeh et al., 2014). Cinnamon EO as an antimicrobial on meat and fish has been studied much less than oregano or thyme EO. Treatment of sheep patties by immersion in 0.25 % *Cinnamomum cassia* (Luo et al., 2007) and chicken breast fillet by immersion in 1 % *Cinnamomum cassia* (Radha Krishnan et al., 2014) were found to be acceptable concerning odor and taste. The observed substantial range of acceptable EO concentrations is explained by the actual concentration of EO that remains on/in the meat/fish tissue after treatment, the variation in compatibility between a certain EO and a certain meat/fish product, and the inherent subjectivity that arises when applying small, moderately trained sensory panels (sensory acceptability is a. o. function of age, gender and cultural background) (Samant et al., 2015). Acceptability of EO treated meat/fish does not imply that the EO does not influence the taste and odor. In the current study, the presence of 0.030 ± 0.002 % EO on pork LTL and 0.018 ± 0.002 % EO on salmon (both due to a 2 min dipping treatment in 1% EO + marinade) resulted in observable but acceptable odors after 24 h storage (and cooking). Treatment through addition of 0.1% *Origanum vulgare* to swordfish fillet, 0.2% to rainbow trout fillet, submerging of chicken breast fillet in 1% *Origanum vulgare*, and addition of 0.2 % *Thymus vulgaris* to chicken kebab and sea bass fillet, all resulted in an acceptable but very noticeable taste and odor (Giatrakou et al., 2008, 2009; Frangos et al., 2010; Kostaki et al., 2009; Khanjari et al., 2013). The use of an active compound instead of the EO (e.g. cinnamaldehyde instead of cinnamon EO) would reduce the total amount of added compounds that have sensorial impact on the foodstuff. Although this would not rule out the sensorial limitations, it could potentially improve the usability of these antimicrobials and is worth investigating.

5. Conclusion

Marinade (10% NaCl, 2% lactic acid, pH 4.5) in itself did not inhibit microbial growth on the food matrices. Cinnamon, oregano and thyme EOs, applied at low concentrations, show potential to slow the growth (extend the microbial shelf life) of some spoilage microorganisms on meat/fish products when applied in a marinade. Of particular interest is cinnamon EO, which is especially efficient for inhibition of fungal growth on meat and fish. Combinations of EOs or specific compounds could be a strategy to increase the antimicrobial spectrum. Comparison of research on the effects of EOs on the shelf life of foodstuffs is hampered by the lack of the use of a method that determines the pick-up (or otherwise stated the active dose). As long as such a method is not adopted, quantitative understanding of these antimicrobial treatments remains limited to the applied experimental setup. Besides the antimicrobial effects, the results in this and other studies also show that the sensorial properties of the meat/fish are inevitably affected (positively, neutrally or negatively) when the necessary EO concentrations to extend the microbial shelf life are applied. This implies that the sensorial effect that results from combining a certain EO with a certain meat/fish product is virtually always a significant factor and not all combinations will be acceptable in commercial use.

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References

Abdollahzadeh, E., Rezaei, M., Hosseini, H., 2014. Antibacterial activity of plant essential oils and extracts: The role of thyme essential oil, nisin, and their combination to control *Listeria monocytogenes* inoculated in minced fish meat. Food Control 35, 177-183.

Andevari, G.T., Rezaei, M., 2011. Effect of gelatin coating incorporated with cinnamon oil on the quality of fresh rainbow trout in cold storage. International Journal of Food Science & Technology 46, 2305-2311.

Badr, H.M., 2005. Chemical properties of chicken muscles and skin as affected by gamma irradiation and refrigerated storage. Journal of Food Technology 3, 1-9.

Bagamboula, C.F., Uyttendaele, M., Debevere, J., 2004. Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri*. Food Microbiology 21, 33-42.

Binks, B.P., Dong, J., 1998. Emulsions and equilibrium phase behaviour in silicone oil + water + nonionic surfactant mixtures. Colloids and Surfaces A: Physicochemical and Engineering Aspects 132, 289-301.

Björkroth, J., 2005. Microbiological ecology of marinated meat products. Meat Science 70, 477-480.

Bonifer, L.J., Froning, G.W., Mandigo, R.W., Cuppett, S.L., Meagher, M.M., 1996. Textural, color, and sensory properties of bologna containing various levels of washed chicken skin. Poultry Science 75, 1047-1055.

Bouchra, C., Achouri, M., Idrissi Hassani, L.M., Hmamouchi, M., 2003. Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against *Botrytis cinerea* Pers: Fr. Journal of Ethnopharmacology 89, 165-169.

Burt, S.A., 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. International Journal of Food Microbiology 94, 223-253.

Burt, S.A., Vlielander, R., Haagsman, H.P., Veldhuizen, E.J.A., 2005. Increase in activity of essential oil components carvacrol and thymol against *Escherichia coli* O157:H7 by addition of food stabilizers. Journal of Food Protection 68, 919-926.

Carlos, A.M.A., Harrison, M.A., 1999. Inhibition of selected microorganisms in marinated chicken by pimento leaf oil and clove oleoresin. The Journal of Applied Poultry Research 8, 100-109.

Carramiñana, J.J., Rota, C., Burillo, J., Herrera, A., 2008. Antibacterial efficiency of Spanish *Satureja montana* essential oil against *Listeria monocytogenes* among natural flora in minced pork. Journal of Food Protection 71, 502-508.

Casaburi, A., Di Martino, V., Ercolini, D., Parente, E., Villani, F., 2014. Antimicrobial activity of *Myrtus communis* L. water-ethanol extract against meat spoilage strains of *Brochothrix thermosphacta* and *Pseudomonas fragi* in vitro and in meat. Annals of Microbiology 65, 841-850.

Chang, S.-T., Chen, P.-F., Chang, S.-C., 2001. Antibacterial activity of leaf essential oils and their constituents from *Cinnamomum osmophloeum*. Journal of Ethnopharmacology 77, 123-127.

Chang, Y., McLandsborough, L., McClements, D.J., 2012. Physical properties and antimicrobial efficacy of thyme oil nanoemulsions: influence of ripening inhibitors. Journal of Agricultural and Food Chemistry 60, 12056-12063.

Chouliara, E., Karatapanis, A., Savvaidis, I.N., Kontominas, M.G., 2007. Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4 °C. Food Microbiology 24, 607-617.

Donsì, F., Annunziata, M., Vincensi, M., Ferrari, G., 2012. Design of nanoemulsion-based delivery systems of natural antimicrobials: Effect of the emulsifier. Journal of Biotechnology 159, 342-350.

Espina, L., Somolinos, M., Lorán, S., Conchello, P., García, D., Pagán, R., 2011. Chemical composition of commercial citrus fruit essential oils and evaluation of their antimicrobial activity acting alone or in combined processes. Food Control 22, 896-902.

Fernández-Pan, I., Carrión-Granda, X., Maté, J.I., 2014. Antimicrobial efficiency of edible coatings on the preservation of chicken breast fillets. Food Control 36, 69-75.

Frangos, L., Pyrgotou, N., Giatrakou, V., Ntzimani, A., Savvaidis, I.N., 2010. Combined effects of salting, oregano oil and vacuum-packaging on the shelf-life of refrigerated trout fillets. Food Microbiology 27, 115-121.

Giatrakou, V., Kykkidou, S., Papavergou, A., Kontominas, M.G., Savvaidis, I.N., 2008. Potential of oregano esssential oil and MAP to extend the shelf life of fresh swordfish: a comparative study with ice storage. Journal of Food Science 73, M167-M173.

Giatrakou, V., Ntzimani, A., Savvaidis, I.N., 2010. Combined chitosan-thyme treatments with modified atmosphere packaging on a ready-to-cook poultry product. Journal of Food Protection 73, 663-669.

Govaris, A., Solomakos, N., Pexara, A., Chatzopoulou, P.S., 2010. The antimicrobial effect of oregano essential oil, nisin and their combination against *Salmonella Enteritidis* in minced sheep meat during refrigerated storage. International Journal of Food Microbiology 137, 175-180.

Gram, L., Dalgaard, P., 2002. Fish spoilage bacteria – problems and solutions. Current Opinion in Biotechnology 13, 262-266.

Gram, L., Ravn, L., Rasch, M., Bruhn, J.B., Christensen, A.B., Givskov, M., 2002. Food spoilage—interactions between food spoilage bacteria. International Journal of Food Microbiology 78, 79-97.

Hashtjin, A., Abbasi, S., 2015. Optimization of ultrasonic emulsification conditions for the production of orange peel essential oil nanoemulsions. Journal of Food Science and Technology 52, 2679-2689.

Hsu, J.-P., Nacu, A., 2003. Behavior of soybean oil-in-water emulsion stabilized by nonionic surfactant. Journal of Colloid and Interface Science 259, 374-381.

Hyldgaard, M., Mygind, T., Meyer, R.L., 2012. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Frontiers in Microbiology 3, 12.

Iturriaga, L., Olabarrieta, I., de Marañón, I.M., 2012. Antimicrobial assays of natural extracts and their inhibitory effect against *Listeria innocua* and fish spoilage bacteria, after incorporation into biopolymer edible films. International Journal of Food Microbiology 158, 58-64.

ISO 4200:2004 (2004). Sensory analysis-Methodology-Triangle test. International Organization for Standardization.

Karabagias, I., Badeka, A., Kontominas, M.G., 2011. Shelf life extension of lamb meat using thyme or oregano essential oils and modified atmosphere packaging. Meat Science 88, 109-116.

Khanjari, A., Karabagias, I.K., Kontominas, M.G., 2013. Combined effect of N,O-carboxymethyl chitosan and oregano essential oil to extend shelf life and control *Listeria monocytogenes* in raw chicken meat fillets. LWT - Food Science and Technology 53, 94-99.

Kostaki, M., Giatrakou, V., Savvaidis, I.N., Kontominas, M.G., 2009. Combined effect of MAP and thyme essential oil on the microbiological, chemical and sensory attributes of organically aquacultured sea bass (*Dicentrarchus labrax*) fillets. Food Microbiology 26, 475-482.

Kykkidou, S., Giatrakou, V., Papavergou, A., Kontominas, M.G., Savvaidis, I.N., 2009. Effect of thyme essential oil and packaging treatments on fresh Mediterranean swordfish fillets during storage at 4 °C. Food Chemistry 115, 169-175.

Lambert, R.J., Skandamis, P.N., Coote, P.J., Nychas, G.J., 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. Journal of Applied Microbiology 91, 453-462.

Lamiri, A., Lhaloui, S., Benjilali, B., Berrada, M., 2001. Insecticidal effects of essential oils against Hessian fly, Mayetiola destructor (Say). Field Crops Research 71, 9-15.

Loeffler, M., Beiser, S., Suriyarak, S., Gibis, M., Weiss, J., 2014. Antimicrobial efficacy of emulsified essential oil components against weak acid-adapted spoilage yeasts in clear and cloudy apple juice. Journal of Food Protection 77, 1325-1335.

Luo, H., Lin, S., Ren, F., Wu, L., Chen, L., Sun, Y., 2007. Antioxidant and antimicrobial capacity of Chinese medicinal herb extracts in raw sheep Meat. Journal of Food Protection 70, 1440-1445.

Mastromatteo, M., Danza, A., Conte, A., Muratore, G., Del Nobile, M.A., 2010. Shelf life of ready to use peeled shrimps as affected by thymol essential oil and modified atmosphere packaging. International Journal of Food Microbiology 144, 250-256.

Mastromatteo, M., Incoronato, A.L., Conte, A., Del Nobile, M.A., 2011. Shelf life of reduced pork back-fat content sausages as affected by antimicrobial compounds and modified atmosphere packaging. International Journal of Food Microbiology 150, 1-7.

Matsuzaki, Y., Kakinoki, Y., Nakamura, M., Nishihara, T., Tsujisawa, T., 2014. Lamiaceae peppermint oil with surfactant showing equal antifungal activity against *Candida albicans* to rosemary chemotype cineol. Advances in Infectious Diseases, 4, 58-65.

McClements, D.J., Rao, J., 2011. Food-Grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. Critical Reviews in Food Science and Nutrition 51, 285-330.

Mexis, S.F., Chouliara, E., Kontominas, M.G., 2009. Combined effect of an oxygen absorber and oregano essential oil on shelf life extension of rainbow trout fillets stored at 4 °C. Food Microbiology 26, 598-605.

Mezzoug, N., Elhadri, A., Dallouh, A., Amkiss, S., Skali, N.S., Abrini, J., Zhiri, A., Baudoux, D., Diallo, B., El Jaziri, M., Idaomar, M., 2007. Investigation of the mutagenic and antimutagenic effects of Origanum compactum essential oil and some of its constituents. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 629, 100-110.

Ntzimani, A.G., Giatrakou, V.I., Savvaidis, I.N., 2010. 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. Innovative Food Science & Emerging Technologies 11, 187-196.

Ouattara, B., Sabato, S.F., Lacroix, M., 2001. Combined effect of antimicrobial coating and gamma irradiation on shelf life extension of pre-cooked shrimp (*Penaeus* spp.). International Journal of Food Microbiology 68, 1-9.

Pacek, A.W., Man, C.C., Nienow, A.W., 1998. On the Sauter mean diameter and size distributions in turbulent liquid/liquid dispersions in a stirred vessel. Chemical Engineering Science 53, 2005-2011.

Pathania, A., McKee, S.R., Bilgili, S.F., Singh, M., 2010. Antimicrobial activity of commercial marinades against multiple strains of *Salmonella* spp. International Journal of Food Microbiology 139, 214-217.

Periago, P.M., Delgado, B., Fernandez, P.S., Palop, A., 2004. Use of carvacrol and cymene to control growth and viability of *Listeria monocytogenes* cells and predictions of survivors using frequency distribution functions. Journal of Food Protection 67, 1408-1416.

Petrou, S., Tsiraki, M., Giatrakou, V., Savvaidis, I.N., 2012. Chitosan dipping or oregano oil treatments, singly or combined on modified atmosphere packaged chicken breast meat. International Journal of Food Microbiology 156, 264-271.

Quelhas, I., Petisca, C., Viegas, O., Melo, A., Pinho, O., Ferreira, I.M.P.L.V.O., 2010. Effect of green tea marinades on the formation of heterocyclic aromatic amines and sensory quality of pan-fried beef. Food Chemistry 122, 98-104.

Radha krishnan, K., Babuskin, S., Azhagu Saravana Babu, P., Sasikala, M., Sabina, K., Archana, G., Sivarajan, M., Sukumar, M., 2014. Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. International Journal of Food Microbiology 171, 32-40.

Salvia-Trujillo, L., Rojas-Graü, M.A., Soliva-Fortuny, R., Martín-Belloso, O., 2013. Effect of processing parameters on physicochemical characteristics of microfluidized lemongrass essential oil-alginate nanoemulsions. Food Hydrocolloids 30, 401-407.

Salvia-Trujillo, L., Rojas-Graü, M.A., Soliva-Fortuny, R., Martín-Belloso, O., 2014. Impact of microfluidization or ultrasound processing on the antimicrobial activity against *Escherichia coli* of lemongrass oil-loaded nanoemulsions. Food Control 37, 292-297.

Samant, S.S., Crandall, P.G., O'Bryan, C., Lingbeck, J.M., Martin, E.M., Seo, H.-S., 2015. Sensory impact of chemical and natural antimicrobials on poultry products: a review. Poultry Science 94, 1699-1710.

Skandamis, P.N., Nychas, G.J.E., 2001. Effect of oregano essential oil on microbiological and physico-chemical attributes of minced meat stored in air and modified atmospheres. Journal of Applied Microbiology 91, 1011-1022.

Soković, M., van Griensven, L.L.D., 2006. Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, *Agaricus bisporus*. European Journal of Plant Pathology 116, 211-224.

Solomakos, N., Govaris, A., Koidis, P., Botsoglou, N., 2008. The antimicrobial effect of thyme essential oil, nisin, and their combination against *Listeria monocytogenes* in minced beef during refrigerated storage. Food Microbiology 25, 120-127.

Suriyarak, S., Weiss, J., 2014. Cutoff Ostwald ripening stability of alkane-in-water emulsion loaded with eugenol. Colloids and Surfaces A: Physicochemical and Engineering Aspects 446, 71-79.

Sánchez-Escalante, A., Djenane, D., Torrescano, G., Beltrán, J.A., Roncales, P., 2003. Antioxidant action of borage, rosemary, oregano, and ascorbic acid in beef patties packaged in modified atmosphere. Journal of Food Science 68, 339-344.

Tao, F., Hill, L.E., Peng, Y., Gomes, C.L., 2014. Synthesis and characterization of β-cyclodextrin inclusion complexes of thymol and thyme oil for antimicrobial delivery applications. LWT - Food Science and Technology 59, 247-255.

Terjung, N., Loffler, M., Gibis, M., Hinrichs, J., Weiss, J., 2012. Influence of droplet size on the efficacy of oil-in-water emulsions loaded with phenolic antimicrobials. Food & Function 3, 290-301.

Thanissery, R., Smith, D.P., 2014a. Effect of marinade containing thyme and orange oils on broiler breast fillet and whole wing aerobic bacteria during refrigerated storage. The Journal of Applied Poultry Research 23, 228-232.

Thanissery, R., Smith, D.P., 2014b. Marinade with thyme and orange oils reduces *Salmonella Enteritidis* and *Campylobacter coli* on inoculated broiler breast fillets and whole wings. Poultry Science 93, 1258-1262.

Unlu, M., Ergene, E., Unlu, G.V., Zeytinoglu, H.S., Vural, N., 2010. Composition, antimicrobial activity and in vitro cytotoxicity of essential oil from *Cinnamomum zeylanicum* Blume (Lauraceae). Food and Chemical Toxicology 48, 3274-3280.

Uyttendaele, M., Jacxsens, L., De Loy-Hendrickx, A., Devlieghere, F., Debevere, J., 2010. Microbiologische richtwaarden en wettelijke microbiologische criteria. Ghent University. Faculty of Bioscience Engineering. Laboratory of Food Microbiology and Biotechnology. ISBN: 9789059893856. Available: http://biblio.ugent.be/publication/1169787/file/6867231.

Wang, S.-Y., Chen, P.-F., Chang, S.-T., 2005. Antifungal activities of essential oils and their constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) leaves against wood decay fungi. Bioresource Technology 96, 813-818.

Warisnoicharoen, W., Lansley, A.B., Lawrence, M.J., 2000. Nonionic oil-in-water microemulsions: the effect of oil type on phase behaviour. International Journal of Pharmaceutics 198, 7-27.

Yang, Y.-C., Lee, H.-S., Lee, S.H., Clark, J.M., Ahn, Y.-J., 2005. Ovicidal and adulticidal activities of *Cinnamomum zeylanicum* bark essential oil compounds and related compounds against *Pediculus humanus capitis* (Anoplura: Pediculicidae). International Journal for Parasitology 35, 1595-1600.

Table 1. Composition of the essential oils *Cinnamomum zeylanicum*, *Origanum compactum*, *Thymus zygis* ct. thymol (expressed as % of the ion signal area) (n=3)

retention time (min)	compound	*Cinnamomum zeylanicum*	*Origanum compactum*	*Thymus zygis* ct. Thymol
7.13	α-thujene	0.18	0.48	0.60
7.41	α-pinene	0.98	0.43	0.81
8.06	camphene	0.45	0.06	0.65
9.40	β-pinene	0.28	0.06	0.15
10.19	β-myrcene		0.91	0.97
10.83	α-phellandrene	0.99	0.17	0.13
11.49	α-terpinene	0.94	1.70	1.12
11.94	p-cymene	2.43	8.53	20.61
12.14	sylvestrene/limonene		0.38	0.53
12.15	β-phellandrene	3.94		
12.26	eucalyptol	0.30	0.05	0.32
13.86	γ-terpinene	0.12	13.44	5.59
15.54	terpinolene	0.12	0.08	0.27
16.26	linalool	1.60	1.05	3.30
18.62	L-camphor			0.35
19.88	borneol		0.13	1.40
19.90	hydrocinnamic aldehyde	0.46		
20.58	terpinen-4-ol	0.66	0.43	0.76
21.39	α-terpineol	0.65	0.19	0.18
23.04	(Z)-cinnamaldehyde	0.64		
23.71	hydrocinnamyl alcohol	0.22		
24.42	thymyl methyl ether		0.12	0.62
25.94	(E)-cinnamaldehyde	66.28		
27.28	thymol		21.41	55.91
27.76	carvacrol	0.12	47.80	2.90
30.51	eugenol	2.25		
31.33	α-copaene	0.38		
33.50	β-caryopyllene	2.40	1.56	1.13
34.95	cinnamyl acetate	10.54		
35.19	α-caryophyllene	1.97	0.07	
41.35	caryophylllene-oxide	0.56		
	Not identified	0.61	0.95	1.74

Table 2. Necessary ratio of Tween 80:EO to emulsify 10 w/w % of the studied essential oils in the presence of NaCl, lactic acid buffer, and sunflower oil (n=2)

EO	NaCl (m%)	lactic acid buffer (w/w %)	sunflower oil (w/w %)	Tween 80:EO	Particle size (µm)	Span
cinnamon	0	0	0	1:100	0.40[A]	2.75
	0	0	0	1:10	0.26	2.62
	0	2	0	1:10	ND[B]	
	10	0	0	2:10	ND	
	10[C]	**2**	**0**	**2:10**	**0.23**	**3.22**
	10	2	0	12:10	0.19	3.29
	10	2	5	1:10	0.52	2.16
oregano	0	0	0	1:10	0.24	6.14*
	0	2	0	7:10	ND	
	10	0	0	12:10	ND	
	10	**2**	**0**	**12:10**	**0.20**	**2.61**
	10	2	0-15	>7:10	ND	
thyme	0	0	0	1:10	0.41	13.0*
	0	2	0	7:10	ND	
	10	0	0	10:10	ND	
	10	**2**	**0**	**12:10**	**0.20**	**2.53**
	10	2	5	7:10	0.21	123.0*

[A] Sauter mean diameter (d_{32}), [B] ND: not determined, [C] lines in boldface denote the EO + marinade emulsions used in the sensorial and antimicrobial experiments, * a bimodal particle size distribution was observed.

Table 3. Pick-up of EO + marinade on the studied food matrices[A] (n=20)

food matrix	pick-up (marinade) g/100 g	estimated pick-up (EO) g/100 g	fat g/100 g	protein g/100 g	water g/100 g
chicken skin	9.0±1.1[B]	0.090±0.011	44.9	9.6	42.9
chicken filet	4.9±0.5	0.049±0.005	1.3	22.8	74
pork back fat	4.2±0.4	0.042±0.004	53.3	10.6	34
pork LTL	3.0±0.2	0.030±0.002	1.9	20.5	76
salmon	1.8±0.2	0.018±0.002	16.5	18.4	63
scampi	0.9±0.4	0.009±0.004	0.1	17.5	79

[A]fat, protein and water content were acquired from the food producer and www.internubel.be and for chicken skin from (Bonifer et al., 1996; Badr, 2005), [B] standard error of mean

Table 4. Microbial counts (log CFU/g) of selected microbial parameters during storage of treated chicken breast filet and chicken skin (n=3)

		chicken breast filet		**chicken skin**	
storage time (days)		**1**	**6**	**1**	**6**
total coliforms	blank	2.1±0.3	2.7±0.6	3.8±0.5	5.2±0.4
	marinade	1.9±0.1	2.3±0.8	3.6±0.2	4.9±0.1
	1% cinnamon + marinade	2.2±0.4	2.9±1.5	3.5±0.1	4.1±0.5[A]
	1% oregano + marinade	1.9±0.1	3.4±0.4	4.6±0.7	4.6±0.7
	1% thyme + marinade	1.9±0.0	2.8±1.3	3.8±0.4	4.4±1.1
E. coli	blank	<2	<2	3.2±0.2	2.8±0.5
	marinade	<2	<2	3.3±0.1	3.0±0.4
	1% cinnamon + marinade	<2	<2	3.1±0.2	2.6±0.4
	1% oregano + marinade	<2	<2	2.9±0.6	2.7±0.3
	1% thyme + marinade	<2	<2	3.2±0.2	2.7±0.4
Y&M	blank	2.2±0.3	3.7±0.4	2.8±0.4	4.1±0.2
	marinade	1.9±0.1	3.5±0.3	2.7±0.1	4.1±0.2
	1% cinnamon + marinade	2.2±0.1	2.7±0.8[A,B]	2.7±0.3	3.4±0.4[A,B]
	1% oregano + marinade	1.9±0.1	3.1±0.1	3.0±0.3	4.0±0.1
	1% thyme + marinade	2.0±0.2	3.1±0.2	3.2±0.4	4.4±0.3
LAB	blank	1.8±0.6	3.6±0.2	3.5±0.4	5.6±0.3
	marinade	1.6±0.2	3.1±0.4	3.5±0.2	5.3±0.5
	1% cinnamon + marinade	1.7±0.9	2.6±0.2[A,B]	3.9±0.3	4.9±0.3[A]
	1% oregano + marinade	1.4±0.6	3.0±1.1	3.4±0.3	5.0±0.6
	1% thyme + marinade	2.0±0.7	3.0±1.0	4.3±0.7	5.3±0.5

[A] significant reduction ($p < 0.05$) compared to the untreated (blank) sample, [B] significant reduction ($p < 0.05$) compared to the marinated (without EO) samples.

Table 5. Microbial counts (log CFU/g) of selected microbial parameters during storage of treated pork LTL and pork back-fat (n=3)

		pork LTL				pork back-fat			
storage time (days)		**1**	**6**	**10**	**16**	**1**	**6**	**10**	**16**
Total	blank	<2	<2	<2	<2	3.8±0.5	5.9±0.4	5.7±1.1	5.9±0.9
	marinade	<2	<2	<2	<2	2.4±0.2^{A}	5.3±0.7	5.1±1.3	5.3±1.0
	1% cinnamon + marinade	<2	<2	<2	<2	2.1±0.2^{A}	3.1±1.0^{A,B}	4.0±1.8	3.3±2.3^{A}
	1% oregano + marinade	<2	<2	<2	<2	2.3±0.6^{A}	3.5±1.5^{A}	5.5±1.3	5.5±0.7
	1% thyme + marinade	<2	<2	<2	<2	2.3±0.6^{A}	3.2±2.1^{A}	3.8±1.9	4.6±2.3
E. coli	all treatments	<2	<2	<2	<2	<2	<2	<2	<2
Y&M	blank	2.3±0.6	4.7±0.5†	6.6±0.4	6.2±0.5	4.1±0.2	6.3±0.1†	6.3±0.4	7.0±0.4
	marinade	2.1±0.2	4.9±0.2†	6.5±0.6	7.3±0.6	4.2±0.2	6.4±0.1†	6.9±0.1	7.3±0.3
	1% cinnamon + marinade	2.0±0.0	2.3±0.5^{A,B}	3.5±0.6^{A,B}	4.9±1.1^{A,B}†	2.4±0.6^{A,B}	2.7±0.9^{A,B}	3.0±1.0^{A,B}	3.4±2.1^{A,B}†
	1% oregano + marinade	2.2±0.2	3.8±0.6^{A,B}	5.7±0.1^{A,B}†	6.1±1.2	3.2±1.1	5.3±1.7†	6.1±0.6	6.2±1.1
	1% thyme + marinade	2.0±0.0	3.8±0.8^{A,B}	5.6±0.4^{A,B}†	6.7±0.5	3.3±1.1	5.0±2.0†	6.0±0.7^{B}	6.9±0.5
LAB	blank	1.8±0.6	5.1±0.5	6.8±0.3	7.2±0.5	2.8±0.4	5.1±0.2	5.7±0.6	5.8±0.9
	marinade	1.2±0.2	4.6±0.4	6.5±0.7	7.0±0.6	2.6±0.1	5.3±0.8	5.7±0.5	5.9±0.6
	1% cinnamon + marinade	1.3±0.3	4.7±0.4	6.1±0.3	7.2±0.7†	2.1±0.4^{A}	4.0±1.3	5.6±0.7	6.4±0.3
	1% oregano + marinade	1.4±0.3	4.6±0.3	5.9±0.5^{A}	6.5±0.4	2.0±0.3^{A}	4.5±0.9	5.9±0.5	5.8±1.5
	1% thyme + marinade	1.0±0.1	4.5±0.6	6.1±0.7	7.1±0.6	2.1±0.5^{A}	5.1±0.5	4.9±1.4	5.4±1.5

^{A} significant reduction ($p < 0.05$) compared to the untreated (blank) sample, ^{B} significant reduction ($p < 0.05$) compared to the marinated (without EO) samples, † the end of shelf life is reached due to the value of this microbial parameter.

Table 6. Microbial counts (log CFU/g) of selected microbial parameters during storage of treated salmon and scampi (n=3)

		salmon			scampi		
storage time (days)		**1**	**3**	**6**	**1**	**3**	**6**
Y&M	blank	3.1±0.3	3.7±0.1	4.7±0.2	<2	<2	<2
	marinade	3.2±0.2	3.8±0.2	5.0±0.1†	<2	<2	<2
	1% cinnamon + marinade	2.1±0.2^{A,B}	3.4±0.6	2.9±0.7^{A,B}	<2	<2	<2
	1% oregano + marinade	2.9±0.2	3.8±0.4	4.8±0.2†	<2	<2	<2
	1% thyme + marinade	3.0±0.3	3.8±0.2	4.7±0.1	<2	<2	2.2±0.4
LAB	blank	<1	3.1±0.2	2.9±0.3	1.1±0.1	1.8±0.1	2.6±0.5
	marinade	<1	2.9±0.1	3.3±0.1	1.7±0.6	2.2±1.0	2.6±0.8
	1% cinnamon + marinade	<1	2.4±0.2	3.1±0.1	1.2±0.2	1.4±0.4	1.8±0.7
	1% oregano + marinade	<1	2.9±0.4	3.1±0.3	1.0±0.0	1.3±0.5	1.2±0.2^{A,B}
	1% thyme + marinade	<1	3.1±0.1	3.0±0.1	1.3±0.2	1.1±0.2^{A}	1.0±0.1^{A,B}
TAP	blank	5.5±0.3	7.3±0.4†	9.3±0.4	5.1±0.2	5.7±0.4	8.0±2.3†
	marinade	5.3±0.3	6.7±0.3†	8.7±0.7	4.7±0.5	5.5±0.1	6.5±0.4
	1% cinnamon + marinade	4.5±0.2^{A,B}	6.2±0.3^{A,B}	8.9±0.6†	4.2±0.2^{A}	4.8±0.4^{A}	6.0±0.3
	1% oregano + marinade	5.1±0.3	6.5±0.1^{A}	9.2±0.5†	4.2±0.3^{A}	3.7±0.7^{A,B}	7.4±2.1†
	1% thyme + marinade	5.1±0.2	7.0±0.2†	9.5±0.0	4.0±0.5^{A}	3.9±0.5^{A,B}	5.6±0.3^{A,B}

^{A} significant reduction ($p < 0.05$) compared to the untreated (blank) sample, ^{B} significant reduction ($p < 0.05$) compared to the marinated (without EO) samples, † the end of shelf life is reached due to the value of this microbial parameter.

Table 7. Results of triangle tests for detecting a difference between raw and fried pork LTL and salmon treated with sunflower oil/EO+marinade

raw pork LTL	**correct**	**α-risk[A]**
sunflower oil 1% *VS* cinnamon 1%	10/10	**<0.1%**
sunflower oil 1% *VS* oregano 1%	9/10	**<0.1%**
sunflower oil 1% *VS* thyme 1%	10/10	**<0.1%**
cinnamon 1% *VS* cinnamon 3%	3/10	>20%
oregano 1% *VS* oregano 3%	4/10	>20%
thyme 1% *VS* thyme 3%	6/10	8%
cinnamon 1% *VS* cinnamon 5%	7/10	**2%**
oregano 1% *VS* oregano 5%	6/10	8%
thyme 1% *VS* thyme 5%	6/10	8%
sunflower oil 1% *VS* EO 1%	29/30	**<0.1%**
EO 1% *VS* EO 3%	13/30	17%
EO 1% *VS* EO 5%	19/30	**<0.1%**
raw salmon	**correct**	**α-risk**
sunflower oil 1% *VS* cinnamon 1%	8/10	**0.3%**
sunflower oil 1% *VS* oregano 1%	10/10	**<0.1%**
sunflower oil 1% *VS* thyme 1%	7/10	**2%**
cinnamon 1% *VS* cinnamon 3%	5/10	>20%
oregano 1% *VS* oregano 3%	3/10	>20%
thyme 1% *VS* thyme 3%	5/10	>20%
cinnamon 1% *VS* cinnamon 5%	7/10	**2%**
oregano 1% *VS* oregano 5%	5/9	>20%
thyme 1% *VS* thyme 5%	3/10	>20%
sunflower oil 1% *VS* EO 1%	25/30	**<0.1%**
EO 1% *VS* EO 3%	13/30	17%
EO 1% *VS* EO 5%	15/29	**3%**
fried pork LTL	**correct**	**α-risk**
sunflower oil 1% *VS* cinnamon 1%	7/8	**0.3%**
sunflower oil 1% *VS* oregano 1%	6/8	**2%**
sunflower oil 1% *VS* thyme 1%	7/8	**0.3%**
cinnamon 1% *VS* cinnamon 5%	4/8	>20%
oregano 1% *VS* oregano 5%	6/8	**2%**
thyme 1% *VS* thyme 5%	5/8	9%
sunflower oil 1% *VS* EO 1%	20/24	**<0.1%**
EO 1% *VS* EO 5%	15/24	**0.3%**
fried salmon	**correct**	**α-risk**
sunflower oil 1% *VS* cinnamon 1%	7/8	**0.3%**
sunflower oil 1% *VS* oregano 1%	5/8	9%
sunflower oil 1% *VS* thyme 1%	4/8	>20%
cinnamon 1% *VS* cinnamon 5%	5/8	9%
oregano 1% *VS* oregano 5%	6/8	**2%**
thyme 1% *VS* thyme 5%	4/8	>20%
sunflower oil 1% *VS* EO 1%	16/24	**<0.1%**
EO 1% *VS* EO 5%	15/24	**0.3%**

[A]probability of false positive result, determined via the binomial distribution

Table 8. Summary of hedonic values for each treatment and food matrix

	pork LTL hedonic value		salmon hedonic value	
raw	**number of tests**	**mean**	**number of tests**	**mean**
sunflower oil1%+marinade	45	6.6±2.2	30	6.6±2.7
cinnamon 1%+marinade	45	5.0±2.3 [A]	45	5.4±2.4 [A]
cinnamon 3%+marinade	22	4.6±2.0[A]	14	5.2±2.6 [A]
cinnamon 5%+marinade	25	4.4±2.6[A]	15	2.5±2.1[A]
oregano 1%+marinade	44	5.1±2.2 [A]	43	5.4±2.5 [A]
oregano 3%+marinade	25	3.7±2.4[A]	16	6.0±2.5
oregano 5%+marinade	25	3.3±2.9[A]	14	2.6±2.4[A]
thyme 1%+marinade	45	4.3±2.5[A]	43	5.1±2.6 [A]
thyme 3%+marinade	22	4.0±2.0[A]	16	4.8±3.3[A]
thyme 5%+marinade	20	3.8±2.1[A]	13	3.6±2.4[A]
fried	**number of tests**	**mean**	**number of tests**	**mean**
sunflower oil1%+marinade	36	6.5±2.3	34	6.5±2.7
cinnamon 1%+marinade	24	5.5±2.0	20	6.0±2.5
cinnamon 5%+marinade	12	5.7±2.4	11	4.8±2.7 [A]
oregano 1%+marinade	24	4.5±2.2 [A]	23	5.7±2.6
oregano 5%+marinade	12	4.7±3.0 [A]	12	4.7±3.0 [A]
thyme 1%+marinade	24	5.4±2.6	24	5.9±2.8
thyme 5%+marinade	12	5.4±2.7	12	4.5±2.4 [A]

[A]significant difference ($p < 0.05$) from the hedonic value of sunflower oil 1% + marinade