Journal of Food Research; Vol. 3, No. 6; 2014
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Effect of Variation in Food Components and Composition on the Antimicrobial Activity of Oregano and Clove Essential Oils in Broth and in a Reformulated Reduced Salt Vegetable Soup Product

Anna M. Witkowska[1], Dara K. Hickey[1] & Martin G. Wilkinson[1]

[1] Department of Life Sciences, University of Limerick, Castletroy, Limerick, Ireland

Correspondence: Martin G. Wilkinson, Department of Life Sciences, University of Limerick, Castletroy, Limerick, Ireland. Tel: 353-061-213-440. E-mail: martin.wilkinson@ul.ie

Received: March 12, 2014 Accepted: July 15, 2014 Online Published: August 14, 2014

doi:10.5539/jfr.v3n6p92 URL: http://dx.doi.org/10.5539/jfr.v3n6p92

Abstract

The objective of this study was to determine and quantify the influence of various food components (carbohydrates, fat, protein or NaCl contents) or chemical properties (pH, water activity levels) on the antimicrobial efficacy of oregano and clove essential oils (EOs). Growth of *Listeria innocua* or *Escherichia coli* treated with oregano or clove EOs was monitored following separate addition of various food components. Antimicrobial activity of EOs was enhanced in presence of NaCl (≥ 0.5 g/100 ml), or in media with low pH values (≤ 5.0), especially when adjusted with organic acids. Enhanced antimicrobial activity was observed following reduction in water activity, which appeared related to the nature of solute used. Antibacterial activity of EOs was reduced in presence of vegetable oil (≥ 1 ml/100 ml), protein (≥ 1 g/100 ml) or starch (≥ 10 g/100 ml). Based on data obtained, the composition of vegetable soup was altered to optimise the efficacy of EOs, by lowering the pH to 5.0 using citric acid. A combination of oregano EO and acidification appeared to control growth of *L. innocua* and *E. coli* during storage at 4 or 10 ºC. Thus, reformulation treatments including EO addition should be considered to improve the shelf-life of chilled ready meals.

Keywords: antimicrobial, essential oils, food ingredients, composition, interactions

1. Introduction

Herbs and spices have been used for centuries to add flavor and extend the shelf-life of a wide variety of foods. However, there has been a renewal of interest in the antimicrobial properties of these compounds as a consequence of changes in legislation and consumer trends that demand foods remain as preservative free as possible but are still safe and convenient to use (Brul & Coote, 1999). Salt has been used routinely as a flavouring and preservative agent in food products. However addition of salt to foodstuffs has become a major issue for the processed food sector, as processed foods, including chilled ready meals are a leading contributor to salt intake worldwide (Desmond, 2006). Recent reports from the Food Safety Authority of Ireland (FSAI, 2005) and the Food Standards Agency in the UK (SACN, 2003) have shown that the average daily sodium (salt) intake from foods in Irish and UK adults has been estimated as 3.3–3.9 g (8.3–10 g salt). It is now recommended by public health agencies that adults reduce their daily salt intake to a target of 6g (FSAI, 2003; SACN, 2003) due to association between excessive sodium intake and the development of osteoporosis, kidney failure, and hypertension, leading to increased risk of cardiovascular disease (MacGregor & de Wardener, 2002). Therefore, it has been recommended by various health organizations worldwide to reduce sodium intake of the general population, through a reduction of the salt content in processed foods (Consensus Action on Salt & Health, 2012; FSAI, 2003). As a result, food manufacturers are attempting to introduce into market new products labelled as "low salt ready meals". However the removal of sodium chloride from complex food systems may have implications for microbiological and sensory quality. Therefore a possible application of herb and spice preparations may be in controlling pathogens and food spoilage bacteria to assure consumer safety and to compensate for any possible loss in flavor impact (Burt, 2004; Prakash, Singh, Kedia, & Dubey, 2012).

Among the many essential oils evaluated in broth media, those from oregano (*Origanum vulgare*) or clove (*Syzygium aromaticum*) have been shown as being particularly active against both Gram-positive and Gram-negative bacteria (Burt & Reinders, 2003; Moreira, Ponce, del Valle, & Roura, 2005). Their antimicrobial

properties have been attributed to phenolic compounds, in particular, carvacrol and eugenol which are principal components of oregano and clove, respectively (Deans & Ritchie, 1987). These phytophenols exert antibacterial effects by disruption of cytoplasmic membrane leading to leakage of ions, ATP, nucleic acids, amino acids, impairment of respiratory activity of bacteria and enzyme inhibition preventing substrate utilization for energy production (Holley & Patel, 2005; Lv, Liang, Quan, & Li; 2011; Witkowska, Hickey, Alonso-Gomez & Wilkinson, 2013). The efficacy of EOs and extracts of herbs and spices *in vitro* is often much greater than in food systems (Burt, 2004; Glass & Johnson, 2004). To produce a similar effect to that reported *in vitro* the following increases in addition rates were required in foods: two-fold in semi-skimmed milk (Karatzas, Kets, Smid, & Bennik, 2001), 10-fold in pork sausages (Pandit & Shelef, 1994), 25- to 100-fold in Spanish soft cheese and 50-fold in mushroom soup (Ultee & Smid, 2001). As most foods consist of carbohydrates, proteins, fats, salt, and water it is important to determine the effect of these components on the antimicrobial properties of any proposed added EO preparation to enable their successful application (Devlieghere, Vermeulen, & Debevere, 2004). Despite the drive towards a reduction in salt content in foods for dietary reasons and consequently the enhanced use of herb and spice preparations such as EOs, there is still very little published information available on the effect of the main food constituents or food chemical properties on the antimicrobial activity of EOs, as well as on application of EOs in combinations with other preservation techniques in reduced salt ready meals.

Hence, the objectives of this study were to: (i) evaluate the antimicrobial effect of commercial oregano and clove EOs against *Listeria innocua* and *Escherichia coli* in broth model media or in a reduced salt chilled vegetable soup product, (ii) to determine and quantify the influence of various food components and food chemical properties on the antimicrobial efficacy of EOs, (iii) to evaluate the effect of type of acid used to adjust the pH of growth medium, (iv) to optimise the antimicrobial efficacy of plant EOs by reformulation of a reduced salt vegetable soup.

2. Materials and Methods

2.1 Materials

Oregano (*Origanum vulgare*) and clove (*Syzygium aromaticum*) EOs, rapeseed oil, sunflower oil, 2M HCl, 2M citric acid, and 2M NaOH were purchased from Sigma-Aldrich (Steinheim, Germany). Whey protein concentrate (WPC) containing 75.8 g/100g protein was kindly supplied by Carbery Food Ingredients (Ballineen, Cork, Ireland). Water soluble starch was purchased from Becton Dickinson (Sparks, Maryland, USA), while NaCl, sucrose, glucose, 85% lactic acid, glycerol, 99.8% acetic acid were purchased from Merck (Darmstadt, Germany).

2.2 Microorganisms and Growth Conditions

Bacterial cultures of *Listeria innocua* ATCC 33090, and *Escherichia coli* ATCC 11303 used in the present studies were obtained from the American Type Culture Collection (ATCC). Stock cultures were maintained at –80 °C in cryovials (Technical Service Consultants Ltd., Lancashire, UK) and sub-cultured twice onto Tryptone Soya Agar (TSA, Oxoid, Basingstoke, UK) followed by incubation at 37 °C for 24 h. Working cultures were prepared from subcultures and grown for 18 h at 37 °C in Tryptone Soya Broth medium (TSB, Oxoid, Basingstoke, UK).

2.3 Minimum Inhibitory Concentration (MIC) of Essential Oilss in Broth Systems

MIC values were determined using a microdilution broth method in 96-well microplates (Sarstedt, Numbrecht, Germany). Briefly, oregano or clove EOs were tested using two-fold serial dilution method over a range of concentrations from 0.5 to 0.015625 ml/100 ml (0.5, 0.25, 0.125, 0.0625, 0.03125 and 0.015625 ml/100 ml) against overnight broth cultures of *Listeria innocua* ATCC 33090, and *Escherichia coli* ATCC 11303 grown to a population 5×10^5 CFU/ml in TSB. Microplates were incubated at 37 °C and growth was monitored by measuring absorbance at 600 nm every 30 min over 18 h, using a microplate reader (Biotek Instruments Inc, Highland Park, VT, USA). MIC was defined as the lowest concentration of the essential oil which completely inhibited bacterial growth.

2.4 Determination of Antimicrobial Effects of Oregano and Clove Essential Oils in a Reduced Salt Vegetable Soup

Antimicrobial efficacy of oregano and clove EOs was evaluated in a reduced salt ready meal vegetable soup, produced on a 500 kg commercial scale by Dawn Fresh Foods (Fethard, Tipperary, Ireland) as described by Mitchell, Brunton and Wilkinson, (2011, 2013). The product was obtained from the factory after production, chilled and stored at 4 °C prior to analysis. The formulation consisted of a blend of ingredients including: potatoes, carrots, turnips, celery, diced onions, vegetable stock, and water. The approximate composition was:

protein 0.79 g/100 g, carbohydrate 6.44 g/100 g, fat 2.72 g/100 g, NaCl 0.46 g/100 g, pH 6.20, a_w 0.994. Vegetable soup was first microwaved for 3 min at 800W to eliminate any background microflora and was immediately transferred to stomacher bags in 25 g portions. Initially, oregano and clove EOs were added to the soup at the range of concentrations used to determine MICs in broth media (0.015625 ml/100 ml – 0.5 ml/100g), however at these levels considerable antimicrobial effect could not be obtained (data not shown), thus oregano and clove EOs were added to achieve higher final concentrations of 0.0625, 0.125, 0.25, 0.5, 1.0, 2.0, and 4.0 ml/100g. Samples were left to cool to ambient temperature (~ 21 ºC) and were then inoculated with overnight cultures of *L. innocua* ATCC 33090 or *E. coli* ATCC 11303 to a final level of approximately 5×10^5 CFU/g. Uniform distribution of the EO was ensured by mechanical treatment for one minute in a stomacher lab-blender (Seward Medical, London, UK). Controls included soup without added EOs as well as uninoculated soup with added EOs to detect any possible contamination by background microflora that might have survived cooking process and microwave treatment. Samples were sealed and stored at a non-restrictive growth temperature of 25 ºC for 24 h. Subsequently, 25 g portions were homogenized for two minutes, in a stomacher lab-blender with 225 ml of sterile solution of 0.1 g/100 ml peptone water (Oxoid, Basingstoke, UK). 0.1 ml of each suspension was then serially diluted up to 10^{-5} in peptone water and 0.1 ml aliquots were spread on TSA plates, which were further incubated for up to 48 h at 37 ºC. Results were expressed as log CFU/g.

2.5 Effects of Food Components, and Chemical Parameters on Antimicrobial Activity of Essential Oils

The effect of different food components, pH or water activity (a_w) on the antimicrobial properties of EOs, was evaluated by following the growth of *L. innocua* ATCC 33090 and *E. coli* ATCC 11303 in TSB medium (30 g/L, pH 7.2) with varying added concentrations of clove or oregano EOs (0.5×MIC, MIC, 2×MIC) with separate addition of the differing levels of protein from whey protein concentrate (WPC), NaCl, starch, rapeseed oil or sunflower oil.

For investigation of the effects of added WPC proteins, the pH of suspensions in distilled water was adjusted to 3.0 with HCl to avoid gel formation during sterilization, and adjusted back to pH 7.2 with sterile 2M NaOH after autoclaving and cooling to room temperature (~ 21 ºC). Sterile WPC suspensions were then mixed with equal amounts of double-strength TSB to give final protein concentrations of 0, 1, 3, 5 g/100 ml.

To investigate the effects of added salt, sterilized salt solutions in distilled water were mixed with equal amounts of double-strength TSB to give final NaCl concentrations of 0, 0.1, 0.5, 1, 3, or 5 g/100 ml.

To investigate the effect of added carbohydrates, solutions of water soluble starch were autoclaved and mixed with equal amounts of double-strength TSB after cooling to room temperature (~ 21 ºC), to give final carbohydrate concentrations of 0, 1, 5, 10 or 20 g/100 ml.

To investigate the effect of fat, rapeseed oil, or sunflower oil were autoclaved separately, mixed with sterile water and double-strength TSB using a sterile hand blender (Moulinex, Paris, France) to give final oil concentrations of 0, 1, 5, 10, and 30 ml/100 ml. Sterile Tween 80 was added at 0.5 ml/100 ml in order to facilitate mixing and to stabilize emulsions (Devlieghere et al., 2004).

To investigate the effect of water activity (a_w), equal amounts of double-strength TSB were mixed with: sterile solutions of NaCl ranging from 0, 4, 8, and 16 g/100 ml to give a_w values of 0.997, 0.975, 0.949, or 0.876, respectively, sucrose solutions ranging from 0.75, 26 or 46 g/100 ml to give a_w values of 0.997, 0.988, 0.975 and 0.952, respectively, and glycerol solutions ranging from 0, 4, 8, 20 or 30 ml/100 ml to give a_w values of 0.997, 0.99, 0.977, 0.948, and 0.911, respectively. The values for a_w of the different samples were measured using an AquaLab water activity meter (Decagon Devices, Pullman, USA).

To evaluate the effect of pH on antimicrobial activity of essential oils, pH of single-strength TSB was adjusted to 4.0, 5.0, 6.0, 7.0, and 8.0 with sterile 2 M HCl or 2 M NaOH solutions. Subsequently, the influence of the particular acid used to adjust the pH of growth medium on antimicrobial properties of EOs was evaluated. In this case, the pH of TSB medium supplemented with oregano or clove EOs at their respective MICs, was adjusted to 5.0 using hydrochloric, citric, lactic or acetic acids.

For all experiments, except that regarding the influence of pH, the pH of each medium was adjusted to 7.2 with either 2 M HCl or 2 M NaOH solutions. 10 ml samples of each of these solutions were finally inoculated with 100 µl of a diluted overnight suspension of bacterial cultures to give approximately 5×10^5 CFU/ml, and incubated at 37 °C for 24 h. Controls included samples with added food ingredients only, to determine whether they had any individual influence on bacterial growth and samples with EOs only. For each suspension 0.1 ml was then serially diluted up to 10^{-6} in peptone water and 0.1 ml aliquots spread on to TSA plates, which were further incubated for up to 48 h at 37 °C. Results were expressed in log CFU/ml.

2.6 Application of Oregano Essential Oil in Vegetable Soup With Reformulated Composition During Chilled Storage

The pH of vegetable soup produced by Dawn Fresh Foods (Fethard, Tipperary, Ireland) was adjusted to 5.0 using citric acid, as this acid is routinely used by the manufacturer to alter pH of all the meals produced, chilled immediately and stored at 4 °C prior to the experiment. Simultaneously, another batch of vegetable soup (pH 6.2) was produced also chilled immediately and stored at 4 ºC prior to the experiment. Oregano oil was added to 25 g portions of soup previously microwaved to a final concentration of 0.25 ml/100 g. Samples were inoculated with *L. innocua* or *E. coli* as previously described (section 2.4) and stored at a refrigeration temperature of 4 ºC or at an abuse temperature of 10 ºC for 9 days. Growth was monitored on day 1, 3, 6, and 9 of storage by plate counts on TSA plates, as described earlier (section 2.4).

2.7 Statistical Analysis

All experiments were replicated three times and each treatment was run in duplicate. Data represents means ± standard deviations. Statistical analysis was performed by Analysis of variance (ANOVA) using GraphPad Prism Version 4.03 statistical software (GraphPad Software, San Diego California USA). Level of significance was set at $P < 0.05$. Means were compared using Tukey's multiple comparision tests to determine the effect of variation in food components and chemical properties on the antimicrobial activity of EOs.

3. Results and Discussion

3.1 Minimum Inhibitory Concentration (MIC) of Eos in Broth Systems

In this study, oregano oil showed MIC values of 0.0625 ml/100 ml against *L. innocua* ATCC 33090 and *E. coli* ATCC 11303, while clove oil showed MIC values of 0.125 ml/100 ml against both strains tested in TSB medium using a broth microdilution method (data not shown). Burt and Reinders (2003) reported similar MIC values (0.0625 ml/100 ml) for oregano oil against *E. coli*, while clove oil displayed bacteriostatic properties against *E. coli* at 0.25 ml/100 ml (Moreira et al., 2005).

3.2 Antimicrobial Effects of Oregano and Clove Essential Oils in a Reduced Salt Vegetable Soup

Data obtained in this study indicated that the antimicrobial activity of EOs was considerably reduced in an actual commercial food product (Figure 1). Oregano and clove EOs did not display any antimicrobial properties in soup when used at their respective MICs ($P > 0.05$), and a 32-fold increase in concentration of both oils was required to maintain the bacterial strains at their initial inoculation level (Figure 1). Although some studies have reported successful or potential application of essential oils as antimicrobial agents in food products such as fish (0.05% v/v oregano and thyme oil) (Harpaz, Glatman, Drabkin, & Gelman, 2003), chicken meat (1-2% v/w clove oil) (Mytle, Anderson, Doyle, & Smith, 2006), cheese (0.5 – 1% v/w clove oil) (Menon & Garg, 2001), in most cases the activity of plant derived antimicrobials was considerably reduced in food systems compared with broth media. For example, Smith-Palmer, Stewart and Fyfe (1998) reported that EOs of clove, cinnamon and thyme were effective against *L. monocytogenes* in TSB with MICs of 0.03-0.075%. However, when subsequently evaluated for their activity in cheese, a 1% concentration of the oils was required to inhibit growth of *L. monocytogenes* (Smith-Palmer, Stewart, & Fyfe, 2001). Overall, the data in this study would also indicate the necessity for significantly elevated levels of EO to be added for antimicrobial activity in food systems.

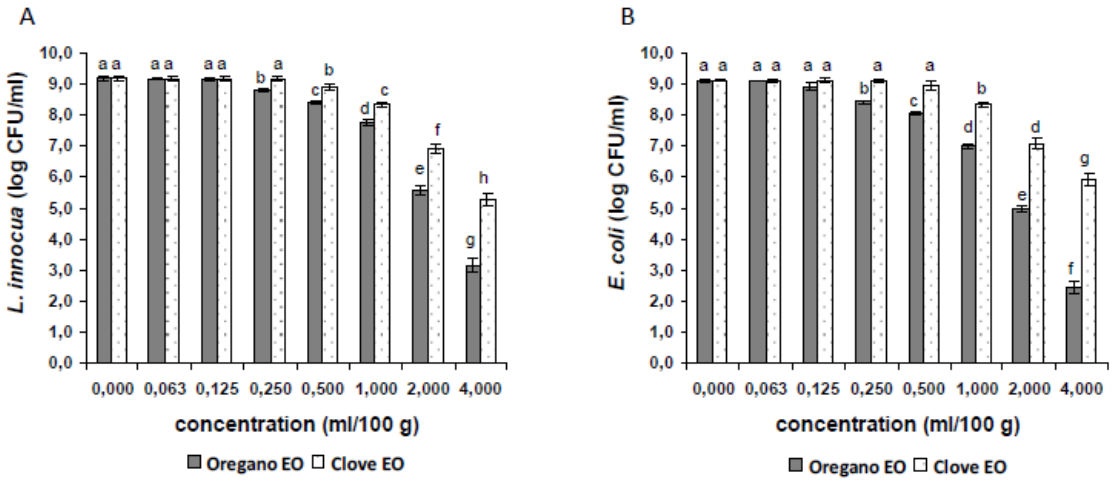

Figure 1. Effect of oregano and clove essential oils on survival of (A) *L. innocua* ATCC 33090 or (B) *Escherichia coli* ATCC 11303 in vegetable soup at 25 °C. Error bars indicate S. D. Different letters signify statistical differences between values ($P < 0.05$)

3.3 Effects of Food Components, and Chemical Parameters on Antimicrobial Activity of Essential Oils

As most foods consist mainly of carbohydrates, proteins, fats, salt, and water it is important to determine effect of these components on the antimicrobial properties of any proposed preparation for successful application in actual food systems (Devlieghere et al., 2004). Generally, antimicrobial activity of oregano and clove EOs, present in medium at 2×MIC and MIC levels was reduced ($P < 0.05$) when proteins were included in the growth medium at levels ≥ 1 g/100 ml (Table 1). At a protein concentration of 5 g/100 ml populations of *L. innocua* or *E. coli* treated with oregano oil at MIC, increased by 2.32 or 1.85 log CFU/ml, respectively compared with samples containing EO only. In the case of clove oil, populations of *L. innocua* or *E. coli* increased by 1.94 or 1.33 log CFU/ml respectively, indicating a possible antagonistic effect of added protein or a species related response to the added protein content (Table 1). Reduced antimicrobial effectiveness of added EO in presence of proteins may be attributed to complexing by hydrogen bonds between phenolic groups and peptides, and by hydrophobic interaction (Spencer et al., 1988). However, Gutierrez, Barry-Ryan and Bourke (2008) reported that the efficacy of oregano and thyme oils was enhanced in a medium containing proteins. These researchers used beef extract as a protein source, which consists mainly of peptones, amino acids and gelatin, while the WPC used in the present study consists mainly of β-lactoglobulin and α-lactalbumin. Therefore, it is possible that differing nitrogenous compounds may influence the antimicrobial activity of phenolic constituents differently, as phenolics-protein complexation appears to depend strongly on the type of protein involved (Juven, Kanner, Schved, & Weisslowicz, 1994).

Table 1. Effect of added protein concentration on the antimicrobial efficacy of oregano and clove essential oils against *Listeria innocua* or *Escherichia coli* incubated at 37 °C for 24 h in TSB medium assessed at three EOs concentrations: MIC, 2×MIC, 0.5×MIC

Protein (g/100 ml)	Oregano oil			Clove oil			Untreated
	2×MIC	MIC[C]	0.5×MIC	2×MIC	MIC[C]	0.5×MIC	
Listeria innocua (Log CFU/ml)[A]							
0	3.55±0.12[a]	6.50±0.12[a]	8.72±0.16[a]	0.00[a,B]	6.98±0.13[a]	8.48±0.19[a]	9.20±0.10[a]
1	6.42±0.24[b]	8.16±0.11[b]	8.84±0.06[ab]	1.06±0.71[b]	8.32±0.27[b]	8.88±0.26[b]	9.04±0.08[a]
3	7.10±0.10[c]	8.85±0.18[c]	8.98±0.08[b]	1.95±0.28[c]	8.85±0.25[c]	8.98±0.12[b]	8.98±0.11[a]
5	7.41±0.17[d]	8.82±0.22[c]	8.97±0.12[b]	3.20±0.20[d]	8.92±0.18[c]	8.99±0.21[b]	9.01±0.21[a]
Escherichia coli (Log CFU/ml)[A]							
0	0.00[a,B]	6.18±0.12[a]	8.20±0.15[a]	0.00[a,B]	7.11±0.16[a]	8.01±0.16[a]	9.14±0.11[a]
1	0.00[a,B]	6.51±0.17[b]	8.47±0.14[a]	1.34±0.38[b]	7.78±0.23[b]	8.19±0.22[a]	9.15±0.09[a]
3	1.23±0.27[b]	7.75±0.20[c]	8.92±0.12[b]	1.85±0.23[c]	8.26±0.20[c]	9.14±0.20[b]	9.21±0.14[a]
5	1.99±0.31[c]	8.03±0.17[c]	9.08±0.12[b]	2.30±0.21[d]	8.44±0.19[c]	9.15±0.28[b]	9.15±0.09[a]

[a-d] Means in the same column for each strain followed by different superscript letters (a-d) differ significantly ($P < 0.05$).

[A] Results are expressed as log CFU/ml (mean±SD).

[B] Less than minimum level of sensitivity of assay procedure (10 CFU/ml).

[C] MICs of oregano EO against *L. innocua* ATCC 33090 and *E. coli* ATCC 11303: 0.0625 ml/100 ml, MICs of clove EO against *L. innocua* ATCC 33090 and *E. coli* ATCC 11303: 0.125 ml/100 ml.

Antimicrobial activity of oregano and clove EOs was strongly decreased in presence of rapeseed oil (Table 2). Significantly higher levels ($P < 0.05$) of viable bacteria were enumerated even at the lowest level of added rapeseed oil irrespective of concentration of EO. Populations of *L. innocua* or *E. coli* treated with clove oil at 2xMIC in presence of 1 ml/100 ml of rapeseed oil were detected at levels of 4.21 or 4.96 log CFU/ml, respectively. In contrast, in the absence of rapeseed oil, surviving viable bacteria could not be detected, indicating a possible antagonistic effect of fat on the efficacy of EOs. These trends were also found for addition of sunflower oil (data not shown), demonstrating strong protective effects of vegetable oils towards bacterial cells when treated with EOs. Smith-Palmer, Stewart and Fyfe (2001) observed that the efficiency of plant EOs against *L. monocytogenes* in high-fat soft cheese was significantly reduced compared with activity in low-fat cheese. Partitioning of essential oil components into the lipid phase of the foods has been suggested as an explanation for reduced activity in food products. It is generally thought that if the hydrophobic components of essential oils are highly absorbed onto a fatty fraction of food, there will be relatively less EO available to act against bacteria growing in the aqueous phase (Mejlholm & Dalgaard, 2002).

Table 2. Effect of added fat concentration on the antimicrobial efficacy of oregano and clove essential oils against *Listeria innocua* or *Escherichia coli* incubated at 37 °C for 24 h in TSB medium assessed at three EOs concentrations: MIC, 2×MIC, 0.5×MIC

Fat	Oregano oil			Clove oil			Untreated
(ml/100 ml)	2×MIC	MIC[C]	0.5×MIC	2×MIC	MIC[C]	0.5×MIC	
Listeria innocua (Log CFU/ml)[A]							
0	3.55±0.12[a]	6.50±0.12[a]	8.72±0.16[a]	0.00 [a,B]	6.98±0.13[a]	8.48±0.19[a]	9.20±0.10[a]
1	6.00±0.22[b]	8.45±0.14[b]	9.04±0.18[b]	3.91±0.48[b]	8.67±0.18[b]	9.08±0.16[b]	9.18±0.09[a]
5	9.09±0.11[c]	9.20±0.11[c]	9.21±0.08[c]	7.30±0.22[c]	9.14±0.21[c]	9.22±0.11[c]	9.20±0.11[a]
10	9.20±0.08[cd]	9.18±0.09[c]	9.23±0.07[c]	9.08±0.16[d]	9.20±0.09[c]	9.20±0.12[c]	9.24±0.12[a]
30	9.23±0.08[d]	9.20±0.07[c]	9.21±0.12[c]	9.11±0.16[d]	9.23±0.11[c]	9.19±0.15[c]	9.21±0.12[a]
Escherichia coli (Log CFU/ml)[A]							
0	0.00 [a,B]	6.19±0.14[a]	8.21±0.16[a]	0.00 [a,B]	7.10±0.15[a]	8.01±0.16[a]	9.11±0.11[a]
1	4.21±0.33[b]	7.38±0.21[b]	8.94±0.19[b]	4.96±0.52[b]	8.23±0.16[b]	9.07±0.20[b]	9.10±0.09[a]
5	8.25±0.26[c]	9.11±0.11[c]	9.09±0.21[b]	7.60±0.31[c]	9.09±0.22[c]	9.10±0.24[b]	9.08±0.14[a]
10	8.82±0.19[d]	9.14±0.06[c]	9.12±0.11[b]	8.94±0.18[d]	9.13±0.09[c]	9.11±0.18[b]	9.12±0.09[a]
30	9.03±0.24[e]	9.11±0.07[c]	9.10±0.09[b]	8.96±0.16[d]	9.12±0.08[c]	9.09±0.12[b]	9.10±0.14[a]

[a-e] Means in the same column for each strain followed by different superscript letters (a-e) differ significantly ($P < 0.05$).

[A] Results are expressed as log CFU/ml (mean ± SD).

[B] Less than minimum level of sensitivity of assay procedure (10 CFU/ml).

[C] MICs of oregano EO against *L. innocua* ATCC 33090 and *E. coli* ATCC 11303: 0.0625 ml/100 ml, MICs of clove EO against *L. innocua* ATCC 33090 and *E. coli* ATCC 11303: 0.125 ml/100 ml.

Addition of NaCl at concentration of 0.1 g/100 ml to growth medium with added EO, had no influence on the efficacy of essential oils ($P > 0.05$) (Table 3). However, the bacterial counts were progressively reduced ($P < 0.05$) with a further increase in NaCl concentration, with respect to samples with EOs only. When NaCl was added at a concentration of 5 g/100 ml to bacterial suspensions treated with any of EOs at respective MICs, bacterial counts were reduced below the detection limit, indicating a positive influence of salt on antimicrobial activity of herb and spice derived preparations (Table 3). Control populations without EOs were quite resistant to the action of NaCl alone, as *L. innocua* and *E. coli* were present at levels of 8.48 and 7.72 log CFU/ml respectively, when grown in TSB with 5 g/100 ml NaCl added (Table 3). Wendakoon and Sakaguchi (1993) proposed a mechanism for synergism between sodium chloride and clove that involves an increase in cell membrane permeability, and subsequently growth inhibition due to NaCl action on intracellular enzymes.

Table 3. Effect of added NaCl concentration on the antimicrobial efficacy of oregano and clove essential oils against *Listeria innocua* or *Escherichia coli* incubated at 37 °C for 24 h in TSB medium assessed at three EO concentrations: MIC, 2×MIC, 0.5×MIC

NaCl	Oregano oil			Clove oil			Untreated
(g/100 ml)	2×MIC	MIC[C]	0.5×MIC	2×MIC	MIC[C]	0.5×MIC	
Listeria innocua (Log CFU/ml)[A]							
0	3.55±0.12[a]	6.50±0.12[a]	8.72±0.16[a]	0.00[B]	6.98±0.13[a]	8.48±0.19[a]	9.20±0.10[a]
0.1	3.60±0.21[a]	6.53±0.16[a]	8.73±0.19[a]	0.00[B]	6.94±0.29[a]	8.50±0.19[a]	9.20±0.09[a]
0.5	2.96±0.36[b]	5.68±0.19[b]	8.34±0.14[b]	0.00[B]	5.72±0.22[b]	8.00±0.21[b]	9.07±0.14[ab]
1	0.00[c,B]	2.08±0.48[c]	7.11±0.11[c]	0.00[B]	0.00[c,B]	7.23±0.27[c]	9.01±0.16[ab]
3	0.00[c,B]	0.00[d,B]	6.12±0.18[d]	0.00[B]	0.00[c,B]	6.15±0.24[d]	8.90±0.11[b]
5	0.00[c,B]	0.00[d,B]	5.01±0.17[e]	0.00[B]	0.00[c,B]	4.91±0.29[e]	8.48±0.18[c]
Escherichia coli (Log CFU/ml)[A]							
0	0.00[B]	6.18±0.14[a]	8.20±0.16[a]	0.00[B]	7.11±0.15[a]	8.01±0.16[a]	9.11±0.11[a]
0.1	0.00[B]	6.21±0.28[a]	8.19±0.23[a]	0.00[B]	7.19±0.21[a]	7.98±0.21[a]	9.12±0.08[a]
0.5	0.00[B]	6.00±0.25[a]	8.03 ±0.41[b]	0.00[B]	7.25±0.27[a]	7.68±0.16[ab]	9.08±0.07[a]
1	0.00[B]	5.39±0.19[b]	7.67±0.20[c]	0.00[B]	6.60±0.31[b]	7.40±0.28[b]	8.64±0.15[b]
3	0.00[B]	4.83±0.23[c]	6.45±0.20[d]	0.00[B]	6.05±0.20[c]	6.78±0.17[c]	8.21±0.19[c]
5	0.00[B]	0.00[d,B]	2.83±0.56[e]	0.00[B]	0.00[d,B]	3.13±0.29[d]	7.72±0.19[d]

[a-e] Means in the same column for each strain followed by different superscript letters (a-e) differ significantly ($P < 0.05$)

[A] Results are expressed as log CFU/ml (mean±SD)

[B] Less than minimum level of sensitivity of assay procedure (10 CFU/ml).

[C] MICs of oregano EO against *L. innocua* ATCC 33090 and *E. coli* ATCC 11303: 0.0625 ml/100 ml, MICs of clove EO against *L. innocua* ATCC 33090 and *E. coli* ATCC 11303: 0.125 ml/100 ml.

Addition of water soluble starch at concentration of 1 g/100 ml to growth medium with added EOs had no influence on antimicrobial efficacy ($P > 0.05$) (Table 4). However, bacterial counts were significantly higher ($P < 0.05$) with a further increase in starch concentration, with respect to samples with EOs only. At a starch concentration of 20 g/100 ml, counts of *L. innocua* or *E. coli* treated with oregano or clove oil at respective MICs, increased on average by approx. 1 log CFU/ml, compared with samples containing EOs only ($P < 0.05$), indicating a degree of inhibition by water soluble starch on the efficacy of the EOs (Table 4). Previously, it was observed that the growth rate of *L. monocytogenes* treated with EOs decreased at high starch concentrations (Gutierrez et al., 2008). Devlieghere et al. (2004) also reported a negative effect of carbohydrates on antimicrobial properties of chitosan in the presence of a high level (30 g/100 ml) of starch. It should be noted however, that various types and grades of carbohydrates and starches are available for use in the food industry, which may also influence their interactions with EOs.

Table 4. Effect of added water soluble starch concentration on the antimicrobial efficacy of oregano and clove essential oils against *Listeria innocua* and *Escherichia coli* incubated at 37 °C for 24 h in TSB medium assessed at three EOs concentrations: MIC, 2×MIC, 0.5×MIC

Starch	Oregano oil			Clove oil			Untreated
(g/100 ml)	2×MIC	MIC[C]	0.5×MIC	2×MIC	MIC[C]	0.5×MIC	
Listeria innocua (Log CFU/ml)[A]							
0	3.55±0.12^{a}	6.50±0.12^{a}	8.72±0.16^{a}	0.00 [B]	6.96±0.12^{a}	8.48±0.19^{a}	9.22±0.10^{a}
1	3.59±0.20^{a}	6.58±0.19^{ab}	8.74±0.22^{a}	0.00 [B]	7.00±0.19^{a}	8.54±0.23^{a}	9.23±0.09^{a}
5	3.71±0.23^{a}	6.99±0.21^{bc}	8.86±0.20^{a}	0.00 [B]	7.15±0.19^{a}	8.86±0.12^{ab}	9.21±0.11^{a}
10	4.28±0.18^{b}	7.29±0.08^{cd}	8.99±0.06^{a}	0.00 [B]	7.73±0.13^{b}	9.02±0.14^{b}	9.19±0.14^{a}
20	4.62±0.30^{b}	7.53±0.11^{d}	9.04±0.11^{a}	0.00 [B]	8.04±0.15^{b}	9.03±0.18^{b}	9.20±0.10^{a}
Escherichia coli (Log CFU/ml)[A]							
0	0.00 [B]	6.18±0.14^{a}	8.20±0.15^{a}	0.00 [B]	7.11±0.15^{a}	8.01±0.16^{a}	9.12±0.11^{a}
1	0.00 [B]	6.26±0.17^{a}	8.28±0.26^{ab}	0.00 [B]	7.18±0.21^{a}	8.08±0.21^{a}	9.11±0.10^{a}
5	0.00 [B]	6.49±0.15^{ab}	8.72±0.28^{bc}	0.00 [B]	7.49±0.15^{a}	8.26±0.12^{a}	9.08±0.21^{a}
10	0.00 [B]	6.82±0.10^{bc}	8.96±0.14^{c}	0.00 [B]	7.89±0.23^{b}	8.71±0.21^{b}	9.11±0.06^{a}
20	0.00 ^{b}	7.09±0.10^{c}	8.94±0.13^{c}	0.00 [B]	8.11±0.10^{b}	8.87±0.28^{b}	9.14±0.09^{a}

[a-d] Means in the same column for each strain followed by different superscript letters (a-d) differ significantly ($P < 0.05$).

[A] Results are expressed as log CFU/ml (mean ± SD).

[B] Less than minimum level of sensitivity of assay procedure (10 CFU/ml).

[C] MICs of oregano EO against *L. innocua* ATCC 33090 and *E. coli* ATCC 11303: 0.0625 ml/100 ml, MICs of clove EO against *L. innocua* ATCC 33090 and *E. coli* ATCC 11303: 0.125 ml/100 ml.

Regarding the effect of water activity (a_w) on the efficacy of EOs, data obtained for clove and oregano oils followed similar trends, therefore, results for clove oil only are described and illustrated in Figure 2. To differentiate between the effect of a_w and the nature of the solute controlling the a_w, NaCl, sucrose or glycerol were used separately to adjust this parameter. Generally, for each of the solutes used, lowering of a_w resulted in enhanced antimicrobial activity of clove EO (Figure 2 A-F). However, depending on the type or concentration of solute used to adjust a_w, the efficacy of clove oil varied considerably, even at the same a_w. For example, populations of *L. innocua* or *E. coli* treated with clove oil at MIC, were completely inactivated at an a_w of 0.975 adjusted with NaCl (Figure 2 A, D). However, when a_w was adjusted using sucrose or glycerol, cell counts were detected at levels of 6.2 or 7.0 log CFU/ml, respectively for *L. innocua* (Figure 2 B, C), and at levels of 3.96 or 7.01 log CFU/ml, respectively for *E. coli* (Figure 2 E, F). Further decreasing of a_w to 0.950 using sucrose resulted in a complete inactivation of both bacteria treated with clove oil (Figure 2 B, E), whereas in presence of glycerol at the same a_w, *L. innocua* and *E. coli* were detected at levels of 6.5 and 3.01 log CFU/ml, respectively (Figure 2 E, F). Therefore, results of this study indicate that the parameter "water activity" *per se* may not determine the antimicrobial efficacy of EOs. Instead, our data suggests that the nature of the a_w controlling solute and its concentration in the suspending medium may considerably influence the antimicrobial activity of EOs (Garcia-Gonzalez et al., 2009).

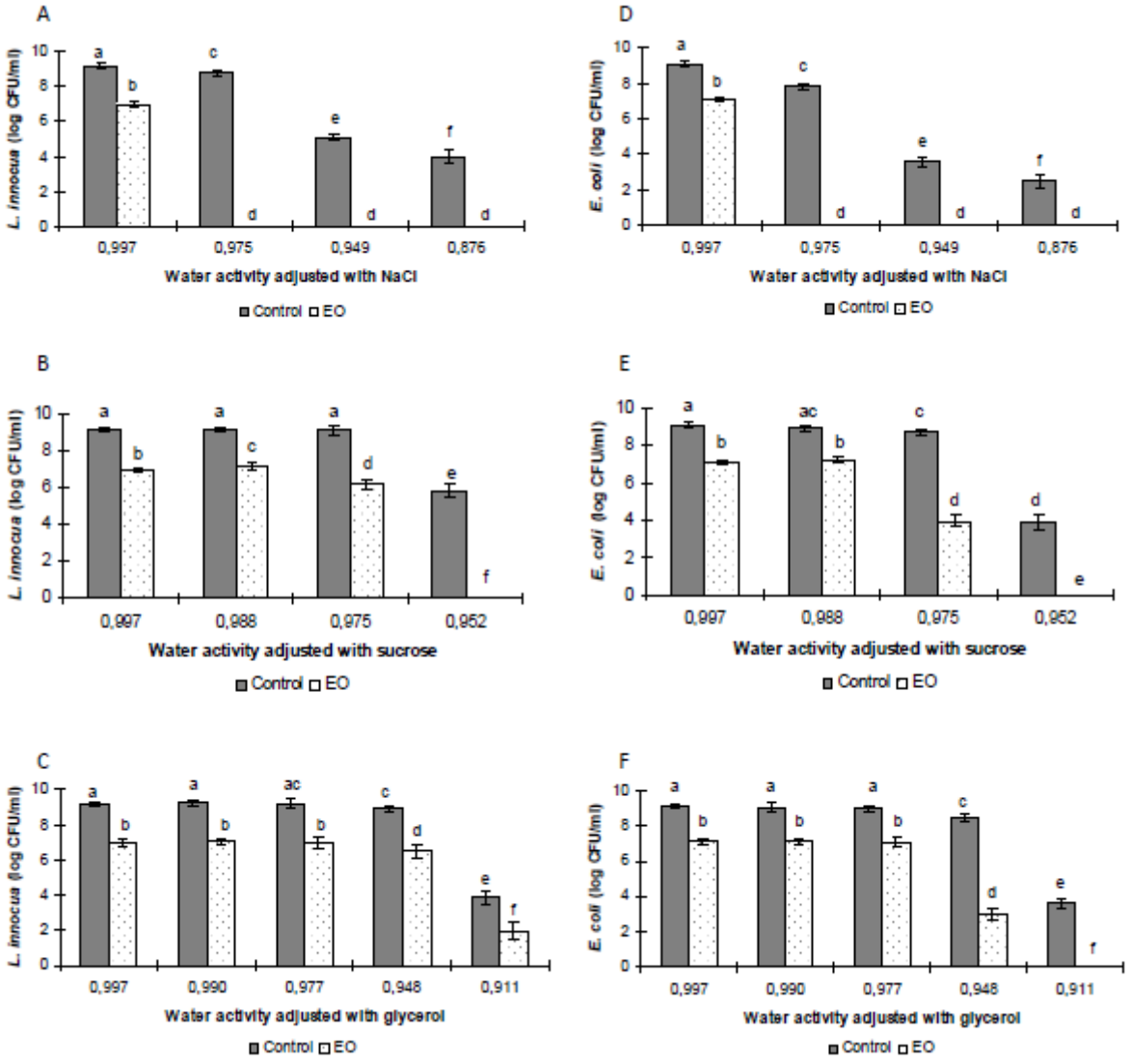

Figure 2. Effect of a_w adjusted with NaCl (A, D), sucrose (B, E), or glycerol (C, F) on growth of *L. innocua* ATCC 33090 (A, B, C) or *Escherichia coli* ATCC 11303 (D, E, F) in the presence of clove essential oil at MIC. Error bars indicate S. D. Error bars indicate S. D. Different letters signify statistical differences between values ($P < 0.05$)

The effect of pH on the antimicrobial activity of oregano and clove oils is shown in Table 5. Survival of either *L. innocua* or *E. coli* was not influenced by alterations in pH over the range 8.0 to 6.0 ($P > 0.05$). However, further acidification of growth medium with HCl to 5.0 or 4.0 resulted in significantly reduced cell counts ($P < 0.05$). This was especially evident at pH 4.0, where in presence of oregano or clove EOs at respective MICs, survivors could not be detected for either of the bacterial strains, indicating synergistic antimicrobial effects of EOs on acidification of growth medium. Hsieh, Mau and Huang (2001) also noted enhanced antimicrobial activity of plant extracts and EOs at acidic pH values. This effect may be attributed either to the direct effect of pH or to an increase in the hydrophobicity of EOs and extracts of herbs and spices at low pH values. While these ingredients tend to partition in the lipid phase of the food, they can also dissolve more easily in the lipids of the cell membranes of bacteria and thereby display greater antimicrobial activity (Holley & Patel, 2005).

Table 5. Effect of pH variation on the antimicrobial efficacy of oregano and clove essential oils against *Listeria innocua* or *Escherichia coli* incubated at 37 °C for 24 h in TSB medium assessed at three EOs concentrations: MIC, 2×MIC, 0.5×MIC

pH	Oregano oil			Clove oil			Untreated
	2×MIC	MIC[C]	0.5×MIC	2×MIC	MIC[C]	0.5×MIC	
Listeria innocua (Log CFU/ml)[A]							
8.0	3.50±0.17[a]	6.49±0.15[a]	8.81±0.17[a]	0[B]	6.99±0.16[a]	8.50±0.11[a]	9.26±0.14[a]
7.0	3.56±0.12[a]	6.51±0.12[a]	8.70±0.19[ab]	0[B]	7.01±0.25[a]	8.52±0.20[a]	9.21±0.14[a]
6.0	3.46±0.24[a]	6.38±0.22[a]	8.47±0.14[b]	0[B]	6.96±0.10[a]	8.20±0.24[a]	8.89±0.21[b]
5.0	2.78±0.37[b]	5.92±0.22[b]	6.44±0.21[c]	0[B]	6.23±0.13[b]	6.59±0.18[b]	8.23±0.14[c]
4.0	0[c,B]	0[c,B]	0[d,B]	0[B]	0[c,B]	0[c,B]	4.65±0.23[d]
Escherichia coli (Log CFU/ml)[A]							
8.0	0[B]	6.14±0.07[a]	8.14±0.12[a]	0[B]	7.08±0.18[a]	7.68±0.20[ab]	9.16±0.11[a]
7.0	0[B]	6.20±0.13[a]	8.23±0.19[a]	0[B]	7.19±0.10[a]	7.90±0.19[a]	9.12±0.08[a]
6.0	0[B]	6.16±0.14[a]	8.18±0.10[a]	0[B]	7.20±0.15[a]	7.81±0.14[a]	9.10±0.11[a]
5.0	0[B]	5.34±0.28[b]	7.68±0.19[b]	0[B]	5.71 ±0.17[b]	7.32±0.19[b]	8.62±0.18[b]
4.0	0[B]	0[c,B]	2.68±0.23[c]	0[B]	0[c,B]	3.42±0.25[c]	5.30±0.15[c]

[a-d] Means in the same column for each strain followed by different superscript letters (a-d) differ significantly ($P < 0.05$).

[A] Results are expressed as log CFU/ml (mean ± SD).

[B] Less than minimum level of sensitivity of assay procedure (10 CFU/ml).

[C] MICs of oregano EO against *L. innocua* ATCC 33090 and *E. coli* ATCC 11303: 0.0625 ml/100 ml, MICs of clove EO against *L. innocua* ATCC 33090 and *E. coli* ATCC 11303: 0.125 ml/100 ml.

Acidity is commonly used to control microbial spoilage in foods. However, some studies indicate that the inhibitory effects of acidic pH depends on the particular acid used to adjust the medium (Buchanan, Golden, & Whiting, 1993). Therefore, the efficacy of oregano and clove oils was evaluated at a constant pH (5.0) which was obtained using either strong inorganic (HCl) or weak organic acids (citric, lactic, acetic acids). Results are presented in Figure 3. Acidification of growth medium with organic acids considerably enhanced the antimicrobial properties of EOs, as demonstrated by a significant ($P < 0.05$) decrease in populations of *L. innocua* or *E. coli* treated with oregano or clove oil, in media acidified using organic acids compared with acidification using hydrochloric acid (Figure 3). Both EOs displayed bactericidal properties against *L. innocua* or *E. coli* when pH of the growth medium was adjusted with lactic and acetic acids, respectively. Bacterial growth in control samples without EOs also appeared to be influenced by the type of acid used to adjust pH of growth medium. Control populations of *L. innocua* or *E. coli* were reduced ($P < 0.05$) by 1.97-2.09 log CFU/ml and 0.81-2.75 log CFU/ml, respectively, when pH was adjusted with lactic or acetic acids compared with HCl (Figure 3). Lehrke, Hernaez, Mugliaroli, von Staszewski and Jagus (2011) studied the responses of *L. innocua* to acid stress applied alone or in combination with green tea extract, and reported a synergistic antimicrobial effect of the extract with acidification, especially when the pH of growth medium was adjusted with lactic or citric acid, compared with medium acidified using HCl. The differences in effect of strong inorganic or weak organic acids may be attributed to different mechanisms of action on bacterial cells. Strong mineral acids dissociate completely into protons and anions. Although the cell membrane has a very low permeability to protons, they can still enter or exit cells by interacting with the cellular proton transport system. To maintain the correct intracellular pH bacterial cells expend energy, and as a consequence nutrient transport, metabolic activity is affected resulting in extended lag phases, reduced growth rates and cell biomass (Lehrke et al., 2011). In comparison, weak organic acids can penetrate cell membranes in an undissociated form. Inside bacterial cells, higher intracellular pH causes dissociation of these molecules, releasing protons and acidifying the cytoplasm, which in turn inactivates some metabolic enzymes, impairs membrane function, nutrient transport and overall metabolic activity (Samelis & Sofos, 2003). Recently nanoencapsulation technology has been used to deliver enhanced antibacterial activity

from EOs in dilute liquid food systems such as fruit juices (Donsì, Annunziata, Sessa, & Ferrari, 2011). However, the application of this technology to more complex viscous food products such as ready meals remains to be investigated.

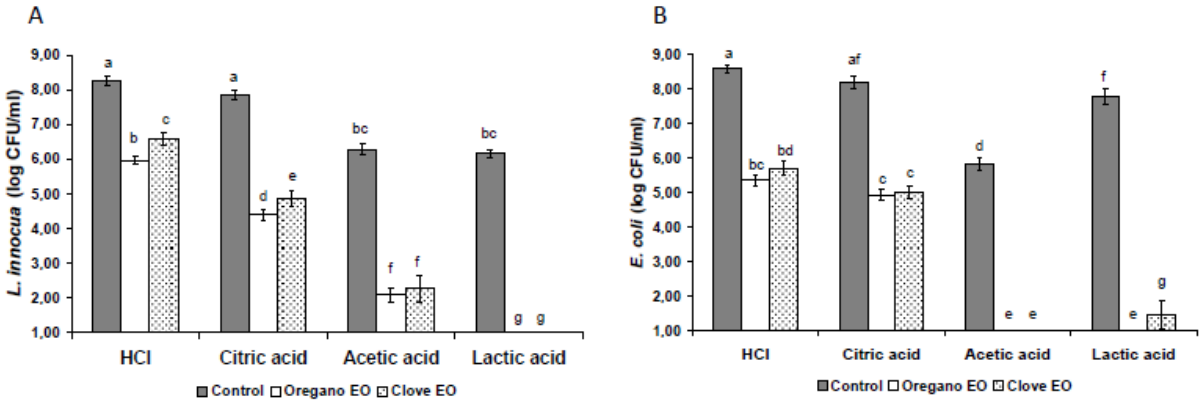

Figure 3. Effect of type of acid used for medium acidification on growth of (A) *L. innocua* ATCC 33090 or (B) *Escherichia coli* ATCC 11303 treated with oregano and clove essential oils at MICs. Error bars indicate S. D. Different letters signify statistical differences between values ($P < 0.05$)

3.4 Application of Oregano Essential Oil in Vegetable Soup With Reformulated Composition During Chilled Storage

The data obtained in this study on the effect of food ingredients and food properties on antimicrobial efficacy of EOs was used to reformulate the composition of the reduced salt vegetable soup for optimised application in a food product. Oregano EO was selected, based on the slightly stronger antimicrobial properties found compared with clove oil, and its' flavour suitability for inclusion into vegetable soup. Among the food ingredients and food chemical properties tested, NaCl addition or acidification of growth medium were found to enhance the activity of EOs. The soup used in this study was produced as a low sodium product (NaCl 0.46 g/100 g), therefore the salt concentration was not further adjusted. However, reformulation of the soup was undertaken by reducing the pH to 5.0 using citric acid, as organic acids had contributed to enhanced antimicrobial activity of essential oils and this acid had also been used by the manufacturer of the soup to adjust pH of other commercial products. A combination of EO with a reduction in pH of the soup to 5.0 using citric acid resulted in a significant reduction ($P < 0.05$) of bacterial counts of *L. innocua* or *E. coli* throughout storage, both at the refrigerated temperature of 4 ºC and at an abuse temperature of 10 ºC (Figure 4). Effectiveness of combination treatments is particularly important at 10 ºC storage, which can favour growth of psychrotrophic bacteria including *L. monocytogenes* to significant numbers food products, making post-process contamination a significant concern for chilled ready meals (Grau & Vanderlinde, 1992). After 9 days of storage, populations of *L. innocua* inoculated into acidified soup and treated with 0.25 ml/ 100g oregano oil, were reduced to 4.73 and 4.12 log CFU/g when stored at 10 ºC or 4 ºC, respectively, while control populations inoculated into unadjusted soup with a pH 6.2 without EO grew to levels of 9.78 or 7.60 log CFU/g when stored at 10 ºC or 4 ºC, respectively. Similarly, populations of *E. coli* inoculated into a reformulated acidified soup and treated with EO, were reduced to 4.87 or 4.48 log CFU/g when stored at 10 ºC or 4 ºC, respectively, while control populations inoculated into an unadjusted soup of pH 6.2 without EO were detected at levels of 9.35 or 5.63 log CFU/g when stored at 10 ºC or 4 ºC, respectively, after 9 days of storage (Figure 4). To our knowledge, the combined effect of EO treatment and acidification with weak organic acid in a reformulated reduced sodium soup product, has not been reported. The antimicrobial efficacy of carvacrol against *Staphylococcus aureus* was enhanced by simultaneous combination with acetic acid in meat broth (de Oliveira, Stamford, Neto, & de Souza, 2010). Gutierriez, Barry-Ryan and Bourke (2009) evaluated a number of EO's in broth and model food based media derived from milk, lettuce and beef ingredients and also noted differences in efficacy between data from broth experiments and food based media against a range of pathogens.

The present study has utilised data from broth experiments and applied them to enhance the microbial quality of a reformulated commercially produced reduced salt vegetable soup ready meal. As salt plays a key role in food

preservation and provides typical organoleptic characteristics in a range of foods, its removal in reduced salt ready meals may have adverse effects on quality of these products. However, our results indicate that combinations of EOs with modification of food composition in reduced sodium chilled ready meal reformulation may allow for application of EOs at low levels for enhanced food safety. This information should be of interest to food manufacturers as targeted reformulation strategies could provide the consumer with safe, high quality, reduced sodium products. Further research will investigate the combined application of essential oils and weak organic acids in different types of chilled meals, as well as sensory acceptability of these reformulated foods.

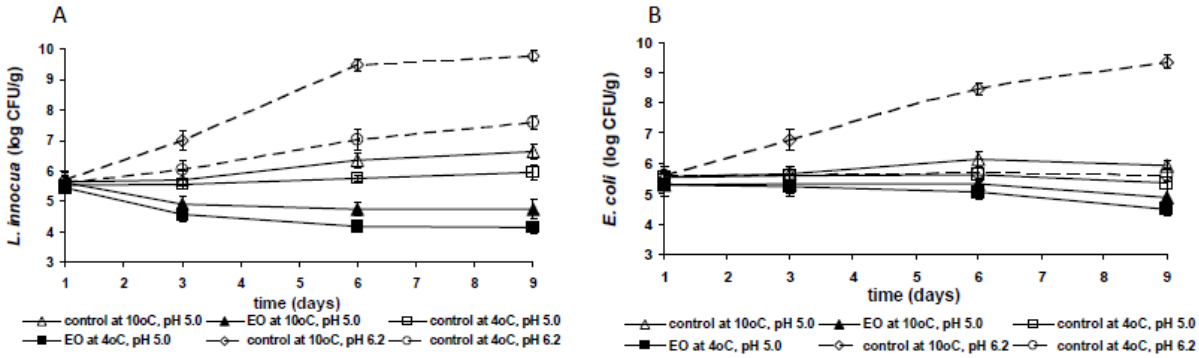

Figure 4. Effect of combined oregano essential oil treatment (0.25 ml/100 g) and reformulation by acidification of a reduced salt vegetable soup on the survival of (A) *L. innocua* ATCC 33090 and (B) *Escherichia coli* ATCC 11303 during 9 days of storage: (—▲—) EO at pH 5.0 at 10 °C, (—■—) EO at pH 5.0 at 4 °C (—Δ—) control at pH 5.0 at 10 °C, (—□—) control at pH 5.0 at 4 °C (--◊--) control at pH 6.2 at 10 °C (--○--) control at pH 6.2 at 4 °C. Error bars indicate S. D

4. Conclusions

Antimicrobial activity of oregano and clove EOs used in this study were considerably affected by the composition of the suspending medium or variation in food matrix ingredient levels. Antimicrobial activity of EOs against *L. innocua* or *E. coli* was enhanced in the presence of NaCl (≥ 0.5 g/100 ml), and in medium with low pH values (≤ 5.0), especially when adjusted with weak organic acids. Antibacterial activity of EOs was reduced in the presence of vegetable oil, protein and higher concentrations of starch. A combination of oregano EO with reformulation by reduction in pH of a reduced salt soup to 5.0 using citric acid was effective in controlling and reduction of bacterial growth of *L. innocua* or *E. coli* during 9 days storage at 4 or 10 °C. Therefore, a combination of reformulation treatments may be considered as a more natural and attractive alternative to food manufacturers to improve preservation of chilled ready meals, without the use of chemical additives.

Acknowledgements

This work was funded by the Department of Agriculture, Fisheries and Food, (Agriculture House, Kildare Street, Dublin 2) under Food Institutional Research Measure (FIRM), National Development Plan (NDP) 2000-2006.

References

Brul, S., & Coote, P. (1999). Preservative agents in foods: Mode of action and microbial resistance mechanisms. *International Journal of Food Microbiology, 50*, 1-17. http://dx.doi.org/10.1016/S0168-1605(99)00072-0

Buchanan, R. L., Golden, M. H., & Whiting, R. C. (1993). Differentiation of the effects of pH and lactic or acetic acid concentration on the kinetics of *Listeria monocytogenes* inactivation. *Journal of Food Protection, 56*, 474-478.

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology, 94*, 223-253. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.03.022

Burt, S., & Reinders, R. D. (2003). Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. *Letters in Applied Microbiology, 36,* 162-167. http://dx.doi.org/10.1046/j.1472-765X.2003.01285.x

de Oliveira, C. E., Stamford, T. L., Gomes, N. J., & de Souza, E. L. (2010). Inhibition of *Staphylococcus aureus*

in broth and meat broth using synergies of phenolics and organic acids. *International Journal of Food Microbiology, 137*, 312-316. http://dx.doi.org/10.1016/j.ijfoodmicro.2009.11.019

Consensus Action on Salt & Health (CASH). (2012). Salt Awareness Week 2012 - Reducing Salt. Preventing Stroke, Retrieved 15 April, 2014, from http://www.worldactiononsalt.com/docs/saltawareness/76906.pdf

Deans, S. G., & Ritchie, G. (1987). Antibacterial properties of plant essential oils. *International Journal of Food Microbiology, 5*(2), 165-180. http://dx.doi.org/10.1016/0168-1605(87)90034-1

Desmond, E. (2006). Reducing salt: A challenge for the meat industry. *Meat Science, 74*, 188-196. http://dx.doi.org/10.1016/j.meatsci.2006.04.014

Devlieghere, F., Vermeulen, A., & Debevere, J. (2004). Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. *Food Microbiology, 21*, 703-714. http://dx.doi.org/10.1016/j.fm.2004.02.008

Donsì, F., Annunziata, M., Sessa, M., & Ferrari, G. (2011). Nanoencapsulation of essential oils to enhance their antimicrobial activity in foods. *LWT-Food Science and Technology, 44*, 1908-1914. http://dx.doi.org/10.1016/j.lwt.2011.03.003

Garcia-Gonzalez, L., Geeraerd, A. H., Elst, K., Van Ginneken, L., Van Impe, J. F., & Devlieghere, F. (2009). Influence of type of microorganism, food ingredients and food properties on high-pressure carbon dioxide inactivation of microorganisms. *International Journal of Food Microbiology, 129*, 253-263. http://dx.doi.org/10.1016/j.ijfoodmicro.2008.12.005

FSAI. (2003). Salt and Health: Review of the Scientific Evidence and Recommendations for Public Policy in Ireland. *Food Safety Authority of Ireland.*

FSAI. (2005). Salt and health: review of the scientific evidence and recommendations for public policy in Ireland. *Food Safety Authority of Ireland.*

Glass, K. A., & Johnson, E. A. (2004). Antagonistic effect of fat on the antibotulinal activity of food preservatives and fatty acids. *Food Microbiology, 21*, 675-682. http://dx.doi.org/10.1016/j.fm.2004.03.002

Grau, F. H., & Vanderlinde, P. B. (1992). Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. *Journal of Food Protection, 55*, 4-7.

Gutierrez, J., Barry-Ryan, C., & Bourke, P. (2008). The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *International Journal of Food Microbiology, 124*, 91-97. http://dx.doi.org/10.1016/j.ijfoodmicro.2008.02.028

Gutierrez, J., Barry-Ryan, C., & Bourke, P. (2009). Antimicrobial activity of plant essential oils using food model media: Efficacy,synergistic potential and interactions with food components. *Food Microbiology, 26,* 142-150. http://dx.doi.org/10.1016/j.fm.2008.10.008

Harpaz, S., Glatman, L., Drabkin, V., & Gelman, A. (2003). Effects of herbal essential oils used to extend the shelf life of freshwater-reared Asian sea bass fish (*Lates calcarifer*). *Journal of Food Protection, 66*, 410-417.

Holley, R. A., & Patel, D. (2005). Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. *Food Microbiology, 22*, 273-292. http://dx.doi.org/10.1016/j.fm.2004.08.006

Hsieh, P.-Ch., Mau, J.-L., & Huang, Sh.-H. (2001). Antimicrobial effect of various combinations of plant extracts. *Food Microbiology, 18*, 35-43. http://dx.doi.org/10.1006/fmic.2000.0376

Juven, B. J., Kanner, J., Schved, F., & Weisslowicz, H. (1994). Factors that interact with the antibacterial action of thyme essential oil and its active constituents. *Journal of Applied Bacteriology, 76*, 626-631. http://dx.doi.org/10.1111/j.1365-2672.1994.tb01661.x

Karatzas, A. K., Kets, E. P. W., Smid, E. J., & Bennik, M. H. J. (2001). The combined action of carvacrol and high hydrostatic pressure on *Listeria monocytogenes* Scott A. *Journal of Applied Microbiology, 90*, 463-469. http://dx.doi.org/10.1046/j.1365-2672.2001.01266.x

Lehrke, G., Hernaez, L., Mugliaroli, S. L., von Staszewski, M., & Jagus, R. J. (2011). Sensitization of *Listeria innocua* to inorganic and organic acids by natural antimicrobials. *LWT - Food Science and Technology, 44*, 984-991. http://dx.doi.org/10.1016/j.lwt.2010.09.016

Lv, F., Liang, H., Yuan, Q., & Li, C. (2011). *In vitro* antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. *Food Research International, 44*, 3057-3064. http://dx.doi.org/10.1016/j.foodres.2011.07.030

MacGregor, G. A., & de Wardener, H. E. (2000). "Salt": A commentary. *American Journal of Hypertension*, *13*, 313-316.

Mejlholm, O., & Dalgaard, P. (2002). Antimicrobial effect of essential oils on the seafood spoilage micro-organism *Photobacterium phosphoreum* in liquid media and fish products. *Letters in Applied Microbiology, 34*, 27-31. http://dx.doi.org/10.1046/j.1472-765x.2002.01033.x

Menon, K. V., & Garg, S. R. (2001). Inhibitory effect of clove oil on *Listeria monocytogenes* in meat and cheese. *Food Microbiology, 18*, 647-650. http://dx.doi.org/10.1006/fmic.2001.0430

Mitchell, M., Brunton., N. P., & Wilkinson, M. G. (2011). Impact of salt reduction on the instrumental and sensory flavor profile of vegetable soup. *Food Research International, 44*, 1036-1043. http://dx.doi.org/10.1016/j.foodres.2011.03.007

Mitchell, M., Brunton., N. P., & Wilkinson, M. G. (2013). The Influence of Salt Taste Threshold on Acceptability and Purchase Intent of Reformulated Reduced Sodium Vegetable Soups. *Food Quality and Preference, 28*, 356-360. http://dx.doi.org/10.1016/j.foodqual.2012.11.002

Moreira, M. R., Ponce, A. G., del Valle, C. E., & Roura, S. I. (2005). Inhibitory parameters of essential oils to reduce a foodborne pathogen. *LWT - Food Science and Technology, 38*, 565-570. http://dx.doi.org/10.1016/j.lwt.2004.07.012

Mytle, N., Anderson, G. L., Doyle, M. P., & Smith, M. A. (2006). Antimicrobial activity of clove (*Syzgium aromaticum*) oil in inhibiting *Listeria monocytogenes* on chicken frankfurters. *Food Control, 17*, 102-107. http://dx.doi.org/10.1016/j.foodcont.2004.09.008

Pandit, V. A., & Shelef, L. A. (1994). Sensitivity of *Listeria monocytogenes* to rosemary (*Rosmarinus officinalis* L.). *Food Microbiology, 11*, 57-63. http://dx.doi.org/10.1006/fmic.1994.1008

Prakash, B., Singh, P., Kedia, A., & Dubey, N. K. (2012). Assessment of some essential oils as food preservatives based on antifungal, antiaflatoxin, antioxidant activities and *in vivo* efficacy in food system. *Food Research International, 49*, 201-208. http://dx.doi.org/10.1016/j.foodres.2012.08.020

SACN. (2003). Salt and Health. Scientific Advisory Committee on Nutrition. Retrieved 23, May 2014, from http://www.sacn.gov.uk/pdfs/sacn_salt_final.pdf

Samelis, J., & Sofos, J. N. (2003). Organic acids. In S. Roller (Ed.), *Natural antimicrobials for the minimal processing of foods* (pp. 98-132). Cambridge, England: Woodhead Publishing Ltd. http://dx.doi.org/10.1093/nar/gkg784

Smith-Palmer, A., Stewart, J., & Fyfe, L. (1998). Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Letters in Applied Microbiology, 26*, 118-122. http://dx.doi.org/10.1046/j.1472-765X.1998.00303.x

Smith-Palmer, A., Stewart, J., & Fyfe, L. (2001). The potential application of plant essential oils as natural food preservatives in soft cheese. *Food Microbiology, 18*, 463-470. http://dx.doi.org/10.1006/fmic.2001.0415

Spencer, C. M., Cai, Y., Martin, R., Gaffney, S. H., Goulding, P. N., Magnolato, D., … Haslam, E. (1988). Polyphenol complexation—some thoughts and observations. *Phytochemistry, 27*, 2397-2409. http://dx.doi.org/10.1016/0031-9422(88)87004-3

Ultee, A., & Smid, E. J. (2001). Influence of carvacrol on growth and toxin production by *Bacillus cereus*. *International Journal of Food Microbiology, 64*, 373-378. http://dx.doi.org/10.1016/S0168-1605(00)00480-3

Wendakoon, C. N., & Sakaguchi, M. (1993). Combined effect of sodium-chloride and clove on growth and biogenic-amine formation of *Enterobacter aerogenes* in mackerel muscle extract. *Journal of Food Protection, 56*, 410-413.

Witkowska, A. M., Hickey, D. K., Alonso-Gomez, M., & Wilkinson, M. G. (2013). Evaluation of Antimicrobial Activities of Commercial Herb and Spice Extracts against selected Food-Borne Bacteria. *Journal of Food Research, 2*, 37-54. http://dx.doi.org/10.5539/jfr.v2n4p37

Copyrights

Copyright for this article is retained by the author(s), with first publication rights granted to the journal.

This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).