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Research Paper

Impedimetric evaluation of the synergistic effects of oregano and cranberry aqueous extract mixtures on the growth of *Salmonella typhimurium*

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Abstract. *Salmonella* is a foodborne, Gram negative and facultative anaerobic bacteria and continues to be a serious public health concern especially in the tropics. *Salmonella typhimurium* is one of the most economically important serotype of *Salmonella* and it is the most common non-typhoid serotype isolated from pork and humans. It is estimated that more than 63.1% of *S*. *typhimurium* infections in humans are due to pork-associated cases. Oregano and cranberry extracts are attested to have potent antimicrobial properties; however, the effect of an oregano-cranberry extract combination has not been assessed on *S*. *typhimurium.* The synergistic efficacy of oregano-cranberry extract mixtures was evaluated against *S*. *typhimurium* in broth systems through the use of a RABIT system. Oregano-cranberry extract mixtures of different proportions were added at 0.5 and 1% v/v into RABIT cells containing 50 µl of bacterial cells and then incubated (30°C). Conductance was recorded automatically at 6 min intervals for 24 h. The impedimetric detection times (TTD) were automatically determined by the RABIT system as indicated when there were three consecutive changes in conductivity greater than 5 µs/6 min. Results obtained showed that the synergies of oregano-cranberry extract combinations produced significant effects ($P < 0.05$) on the TTD. Oregano-cranberry (50-50% w/w, 1% v/v) treatment was most effective at inhibiting the growth of *S*. *typhimurium* with the longest TTD of 9.36 h. In conclusion, synergies of the combined plant extracts were significantly more effective in inhibiting growth of *S*. *typhimurium* than were individual extracts of the same plants. The results show a promising potential for the application of the combined extracts in pork systems.

Keywords: Phytochemical synergies, oregano, cranberry, TTD, *S*. *typhimurium.*

INTRODUCTION

Salmonella is a foodborne bacterial pathogen (Krieg and Holt, 1984). Salmonellosis is a serious public health concern as several cases of human infection are reported globally every year (Oliveira et al., 2006). The antibiotic resistance potential of *Salmonella* is a major driver of the on-going research interest in plant antimicrobial molecules. Also, increasing consumer demand for more clean-label products due to health concerns associated with chemical preservatives has necessitated the food industry's drive to look at possible use of natural preservatives in a range of food products (Sullivan et al., 2012; Dorman and Deans, 2000). *S*. *typhimurium* is one of the most important serotype of *Salmonella*. This is borne out of the fact that recent studies have reported that the serotype is the most common non-typhoid serotype isolated from pork and humans. Also more than 63.1% of *S. typhimurium* infections are pork-associated cases (Boyen et al., 2008; Perugini et al., 2010). Traditional plate-counting, disk assay and broth micro-dilutions are common techniques used in determination of the antibacterial efficacy of an antimicrobial or of the synergy of antimicrobial mixtures. In recent times however, the use of rapid impedance techniques in quantifying bacterial growth and assessing the potency of antimicrobials is increasingly gaining ground and is becoming an important tool for use to food scientists and microbiologists (Silley and Forsythe, 1996).

Recent researches have shown that the antibacterial effect of the synergies of extracts of oregano (*Origanum vulgare*) and cranberry (*Vaccinium macrocarpon*) are stronger compared to pure extracts of each plant (Lin et al., 2004; Lin et al., 2005). Oregano extracts were reported to have a slightly inhibitory effect on *S. typhimurium* (Tayel et al., 2012). Therefore, the application of the plausible synergistic effects of oregano and cranberry combinations to curtail *Salmonella* growth is an interesting area worth exploring.

The objective of this study is to determine the antimicrobial effectiveness of the phytochemical synergies of oregano and cranberry extracts on *S. typhimurium* using impedance techniques.

MATERIALS AND METHODS

Maintenance of bacteria

S. typhimurium (NCTC 74) was used throughout this project. The organism was maintained on Nutrient Agar (NA) which was kept in a refrigerator at 5°C (+/-1°C).

Preparation of bacterial inoculum

From a 24 h *S*. *typhimurium* streak culture, a typical colony was aseptically transferred to a universal bottle containing 10ml of Nutrient Broth (CM0001, Oxoid) and incubated at 37°C for 18 h. The culture grew overnight to a level of approximately 7.0 log CFU/ml. This level was confirmed by serially diluting the inoculum using sterile MRD (CM0733, Oxoid) and then spread plating onto NA (CM0003, Oxoid) plates and incubating at 37°C for 24 h before colonies were enumerated by counting on a colony counter (Gallenkamp, England).

Preparation of aqueous extracts of cranberry and oregano

Cranberry and oregano extracts were evaluated for their antibacterial activity. The cranberry was obtained from Healthy Supplies Limited while the oregano was purchased from Hampshire Foods Limited. The extracts were stored at 20 to 25°C until needed. The dried plant parts were finely ground for 5 min using a blender (Moulineux, France). The ground powder was mixed in different proportions -100% cranberry, 75%-25% cranberry-oregano, 50%-50% cranberry-oregano, 25%-75% cranberry–oregano and 100% oregano powder respectively. The powdered mixtures (5 g) were diluted in 50 ml de-ionized water in beakers and heated for 10 min on a magnetic stirrer hotplate (Gallenkamp, England) until the temperature reached 95°C. Subsequently, the mixtures were allowed to cool for 10 min to increase extraction of active compounds. Extracts were then filtered through filter paper (Whatmann size 41) to remove smaller particles using a Buchner funnel. Each final extract was stored in a screw-cap sterile container protected from light (this is done because phenolics are photo-sensitive) by wrapping with foil and then stored at 4°C for 24 h before use.

Assessment of antibacterial activity using the impedance method

Inhibitory activity was investigated using a Rapid Automated Bacteria Impedance Technique (RABIT) system from Don Whitley Scientific (Shipley, United Kingdom). Direct impedance technique was used whereby test organisms are in direct contact with the system electrodes. The changes in conductance of the growth medium were directly caused by the changes taking place in the bulk electrolyte. 50 µl aliquots of an 18 h *S*. *typhimurium* culture were inoculated into 4.5 ml of Whitley impedance broth (G50001, Whitley Scientific) in RABIT test tubes to achieve a 10^5 cfu level of inoculum. 23 and 46 µl aliquots of each extract treatment were then added to duplicate RABIT test tubes inoculated with bacterial cultures to achieve a final extract concentration of 0.5 and 1% v/v respectively in the RABIT test tubes. A control experiment was set up with a RABIT tube containing 4.5 ml of the impedance broth inoculated with 50 µl (10^5 cfu) of bacterial cultures and no extract was added. Conductance was recorded automatically at 6 min intervals for 24 h at 30°C. The impedance time to detection (TTD) was automatically determined by the RABIT system as indicated when there were three consecutive changes in conductivity greater than 5 µs/6 min.

Statistical analysis

Statistical analysis was done by analysis of variance (ANOVA) (two-factor F test; $P < 0.05$) to determine differences in treatments along column and within rows. Graphs and charts were used to illustrate the results. These analyses were performed using Microsoft Excel spread sheet package (version 5.0; Microsoft Corp., Redmond, WA).

RESULTS AND DISCUSSION

The antimicrobial effects of five different extract proportions (at concentrations of 0.5 and 1% v/v) on *S*. *typhimurium* were determined impedimetrically using the

Table 1. Effect of oregano and cranberry extracts on growth of *S. typhimurium* as investigated by automated conductimetry at 30°C.

Treatment	0%		0.5%		1%	
	TTD (h)	Conductivity (µS)	TTD (h)	Conductivity (µS)	TTD (h)	Conductivity (µS)
Control	1.24	3593				
(100)% oregano			4.0	3356	4.54	2198
(75:25)% oregano-cranberry			4.42	2770	6.06	2347
(50:50)% oregano-cranberry			6.18	2790	9.36	1685
(25:75)% oregano-cranberry			5.0	3280	6.30	1690
100% cranberry			4.3	2198	5.30	2068

Detection times are means of duplicates

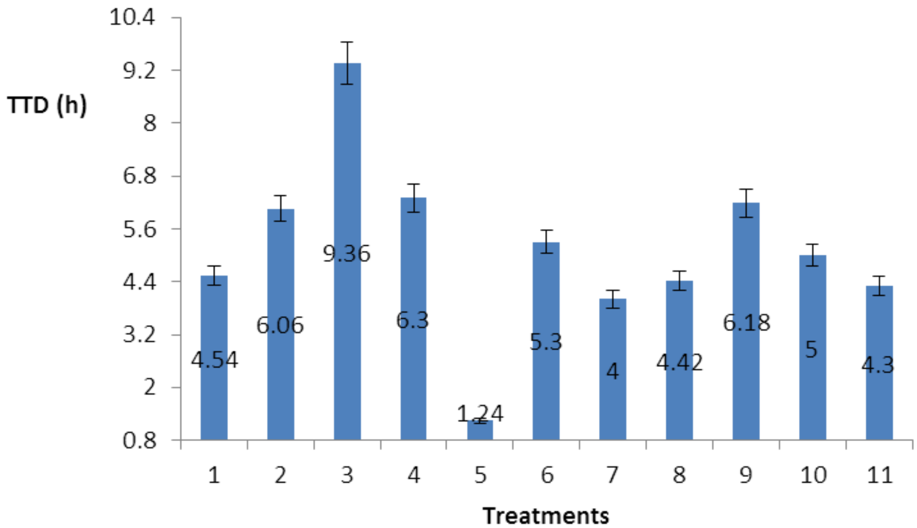

Figure 1. Graph showing plots of TTD (h) against treatments. O, Oregano; C, Cranberry. Treatment 1: oregano (100% w/w, 1% v/v extract) 6: cranberry (100% w/w, 1% v/v); Treatment 2: O-C (75-25% w/w, 1% v/v extract) 7: oregano (100% w/w, 0.5% v/v); Treatment 3: O-C (50-50% w/w, 1% v/v extract) 8: O-C (75-25% w/w, 0.5% v/v); Treatment 4: O-C (25-75% w/w, 1% v/v extract) 9: O-C (50-50% w/w, 0.5% v/v); Treatment 5: control (0% v/v extract) 10: 0-C (25-75% w/w, 0.5% v/v) Treatment 11: cranberry (100% w/w, 0.5% v/v).

RABIT system. The tests were carried out over a 24 h duration. Impedimetric times to detect (TTD) and total conductivity changes were parameters used in assessing the antimicrobial activity of oregano and cranberry extracts. The addition of extracts in all the ratios tested and at all concentrations inhibited the growth of the organism.

Effect of individual extracts of oregano and cranberry on TTD

Detection times (Table 1) obtained from the RABIT study shows the inhibitory properties that oregano and cranberry extracts had against the growth of *Salmonella*. A TTD of 1.24 h was obtained for the control sample containing bacterial cultures alone, indicating that the short detection time could have been due to optimal growth conditions (nutrients, temperature, and absence of inhibitory compounds) in the culture medium and also the fast-growing nature of *Salmonella* spp., growth was detected in the samples containing 0.5% v/v of pure oregano extract at a TTD of 4 h, an increase in extract concentration to 1% v/v resulted in a longer TTD (4.54 h). In the samples containing 0.5% v/v of pure cranberry extracts, the TTD was 4.30 h which increased to 5.30 h when concentration was increased to 1% v/v (Figure 1). At equal concentrations of 0.5 and 1% v/v respectively, cranberry extracts were more effective at inhibiting growth of *S. typhimurium* as longer TTDs were measured. The study by Lin et al. (2004) on the effect of oregano and cranberry extracts on *L. monocytogenes* showed that cranberry extracts were more effective in inhibiting growth of the pathogen than oregano extracts at pH 7 (at the same phenolic concentration). It was stated that the acidic character of cranberry phenolics could be the reason.

In the experiment, addition of pure oregano and cranberry extracts at concentrations of 0.5 and 1% v/v resulted in a significant difference in TTDs as compared with the control sample (*P* < 0.05). This indicates that oregano and cranberry contain antibacterial compounds with bacteriostatic properties as reported by Lin et al. (2004). Pure cranberry extracts resulted in a TTD of 4.3 h which was 3.06 h (at 0.5% v/v) longer than that obtained in the control experiment and a TTD of 5.3 h (at 1% v/v) which was 4.06 h longer than that obtained for the control experiment. Pure oregano extracts resulted in a TTD of 4.0 h at (0.5% v/v) which was 2.42 h longer than that obtained for the control experiment and a TTD of 4.54 h (at 1% v/v) which was 3.30 h longer than that obtained for the control experiment (Figure 1). Increasing the concentration of cranberry and oregano extracts in RABIT tests cells resulted in a statistically significant difference in TTDs as there was significant difference across rows in Table 1 (ANOVA; *P* < 0.05).

As stated earlier, the superior inhibitory effect of pure cranberry extracts on the growth of *S*. *typhimurium* could be as a result of the sensitivity of the pathogen to cranberry phenolics (Lin et al., 2004). Phenolic compounds are able to delay growth and hence prolong TTD by inhibiting the activity of ATPase, an enzyme that is important in the breakdown of ATP to release energy for cellular reactions. Lambert et al. (2001) reported this ATPase-inhibiting phenomenon in *Salmonella* spp.

Synergistic effects of oregano-cranberry extract mixtures on TTD

Oregano-cranberry extract mixtures had a significant synergistic effect on the TTD for *Salmonella* (ANOVA; *P* < 0.05). Oregano-cranberry (75:25% w/w, 0.5% v/v) treatment resulted in a TTD of 4.42 h which was 42 min longer than that obtained in the presence of pure oregano extracts (0.5% v/v) but only 12 min longer than when pure cranberry extracts (0.5% v/v) was present. An increase in concentration of oregano-cranberry (75:25% w/w) to 1% v/v resulted in a TTD of 6.06 h which was 1.12 h longer than that obtained in the presence of pure oregano (1% v/v) but only 30 min longer than when pure cranberry extracts (1% v/v) was present. Oregano-cranberry (50:50% w/w, 0.5% v/v) treatment resulted in a TTD of 6.18 which was 2.18 h longer than that obtained in the presence of pure oregano (0.5% v/v) and 1.48 h longer than when pure cranberry extract (0.5% v/v) was present. An increase in concentration of Oregano-cranberry (50:50% w/w) to 1% v/v resulted in a TTD of 9.36 h which was 4.42 h longer than that obtained in the presence of pure oregano extracts (1% v/v) and 4.06 h longer than when pure cranberry extracts (1% v/v) was present. Oregano-cranberry (25:75% w/w, 0.5% v/v) resulted in a TTD of 5.0 h which was 1 h longer than that obtained when pure oregano (0.5% v/v) was used and was 30 min longer than when pure cranberry (0.5% v/v) was used. An increase in concentration of oregano-cranberry (25:75% w/w) to 1% v/v resulted in a TTD of 6.3 h which was 1.36 h longer than when pure oregano was used (1% v/v) and 1 h longer than that obtained in the presence of pure cranberry (1% v/v) (Table 1).

These results agree with the work of Lin et al. (2004) and Lin et al. (2005) on the superior antimicrobial efficacy of oregano-cranberry extract mixtures when compared to individual extracts of the same plants. While oregano-cranberry (75:25% w/w) and oregano-cranberry (25:75% w/w) treatments had a significant synergistic effect (*P* < 0.05) compared to pure oregano extracts, this was not the case with cranberry extracts. As shown earlier, pure cranberry extracts showed a superior inhibitory effect on *S*. *typhimurium* when compared with pure oregano extracts as demonstrated by the individual TTDs (Figure 1). Hence, pure cranberry extract treatments had TTDs that compared favourably with oregano-cranberry (75:25% w/w) and oregano-cranberry (25:75% w/w) treatments. Oregano-cranberry (50:50% w/w) treatments at 0.5 and 1% v/v respectively had the most significant synergistic effect on the growth of *S*. *typhimurium* as they resulted in the longest TTD of 6.18 h and 9.36 h respectively at 0.5% and 1% v/v (Figure 1). Oregano and cranberry extracts can effectively inhibit *S. typhimurium* growth when applied individually but the results showed that the antimicrobial effect can be significantly enhanced when both extracts are mixed together in the proportion of (50-50) % w/w at concentrations of 0.5% and 1% v/v. The synergy obtained from combined extracts significantly affected the TTDs as the antimicrobial effect of the extracts become enhanced when they are combined in different proportions. Oregano and cranberry contain a variety of phenolic compounds including rosmarinic acid, carvacrol, thymol, anthocyanins and proanthrocyanidins. These are compounds that have strong antibacterial and antioxidant properties which are effective against a wide array of pathogenic bacteria. Phenolic diversity offered by combined plant extracts thus helps to provide a broader antibacterial spectrum. It can also be observed that the different extract ratios used had varying effects on the TTD values for *S*. *typhimurium*; this could be as a result of the sensitivity of the pathogen to different ratios of the extract mixtures used. These differences in TTD values may be as a result of the phenolic composition of a particular extract. Lin et al. (2004) reported that the phenolic profile of a specific extract may cause membrane disruption making cells more vulnerable to hyperacidification by acid phenolics. Phenolic compounds in oregano could cause membrane damage in *S*. *typhimurium* which could make the cells more sensitive to acid phenolics of cranberry extracts. Exposure of pathogens to low pH can lead to cell

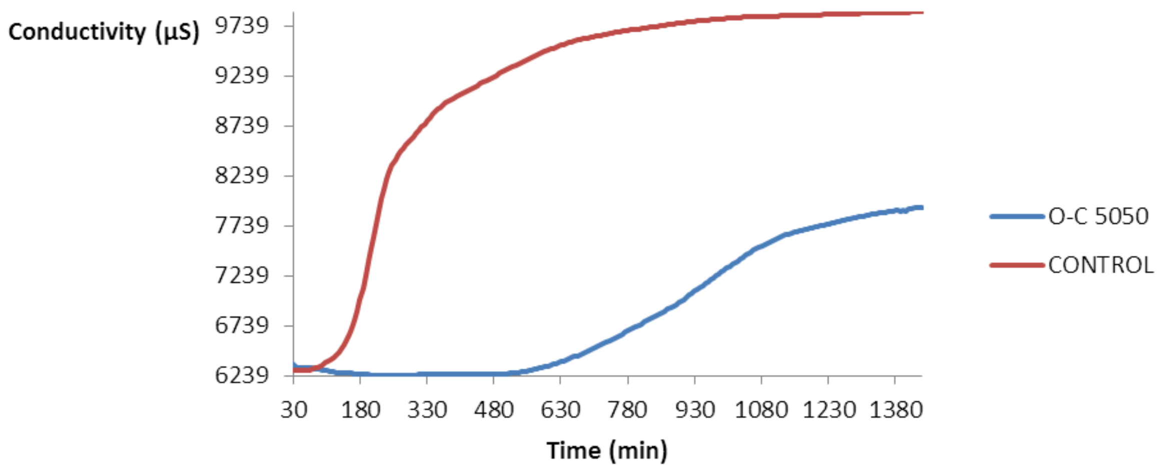

Figure 2. Relative comparative graph comparing GRVs of O-C 5050 and control treatment curves. GRVs, growth response curves; O-C 5050, oregano-cranberry (50-50% w/w, 1% v/v); Control: (0% v/v).

membrane disruption which can in turn cause loss of protons (H^+) and inhibition of ATPase activity. The results of the experiment could be possibly explained by the oregano-cranberry (50:50% w/w) at a higher concentration of 1% v/v creating a low pH due to the H^+ ions present and resulting in membrane damage (due to the action of rosmarinic acid in both extracts) which had a greater inhibitory effect on the growth of *S*. *typhimurium*. Lin et al. (2004) reported that an oregano-cranberry mixture (75:25%, w/w) was more effective at inhibiting *L. monocytogenes* growth than individual extracts at same phenolic concentration while Lin et al. (2005) reported that an oregano-cranberry mixture (25-75%, w/w) was more effective at inhibiting the *H. pylori* growth than individual extracts at the same phenolic concentration. Results obtained from the RABIT experiment show that oregano-cranberry mixture (50-50%, w/w) was significantly more effective at inhibiting *S*. *typhimurium* growth than were individual extracts at same extract concentrations of 0.5 and 1%.

Conductimetric evaluation of growth-inhibition of *S. typhimurium* by oregano and cranberry extracts

Conductance change during the test period is an important factor in RABIT research; a large total change indicates good and smooth growth. Table 1 shows the maximum conductivity change obtained for each treatment. The growth response curve of oregano-cranberry (50:50%, 1% v/v) treatment which had the longest TTD and hence superior growth inhibition was compared with other sets of extract treatments on relative comparative graphs. Figure 2 shows the comparison of the control sample (TTD at 1.24 h) with the oregano-cranberry (50:50%, 1% v/v) treatment (TTD at 9.36 h) over a 24 h (1440 min) test period. The maximum conductance change for the control sample was 3593 µS indicating good and smooth growth. The growth response curve was typical, smooth and sigmoid (Figure 2). The maximum conductance change obtained for the oregano-cranberry (50:50%, 1% v/v) treatment was 1685 µS; the low conductance change indicates poor growth. The growth response curve was atypical and weakly sigmoidal.

There was minimal conductance change over the first 9 h of the test period suggesting that bacterial growth was being inhibited, while a steady conductance increase continued throughout the remaining 15 h. The control treatment growth response curve showed a steady conductance increase throughout (Figure 2). This suggests that culture media conditions were optimal. The fall in total conductance change from 3593 to 1685 µS shows the significant synergistic effect of oregano-cranberry (50:50% w/w, 1% v/v) treatment ($P < 0.05$) on the inhibition of growth of *S*. *typhimurium* as fewer molecules were being converted into ions, indicating that metabolism rates had fallen drastically (Silley and Forsythe, 1996).

In Figure 3, the growth response curves of oregano-cranberry (50:50% w/w, 1% v/v), pure cranberry extract (1% v/v) and pure oregano extract (1% v/v) treatments were compared. The total conductance change obtained for the oregano-cranberry (50:50% w/w, 1% v/v) treatment was 1685 µS, that of pure oregano extract (1% v/v) was 2198 µS while that of pure cranberry extract was 2068 µS (Figure 3). The total conductance change for the pure cranberry extract (1% v/v) treatment was lower than that of pure oregano extract (1% v/v) which corroborates the evidence obtained from TTD values that indicated that cranberry was a better inhibitor of the growth of *S*. *typhimurium* than oregano at the same extract concentration. Growth response curves from Figure 3 show that the conductance change for the pure cranberry extract (1% v/v) treatment and pure oregano extract (1% v/v) treatment increased steadily and that the growth curve

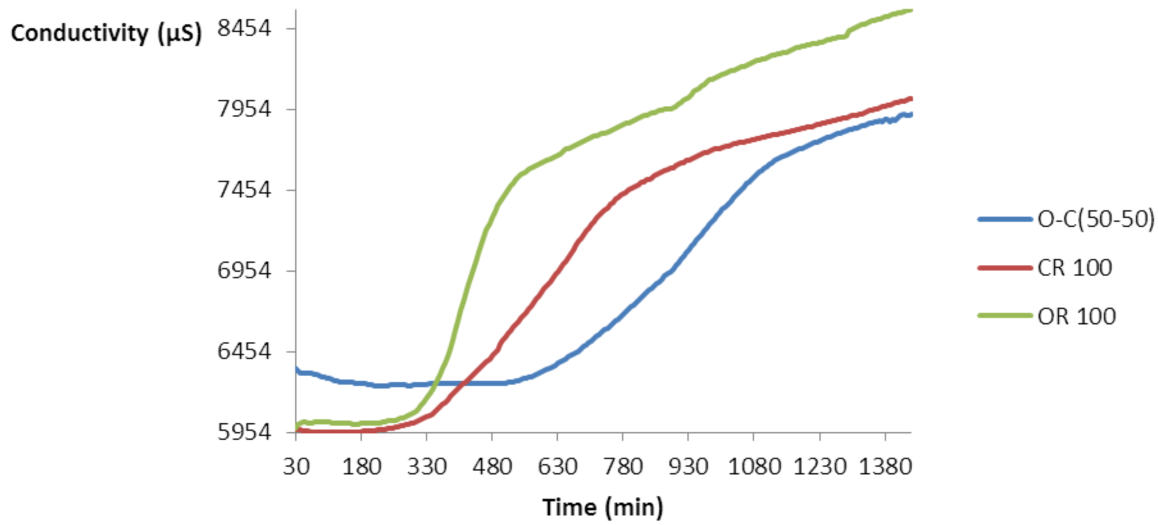

Figure 3. Relative comparative graph comparing GRVs O-C 5050, CR 100 and OR 100. GRVs, growth response curves; O-C 5050, oregano-cranberry (50-50% w/w, 1% v/v); CR100, cranberry (100% w/w, 1% v/v); OR100, oregano (100% w/w, 1% v/v).

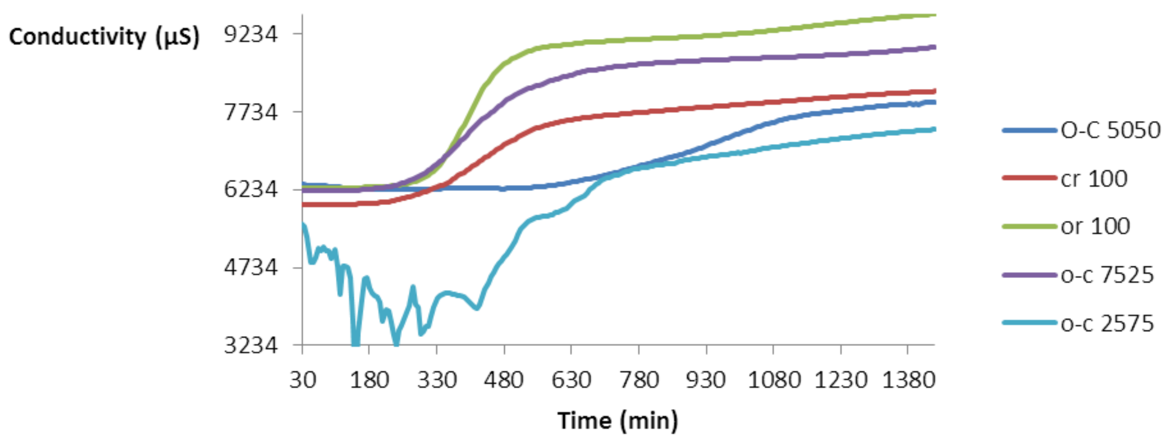

Figure 4. Relative comparative graph comparing GRVs of O-C 5050, cr 100, or 100, o-c2575 and o-c 7525. GRVs, growth response curves; O-C 5050, oregano-cranberry (50-50% w/w, 1% v/v); cr100: cranberry (100% w/w, 0.5% v/v); or100, oregano (100% w/w, 0.5% v/v); o-c 7525, oregano-cranberry (75-25% w/w, 0.5% v/v); o-c 2575, oregano-cranberry (25-75% w/w, 0.5% v/v).

was sigmoidal whereas the growth curve for oregano-cranberry (50:50% w/w, 1% v/v) treatment was weakly sigmoidal and the conductance change was low. This indicates that oregano-cranberry (50:50% w/w, 1% v/v) was a more effective inhibitor of bacterial growth. It is important to note that the shape of the curve is a likely indicator of the culture media conditions as a sigmoidal growth response curve indicates smooth and unhindered growth.

The growth response curves of five different treatments comprising oregano-cranberry (50-50% w/w, 1% v/v) with a conductance change of 1685 µS, cranberry (100% w/w, 0.5% v/v) with a total conductance change of 2198 µS, oregano (100% w/w, 0.5% v/v) with a total conductance change of 3356 µS, oregano-cranberry (75-25% w/w, 0.5% v/v) with a total conductance change of 2770 µS and oregano-cranberry (25-75% w/w, 0.5% v/v) with a total conductance change of 3280 µS were compared (Figure 4). Oregano-cranberry (50-50% w/w, 1% v/v) had the lowest conductance change and a weakly sigmoidal curve while oregano-cranberry (25-75% w/w, 0.5% v/v) was fairly sigmoidal though the conductance change fluctuated for the first 7 h. The other treatments had steady growth curves and were sigmoidal. Oregano (100% w/w, 0.5% v/v) had the highest conductance change of the five treatments but the shortest TTD (4 h) of the selected treatments. It can be concluded that treatments with higher conductance changes had shorter TTD values. Conversely, treatments with lower conductance changes had higher TTD values.

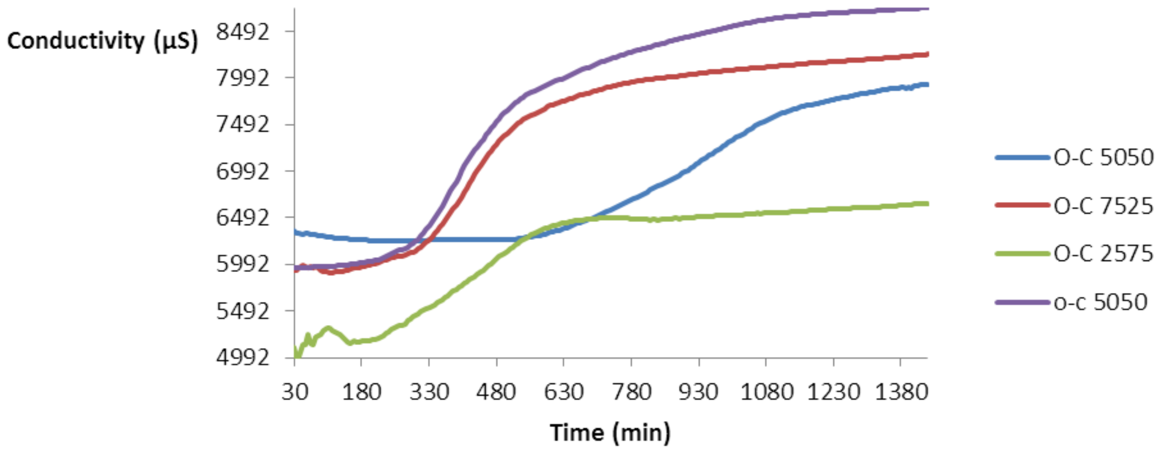

Figure 5. Relative comparative graph comparing GRVs of O-C 5050, O-C 7525, O-C 2575 and o-c 5050. GRVs, growth response curves; O-C 5050, oregano-cranberry (50-50% w/w, 1%v/v); O-C 7525, oregano-cranberry (75-25% w/w, 1% v/v); O-C 2575, oregano-cranberry (25-75% w/w, 1% v/v); o-c 5050, oregano-cranberry (50-50% w/w, 0.5% v/v).

In Figure 5, the growth response curves of five different treatments were compared. The treatments were oregano-cranberry (50-50% w/w, 1%v/v) with a total conductance change of 1685 µS, oregano-cranberry (75-25% w/w, 1% v/v) with a total conductance change of 2347 µS, oregano-cranberry (25-75% w/w, 1% v/v) with a total conductance change of 1690 µS, oregano-cranberry (50-50% w/w, 0.5% v/v) with a total conductance change of 2790 µS. The two treatments with the lowest conductance changes; oregano-cranberry (50-50% w/w, 1% v/v) and oregano-cranberry (25-75% w/w, 1% v/v) had the highest TTD values of the four samples analysed. Hence, it can be stated that the higher the conductance change, the shorter the TTD. Conductance changes are powerful parameters in RABIT studies and this research has shown that conductance change is almost always inversely proportional to detection time.

Application of the synergy of oregano-cranberry extracts mixtures towards the inhibition of *S. typhimurium* growth in the pork industry - potentials and challenges.

Research has been done on the application of plant extracts in meat systems (Lin et al., 2004; Tayel et al., 2012). Lin et al. (2004) discovered that the application of oregano and cranberry in meat and fish systems was effective in inhibiting growth of *L. monocytogenes*. Tayel et al. (2012) also worked on a number of plant extracts including oregano and their antibacterial effect on meat steaks. While all the plant extracts were effective growth inhibitors, oregano extracts had only a moderately inhibitory effect on *S. typhimurium* growth on meat surfaces. It is important to state that while these experiments were conducted in the laboratory, the application of plant extracts as a decontamination strategy to reduce the load of both pathogen and spoilage microorganisms in meat products in production lines is becoming a priority in the food industry (Sullivan et al., 2012). Lin et al. (2004) stated that the application of plant antimicrobials to food products may provide an extra hurdle to inhibit the growth of foodborne pathogens in food. The synergistic effect of oregano and cranberry extracts on the inhibition of the growth of *L. monocytogenes* in broth and meat (Lin et al., 2004) has raised the possibility of the combined plant extracts having the same or even superior effects on *S. typhimurium* in pork products. The results from this experiment have shown that all the different extract mixture combinations produced synergy that had a greater inhibitory effect on growth of *S. typhimurium* than individual oregano and cranberry extracts. Oregano-cranberry mixture (50:50% w/w, 1% v/v) treatment with a TTD of 9.36 h was the best extract combination. The combination with a TTD of 9.36 h was able to delay TTD by more than 8 h (Control = TTD of 1.24 h).

Possible challenges that could be encountered in the application of oregano-cranberry extract mixture to pork could be the high fat content of the meat which could reduce sensitivity of the pathogen to the phytochemicals and also consumer perceptions of extract-treated meat. However, Lin et al. (2004) reported that due to the partial hydrophobic property of most of the phenolic compounds present in oregano and cranberry, they were more effective ingredients to use in meat studies due to their ability to work between the lipid and water-compatible parts of the meat. The appropriate concentration of extract to be applied is also important so as not to impact on the taste and other organoleptic properties of the meat. Sullivan et al. (2012) in a study reported that there was no significant difference between consumer perception of antimicrobial-treated ham and non-treated ham in terms of taste and other sensory quality.

CONCLUSIONS, CHALLENGES AND RECOMMENDATIONS

The antimicrobial effectiveness of the combined extract mixtures of oregano and cranberry was assessed using a RABIT system. All the extract mixture combinations; oregano-cranberry (75:25% w/w), oregano-cranberry (50:50% w/w), oregano-cranberry (25:75% w/w) exerted inhibitory effects that were superior to those obtained from individual oregano and cranberry extracts at concentrations of 0.5% and 1% v/v. Oregano-cranberry (50:50% w/w, 1% v/v) with a TTD of 9.36 h was the most effective combination as it delayed the growth of *S. typhimurium* for more than 8 h (control: TTD of 1.24 h).

It is recommended that oregano-cranberry (50-50% w/w) be introduced into pork systems in production lines as a decontamination strategy for the reduction of *S. typhimurium* load on pork and pork products. Before this is done, it is important that laboratory trials are done in vitro for the application of oregano-cranberry (50-50% w/w) on pork systems, taking into account the high fat content (>15%) of pork which could reduce the sensitivity of the pathogen to the antibacterial action of the phytochemicals (Lin et al., 2004). This is essential so as to get the right balance of concentration which will not only guarantee food safety (significant reduction of *S. typhimurium* load) but also not impact negatively on taste and other sensory perceptions (food quality). A taste panel made up of selected personnel should be set up for this purpose and scores given for each sensory assessment done by the personnel on both extract-treated and non-treated pork (Sullivan et al., 2012; Tayel et al., 2012).

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