Disinfection of Produce Using Stabilized Emulsions of White Mustard Essential Oil and Chlorine Wash

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Abstract. Fresh produce has been linked to more than 30 outbreaks per year on average in the US from 1998-2016. Essential oils possess antimicrobial properties and are screened as a potential alternative to conventional disinfectants. The aim of the study was to determine the optimum sucrose palmitate: soy lecithin (SP:SL) ratio to disperse White mustard essential oil (WMEO) and evaluate the inhibitory effect of WMEO microemulsions against *Escherichia coli* in fresh lettuce compared to chlorine wash. The experiment was divided in three phases: formulation of emulsions using WMEO and SP:SL, macrodilution assay against *E. coli* in tryptic soy broth (TSB) and in fresh lettuce. In phase I stability of WMEO concentrations was measured visually in different SP:SL ratios ordered in a factorial arrangement in a 24-hour period. A Randomized Complete Block Design was used for phase II and III with duplicate for treatments and performed in triplicate for each phase. Results showed that a 5:5 ratio of SP:SL was able to disperse 3 and 5% of WMEO and maintain an emulsion the longest in a 24 hr period. In addition, 0.84% WMEO was able to reduce 2.7-2.9 Log CFU in the population of *E. coli* in TSB at 22 °C. Finally, 1% WMEO and Sodium hypochlorite (150 ppm) were the most effective treatments and caused a 2 Log reduction in *E. coli* population after simulating a commercial wash of lettuce. Results showed that WMEO is a suitable disinfection alternative to conventional chlorine solutions for lettuce.

Key words: Antimicrobial, *Escherichia coli*, fresh produce.

Resumen. Los vegetales frescos han sido ligados a 30 brotes de enfermedades transmitidas por alimentos (ETAS) en promedio anuales en EUA durante 1998-2016. Los aceites esenciales poseen propiedades antimicrobianas y apuntan a ser una alternativa potencial a los desinfectantes convencionales. El estudio tenía como objetivo determinar la proporción óptima de palmitato de sacarosa y lecitina de soya (PS:LS) que mejor disperse el aceite esencial de mostaza (AEM) y evaluar el efecto inhibitorio de AEM en emulsiones contra *Escherichia coli* en lechuga fresca comparado a soluciones cloradas. El experimento fue realizado en tres fases: formulación de emulsiones utilizando PS:LS; ensayo de macrodilución contra *E. coli* en caldo de soya tripticasa (CST) y en lechuga fresca. En la fase I la estabilidad de concentraciones de AEM fue medida visualmente en proporciones de PS:LS durante 24 horas ordenados en un arreglo factorial. Se utilizó Bloques Completos al Azar para la fase II y III con duplicado para tratamientos y cada fase fue realizada por triplicado. Los resultados mostraron que una proporción de 5:5 de PS:LS logró dispersar 3 y 5% de AEM y mantener una emulsión en un periodo de 24 horas. Una concentración de 0.84% AEM logró reducir 2.7-2.9 Log UFC/ml la población de *E. coli* en CST después de 24 horas. Finalmente, 1% AEM e hipoclorito de sodio (150 ppm) fueron los tratamientos más efectivos al reducir 2 Log UFC/ml en la población de *E.coli* mediante un lavado por inmersión. Los resultados mostraron que el AEM es una alternativa a soluciones de cloro para desinfección de lechuga.

Palabras clave: Antimicrobiano, *Escherichia coli*, vegetales frescos.

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1. INTRODUCTION

Currently, consumers have concerns about their food and food additives and are driving trend of natural derived products and ingredients. One of the main challenges to the food industry is the preservation of food against spoilage and pathogens using natural preservatives to meet the consumers' desire. When referring to food safety, *Escherichia coli*, *Salmonella enterica* subsp. enterica serovar Enteritidis, *Staphylococcus aureus*, and *Listeria monocytogenes* are the microorganisms of concern to the industry, regulation agencies and consumers (Scallan *et al*. 2011). Examples of microorganisms that cause spoilage during growth are *Brochothrix thermosphacta* (meat and meat products), *Carnobacterium* spp. (Modified atmosphere packed meat and dairy products), *Lactobacillus* spp. (dairy products), and *Leuconostoc* spp. (chill-stored meats) (Lorenzo *et al*. 2018).

In the United States, approximately 9.4 million illnesses are caused from foodborne diseases by known pathogens (Scallan *et al*. 2011). During 2016, 839 foodborne disease outbreaks were reported, resulting in 14,259 illnesses, 875 hospitalizations, 17 deaths, and 18 food product recalls (CDC 2016). Outbreaks associated with fresh produce ranged from 30-60 per year and sickened from 900-3,000 people anually between 1998-2016. Leafy greens accounted between 20-40% of these outbreaks and 10-40% of the illnesses. Multiple foodborne illness and outbreak investigations involved *E. coli* O157:H7 illnesses that were linked to leafy greens (Johnson 2019). *E. coli* is a diverse group of bacteria in which most strains are harmless, however some strains cause disease by producing Shiga toxin and are called "Shiga toxin-producing *E. coli* " (STEC) (CDC 2016).

To enhance shelf-life and safety in fresh produce, disinfection processes that incorporate chlorine are used, however, the chronic exposure to chlorinated compunds such as sodium hypochlorite, which is known to be corrosive, can cause sever injuries to the digestive system and dermis (European Chemicals Agency 2007, Public Health England 2015). Consumers are minimizing or excluding the use of synthesized antimicrobials and prefering the use of natural antimicrobials (Gutierrez *et al.* 2008). One of the main sources of natural antimicrobials are essential oils and extracts from plants (Safaei-Ghomi and Ahd 2010). Essential oils are plant-derived compounds that have antibacterial, antiviral and antifungal properties, containing a mixture of terpenes and oxygenated hydrocarbon compounds such as aldehydes, ketones, esters, alcohols, lactones and phenols (Barbieri *et al*. 2017). They are aromatic liquids obtained from different plant materials such as flowers, seeds, wood, leaves and fruits.

Essental oils have been studied as natural sanitizers on minimally processed fruits and vegetables against foodborne pathogens and spoilage bacteria (Gutierrez *et al*. 2008). Apart from their application in fresh produce, essential oils are promising to the meat industry. Oxidation of food components such as lipids, proteins and pigments decrease product quality and acceptance. Phenolic compounds of essential oils are responsible for strong antioxidant activity (Pateiro *et al*. 2018). Zeningn and Baysal (2015) proposed to enhance oxidative stability in minced beef by incorporating thyme essential oil (TEO). They used 2 minimum inhibitory concentrations (MIC) doses; lipid oxidation was evaluated through a thiobarbituric acid reactive substances (TBARS) assay and their results pointed that TEO retarded lipid and color oxidation for nine days of storage at 4 °C.

One of the main issues regarding essential oils is their hydrophobicity in other solutions. To overcome this immiscibilty, hydrophobic compounds are incorporated into emulsions. Micro emulsions are a mixture of water, oil and surfactants that assemble in stable structures (Lawrence & Rees 2000). Zhang *et al*. (2014), formulated essential oil micro emulsions for organic fresh produce using lecithin and sucrose octanoate ester (SOE) as surfactants and organic essential oils of clove bud, cinnamon bark, and thyme. Their results showed that micro emulsions can be formed at lecithin: SOE mass ratios of 1:9–5:5 to dissolve up to 4.5, 4.5 and 3.5% w/w of clove bud oil, cinnamon bark oil, and thyme oil, respectively. In another study micro emulsions were formulated with clove, thyme oil and arabic gum where 0.5% thyme oil and 0.5% clove oil were the most effective treatments; however, there was a low inactivation of *Salmonella* (Dunn *et al*. 2019).

Several studies have indicated that mustard extracts are capable to work as antimicrobials against pathogenic bacteria. The active component of white mustard essential oil (WMEO) is 4-hydroxybenzyl isothiocyanate, which is an oily compound that is the result of moistening or grinding the seed (Ekanayake 2016). Chemically, when the plant´s tissue is damaged, an endogenous enzyme called myrosinase degrades glucosinolates. This reaction mainly depends on metal ions and pH. Monu *et al*. (2014), determined the *in-vitro* antimicrobial activity of white mustard essential oil against *Escherichia coli*, *Salmonella enterica* serovar Enteritidis, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Listeria monocytogenes, Bacillus cereus*, *Lactobacillus fermentum* and *Schizosaccharomyces pombe*. In their results, all microorganisms were inhibited by 8.3 g/L of WMEO.

The objectives of this study were:

- To determine the optimum sucrose palmitate: soy lecithin ratio of mixture that maintains an antimicrobial emulsion of WMEO.

- To evaluate the inhibitory effect of WMEO emulsion against *Escherichia coli* in fresh lettuce compared to other disinfectant solutions.

2. MATERIALS AND METHODS

Location.
This project was developed in a microbiology laboratory at Auburn University in Auburn, Alabama, USA.

Phase 1. Emulsion stability test.
Stability of White Mustard essential oil (WMEO) in sucrose palmitate: soy lecithin (SP:SL) mixtures was tested at different surfactant ratios and WMEO concentrations. For a better dispersion, 5% sucrose palmitate and 2% soy lecithin stock solutions were prepared. Sucrose palmitate and soy lecithin stock solutions were mixed with sterile water to achieve ratios of 1:9, 2:8, 3:7, and 5:5, where the combined amount of SP:SL represent the 5% of the mixture. WMEO was thawed and added to SP:SL mixtures obtaining concentrations of 1, 2, 3 and 5% WMEO. Treatment description can be observed in Chart 1. The procedure was repeated for all ratios of SP:SL being 16 treatments in total and were assessed at 24 hr.

Chart 1. Description of emulsion treatments using WMEO and Sucrose palmitate: soy lecithin.

	Sucrose Palmitate: Soy lecithin ratio			
WMEO concentration (%)	01:09	02:08	03:07	05:05
1	A_1	A_2	A_3	A_4
2	B_1	B_2	B_3	B_4
3	C_1	C_2	C_3	C_4
5	D_1	D_2	D_3	D_4

WMEO (White mustard essential oil)
ABCD (WMEO concentration)
$_{1234}$ (Sucrose Palmitate: Soy lecithin ratio)

Experimental design. WMEO concentrations and SP:SL ratios were ordered in a 4×4 factorial arrangement in a Randomized Block Design and measured visually. Experiment was performed in triplicate.

Phase 2. Macrodilution assay against *Escherichia coli* ATCC BAA-2196.

Bacterial culture preparation. The bacterial strain used for this study was *Escherichia coli* 2196. Frozen culture of *E. coli* was grown in tryptic soy broth (TSB) at a starting pH

of 7.2 and incubated at 37 °C for 18-22 hr. The culture was transferred again under the same conditions. Culture was streaked on TSA, incubated at 37 °C for 20-24 hours, sealed with Para film and stored at 4 °C.

Assay in TSB. *Escherichia coli* 2196 was inoculated in TSB and incubated at 37 °C for 20-24 hr. The culture was transferred again under the same conditions. The surfactants used for this assay were SP:SL mixture and dimethyl-sulfoxide (DMSO) for comparison of results in previous studies. A 5:5 ratio of SP:SL mixture was prepared. Solutions with 42% and 25% WMEO were prepared in 2 mL centrifuge tubes for both DMSO and SP: SL. From these, an aliquot of 200 µL of each solution was added to tubes with 9.8 mL of TSB and vortexed thoroughly to achieve final concentration of 0.84% WMEO. Controls were prepared by adding a volume of 200 µL of SP:SL or DMSO to tubes with 9.8 mL of TSB to ensure that the dispersing agent had no inhibitory effect and the final control was TSB by itself. All treatments were performed in duplicate and are described in Chart 2. The overnight culture of *E. coli* was diluted once resulting in an approximate 8 Log CFU/ml concentration. A volume of 20 µL of culture was added to each tube and vortexed thoroughly. For both 0 and 24-hour assay, the effect of the treatments was measured by serially diluting in 0.1% peptone water; spread plating in duplicate on TSA and incubating at 37 °C for 24 hr before counting.

Chart 2. Description of treatments of *Escherichia coli* ATCC BAA-2196 with WMEO in Tryptic soy broth.

Dispersing agent	**WMEO concentration**	
	0.84%	**0%**
Sucrose palmitate: lecithin	T_1	T_2*
Dimethyl-sulfoxide	T_3	T_4*
No Dispersing agent	NA	T_4*

*Control treatments
NA (Not applicable)
WMEO (White mustard essential oil)

Experimental design. The complete experiment was performed in triplicate and arranged in a Complete Randomized Block design using two surfactants, two WMEO concentrations and two-time assays (0 and 24 hour). Data was analyzed with a one-way ANOVA (SAS 9.4) with a significance level of $P < 0.05$. Means were separated through a Duncan's Multiple Range test (DMRT).

Phase 3. Assay against *Escherichia coli* 2196 ATCC BAA-2196 in fresh lettuce.

Induction to bacterial resistance. *E. coli* was induced to antibiotic resistance for more accurate results and to eliminate background microflora on lettuce. Pure culture was inoculated in TSB and incubated at 37 °C for 20-24 hr. Then, culture was transferred to

other TSB tube plus the addition of nalidixic acid (NAL) and incubated at the same conditions. The NAL concentration increased incrementally from 25 ppm to a final concentration of 100 ppm. The 100 ppm NAL resistant *E. coli* was grown for 24 hr at a temperature of 37 °C in TSB. Cells were centrifuged at 5000 × *g* for 10 minutes and washed twice with peptone water. Finally, pellet was resuspended in 10 mL of peptone water.

Treatment of produce. The inoculation solution was prepared by transferring 10 mL of culture (pellet resuspended in peptone water) to 200 mL of sterile distilled water to achieve an average concentration 6-7 Log CFU/ml. Dip-inoculation method was adopted as described by Moutacho *et al*. (2017) with some modifications. The dipping solution of WMEO: SP:SL (1% WMEO) was prepared in sterile water and mixed thoroughly (|Moore-Neibel *et al*. 2011). Two chlorine solutions were prepared, one with sodium hypochlorite (150 ppm) and another with chlorine dioxide (5 ppm). A volume of 1 liter of tap water was used as a control. Fresh romaine lettuce was acquired from a local market and stored at 4 °C. Lettuce was cut into 25 g pieces with a sterile knife and immersed in the inoculated solution for one-minute simulating a normal wash. Samples were dried for 30 minutes under a bio safety hood at room temperature (25 °C). Inoculated lettuce was then dipped for one min in the aforementioned treatment solutions and can be observed in Chart 3. Once washing was complete, samples were placed in stomacher bags with peptone water and stomached for one minute. Finally, samples were serially diluted and spread plated in duplicate in TSA+100 ppm of NAL and incubated at 37 °C for 24 hours before counting.

Chart 3. Description of washing treatments against *Escherichia coli* ATCC BAA-2196 inoculated in fresh lettuce.

Disinfection solution	**Treatment**
1% WMEO + Sucrose Palmitate: Soy Lecithin (5:5)	A
Sodium hypochlorite (150 ppm)	B
Chlorine dioxide (5 ppm)	C
Tap Water	D*
No Wash	E*

*Control treatments Data was taken at 0 hour for each washing treatment.
WMEO (White mustard essential oil)

Experimental design. The complete experiment was performed in triplicate and arranged in a Complete Randomized Block design with duplicate for treatments and controls. Data were analyzed with a one-way ANOVA (SAS 9.4) with a significance level of $P < 0.05$. Means were separated through a Tukey test.

3. RESULTS AND DISCUSSION

Emulsion stability test.

Emulsion stability was measured visually due that it is the simplest and quickest method to assess gravitational separation without the use of any analytical instruments. Level of separation was categorized into two groups: "separation" which accounted > 25% of oil separated and "minor" or no "separation" where < 25% of oil was separated. Overall, ratios of 3:7 and 5:5 were the most effective in maintaining the emulsion for the longest amount of time in a period of 24 hr and these results can be observed in Chart 4. The highest amount of WMEO that can be dispersed was achieved by the 5:5 SP:SL ratio which coincides with Zhang e*t al.* (2014) in which clove bud, cinnamon bark and thyme oil were used. They found that noted a 5:5 ratio of sucrose octanoate: soy lecithin could disperse the greatest amount of essential oils but that it had limitations such as a high viscosity and poor ability to wet produce. However, stock solutions were made for both sucrose palmitate and soy lecithin, surfactants were less viscous and able to wet. Ratio 3:7 of SP:SL was able to disperse between 2-3% of WMEO making it the second most effective. However, lower ratios of SP:SL were not able to disperse 1-5% of WMEO. Visual appearance of emulsions was affected by its composition and oil type, emulsions were turbid and long periods of vortexing were used to ensure a correct mixture. Emulsion composition was affected by the amount dispersing agents used. In previous studies the emulsion tends to separate with higher ratios of soy lecithin (Zhang *et al*. 2014).

Chart 4. Stability of emulsions with WMEO and Sucrose palmitate: soy lecithin after 24 hr.

	Sucrose Palmitate: Soy lecithin ratio			
WMEO concentration	01:09	02:08	03:07	05:05
1%	Separation[1]	Separation[1]	Separation[1]	Minor Separation[2]
2%	Separation[1]	Separation[1]	Minor Separation[2]	Minor Separation[2]
3%	Separation[1]	Separation[1]	Minor Separation[2]	Minor Separation[2]
5%	Separation[1]	Separation[1]	Separation[1]	Minor Separation[2]

WMEO (White mustard essential oil)

[1] Separation (> 25% of oil separated)

[2] Minor Separation (< 25% of oil separated)

In a similar study, nanoemulsions were prepared using Tween 40 and Tween 80 as surfactants and propylene glycol as co-surfactant for dispersing up to 3% orange oil where a ratio of 1:1 (surfactant:co-surfactant) was the optimum and then incorporated into films against *S. aureus* and *P. acnes* (Jantrawut *et al*. 2018). Tahlan (2014) prepared essential oil emulsions using Tween-80 at a ratio of 1:0.5 resulting in a 2% oil concentration and screened against a series of pathogens in which cinnamon and oregano EOs had the highest antimicrobial activity. The use of sucrose palmitate and soy lecithin as surfactants allowed the stabilization of the emulsion overcoming hydrophobic properties. Combinations of WMEO with other essential oils must be screened to determine synergies and if higher antimicrobial activity can be achieved.

Effect of WMEO against ATCC *Escherichia coli* BAA-2196.

Growth of *E. coli* was evaluated in the presence of WMEO in TSB at 22 °C. Analysis showed that there were no statistical differences among treatments and controls at 0 hr. The use of blocks was justified, and repetitions were a source of variation. At 24 hours, significant statistical differences were observed among treatments and controls with a P value of < 0.05. The use of blocks was justified, and repetitions were a source of variation. The use of both surfactants SP:SL and DMSO along with WMEO showed effectiveness in reducing *E. coli* counts in TSB after 24 hr. Significant reductions were observed with 0.84% WMEO (with SP:SL or DMSO); populations of *E. coli* were decreased by 2.7-2.9 Log CFU/ml average. Results can be observed in Chart 5 which are similar to a previous study where 0.84% WMEO was able to eliminate *Salmonella* Enteriditis and *E. coli* within 3 and 48 hours of exposure respectively (Monu *et al.* 2014). This study indicates that if a 48-hour assay was done, more inhibition would have been observed. Controls showed that either dispersing agent by itself did not had an antimicrobial effect on *E. coli* populations. Additionally, analysis showed that 0.84% WMEO (with SP:SL or DMSO) compared to positive controls had approximately a 7.2-7.6 Log CFU/ml difference.

Chart 4. Survival of *Escherichia coli* (Log CFU/ml) according to exposure to different WMEO concentrations, dispersing agents and controls.

Treatment	**Exposure time (Log Means±SD)**	
	0 h	**24 h**
0.84% WMEO + SP:SL (5:5)	4.74 ± 0.38^{Ax}	1.84 ± 0.36^{Ay}
0.84% WMEO + Dimethyl sulfoxide	4.90 ± 0.20^{Ax}	2.18 ± 0.17^{Ay}
Sucrose Palmitate: Soy lecithin*	4.92 ± 0.17^{Ax}	9.44 ± 0.59^{By}
Dimethyl sulfoxide*	4.94 ± 0.14^{Ax}	9.49 ± 0.60^{By}
No Dispersing Agent*	5.05 ± 0.13^{Ax}	9.42 ± 0.67^{By}
R^2	0.78	0.99
CV	2.66	4.28

^{ABC} Means with different uppercase letters denote differences between treatments ($P < 0.05$).

^{xyz} Means with different lowercase letters denote differences between time ($P < 0.05$).

WMEO (White mustard essential oil)

*Control treatments

In the present study, *E. coli* expressed more resistance to lower concentrations of WMEO due that no inhibition was observed with 0.5% WMEO with either of the dispersing agents after 24 hours. This differs from previous studies in which 0.42% WMEO was able to inhibit growth. Generally, Gram-negative bacteria are more resistant to essential oils than Gram-positive and this is mainly due to structural differences; *E. coli* possess a peptidoglycan layer that is covered by an outside membrane that contains several proteins. These proteins serve as hydrophilic transmembrane channels and only enables small hydrophilic solutes to pass through the membrane making the bacteria resistant against hydrophobic antibiotics and other drugs (Nazzaro *et al.* 2013). Essential oils in combination with a surfactant agent for overcoming immiscibility, disturb cell structures causing bacteria cells to be more permeable. Leakage of important ions and molecules result in the death of bacterial cell (Devi *et al*. 2010)

Using these results, the next step was to determine the antimicrobial efficacy of 1% WMEO emulsions in a food matrix (lettuce) as it is known that antimicrobial activity can be reduced via the interaction of food components like fat or starch and that higher concentrations of essential oils are needed to achieve an inhibitory effect (Gutierrez *et al.* 2008).

Antimicrobial activity of WMEO, sodium hypochlorite (150 ppm) and chlorine dioxide (5 ppm) against *E. coli* BAA-2196 in fresh produce.

Significant statistical differences were observed due that measured variable had P value of < 0.05. However, the use of blocks was not justified, and repetitions were a source of variation. At 0-hour assay (instant contact) all washing treatments caused changes in *E. coli* populations and can be observed on Chart 6. Dip-inoculation method resulted in an initial count of approximately 6 Log CFU/mL. Washing treatments with 1% WMEO in SP:SL and 150 ppm sodium hypochlorite were effective in reducing 2 Log CFU/ml in average on *E. coli* compared to chlorine dioxide (ClO_2) that reduced an average of 1.35 Log CFU/ml. Studies have shown that tap water wash achieves a 0.5 – 2 Log CFU/ml reduction (Inatsu 2005). Under the washing scenario mimicked in this study, results showed the population of *E. coli* was reduced by 0.92 – 1 Log CFU/ml with tap water.

Results showed that washing treatments used in this experiment were not able to totally eliminate *E. coli* from lettuce. Sodium hypochlorite is normally used for water disinfection and as a bleaching agent. It is known to be corrosive causing skin irritation, pain, blisters or inflammations. The maximum permitted amount of active chlorine is 75-200 ppm according to the Code of Federal Regulations Title 21 Part 178 (FDA 2018). It is known that chlorinated compounds lose their efficiency when there is an interaction with organic matter however there have been no studies on the effect of organic matter on the antimicrobial activity of WMEO during produce disinfection.

Chart 5. *Escherichia coli* recovered counts (Log CFU/ml) on inoculated lettuce after different washing treatments.

Treatment	Population recovered
	Log Means ± SD
1% WMEO: SP:SL (5:5)	3.67 ± 0.12^{A}
Sodium hypochlorite	3.66 ± 0.51^{A}
Chlorine dioxide	4.40 ± 0.42^{B}
Tap Water	$4.85 \pm 0.27^{B*}$
No Wash	$5.77 \pm 0.12^{C*}$
R^2	0.94
CV	6.05

^{ABC} Means with different uppercase letters in each row denote differences between treatments ($P < 0.05$).
*Controls
WMEO (White mustard essential oil)
SP:SL (Sucrose palmitate: soy lecithin ratio)

Günduz *et al.* 2009, suggested that myrtle oil might be a suitable disinfection alternative to chlorine for lettuce and tomato. In his results, 1000 ppm of myrtle EO was comparable to 50 ppm in reducing *Salmonella* population and achieved a 1.66 Log CFU/ml and 1.89 Log CFU/ml reduction. Intrinsic properties of food such as protein, water content, antioxidants, pH and extrinsic factor like temperature, packaging method influence directly the bacterial sensitivity to antimicrobial materials in food matrices (Burt 2004). Lettuce is often consumed fresh and washed with water or with chlorinated solutions and has a very limited shelf life of 5-7 days at 10 °C. One of the main issues with chlorine disinfection systems is the maintenance of the quality of the process water due that it can serve as a cross contamination source. The reuse of water results in a buildup of microbial loads including pathogens. Most studies on disinfection agents are focused on alternatives to chlorine mainly because of the several environmental and human health effects of hyperchlorination.

With consumers demanding more natural products, the use of natural antimicrobials to ensure safety and shelf life is turning into a stronger trend. Synthetic preservatives are causing negative perceptions in consumers therefore essential oils application in food preservation is expanding. Essential oils can provide a large sum of antimicrobial, antioxidant, antifungal benefits. One of the main issues emerging with the use of synthetic sanitizers and antibiotics is the generation of bacterial resistance against them resulting in a demand for new antimicrobials. *Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella* spp., coagulase-negative *Staphylococcus, Shigella, Enterococcus sp.* and *Escherichia coli* are amongst some of the main bacteria with multidrug resistance (Fisher *et al.* 2008). Results screen WMEO as a potential disinfectant of fresh lettuce ensuring the safety of the consumer and extending the shelf life. WMEO can be introduced as a final step before packaging to achieve higher inhibition on potential pathogens and spoilage microorganisms.

4. CONCLUSIONS

- The optimum ratio of sucrose palmitate and soy lecithin with WMEO had the same efficacy as the emulsion in DMSO. The dispersing solution with SP:SL has the advantage that it can be used in food as emulsifier, contrasting from DMSO and propylene glycol used in previous studies.

- The more efficient disinfectants were 1% WMEO and 150 ppm of sodium hypochlorite and responds to consumer demands that are in favor of using natural antimicrobials for food disinfection.

5. RECOMENDATIONS

- Conduct a shelf-life investigation for lettuce storage at 4 °C to study WMEO antimicrobial activity behavior over time and an evaluation of the effect of organic matter in the efficiency of WMEO.

- Carry on a sensory analysis to evaluate the use of WMEO in the acceptance of consumers due that high concentrations of WMEO can cause negative sensory attributes.

- Evaluate the combination of WMEO with other essential oils to determine synergies and antimicrobial activity.

6. REFERENCES

Barbieri R, Coppo E, Marchese A, Daglia M, Sobarzo-Sanchez E, Nabavi SF, Nabavi S. 2017. Phytochemicals for human disease: an update on plant-derived compounds antibacterial activity. Microbiol Res 196:44–68.

Burt S. 2004. Essential oils: their antibacterial properties and potential applications in foods--a review. Int J Food Microbiol. 94(3):223–253. eng. doi:10.1016/j.ijfoodmicro.2004.03.022.

Centers for Disease Control and Prevention (CDC). 2016. Surveillance for Foodborne Disease Outbreaks, United States. Annual Report. Atlanta, Georgia: U.S. Department of Health and Human Services, CDC.

Devi KP, Nisha SA, Sakthivel R, Pandian SK. 2010. Eugenol (an essential oil of clove) acts as an antibacterial agent against Salmonella typhi by disrupting the cellular membrane. J. Ethnopharmacol;130:107–115. doi: 10.1016/j.jep.2010.04.025.

Dunn L, Harness M, Smith D, Gorman S, Zhong Q, Davidson P, Critzer F.2019. Essential Oil Emulsions as Postharvest Sanitizers To Mitigate Salmonella Cross-Contamination on Peppers. Journal of Food Protection, 82(1):159–163. doi:10.4315/0362-028x.jfp-18-190.

Ekanayake A, Strife R, Zehentbauer G, David J. 2016. Yellow or White Mustard (*Sinapis alba L.*) Oils. Essential Oils in Food Preservation, Flavor and Safety, 857–863. doi:10.1016/b978-0-12-416641-7.00098-5

European Chemicals Agency (ECHA). 2007. EU RISK ASSESSMENT - CHLORINE CAS 7782-50-5. Italy: Luxembourg: Office for Official Publications of the European Communities. 36 p.

FDA. 2018. Code of Federal Regulations: Title 21, Volume 3. Silver Spring, MD. [accessed June 2019].

Fisher K, Phillips C. 2008. Potential antimicrobial uses of essential oils in food: Is citrus the answer? Trends Food Sci. Technology;19:156–164. doi: 10.1016/j.tifs.2007.11.006

Gündüz GT, Gönül ŞA, Karapinar M. 2009. Efficacy of myrtle oil against Salmonella Typhimurium on fresh produce. International Journal of Food Microbiology, 130(2), 147–150. doi:10.1016/j.ijfoodmicro.2009.01.010

Gutierrez J, Barry-Ryan C, Bourke P. 2008. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. Int J Food Microbiol. 124(1):91–97.

Inatsu Y, Bari ML, Kawasaki S, Kawamoto S. 2005. Effectiveness of Some Natural Antimicrobial Compounds in Controlling Pathogen or Spoilage Bacteria in Lightly Fermented Chinese Cabbage. Journal of Food Science. 70(9):M393-M397. doi:10.1111/j.1365-2621.2005.tb08323.x.

Jantrawut P, Boonsermsukcharoen K, Thipnan K, Chaiwarit T, Hwang KM, Park ES. 2018. Enhancement of Antibacterial Activity of Orange Oil in Pectin Thin Film by Microemulsion. Nanomaterials (Basel). 8(7). eng. doi:10.3390/nano8070545.

Johnson R. 2019. Foodborne Illnesses and Outbreaks from Fresh Produce. USA: Library of Congress. Congressional Research Service. 2 p.

Lawrence M, Rees G. 2000. Microemulsion-based media as novel drug delivery systems. Advanced Drug Delivery Reviews, 45: 89–121.

Lorenzo J, Munekata P, Dominguez R, Pateiro M, Saraiva J, Franco D. 2018. Main Groups of Microorganisms of Relevance for Food Safety and Stability. In: Innovative Technologies for Food Preservation. Elsevier; p. 53–107. doi:10.1016/b978-0-12-811031-7.00003-0.

Monu E, David J, Schmidt M, Davidson P. 2014. Effect of white mustard essential oil on the growth of foodborne pathogens and spoilage microorganisms and the effect of food components on its efficacy. Journal of food protection, 77 12: 2062-8.

Moore-Neibel K, Gerber C, Patel J, Friedman M, Ravishankar S. 2012. Antimicrobial activity of lemongrass oil against Salmonella enterica on organic leafy greens. Journal of Applied Microbiology, 112(3):485–492. doi:10.1111/j.1365-2672.2011.05222.

Mouatcho J, Tzortzakis N, Soundy P, Sivakumar D. 2017. Bio-sanitation treatment using essential oils against E. coli O157:H7 on fresh lettuce. New Zealand Journal of Crop and Horticultural Science, 45(3): 165–174. doi:10.1080/01140671.2016.1269813.

Nazzaro F, Fratianni F, Martino L de, Coppola R, Feo V de. 2013. Effect of essential oils on pathogenic bacteria. Pharmaceuticals (Basel). 6(12):1451–1474. eng. doi:10.3390/ph6121451

Pateiro M, Barba F, Domínguez R, Sant'Ana A, Mousavi Khaneghah A, Gavahian M, Lorenzo J. 2018. Essential oils as natural additives to prevent oxidation reactions in meat and meat products: A review. Food Research International, 113:156–166. doi:10.1016/j.foodres.2018.07.014

Public Health England (PHE). 2015. Compendium of Chemical Hazards: Sodium Hypochlorite. United Kingdom: PHE Publishing. 10 p.

Safaei-Ghomi J, Ahd A. 2010. Antimicrobial and antifungal properties of the essential oil and methanol extracts of Eucalyptus largiflorens and Eucalyptus intertexta. Pharmacogn Mag 2010, 6:172-175.

Scallan E, Hoekstra R, Angulo F, Tauxe R, Widdowson M, Roy S, Jones J, Griffin P. 2011. Foodborne Illness Acquired in the United States—Major Pathogens. Emerg. Infect. Dis. 17:7–15.

Tahlan V. 2014. Antimicrobial Activity Of Essential Oil Emulsions And Possible Synergistic Effect On Food Borne Pathogens. Wayne State University Theses.Paper 316

Zengin H, Baysal A. 2015. Antioxidant and antimicrobial activities of thyme and clove essential oils and application in minced beef. Journal of Processing and Preservation, 39:261-1271.

Zhang L, Critzer F, Davidson PM, Zhong Q. 2014. Formulating essential oil microemulsions as washing solutions for organic fresh produce production. Food Chem. 165:113–118. eng. doi:10.1016/j.foodchem.2014.05.115.

7. APPENDICES

Appendix 1. Summary of statistical analysis at 0 hour assay for phase 2.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.50104000	0.08350667	4.90	0.0216
Error	8	0.13640000	0.01705000		
Corrected Total	14	0.63744000			

R-Square	Coeff Var	Root MSE	Log Mean
0.786019	2.658299	0.130576	4.912000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	4	0.15204000	0.03801000	2.23	0.1554
BLK	2	0.34900000	0.17450000	10.23	0.0062

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	4	0.15204000	0.03801000	2.23	0.1554
BLK	2	0.34900000	0.17450000	10.23	0.0062

Appendix 2. Summary of statistical analysis at 24-hour assay for phase 2.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	201.6692000	33.6115333	436.82	<.0001
Error	8	0.6155733	0.0769467		
Corrected Total	14	202.2847733			

R-Square	Coeff Var	Root MSE	Log Mean
0.996957	4.284276	0.277393	6.474667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	4	199.6575067	49.9143767	648.69	<.0001
BLK	2	2.0116933	1.0058467	13.07	0.0030

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	4	199.6575067	49.9143767	648.69	<.0001
BLK	2	2.0116933	1.0058467	13.07	0.0030

Appendix 3. Summary of statistical analysis for phase 3

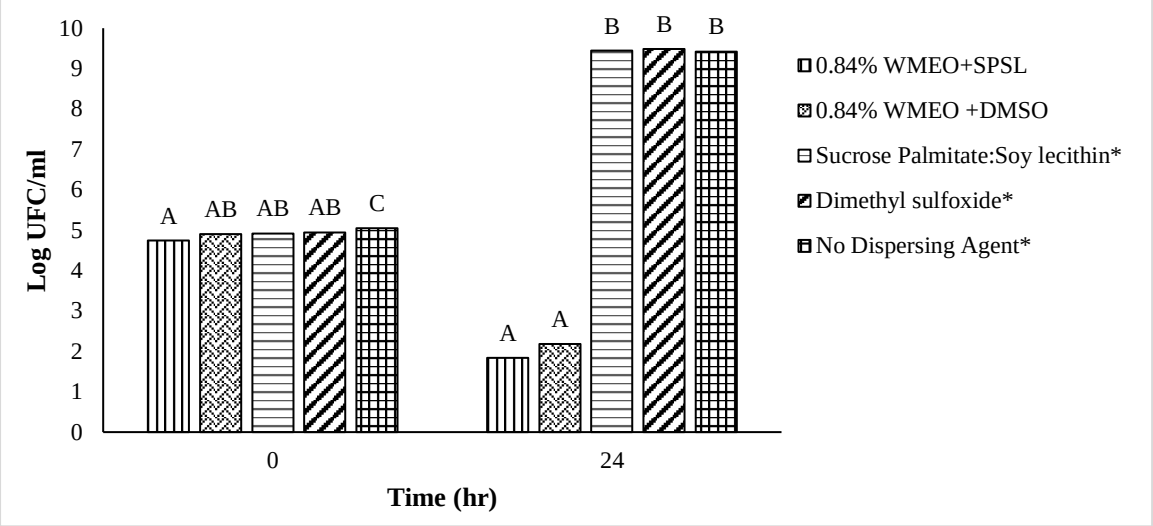
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	12.30556743	1.75793820	23.73	<.0001
Error	10	0.74066035	0.07406603		
Corrected Total	17	13.04622778			

R-Square	Coeff Var	Root MSE	Log Mean
0.943228	6.056019	0.272151	4.493889

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	4	11.77308611	2.94327153	39.74	<.0001
BLK	3	0.53248132	0.17749377	2.40	0.1291

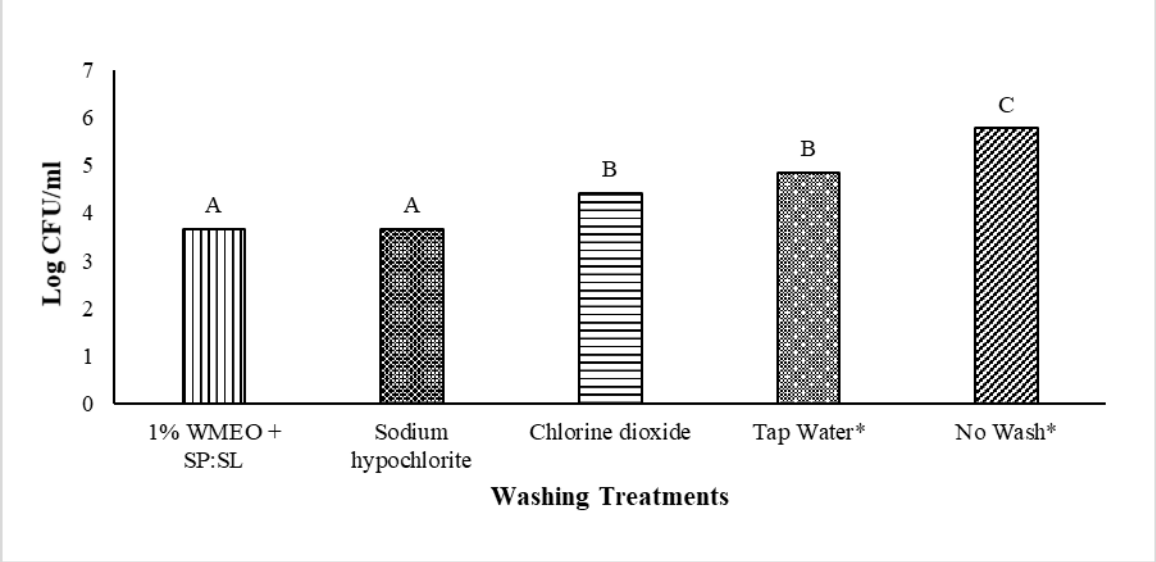
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	4	11.32475965	2.83118991	38.23	<.0001
BLK	3	0.53248132	0.17749377	2.40	0.1291

Appendix 4. *Escherichia coli* survival counts (Log CFU/ml) at 0 and 24 hr assay in TSB.



*Controls
WMEO (White mustard essential oil)
SP:SL (Sucrose palmitate: soy lecithin)
ABC- Different uppercase letters denote differences between treatments.

Appendix 5. *Escherichia coli* survival counts (Log CFU/ml) in inoculated lettuce after washing treatments.



*Controls
WMEO (White mustard essential oil)
SP:SL (Sucrose palmitate: soy lecithin