5-2014

Characterization of Lactose Esters for Their Antimicrobial and Emulsification Properties

Guneev Sandhu
Utah State University

CHARACTERIZATION OF LACTOSE ESTERS FOR THEIR ANTIMICROBIAL AND EMULSIFICATION PROPERTIES

by

Guneev Sandhu

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Nutrition and Food Sciences

Approved:

Marie K. Walsh Major Professor	Brian A. Nummer Committee Member
Robert E. Ward Committee Member	Mark R. McLellan Vice President for Research and Dean of the School of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2014

ABSTRACT

Characterization of Lactose Esters for Their Antimicrobial and Emulsification Properties

by

Guneev Sandhu, Master of Science

Utah State University, 2014

Major Professor: Dr Marie K Walsh
Department: Nutrition and Food Sciences

Sugars esters are widely used as food grade emulsifiers with sucrose esters being the most common. This research describes the synthesis and characterization of the lactose-based esters lactose monodecanoate (LMD) and lactose monomyristate (LMM).

The effects of LMM and LMD in 100 % dimethoxy sulfoxide (DMSO) were evaluated on seven different bacteria. LMM/DMSO and LMD/DMSO proved bactericidal against *Bacillus cereus*, *Mycobacteria KMS* and *Streptococcus suis*. LMM/DMSO was bactericidal against *B. cereus* at concentrations between 1 and 3 mg/ml and bactericidal against *M. KMS* and *S. suis* at concentrations between 3 and 5 mg/ml. LMD/DMSO was bactericidal against *B. cereus* and *S. suis* at concentrations between 1 and 3 mg/ml and against *M. KMS* at concentrations between 0.1 and 1 mg/ml. LMM/DMSO and LMD/DMSO were not effective in inhibiting the growth of *Listeria monocyotgenes*, *Enterococcus faecalis*, *Streptococcus mutans* and *Escherichia coli* O157:H7.

The antimicrobial activity of LMD in 30% ethanol was evaluated to compare to previous sugar ester studies. LMD/ETOH was effective in inhibiting the growth of *B. cereus, S. suis, L. monocytogenes* and *E. faecalis*. LMD/ETOH proved bactericidal against *B. cereus* and *L. monocytogenes* at concentrations between 1 and 3 mg/ml. LMD/ETOH proved bactericidal against *E. faecalis* and *S. suis* at concentrations between 3 and 5 mg/ml. The growth of *S. mutans* and *E. coli* O157:H7 was not inhibited by LMD/ETOH. There was no cell growth of *M. KMS* at 5 mg/ml concentration when treated with LMD/ETOH in the control cells as well as treatment cells, emphasizing that ethanol itself at 5mg/ml concentration (6% ethanol) inhibited the growth of *M. KMS.*

Emulsions were prepared with 80:20 (water: oil) and the emulsion stabilization properties of LMM were evaluated. The rate of destabilization in the emulsion with 0.5% LMM as emulsifier was 1.1 mm/day, which was approximately five-fold lower than the negative control, showing that LMM was effective as an emulsifier at this concentration. It was also found that time had no significant difference statistically on the droplet size when analyzed over five consecutive days, which again indicates the stability of the emulsion.

(73 pages)

PUBLIC ABSTRACT

Characterization of Lactose Esters for Their Antimicrobial and Emulsification Properties

Guneev Sandhu

Sucrose esters have an established use in food industry as emulsifiers. Two novel lactose esters (lactose monodecanoate and lactose monomyristate) were synthesized and studied for their antimicrobial and emulsification properties. Lactose is a byproduct in cheese production and is an inexpensive carbohydrate source.

The antimicrobial activity of lactose monodecanoate (LMD) and lactose monomyristate (LMM) was tested against the growth of seven different bacteria. Both esters, when dissolved in dimethoxy sulfoxide (DMSO), proved bactericidal against *Bacillus cereus, Mycobacteria KMS* and *Streptococcus suis*. LMM/DMSO was bactericidal against *B. cereus* at concentrations between 1 and 3 mg/ml. LMM/DMSO was bactericidal against *M. KMS* and *S. suis* at concentrations between 3 and 5 mg/ml. LMD/DMSO was bactericidal against *B. cereus* and *S. suis* at concentrations between 1 and 3 mg/ml and against *M. KMS* at concentrations between 0.1 and 1 mg/ml. LMM/DMSO and LMD/DMSO were not effective in inhibiting the growth of *Listeria monocyotgenes* and *Enterococcus faecalis*.

The antimicrobial effects of LMD on the growth of *Listeria monocytogenes* and *Enterococcus faecalis* were found to be solvent dependent. LMD, when dissolved in 30% ethanol, was able to inhibit the growth of *L. monocytogenes* at concentration between 1 and 3 mg/ml and *E. faecalis* at concentration between 3 and 5 mg/ml. The growth of *Streptococcus mutans* and *Escherichia coli* O157:H7 remained unaltered in the presence of LMD/ETOH, LMD/DMSO and LMM/DMSO up to 5 mg/ml.

LMM was also analyzed for its emulsification properties. The destabilization rates and droplet size of the emulsion were determined for five consecutive days. At a concentration of 0.5%, LMM produced 20% oil in water emulsion with destabilization rate of 1.1 mm/day, which can be considered a stable emulsion. The droplet size of the emulsion was also within the range of 0-10 μm. Lower droplet size range signifies the effective work of the LMM as an emulsifier. Also the droplet size of the emulsion was found to be consistent over five days, which is indicative of a stable emulsion.

ACKOWLEDGMENTS

I would like to express my gratitude to my advisor, Dr. Marie K. Walsh, for providing me with this priceless opportunity to work with her and for guiding me throughout my research and degree program. I express my gratitude to Dr. Robert E. Ward and Dr. Brian A. Nummer for their valuable time and productive suggestions on my research. I want to thank Dr. Silvana Martini for her guidance. I also want to thank Ashwini Wagh and Yao Chen for training me in preliminary experiments. I want to thank Seungmin Lee for helping me in some experiments.

I could not have accomplished all this work without the support of my parents, Sukhjinder Singh and Satinder Kaur. I also want to thank my fiance, Harjinder Singh, for always being there to support and advise me during my degree program. Finally, I want to thank all my friends and family for their motivation and encouragement.

Guneev Sandhu

CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BHI	Brain heart infusion
LB	Luria bertani
HPLC	High performance liquid chromatography
LML	Lactose monolaurate
LMD	Lactose monodecanoate
LMM	Lactose monomyristate
DMSO	Dimethyl sulfoxide
ETOH	Ethanol
LMD/DMSO	Lactose monodecanoate in DMSO
LMD/ETOH	Lactose monodecanoate in ethanol
LMM/DMSO	Lactose monomyristate in DMSO
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
SML	Sucrose monolaurate
HLB	Hydrophilic lipophilic balance

LITERATURE REVIEW

Overview

Sugar fatty acid esters that are synthesized from fatty acids and carbohydrates have broad applications in the food industry (Nakamura 1997). Various sugar esters are commercially available and are used in variety of applications in the food, pharmaceutical and personal care industries generally functioning as non ionic emulsifiers. Sugar esters can be synthesized by either chemical or enzymatic means. The chemical method leads to formation of di and tri esters as by products while the enzymatic means are better for synthesizing sugar monoesters. Lipase catalysed regioselective esterification of sugars is a better alternative to chemical synthesis as it requires lower reaction temperatures comparatively, thereby producing higher yield (Kennedy *et al*. 2006). Sugar esters of long chain fatty acids are non-ionic surfactants and sorbitan monostearate, sucrose palmitate and sucrose oleate are examples.

In 2011, 10.6 billion pounds of cheese was produced in the US (Geisler 2011) and lactose is obtained as the byproduct and is an inexpensive carbohydrate. This research investigated the enzymatic synthesis and characterization of the lactose fatty acid esters for their use as antimicrobial agents and as emulsifiers. Walsh et al. (2009) synthesized a novel sugar ester, lactose monolaurate (LML) and showed that it had microbial inhibitory and emulsification properties (Wagh *et al*. 2012; Wagh 2013). LML was synthesized using immobilized lipases in an organic solution where vinyl laurate and lactose were used as the substrate and the molar ratio of sugar: fatty acid was 1:3.8. LML has been proven to be bactericidal against some Gram-positive microorganisms in growth media, namely *Listeria monocytogenes* and *Mycobacteria* at concentrations ranging from 1 to 5

mg/ml (Wagh *et al*. 2012).

Foodborne Illness

According to the center for disease control and prevention (2011) roughly 1 in 6 Americans or 48 million people get sick, 128,000 are hospitalized and 3000 die due to foodborne diseases. There are 31 known pathogens that cause the foodborne illness. Among these 31 pathogens, the following few caused the most serious problems;

- Nontyphoidal *Salmonella*, *Listeria*, *Toxoplasma* and *norovirus* caused the most deaths.
- Nontyphoidal *Salmonella*, *Campylobacter*, *norovirus* and *Toxoplasma* caused the most hospitalizations.

Mainly three pathogens, *Salmonella, Listeria*, and *Toxoplasma*, are responsible for 1,500 deaths each year. Scallan et al. (2011) collected the data that showed nontyphoidal *Salmonella* spp., *T. gondii*, *L. monocytogenes*, and *norovirus* caused the most deaths due to food borne illness. According to the CDC (2011) about 28% of food related deaths are caused by *Salmonella*, followed by *Toxoplasma gondii* (24%), *L. monocytogenes* (19%), *norovirus* (11%) and *Campylobacter* (6%).

Dalton *et al.* (1997) stated that *L. monocytogenes* is not commonly diagnosed as the cause of gastroenteritis and fever, as the presence of this organism is not detected by routine stool culture. *L. monocytogenes* has a diversity of strains of varying pathogenicity, therefore it is important to track which strains are involved in causing listeriosis. This can help in control and prevention of further occurrence of listeriosis cases (Liu 2006). Listeriosis is also associated with early spontaneous abortion or miscarriage that may be under diagnosed. In total, 1,651 cases of listeriosis were

reported in the United States during 2009- 2011, out of which 21% of cases resulted in deaths. *B. cereus* causes about 2% of the food related diseases outbreaks with confirmed etiology every year.

A total of 9,588 new tuberculosis cases were reported in the United States (CDC, 2013). This deadly disease is caused by *Mycobacterium tuberculosis*. This bacterium affects about one- third of the world's population. Multidrug resistant TB poses a serious problem as it is difficult to treat and also the prescribed drugs are very expensive and toxic. *Streptococcus suis* is a zoonotic microbe that can exist in pigs without causing illness, however serotype 2 is a pathogenic strain that can result in illness in the pigs. Sometimes direct contact with the diseased pigs leads to illness in human beings. Human *S. suis* infections (66 confirmed cases) were reported in Sichuan, China in 2005 (Yu *et al*. 2006).

Antimicrobial properties of sugar esters

Sugar esters are used in Japan as antibacterial agents in the canned drinks (Ferrer *et al*. 2005) specifically for controlling the growth of *B. cereus*. Research on the microbial inhibitory properties of many sugar esters is given in Table 1. Many studies showed that esters containing laurate were microbial inhibitory, although there are some that showed esters of palmitate and myristate also had microbial inhibitory properties (Yang *et al*. 2003; Habulin *et al*. 2008). It was shown by Habulin *et al*. (2008) that growth of *B. cereus* was inhibited by sucrose laurate at a concentration of 9.375 mg/ml. Ferrer *et al*. (2005) reported that 6-*O*-lauroylsucrose and 6'-*O*-lauroylmaltose inhibited the growth of *Bacillus* sp. at a concentration of 0.8 mg/ml. Combined effects of sucrose laurate, pressure and mild heat caused 3 to 5.5 log reductions in *Bacillus* sp. and

Alicyclobacillus spores in various food sources (Shearer *et al*. 2000). In salad dressing, 1% sucrose monoesters of lauric, myristic or palmitic acid inhibited the growth of *Zygosacchromyces bailii* and *Lactobacillus fructivorans* (Yang *et al*. 2003). Yang *et al*. (2003) also reported that sucrose monoesters with esterified fatty acid as myristic or palmitic acid displayed greater antimicrobial properties than those of laurates. It was reported by Xiao *et al*. (2011) that the efficacy of sodium hypochlorite was improved when used in combination with sucrose monolaurate against the growth of *E. coli* O157:H7 on baby spinach.

Ferrer *et al*. (2005) stated that antimicrobial properties of the sugar ester are affected by the sugar head group, length of the fatty acid and degree of substitution. Smith *et al*. (2008) and Nobmann *et al*. (2009) suggested that carbohydrate moiety might also be involved in the antimicrobial activity of fatty acid derivatives. Devulapalle *et al*. (2004) and Watanabe *et al*. (2000) stated that fatty acids with more than 8 carbon atoms do not have an inhibitory effect on the growth of Gram negative bacteria. Less resistance was shown by Gram positive bacteria to the treatment of fatty acid esters with slightly longer chains (Ferrer *et al*. 2005).

Table 1: Recent publications reporting the antimicrobial effects of sugar esters

Ref	Esters	Organism	Effect	Medium
Chen *et al*. 2013	Synthesized lactose monolaurate	*L. monocytogenes*	Bactericidal against *L. monocytogenes*	Milk, yogurt and cheese
Wagh *et al*. 2012	Synthesized lactose monolaurate	Various Gram positive and Gram negative	Bactericidal against *Listeria monocytogenes* and *Mycobacteria*	Growth media
Xiao *et al*. 2011	Commercial sucrose monolaurate	*E. coli* 0157:H7	Strong inhibition at 10mg/ml with sodium hypochlorite	Spinach
Habulin *et al*. 2008	Commercial and synthesized sucrose and fructose palmitate and laurate	*Bacillus cereus* *E. coli* K12	Strong inhibition (75-96%) against *B. cereus* with sucrose laurate at 1% concentration at 3 days Limited (10%) inhibitation against *E. coli* with all esters	Growth media
Piao *et al*. 2006	Various synthesized erythritol and xylitol esters	Various Gram positive and negative	Strong inhibitory effect with xylitol monolaurate against *B. cereus*. All esters were ineffective against *E. coli*	Growth media and plates
Ferrer *et al*. 2005	Various synthesized sugar esters	Various Gram positive and negative	Sucrose and maltose laurate inhibited *Bacillus* at 0.5%. Limited inhibitation (26%) against *E. coli* at 0.4%.	Growth media
Devulapalle *et al*. 2004	Maltose laurate, maltotriose laurate, sucrose laurate	*Streptococcus mutans*	All esters suppressed the growth at 0.05- 2% concentration of esters	Growth media and plates
Yang *et al*. 2003	Sucrose and glucose esters	Spoilage organisms *Z. bailii* and *L fructivorans*	1% sucrose esters of laurate, myristate or palmitate inhibited the growth of the organisms and were more effective than 0.1% sodium benzoate	Salad dressing
Watanabe *et al*. 2000	23 different synthesized sugar esters	*Streptococcus mutans*	Galactose and fructose laurates inhibited growth at <0.05%	Microbial media
Shearer *et al*. 2000	Sucrose laurate, palmitate and stearate	*Bacillus* and *Clostridium spores*	A combined treatment of sucrose laurate (1%), 392 MPa pressure provided a 3-5.5 log10 DFU/ml reduction of *Bacillus* in milk and beef	Various foods

LML has been proved to have antimicrobial properties. Wagh *et al*. (2012) reported that Gram-positive bacteria are more susceptible to LML than Gram-negative bacteria with minimum bactericidal concentrations (MBC) between 5 and 9.5 mM for *L. monocytogenes* isolates and those for *Mycobacterium* isolates were 0.2 to 2 mM. In another study by Chen *et al*. (2013) the microbial inhibitory activity of LML in dairy products inoculated with *L. monocytogenes* was conducted. Addition of LML at a concentration of 5 mg/ml resulted in 4.4, 4.0 and 4.2 log reductions in 0.5% fat, 1% fat and 3.25% fat milks, respectively. Also 4.1, 4.4 and 3.5 log reductions in non fat, 1% fat and 1.5% fat yogurts, respectively, were reported. In both non fat and 2% fat cottage cheese LML showed 4 log reductions. The antimicrobial properties of two novel lactose esters LMD and LMM were investigated in this research. The proposed structures of LMD and LMM are shown in figure 1 and 2, respectively.

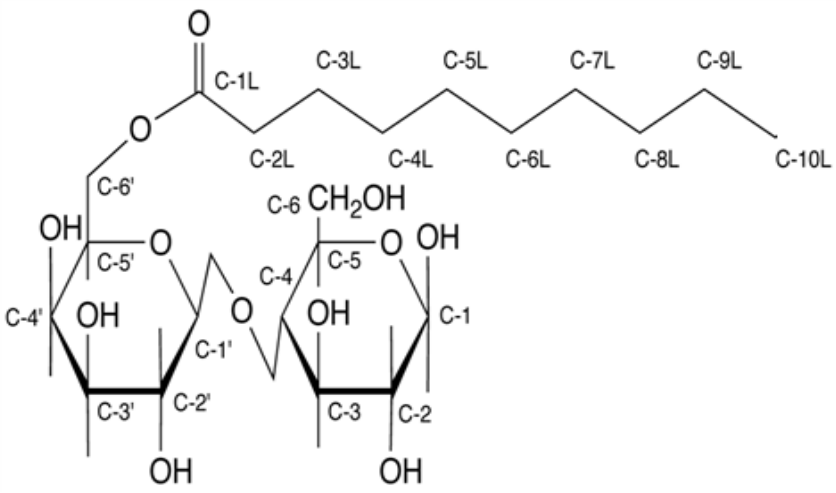


Figure 1: Proposed atomic numbering scheme of LMD

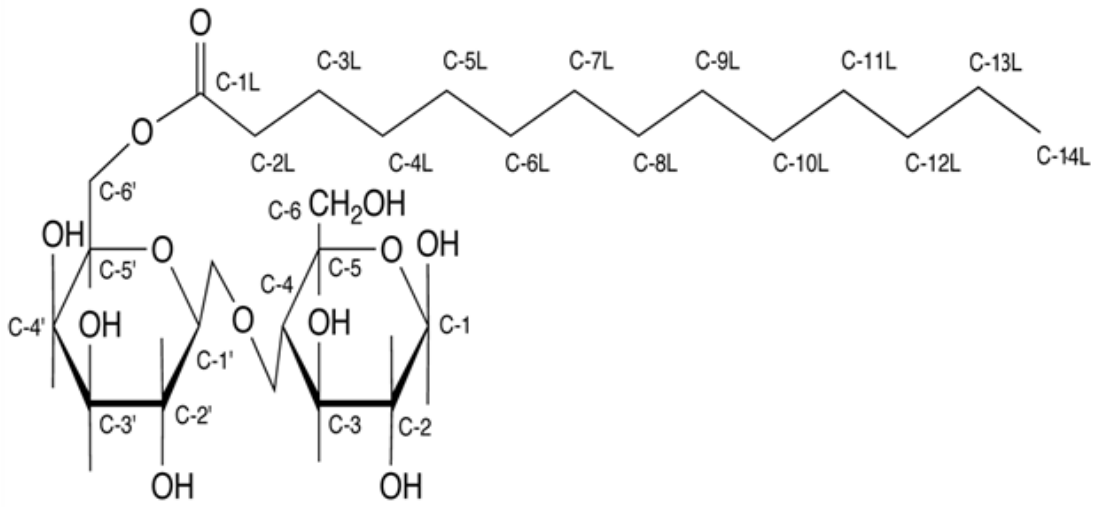


Figure 2: Proposed atomic numbering scheme of LMM

Emulsifying properties of sugar esters

An emulsifier is a substance that increases the stability of an emulsion. Sugar based esters are a class of non-ionic emulsifiers that are environment friendly and can be synthesized using renewable resources (Hill and Rhode 1999). Sugar fatty acid esters have been used in foods since the mid 1950s (Becerra *et al*. 2008). Sucrose esters have a wide range of hydrophilic lipophilic balance (HLB) values. HLB is calculated using "hydrophilic group numbers" that are assigned to various hydrophilic and lipophilic moieties appearing in the surfactants. Sugar esters have HLB scales ranging from 0 to 20 (Gupta *et al*. 1983), which makes them popular emulsifiers in foods since they have a broad HLB range. Surfactants with HLB ranging between 3.5 and 6.0 are more suitable for use in water-in-oil (w/o) emulsions, while those with HLB values ranging from 8 to 18 are mostly used in oil-in-water (o/w) emulsions (Del Vecchio 1975). The surfactant properties depend upon the degree of esterification, the chain length of the fatty acid and also on degree of saturation. Erythritol, ribitol, xylitol and sorbitol esters with monoacyl

chains with 10 to 16 carbon numbers were used by Piao and Adachi (2006), these esters showed an emulsifying ability for preparation of an o/w emulsions where soybean oil was used as oil phase and they also concluded that sugar alcohol type governs the emulsion stabilization.

Lactose monolaurate (LML) has a calculated HLB of 16. LML was shown to be an effective emulsifier in o/w emulsions when used at 0.5% (7.62 mM) in 20% o/w emulsions (Wagh 2013). The emulsification properties of novel ester LMM are demonstrated in this thesis. The calculated molecular weight of LMD was 496.55 g and that of LMM was 552.66 g. The calculated HLB of LMM is 14.4 and that for LMD is 15.6 using the formula HLB = [(L/T) *20] where L is the hydrophilic part of the molecule and T is the total weight of the molecule (Ritthitham 2009). The HLB scale is the basic indicator of the emulsifier's solubility and is the primary criteria for selecting an emulsifier in simpler food systems.

Sugar fatty acids can be added to food as emulsifiers as stated in the Code of Federal Regulations title 21 section 170.3 (o) (8). The use of sugar esters as emulsifiers in food is permissible at 0.1% (1 mg/ml) and must not exceed 5% (50 mg/ml) in finished food products (U.S. Code of Federal Regulation title 21 section 172.859 (b) (2)).

HYPOTHESIS AND OBJECTIVES

Hypothesis of this study were

Lactose esters of myristate and decanoate will have microbial inhibitory properties and LMM will also have emulsification properties.

Objectives

1. Enzymatic synthesis and purification of novel lactose esters (LMD and LMM) using immobilized lipase enzyme, molecular sieves, lactose, vinyl decanoate/ vinyl myristate and tertiary amyl alcohol will be done.
2. Characterizing LMD and LMM for antimicrobial properties against *B. cereus, M. KMS, S. suis, L. monocytogenes, E. faecalis, S. mutans* and *E. coli* O157:H7 at concentrations up to 5 mg/ml and determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) concentration of the esters for each bacterium.
3. Investigating LMM for emulsifying properties in 20% o/w emulsion by determining the destabilization rate of the emulsion and comparing the change in oil droplet size of the emulsion with different concentration of emulsifier over five consecutive days.

MATERIALS AND METHODS

Bacterial strains

E. faecalis V538 and *L. monocytogenes* EGDe were received from Dr. Andy Benson of the University of Nebraska, Lincoln. Different clinical isolates of listeria (FSL J1-177, FSL N3-013, FSL R2-499 and FSL N1-227) were obtained from Dr. Martin Wiedmann, director of the international Life Sciences Institute North American Database at Cornell University. *S. suis* 89/159 was received from Dr. Richard Higgins of University of Montreal, Qubec, Canada. *M. KMS* was isolated by Utah State University from treatment soils in Champion International Superfund Site, Libby, Montana. *B. cereus* ATCC 13061, *S. mutans* ATCC 25175 and *E. coli* O157:H7 EDL 931 stains were obtained from ATCC (Manassas, VA).

Materials and equipment

Materials and equipment included a HPLC (Beckman System Gold 125 Solvent Module, ON, Canada) equipped with Luna 5u C18 100Å (250mm x 4.6mm, Phenomenex, Torrance, CA, USA), an automatic environmental speedvac system (Savant), incubator shaker, spectrophotometer (Beckman, USA), turbiscan (MA2000, Toulouse, France), ultraturrax T 25 (Janke and Kunkel, Staufen, Germany), microfluidizer (Microfuidics Corporation, Newton, MA, USA), coulter particle size analyzer (LS 230, Coulter Corporation, Miami, FL, USA), 48 microtitre well plates (Becton Diskinson, NJ, USA), BHI media (BD, NJ, USA), LB media, granulated agar (BD, NJ, USA), phosphate buffer saline (7.4) (Thermo Fisher, MA, USA), lactose (Proliant, Iowa, USA), vinyl myristate, vinyl decanoate, lipase (immobilized from

Pseudomonas cepacia) (Sigma Aldrich, MO, USA), molecular sieves (3A), 2-methyl-2-butanol (dried using 10% 3A molecular sieves), ethanol, acetonitrile (HPLC grade, Thermo Fisher, MA, USA), tween 80 (Sigma Aldrich, MO, USA), vegetable oil (Western family Inc., Madison, WI, USA), sucrose monolaurate (SML) (Sisterna, The Netherlands); chromatogram profile of SML is given in appendix C.

LMM and LMD synthesis

Synthesis of LMM was carried out using lactose, vinyl myristate, molecular sieves and immobilized lipase enzyme (immobilized from *Pseudomonas cepacia*) (PC2). The solvent used in the reaction was tertiary amyl alcohol (2M2B). To set up a 60 ml reaction, 0.88 g of lactose was mixed with 6 g of dried molecular sieves. Further, 1.8 g of the PC2 enzyme was added to the mixture of lactose and molecular sieves (10%) in which 60 ml of the 2M2B solvent along with 1.4 ml of the vinyl myristate was added (lactose to fatty acid ratio was 1: 2.14). This reaction was set up in a 100 ml glass bottle. The lid of the bottle was covered with teflon and closed tightly with the cap of the bottle. It was then kept in the incubator shaker at 60°C.

When the lipase was used for the first time the formation of product was achieved within 48 hrs while when the enzyme was reused, it took approximately 5 days to synthesize the product. The amount of LMM formed was determined using high performance liquid chromatography at 60°C with a nitrogen gas pressure of 3.55 bar. There was a gradient from 10% acetonitrile: water (40:60) to 100% acetonitrile: water (95:5) in the mobile phase of the HPLC. The amount of desirable product formed in each sample was determined by comparing the peak areas to the control.

Synthesis of LMD was done as described above for LMM but using vinyl decanoate instead of vinyl myristate (lactose to fatty acid ratio was 1:1).

Purification of the product

The reaction solution was collected in a beaker and dried in the hood. After the contents of the beaker were fully dry, 50% ethanol (50% ethanol water solvent) at 60°C was added to the dried product. This solution was then kept in a separating funnel overnight to separate the fat layer. After separation, the bottom layer was carefully decanted into a beaker and dried in the hood. When the contents of the beaker were completely dry, acetone was added to it and the supernatant was run in the HPLC after centrifugation. This acetone extraction was done at least six times for the final purification of the product.

Bacterial treatment with LMD and LMM

Stock (25 mg/ml) of LMD in 30% ethanol was made and used as treatment. Ethanol (30%) was used as control. However, LMM was not soluble in ethanol up to 50% ethanol; hence stocks (60 mg/ml) of LMD and LMM were also made in 100% DMSO. All three stocks of esters were tested on seven different pathogens.

Inoculum preparation and treatment

Five strains of *L. monocytogenes* (C1-056, J1-177, N1-227, N3-013 and R2-499) were used in antimicrobial testing in media. Freezer stocks were kept at -80°C. Then 20 µl of each of the freezer stock was transferred separately into 15 ml of fresh BHI media, these were then kept in the shaker at 37°C for 24 h. The 5-strain cocktail was then prepared by mixing 2 ml aliquot from each strain. Then 315 µl from this mixture was

subcultured in 12 ml of BHI media, which was then incubated in the shaker at 37°C for 4 h. This was then stored as the freezer stock of 5-strain cocktail of *L. monocytogenes*.

The freezer stocks of all microorganisms were stored at -20°C. The individual freezer stocks were held at 37°C for 1 h. After that, 100 µl of the culture was inoculated in 15 ml BHI media. This 15 ml media with the cells was then kept in the incubator shaker at 37°C for 24 h. Three hundred microlitres from this culture was then subcultured into 12 ml of fresh BHI media and incubated in the shaker at 37°C for 4 h. The optical density (OD) of the culture was measured at 595 nm and the cells were allowed to grow to an OD of 0.2 nm (corresponding to10^8 cfu/ml). Thirty microliters of this culture and 0.1% tween-80 was then added to 30 ml BHI media (LB media for *E. coli* O157: H7 and *M. KMS*), this was then used as inoculums in the experiment. The initial concentration of the cells in these inoculums was 10^5cfu/ml. There were 6 replicates of each control and treatment. The concentrations at which the treatment was done were 1, 3 and 5 mg/ml for all bacteria with few exceptions as in case of treatment of *B. cereus* with LMM/DMSO a lower concentration of 0.5 mg/ml and for treatment of *M. KMS* with LMD/DMSO a lower concentration of 0.1 mg/ml were also tested. The highest concentration of the treatments was limited to 5 mg/ml to compare with the previous research done with lactose esters. The experiment was carried out in 48 well microtitre plates and the total volume in each well was 0.5 ml. The treatments and corresponding controls were plated on the BHI agar (LB media agar for *E. coli* O157: H7 and *M. KMS*) after 24 h at appropriate dilutions in phosphate buffer saline. The numbers of cells in each treatment were determined by enumeration via plate count after 24 h of incubation

of the plates at 37°C. Final cfu/ml was calculated using appropriate dilution factors and final values were reported as $\log_{10}$ cfu/ml.

A paired T- test was used to compare the treatments with the controls at each concentration to determine if the treatments were significantly different from the controls. The MIC of each compound was determined as the lowest concentration which showed a significant difference in the number of cells in treatments as compared to those in controls. Similarly, the MBC of each compound for each organism was reported as the minimum concentration of ester at which there was no cell growth.

Emulsion preparation and stability

Oil-in-water emulsions were prepared using 40 ml water and 10 ml vegetable oil along with 0%, 0.1%, 0.25% and 0.5% emulsifier each with LMM and sucrose monolaurate (SML 89% pure) as emulsifiers. SML (Appendix C for purity) is a commercially available emulsifier. The emulsifier was stirred in 40 ml water for 15 minutes before adding 10 ml of oil. The water and oil phases were then mixed using a high speed blender (Ultra-turrax) at 18,000 rpm for 5 minutes and then was passed immediately through a microfluidizer at 17.4±1.6 MPa (~25000 psi) three times. The emulsion destabilization and oil droplet size were measured for five consecutive days (day zero to day four) in triplicate.

The stability of the emulsion or the destabilization rate was determined using turbiscan which is a vertical scan microscopic analyzer. A glass tube with 11cm of length was used and about 5 ml of the emulsion was dispensed into it to measure the change in thickness of the clarification layer in the bottom of the tubes over five consecutive days. This method was described by Garg *et al*. (2010). The replicates were

pooled after individual evaluation of each sample. The percent backscattering was plotted against number of days and the slope of this graph gave the destabilization of emulsion in mm/day.

Droplet size measurement

The diameter of the droplets of the emulsion was measured using LS Beckman Coulter droplet size analyzer with polarization intensity differential scanning small fluid module. The samples were analyzed for five consecutive days. The angular dependence of the intensity of laser light scattered by emulsions generated the mean oil droplet size as the surface-volume mean particle diameter ($D_{(3, 2)}$) as described by Garg *et al*. (2010). The data obtained was statistically analyzed and reported as the mean ± standard deviations of $D_{(3, 2)}$ with respect to the type and concentration of the emulsifier. Droplet diameter curves were obtained as a function of volume percentage of droplets against droplet diameter and the droplet size distribution of all the emulsions made using LMM and SML were analyzed.

RESULTS

Effects of LMD and LMM on bacterial growth

Effects of LMD and LMM were tested in growth media on the growth of six Gram positive bacteria (*L. monocytogenes* (5-strain cocktail), *E. faecalis*, *S. mutans*, *S. suis*, *B. cereus*, *M. KMS*) and one Gram negative bacteria (*E. coli* O157:H7). Initially, a stock solution of LMD (25 mg/ml) was prepared in 30% ethanol since this compound is not soluble in water. LMM was not soluble in ethanol solutions up to 50% so we changed the solvent to 100% DMSO and in order to compare both esters, we prepared 60 mg/ml stock solutions of both esters in 100% DMSO. DMSO at concentrations up to 10% was not growth inhibitory against *Listeria monocytogenes* (data not shown). Previous research (Wagh *et al*. 2012; Chen *et al*. 2013) with LML in 50% ethanol showed decreased growth in the control samples at 5 mg/ml LML which corresponded to 12.5% ethanol.

LMD and LMM showed bactericidal effects against the growth of *B. cereus* (Fig. 3). The MIC and MBC for LMM with *B. cereus* were 1 mg/ml and between 1 and 3 mg/ml, respectively (Fig. 3A). With LMD/ DMSO the MIC and MBC for *B. cereus* were between 1 and 3 mg/ml (Fig. 3B) while the MBC for *B. cereus* with LMD/ETOH was between 3 and 5 mg/ml (Fig. 3C). LMM was a more effective bactericidal agent than LMD since it gave a lower MIC. Ethanol and DMSO controls at the 5 mg/ml LMD treatment lead to a 2 (Fig. 3C) and 1.77 (Fig. 3B) log reductions than the control cells at 1 mg/ml. While a 1.46 log reduction was observed in control cells at 5 mg/ml compared to control cells at 1 mg/ml on treatment with LMM/ DMSO (Fig. 3A). There were about 7 log reductions in the treatments at 3 mg/ml with LMD/DMSO and LMM.

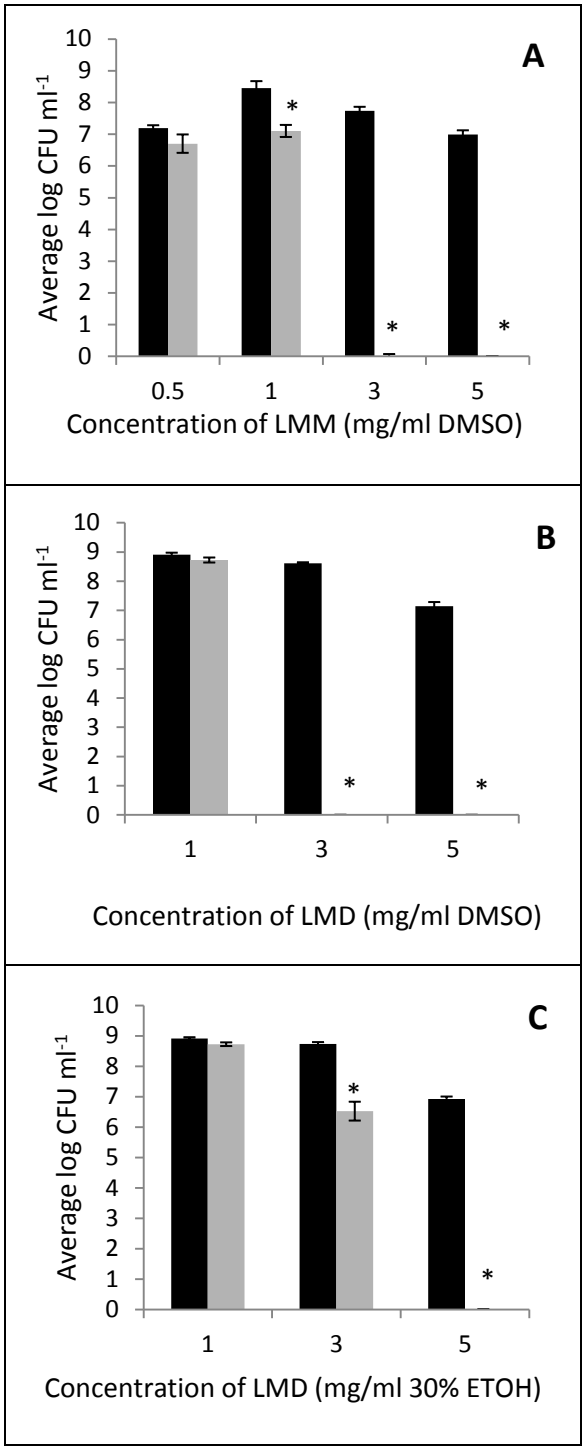


Figure 3: Average log cfu/ml results of *B. cereus* after 24 h of incubation at 37ºC. The black bars indicate the controls and light bars are treatments. Error bars represent the standard errors and asterisks indicate significant difference from the control. A: *B. cereus* treated with LMM/DMSO.; B: *B. cereus* treated with LMD/DMSO; C: *B. cereus* treated with LMD/ETOH.

The MBC of LMM with *M. KMS* (Fig. 4A) was between 3 and 5 mg/ml while the MBC for LMD/DMSO was between 0.1 and 1 mg/ml (Fig. 4B) showing that LMD was a more effective bactericidal agent against *M. KMS*. The DMSO control cells did not show a significant change when compared to those at 1 mg/ml. When using LMD in ethanol (Fig. 4C), no cell growth was shown for the 5 mg/ml treatment (which represents 6% ethanol) and there was no growth inhibition at 3 mg/ml. Therefore, the stock solvent influenced the effectiveness of the inhibitory action and itself inhibited the growth of *M. KMS*.

The growth of *S. suis*, a pig pathogen was also inhibited by LMM (Fig. 5A) and LMD/DMSO (Fig. 5B) at 5 mg/ml and 3 mg/ml, respectively. However, a 6 log reduction from the 1 mg/ml control was observed in the control cells at 5 mg/ml (which represents 8.33 % DMSO). A 4.33 log reduction in the control cells at the 3 mg/ml LMD/DMSO treatment compared 1 mg/ml control was also observed (Fig. 5B). These results indicate a susceptibility of *S. suis* to DMSO at concentrations greater than 1.67%. The MBC for LMD/ETOH was between 3 and 5 mg/ml showing a 5.09 log reduction at 5 mg/ml, but there was also a 3 and 3.8 log reductions in the 3 and 5 mg/ml control cells compared to the 1 mg/ml control (Fig. 5C). Therefore, S. suis was susceptible to ethanol at concentrations greater than 1.2%.

LMM and LMD/DMSO were not effective in inhibiting the growth of *L. monocytogenes* at concentrations up to 5 mg/ml (Fig. 6A and 6B). However, the MBC of LMD/ETOH for *L. monocytogenes* was between 1 and 3 mg/ml (Fig. 6C) showing a 6.42 log reduction at 3 mg/ml. Ethanol (at concentrations greater than 1.2%) also inhibited the growth of *L. monocytogenes* with 2.41 and 4.72 log reductions in the control

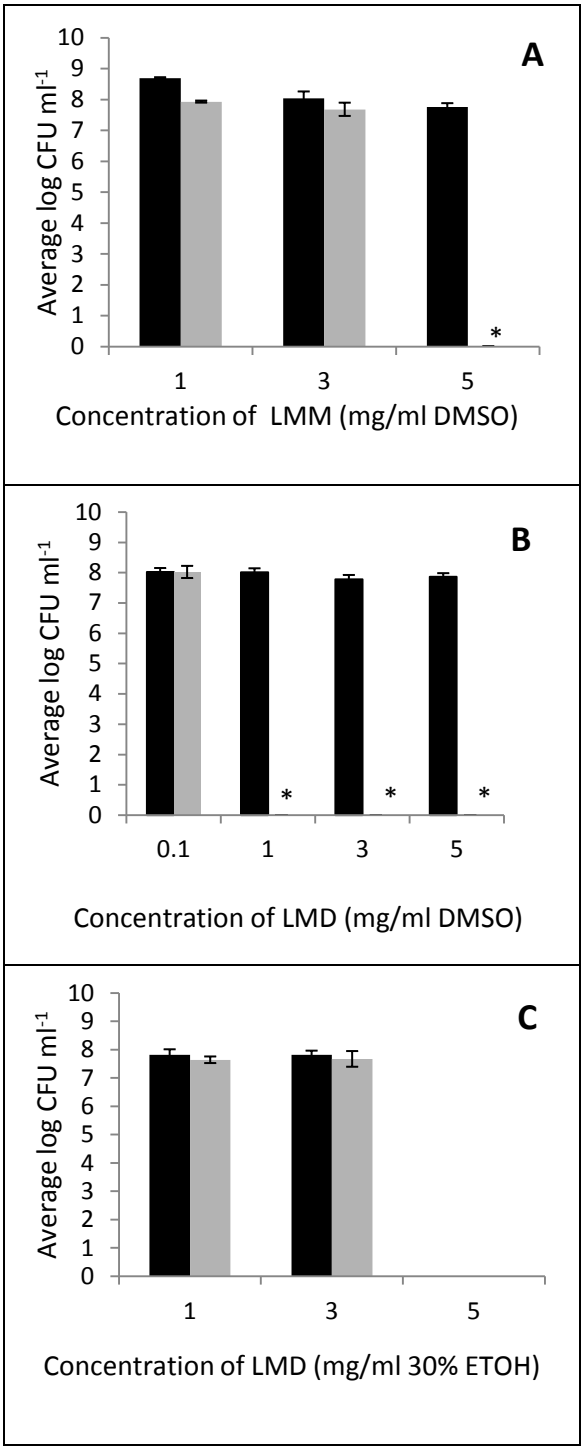


Figure 4: Average log cfu/ml results of *M.KMS* after 24 h of incubation at 37ºC. The black bars indicate the controls and light bars are treatments. Error bars represent the standard errors and asterisks indicate significant difference from the control. A: *M. KMS* treated with LMM/DMSO.; B: *M. KMS* treated with LMD/DMSO; C: *M. KMS* treated with LMD/ETOH.

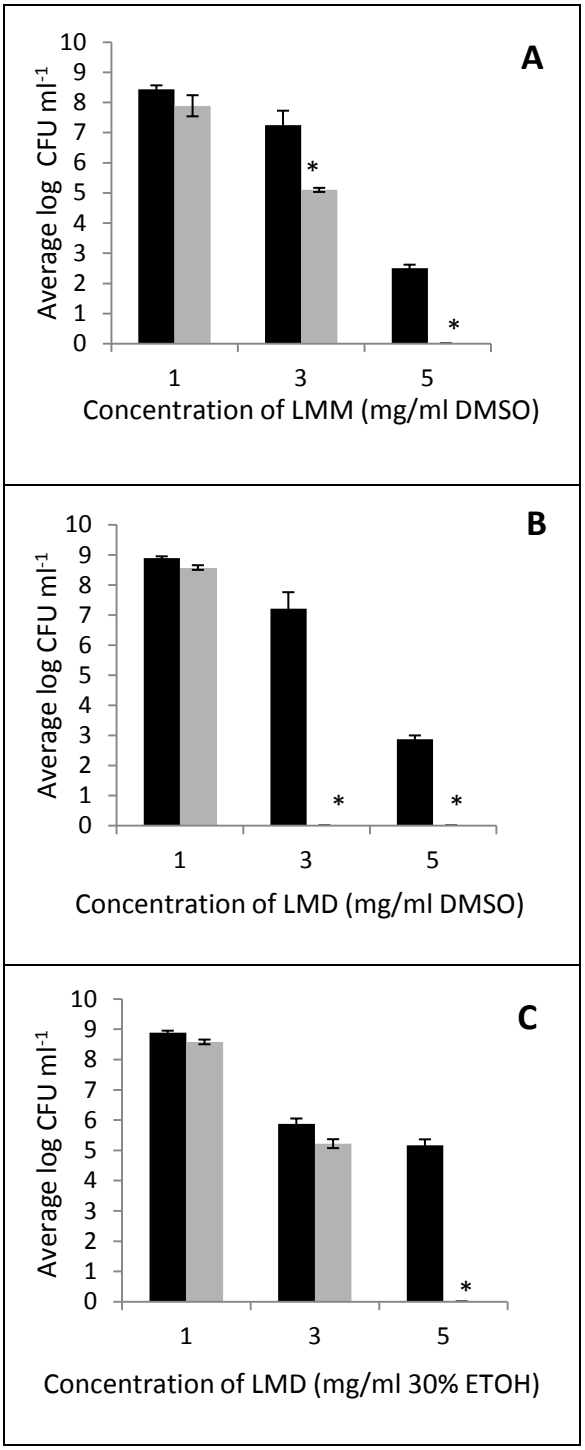


Figure 5: Average log cfu/ml results of *S. suis* after 24 h of incubation at 37ºC. The black bars indicate the controls and light bars are treatments. Error bars represent the standard errors and asterisks indicate significant difference from the control. A: *S. suis* treated with LMM/DMSO.; B: *S. suis* treated with LMD/DMSO; C: *S. suis* treated with LMD/ETOH.

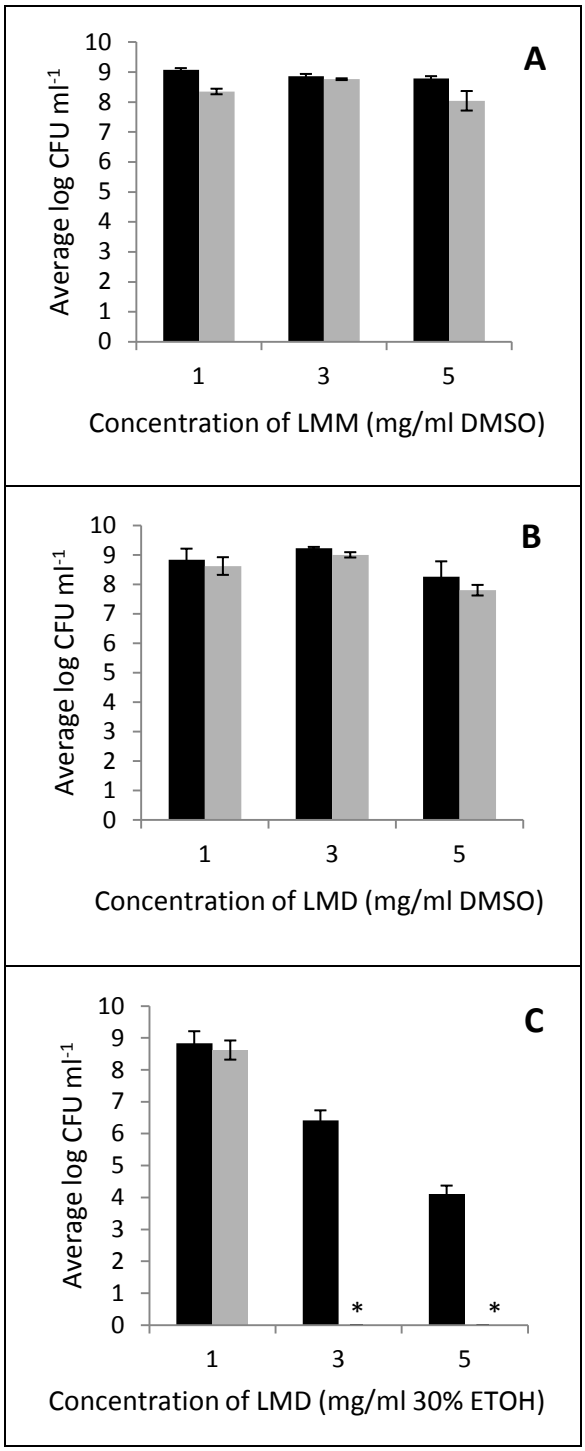


Figure 6: Average log cfu/ml results of *L. monocytogenes* (5-strain cocktail) after 24 h of incubation at 37ºC. The black bars indicate the controls and light bars are treatments. Error bars represent the standard errors and asterisks indicate significant difference from the control. A: *L. monocytogenes* (5-strain cocktail) treated with LMM/DMSO.; B: *L. monocytogenes* (5-strain cocktail) treated with LMD/DMSO; C: *L. monocytogenes* (5-strain cocktail) treated with LMD/ETOH.

cells at 3 and 5 mg/ml, respectively, compared to the control cells at 1 mg/ml (Fig. 6C). A similar effect was observed with *E. faecalis* (Fig. 7). Neither ester in DMSO stock was able to inhibit the growth of the bacterium at concentrations up to 5 mg/ml (Fig. 7A and 7B) while LMD/ETOH inhibited the growth of *E. faecalis* at 5 mg/ml, which is 6% ethanol (Fig. 7C). Ethanol inhibited the growth in control cells showing a 5 log reduction from the 1 mg/ml control. *L. monocytogenes* and *E. faecalis* were not susceptible to the treatment of LMD and LMM at the concentrations used while the solvent in LMD/ETOH stock influenced the inhibitory action of the ester.

LMD and LMM showed no growth inhibitory effects against *E. coli* O157:H7 (Fig. 8) and *S. mutans* (Fig. 9). Previous research has shown that LML and SML did not inhibit the growth of the Gram negative bacteria *E. coli*, *K. pneumonia* or *S. typhimurium* (Wagh *et al*. 2012).

Table 2 shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of LMD and LMM (in 100% DMSO) against *B. cereus, M. KMS, S. suis, L. monocytogenes, E. faecalis, E. coli* O157:H7 and *S. mutans*. LMM was more effective in inhibiting the growth of *B. cereus* with an MIC between 0.9 and 1.8 mM and MBC between 1.8 and 5.4 mM being a lower range than the MIC and MBC of LMD which was between 2 and 6 mM. The MIC and MBC range of LMD for *M. KMS* were between 0.2 and 2 mM while that of LMM were between 5.4 and 9 mM showing that *M. KMS* was more susceptible to LMD. The MIC and MBC of LMD for *S. suis* were found to be between 2 and 6 mM and that of LMM were between 1.8 and 5.4 mM. The MIC of LMD for *L. monocytogenes*, *E. faecalis*, *E. coli* O157:H7 and *S. mutans* was above 10 mM as it was not effective in inhibiting the growth of these bacteria

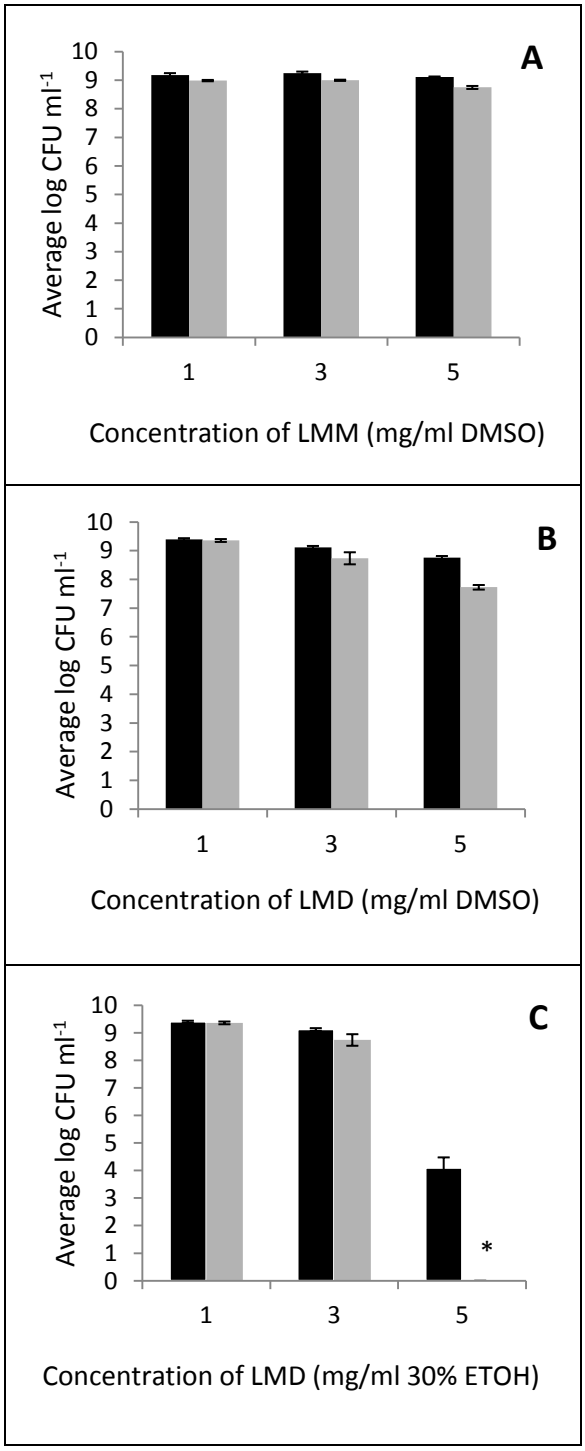


Figure 7: Average log cfu/ml results of *E. faecalis* after 24 h of incubation at 37ºC. The black bars indicate the controls and light bars are treatments. Error bars represent the standard errors and asterisks indicate significant difference from the control. A: *E. faecalis* treated with LMM/DMSO.; B: *E. faecalis* treated with LMD/DMSO; C: *E. faecalis* treated with LMD/ETOH.

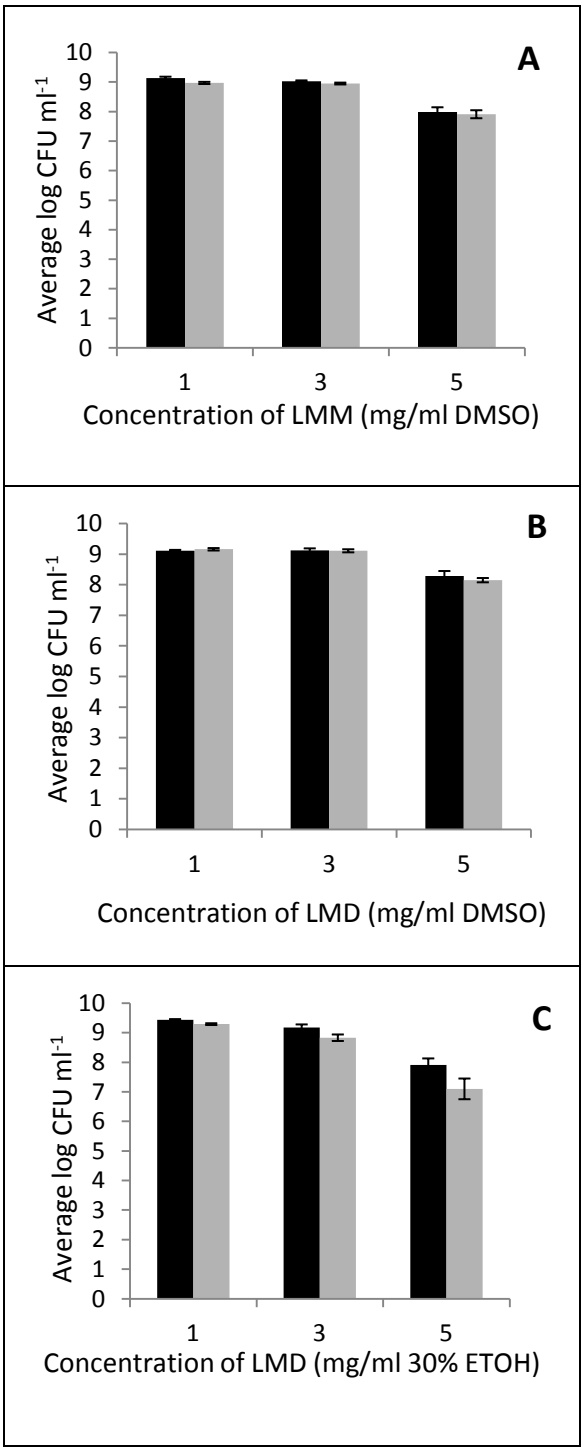


Figure 8: Average log cfu/ml results of *E. coli* O157:H7 after 24 h of incubation at 37ºC. The black bars indicate the controls and light bars are treatments. Error bars represent the standard errors and asterisks indicate significant difference from the control. A: *E. coli* O157:H7 treated with LMM/DMSO.; B: *E. coli* O157:H7 treated with LMD/DMSO; C: *E. coli* O157:H7 treated with LMD/ETOH.

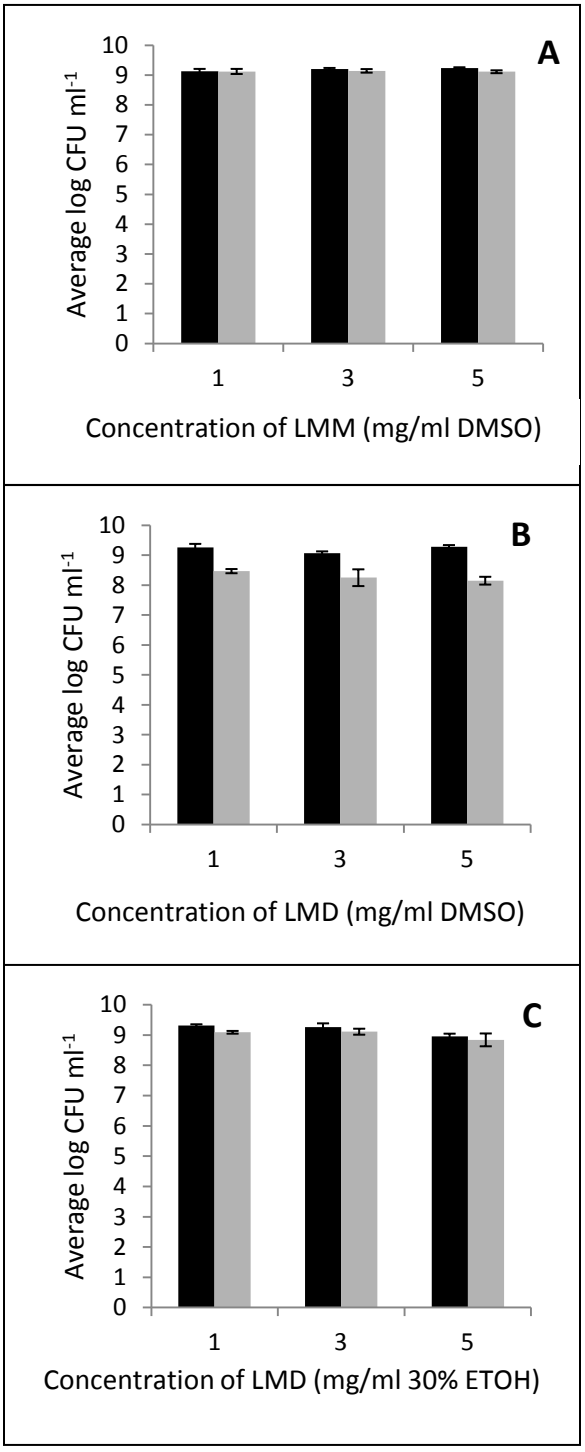


Figure 9: Average log cfu/ml results of *S. mutans* after 24 h of incubation at 37ºC. The black bars indicate the controls and light bars are treatments. Error bars represent the standard errors and asterisks indicate significant difference from the control. A: *S. mutans* treated with LMM/DMSO.; B: *S. mutans* treated with LMD/DMSO; C: *S. mutans* treated with LMD/ETOH.

Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) concentrations (mg/ml and mM) of (LMD and LMM in DMSO) for Gram-positive and Gram-negative bacteria tested.

Organism	MIC (LMD)	MBC (LMD)	MIC (LMM)	MBC (LMM)
B. cereus	1-3 mg/ml 2.01-6.04 mM	1-3 mg/ml 2.01-6.04 mM	0.5-1 mg/ml 0.90-1.81 mM	1-3 mg/ml 1.81-5.42 mM
M. KMS	0.1-1 mg/ml 0.20-2.01 mM	0.1-1 mg/ml 0.20-2.01 mM	3-5 mg/ml 5.42-9.04 mM	3-5 mg/ml 5.42-9.04 mM
S. suis	1-3 mg/ml 2.01-6.04 mM	1-3 mg/ml 2.01-6.04 mM	1-3 mg/ml 1.81-5.42 mM	3-5 mg/ml 5.42-9.04 mM
L. monocytogenes	>5 mg/ml > 10.07 mM	>5 mg/ml > 10.07 mM	>5 mg/ml >9.04 mM	>5 mg/ml >9.04 mM
E. faecalis	>5 mg/ml > 10.07 mM	>5 mg/ml > 10.07 mM	>5 mg/ml >9.04 mM	>5 mg/ml >9.04 mM
E. coli O157:H7	>5 mg/ml > 10.07 mM	>5 mg/ml > 10.07 mM	>5 mg/ml >9.04 mM	>5 mg/ml >9.04 mM
S. mutans	>5 mg/ml > 10.07 mM	>5 mg/ml > 10.07 mM	>5 mg/ml >9.04 mM	>5 mg/ml >9.04 mM

up to 5 mg/ml. Similarly, the MIC of LMM for *L. monocytogenes*, *E. faecalis*, *E. coli* O157:H7 and *S. mutans* was greater than 9 mM as LMM was also ineffective in controlling the growth of these bacteria.

Table 3 shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of LMD (in 30% ETOH) against *B. cereus*, *M. KMS*, *S. suis*, *L. monocytogenes*, *E. faecalis*, *E. coli* O157:H7 and *S. mutans*. The MBC of LMD/ETOH for *L. monocytogenes* was between 2 and 6 mM. LMD/ETOH had an MBC between 6 and 10 mM for *B. cereus*, *M. KMS*, *S. suis* and *E. faecalis*. LMD/ETOH was not effective in inhibiting the growth of *E. coli* O157:H7 and *S. mutans*. Raw data of antibacterial effects of LMD and LMM on all above said bacteria is given in appendix A.

Emulsion stability

Emulsion stability is the measure of rate at which creaming, flocculation or coalescence occurs in an emulsion. The rate at which these changes occur in an emulsion can be measured by determining the size and distribution of oil droplets in the emulsion. An emulsifier coats the surface of oil in an emulsion thereby keeping the oil droplets evenly dispersed in water phase rather than forming clumps. The optimal functioning of the emulsifier can be determined by following correct food processing conditions with most important factors to be noted as temperature and amount of energy applied in mixing or shear force applied. If the conditions of food processing are not optimum, then the emulsifiers added are ensured to be in functional state before being incorporated into the food system. The type of emulsifier used depends on the type and properties food being manufactured and also on the ingredients used in the processing. The emulsifying properties of LMM and SML were analyzed in this research.

Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) concentrations (mg/ml and mM) of (LMD in 30% ethanol) for bacteria tested.

Organism	MIC (LMD/ETOH)	MBC (LMD/ETOH)
B. cereus	1-3 mg/ml 2.01-6.04 mM	3-5 mg/ml 6.04-10.07 mM
M. KMS	Inhibition due to ETOH (5 mg/ml)	Inhibition due to ETOH (5 mg/ml)
S. suis	3-5 mg/ml 6.04-10.07 mM	3-5 mg/ml 6.04-10.07 mM
L. monocytogenes	1-3 mg/ml 2.01-6.04 mM	1-3 mg/ml 2.01-6.04 mM
E. faecalis	3-5 mg/ml 6.04-10.07 mM	3-5 mg/ml 6.04-10.07 mM
E. coli O157:H7	>5 mg/ml > 10.07 mM	>5 mg/ml > 10.07 mM
S. mutans	>5 mg/ml > 10.07 mM	>5 mg/ml > 10.07 mM

The destabilization profile of LMM emulsions was characterized by two slight clarifications each at top and bottom of the tube. Slight creaming was also observed near the top of the tube (Fig. 10A). The clarification observed at the bottom of the tube in destabilization profile of SML emulsions was more pronounced than that observed in the emulsions made using LMM. However, second clarification near the top of the tube in SML profile was very slight similar to that of LMM. In addition, creaming observed in SML emulsions was much more pronounced than that for LMM (Fig. 10B).

The thickness in mm of the clarification at the bottom of the tube was plotted against number of days (Fig. 11) at three different concentrations. The slope of the graph indicates the rate of destabilization of the emulsion. If the value of slope is less than 1 mm/day, the emulsion is stable (Kroll 1992). LMM at concentration of 0.1%, 0.25% and 0.5% produced emulsions with destabilization rates of 2.14 mm/day, 1.63 mm/day and 1.1 mm/day respectively (Fig. 11A). LMM at 0.5% concentration produced a nearly stable emulsion while the emulsions made using lower concentration of LMM were more unstable. The control emulsion with no emulsifier had a high destabilization value which was 5.6 mm/day. SML on the other hand at concentration of 0.1%, 0.25% and 0.5% produced emulsions with destabilization rates of 2.96 mm/day, 1.99 mm/day and 0.57 mm/day respectively (Fig. 11B). The emulsion made using 0.5% SML was a very stable emulsion. Statistical analysis indicated that the higher concentration of emulsifier significantly affected the stability of the emulsion (α=0.05).

Droplet Size measurement and Droplet size distribution

The mean $D_{(3, 2)}$ values of each sample with standard deviations are shown in Table 4. It was observed that time had no significant difference on the mean droplet size

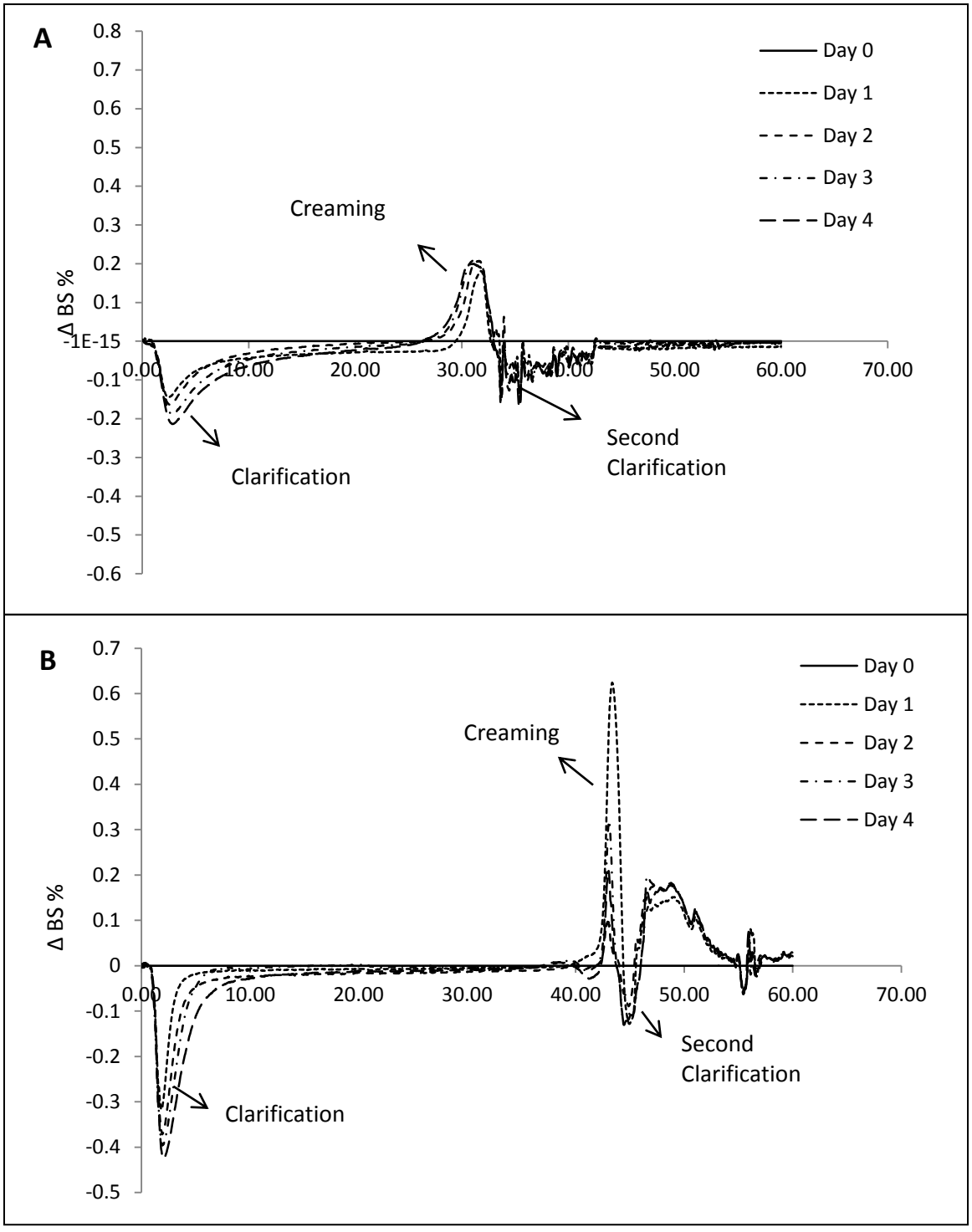


Figure 10: Creaming and clarification profiles of the emulsions at five consecutive days as a function of percent backscattering (BS %). A: Profile of emulsion with 0.5% LMM at 22ºC; B: Profile of emulsion with 0.5% SML at 22ºC.

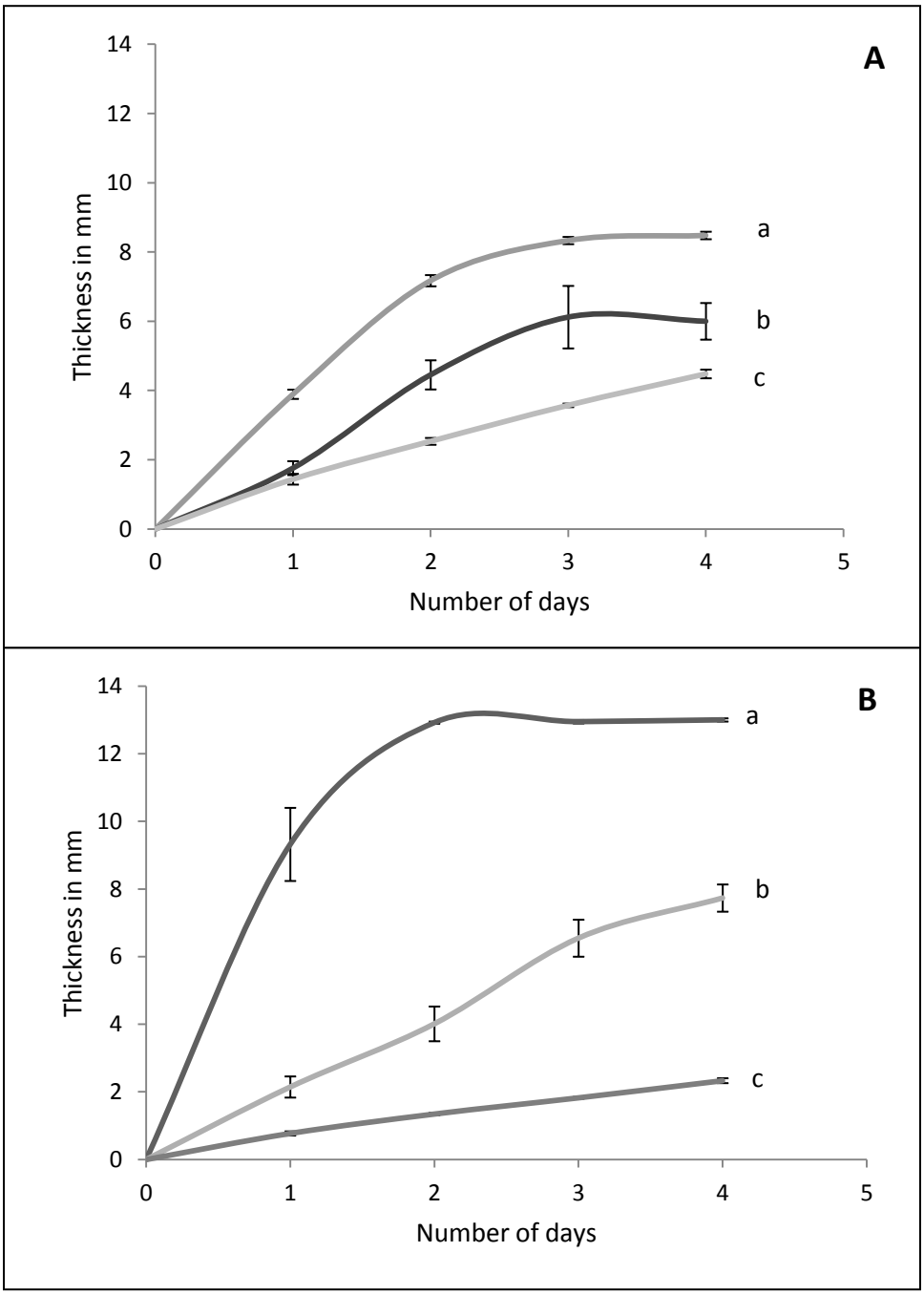


Figure 11: Average rate of destabilization of all emulsions with three different concentrations of emulsifiers at 22ºC. The error bars indicate the standard errors. A: Destabilization profile of emulsions made using LMM; B: Destabilization profile of emulsions made using SML. a: Emulsion with 0.1% emulsifier; b: emulsion with 0.25% emulsifier; c: emulsion with 0.5% emulsifier.

diameter of the emulsions made using either emulsifier. Emulsions with either emulsifier had lower droplet diameter value at 0.5% concentration of emulsifier than at 0.1% concentration of the emulsifier. Droplet diameters of emulsions with 0.5% emulsifier were significantly different from the emulsions made using lower concentration of emulsifier. The statistically different numbers do not indicate differential stability of the emulsions. Statistical data of droplet size measurement is given in Appendix B.

LMM at 0.1% concentration (Fig. 12A), on day zero shows roughly 12% of the volume of droplets in the range of 1-5 µm diameter and 6% of the droplets in the range of 0.5-1 µm. On day two, 13% of the droplets were in the range of 1-5 µm and 9% in the range of 0.5-1 µm. On day four, the percent volume of the droplets in the range 1-5 µm was found to be 15% while those in range 0.5-1 µm was 7%. The volume percent of the droplets measured for 5 days is almost the same which provides evidence of a stable emulsion. SML at 0.1% concentration had 6.5% of the droplets in the diameter range of 0.5-7 µm on day zero. On day two, there were about 7% droplets with in diameter range of 0.5-7 µm and the same pattern was observed on day four (Fig. 12D).

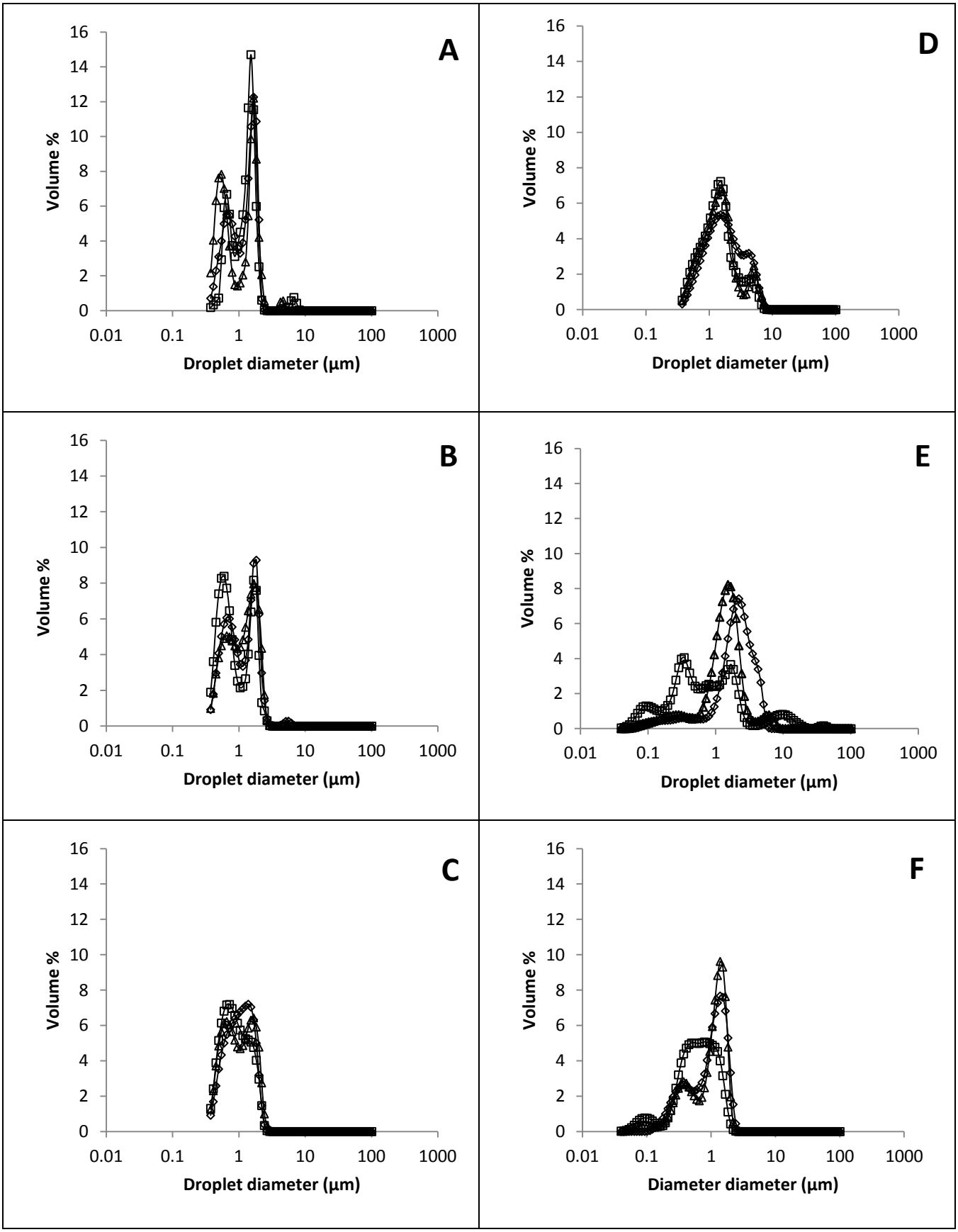
LMM at 0.25% concentration, on day zero 10% of the droplets were in the range of 1-5 µm diameters and 6% in the range of 0.5-1 µm, similar pattern was observed on day two. On day four, 8.5% of the droplets were in the range of 0.5-1 µm and 8.3% lied in the range between 1-5 µm (Fig. 12B). SML at 0.25% concentration, showed 8% of the droplets in the diameter range of 0.5-5 µm on both day zero and day two. While on day four a wider peak was observed, roughly 4.5% of the droplets were in the range of 0.1-5 µm diameter (Fig. 12E).

Table 4: Average $D_{(3, 2)}$ (surface area to volume ratio) of emulsions (at 22ºC) prepared using SML (0.1%, 0.25%, 0.5%) and LMM (0.1%, 0.25% and 0.5%) from day zero to day four with standard deviations. Significant differences across rows and columns are indicated by superscripts x,y and A,B respectively.

Types and concentration	Day 0	Day 1	Day 2	Day 3	Day 4
SML 0.1 %	1.46 ± 0.05^{Ax}	1.07 ± 0.38^{Ax}	1.25 ± 0.14^{Ax}	1.26 ± 0.17^{Ax}	1.24 ± 0.22^{Ax}
SML 0.25 %	1.30 ± 0.11^{Ax}	1.62 ± 0.52^{Ax}	1.01 ± 0.17^{Ax}	1.64 ± 0.03^{Ax}	1.81 ± 0.12^{Ax}
SML 0.5 %	0.76 ± 0.01^{ABx}	0.83 ± 0.04^{ABx}	1.23 ± 0.13^{ABx}	0.94 ± 0.02^{ABx}	0.91 ± 0.11^{ABx}
LMM 0.1 %	0.94 ± 0.24^{ABx}	1.00 ± 0.24^{ABx}	0.80 ± 0.22^{ABx}	0.81 ± 0.16^{ABx}	1.12 ± 0.12^{ABx}
LMM 0.25 %	0.98 ± 0.15^{ABx}	0.89 ± 0.17^{ABx}	1.00 ± 0.10^{ABx}	0.75 ± 0.03^{ABx}	0.81 ± 0.05^{ABx}
LMM 0.5 %	0.95 ± 0.06^{Bx}	0.83 ± 0.03^{Bx}	0.94 ± 0.24^{Bx}	0.76 ± 0.02^{Bx}	0.87 ± 0.15^{Bx}

LMM at highest concentration used (0.5%) at day zero showed 7% of the volume of the droplets with droplet diameter in the range of 0.5-5 µm. A similar pattern was observed in the droplet diameter on day two and day four (Fig. 12C). SML at this concentration showed 8% of the droplets in diameter range of 0.8-4 µm. The pattern was same on day 2. However day 4 showed a wider peak with 5% of the droplets in the range of 0.2-3 µm (Fig. 12F)

Figure 12: Droplet diameter (µm) distribution of different emulsions on day zero, two and four at three different concentrations (0.1%, 0.25% and 0.5%) at 22ºC. A: Droplet diameter distribution of emulsion with 0.1% LMM. B: Droplet diameter distribution of emulsion with 0.25% LMM. C: Droplet diameter distribution of emulsion with 0.50% LMM. D: Droplet diameter distribution of emulsion with 0.1% SML. E: Droplet diameter distribution of emulsion with 0.25% SML. F: Droplet distribution of emulsion with 0.50% SML. Day 0- points are highlighted by diamonds; Day 2- points are highlighted by triangles and Day 4- points are highlighted by square.


A
Volume %
Droplet diameter (µm)
D
Volume %
Droplet diameter (µm)
B
Volume %
Droplet diameter (µm)
E
Volume %
Droplet diameter (µm)
C
Volume %
Droplet diameter (µm)
F
Volume %
Diameter diameter (µm)

DISCUSSION

Effects of LMD and LMM on Bacterial growth

Fatty acid derivatives of carbohydrates are biodegradable, non toxic compounds and are currently used as nonionic surfactants in the food and health care industries. The antimicrobial properties of these derivatives are increasingly of interest. Wagh *et al*. (2012) showed the antimicrobial effect of lactose monolaurate (LML) on *L. monocytogenes*, *M. KMS*, *S. suis* and *E. faecalis*. LML inhibited the growth of *L. monocytogenes* in milk, low fat yogurt and cheese (Chen *et al*. 2013) at 5 mg/ml. Both studies used LML in a 50% ethanol stock solution.

B. cereus is a gram positive, rod shaped toxin producing bacterium. It is considered a food pathogen. Inhibition of *Bacillus* sp., *E. coli* and *L. plantarum* using 6-O- lauroylsucrose and 6'-O- lauroylmaltose was reported by Ferrer *et al*. (2005). Shearer *et al*. (2000) showed inhibitory effects of the sucrose laureates on *Bacillus* and *Alicyclobacillus* spores. A log reduction of 7 and above in the *B. cereus* cells was observed in the treatments at 3 mg/ml with both LMD/DMSO and LMM, which shows that esters were very effective in inhibiting the growth of *B. cereus*.

Mycobacteria are aerobic, Gram positive bacteria with unique waxy cell walls. Some species of the bacterium are pathogenic to humans, most deadly being *M. tuberculosis*. *M. tuberculosis* causes tuberculosis which is the leading cause of death in the world from a bacterial infectious disease. Some other strains of *mycobacteria* are used as surrogates in the preliminary research of novel TB antibiotic development. In our study we used *M. KMS* to study the antimicrobial effect of LMD and LMM. Both the esters proved bactericidal against the bacterium. *M. KMS* was found to be more

susceptible to LMD/DMSO with a MBC between 0.20 and 2.01 mM than LMM (MBC between 5.42 and 9.04 mM). This bacterium was not susceptible to DMSO.

No growth was reported in the control cells of *M. KMS* at 10 mM concentration of LMD/ETOH which corresponds to 6% ethanol, while there was no inhibition in the growth of *M. KMS* at 6 mM concentration of LMD/ETOH (corresponding to 3.6% ethanol). Thus, higher percentage of ethanol at 5 mg/ml treatment in LMD/ETOH resulted in no growth of *M. KMS*. Previous research done by Wagh et al. (2012) showed that LML was bactericidal against *M. KMS* at concentrations between 0.2 and 2 mM. At 2 mM concentration of LML, there was little or no inhibition in the control cells due to ethanol (corresponding to 2.8% ethanol). Hence, *M. KMS* is susceptible to ethanol concentrations above 3.6%.

The growth of *S. suis*, a pig pathogen was also inhibited by LMD/DMSO and LMM at 3 mg/ml and 5 mg/ml concentration respectively (Fig. 5). However, a 6 log reduction was observed in the control cells at 5 mg/ml (which represents 8.33 % DMSO). These results indicate a susceptibility of *S. suis* to DMSO.

LMD/DMSO and LMM were not effective in inhibiting the growth of *L. monocytogenes* (Fig. 6 A and B). Similar effect was observed with *E. faecalis* (Fig. 7). Neither ester in DMSO stock was able to inhibit the growth of the bacterium while the MBC of LMD/ETOH for *E. faecalis* was between 6 and 10 mM and that for *L. monocytogenes* was between 2 and 6 mM. This leads to a conclusion that *L. monocytogenes* and *E. faecalis* were not susceptible to the treatments of LMD and LMM up to the concentrations used while the solvent in LMD/ETOH stock influenced the inhibitory activity of the ester.

Wagh *et al*. (2012) showed that LML had an MBC between 5.7 and 9.5 mM for *L. monocytogenes*, where LML was dissolved in 50% ethanol. This MBC is higher than the MBC of LMD/ETOH, which leads to a conclusion that LMD in 30% ethanol was more effective bactericidal agent against *L. monocytogenes* than LML in 50% ethanol.

Watanabe *et al*. (2000) studied the effect of series of monosaccharide esters synthesized by lipases and proteases on *S. mutans* and showed that only galactose and fructose laurates suppressed the growth of *S. mutans* to a significant extent while other hexose laurates showed no antimicrobial activity, indicating the marked effect of configuration of the hydroxyl group in carbohydrate moiety in the inhibitory activity. These results were however obtained by OD measurement at 660 nm while we estimated the results via plate counts in our experiment. LMD and LMM did not affect the growth of *S. mutans* in our study.

Neither of the ester could inhibit the growth of *E. coli* O157:H7. Our results were similar to previous studies done by Wagh *et al*. (2012) where LML was shown to inhibit the growth of *L. monocytogenes* and *Mycobacteria* isolates but did not inhibit the growth of Gram negative bacteria. SML, fructose dilaurate and sucrose laurate were ineffective in causing any decrease in the growth of *E. coli* K-12 (Habulin *et al*. 2008). It was shown by Piao *et al*. (2006) that erythritol and xylitol esters were ineffective at inhibiting the growth of *E. coli*. These findings were in contrast to a recent study by Xiao *et al*. (2011) which stated that SML along with sodium hypochlorite inhibited the growth of *E. coli* O157:H7 on spinach.

The mechanism of action of these sugar esters is still unclear. It has been postulated that sugar esters recognize the cellular membrane and thereby cause death of

the microbe by altering the permeability of the membrane which leads to loss of important metabolites (Iwami *et al*. 1995). The antimicrobial activity of sucrose esters is assumed to be due to the interaction of the esters with the cell membrane of the bacteria which leads to autolysis of the cell (Wang 2004). This lytic action is assumed to be the result of the stimulation of the autolytic enzymes rather than the actual solubilization of the cell membranes of the bacteria.

The mechanism of action of biocides is unique. They have multiple target sites within the bacterial cell and these reach the target site penetrating the cell membrane. The overall damage to the target sites results in the bactericidal effect on the cell. Gram positive bacteria are more susceptible to most of the biocides as compared to Gram negative bacteria due to the structure of their outer wall. The difference in activity of same ester in different solvents can be explained due to different properties and target sites of the solvents. Ethanol is known as membrane disruptor. It is known to cause rapid release of intracellular components by penetrating into the hydrocarbon part of the phospholipid bilayer (Seiler and Russell 1991). Ethanol at lower concentrations can be used as a preservative and also to increase the activity of other biocides.

It is expected that extensive screening of carbohydrate esters with different core carbohydrate structures, acyl chains and right solvent which can enhance the activity of the ester can lead to further application of these esters as promising antimicrobial agents in various industrial fields.

LMM as an emulsifier

Sugar esters are capable of reducing surface tension and thereby promoting the emulsification of the immiscible liquids. According to Stoke's law the velocity at which

a droplet moves is proportional to the square of its radius. Hence, the stability of an emulsion to gravitational separation can be enhanced by reducing the size of the droplets (McClements 1999). Lactose ester (synthesized by esterification reactions using oleic acid, lactose, immobilized lipase, sodium sulfate anhydrous and ethanol) was found to be effective in decreasing the surface tension of the fresh coconut milk and stabilizing the emulsion (Neta *et al*. 2012). LML at 0.5% produced a stable emulsion similar to that produced by the emulsifier Tween- 20 (Wagh 2013).

Emulsion with 0.5% LMM as emulsifier had 1.1 mm/day as rate of destabilization which is about five folds less than the destabilization rate of the emulsion without any emulsifier (5.6 mm/day) signifying that LMM at this concentration forms a stable emulsion.

The process of creaming and clarification as seen in Figure 10, are responsible for the change in droplet diameter and thereby resulting in the instability of the emulsion over time. According to Figure 10, the clarification and creaming observed in emulsions with LMM (0.5%) was very slight as compared to those with SML (0.5%). At lower concentrations of the emulsifier, there may not have been enough emulsifier to cover the surface of oil droplets which might have caused the higher rate of destabilization of the emulsion. The lower droplet size over time can be explained as when the emulsion is passed through high turbulence of water in the particle size analyzer, the oil droplets break apart thereby leading to smaller droplet size (Hartel and Hasenhuettl 2008). The droplet size is also seen to increase in some cases which are due to the tendency of the oil droplets to coalesce and form larger oil droplets over time.

The droplet size distribution in an emulsion determines the stability of the emulsion to a certain extent; if the size of the dispersed water droplets is smaller the emulsion is tighter and therefore more stable. Physical instability of the emulsions due to creaming, flocculation, coalescence, partial coalescence, phase inversion and Oswald's ripening results in a change in the droplet diameter distribution (Kroll 1992). Most of the droplets were sized between 0 and 10 μm which is in accordance with experiments conducted by Neta *et al*. (2012) on coconut milk using sugar esters as emulsifiers(used in a ratio 1:10; biosurfactant: coconut milk, v/v). This explains the stability of the emulsion formed. The probability of the coalescence is low since the droplets in this range are very small and uniform regarding the low volume of the dispersed phase.

CONCLUSIONS

LMD and LMM were very effective in inhibiting the growth of *B. cereus*, *M. KMS* and *S. suis*. The effect of LMD on *L. monocytogenes* and *E. faecalis* was found to be solvent dependent. LMM was not soluble in ethanol however; LMD in an ethanol stock was efficient in controlling the growth of *L. monocytogenes* as well as *E. faecalis*. Both esters in DMSO were inefficient in inhibiting the growth of *L. monocytogenes* and *E. faecalis* at concentrations up to 5 mg/ml. The growth of *S. mutans* and *E. coli* O157:H7 were also not inhibited by either ester up to 5 mg/ml concentration.

LMM/DMSO with an MBC between 1.8 and 5.4 mM was most effective in inhibiting the growth of *B. cereus*. LMD/DMSO was most effective against *M. KMS* with an MBC between 0.2 and 2 mM. The growth of *S. suis* was also inhibited by LMD/DMSO most effectively with an MBC between 2 and 6 mM. The effect of LMD on *L. monocytogenes* and *E. faecalis* was solvent dependent; LMD/ETOH being most effective bactericidal agent against *L. monocytogenes* with an MBC between 2 and 6 mM and against *E. faecalis* with an MBC between 6 and 10 mM.

The o/w emulsion prepared using a 0.5% concentration of LMM had a destabilization rate of 1.1 mm/day. The droplet size of the emulsion was within the range of 0-10 µm which demonstrates the stability of the emulsion formed. No statistical difference in the droplet diameter was recorded over five consecutive days while the droplet diameter of the emulsion with 0.5% emulsifier was significantly different from that of emulsion with 0.1% and 0.25% emulsifier.

FUTURE RESEARCH

DMSO proved promising solvent for LMD and LMM. The antibacterial effects of LMD and LMM can be further tested on other pathogenic bacteria. LMD and LMM can further be tested in food products. The activity of LMD and LMM in food can be tested by homogenization of the esters in food. Sugar esters have been reported as tasteless and odorless but their taste in food products is still not known. A sensory test to evaluate the taste and odor of LMD and LMM in food products must be done.

Ester solvent combination strongly affects the antibacterial properties of the esters. Hence, right solvent combination for particular bacteria to enhance the antibacterial properties of LMD and LMM is necessary for future research.

LMM can be compared to another commercially available emulsifier with similar structure to further analyze its emulsifying properties. LMD can also be analyzed for its emulsifying properties.

REFERENCES

Becerra, N., Toro, C., Zanocco, A.L., Lemp, E. and Gunther, G. (2008) Characterization of micelles formed by sucrose 6-O-monoesters. Colloids Surf A 327, 134-139.

Centers for Disease Control and Prevention. (2011). CDC estimates of food borne illness in the United States. Retrieved from http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html

Chen Y., Nummer, B.A. and Walsh, M.K. (2013) Antilisterial activity of lactose monolaurate in milk, drinkable yogurt and cottage cheese. *Ltt Appl Microbiol* **58**, 156-162.

Dalton, C.B., Austin, C.C., Sobel, J., Hayes, P.S., Bibb, W.F., Graves, L.M., Swaminathan, B., Proctor, M.E. and Griffin, P.M. (1997) An outbreak of gastroenteritis and fever due to *Listeria monocytogenes* in milk. *N Engl J Med* **336**, 100–5.

Del Vecchio, A.J. (1975) Emulsifiers and their use in soft wheat products. *Bakers Digest* **49(4)**, 28-36.

Devulapalle, K.S., Gómez de Segura, A., Ferrer, A., Alcalde, M., Mooser, M. and Plou, G. (2004) Effect of carbohydrate fatty acid esters on *Streptococcus sobrinus* and glucosyltransferase activity. *Carbohydrate Res* **339**, 1029-1034.

Ferrer, M., Soliveri, J., Plou, F. J., Cortez, N.L., Duarte, D.R., Christensen, M., Copa Patino, J.L. and Ballesteros, A. (2005) Synthesis of sugar esters in solvent mixtures by lipases from *Thermomyces lanuginosus* and *Candida antarctica* B, and their antimicrobial properties. *Enzyme Microbial Technol* **36**, 391-398.

Garg, N., Martini, S., Britt, D.W. and Walsh, M.K. (2010) Emulsifying properties of lactose- amines in oil- in-water emulsions. *Food Res Inter* **43**, 1111-1115.

Geisler, M., Content specialist, AgMRC, Iowa State University (2011) http://www.agmrc.org/commodities__products/livestock/dairy/cheese-industry-profile/

Gupta, R. K., James, K. and Smith, F. J. (1983) Sucrose esters and sucrose ester/glyceride blends as emulsifiers. *J Am Oil Chem Sot* **60(4)**, 862-869.

Habulin, M., Sabedar, S. and Knez, Z. (2008) Enzymatic synthesis of sugar fatty esters in organic solvent and in supercritical carbon dioxide and their antimicrobial activity. *J Supercritical Fluids* **45**, 338-345.

Hartel, R.W. and Hasenhuettl, G.L. (2008) *Food Emulsifiers and their Applications*, 2nd

Ed., New York, Springer.

Hill, K. and Rhode, O. (1999) Sugar based surfactants for consumer products and technical applications. *Lipid / Fett* **101**, 25-33.

Iwami, Y., Schachtele, C.F. and Yamada, T. (1995) Effect of sucrose monolaurate on acid production, levels of glycolytic – intermediates, and enzyme – activities of *Streptococcus mutans* NCTC -10449. *J Dent Res* **74**, 1613-7

Kennedy, J.F., Kumar, H., Panesar, P.S., Marwaha, S.S., Goyal, R., Parmar, A. and Kaur, S. (2006) Enzyme- catalyzed regioselective synthesis of sugar esters and related compounds. *J Chem Technol Biotechnol* **81**, 866-876.

Kroll, J. (1992): *An introduction to food colloids*. Food /Nahrung 36, 514-514. Oxford, New York, Tokyo: Oxford University Press.

Liu, D. (2006) Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen. *J Med Microbiol* Vol. **55 (6)**, 645-659.

McClements, D. J.(1999). *Food emulsions*, New York: CRC Press.

Nakamura, S. (1997) Using sucrose esters as food emulsifiers. *Inform* **8**, 866-74

Neta, N.D.A.S., Santos, J.C.S.D., Sancho, S.D.O., Rodrigues, S., Goncalves, L.R.B., Rodrigues, L.R. and Teixeira, J.A. (2012) Enzymatic synthesis of sugar esters and their potential as surface- active stabilizers of coconut milk emulsions. *Food Hydrocolloid* **27**, 324-331.

Nobmann, P., Smith, A., Dunne, J., Henehan, G. and Bourke, P. (2009) The antimicrobial efficacy and structure activity relationship of novel carbohydrate fatty acid derivatives against Listeria spp. and food spoilage microorganisms. *Int J Food Microbiol* **128**, 440-445.

Piao, J. and Adachi, S. (2006) Stability of O/W emulsions prepared using various monoacyl sugar alcohols as an emulsifier. *Inno Food Sc. Technol* **7**, 211-216.

Piao, J., Kawahara, Y., Inoue, T. and Adachi, S. (2006) Bacteriostatic activities of monoacyl sugar alcohols against thermophilic sporeformers. *Biosci Biotechnol Biochem* **70**, 263-265.

Ritthitham, S. (2009) Synthesis of sucrose fatty acid esters as catalyzed by alkaline protease AL 89 and *Candida antarctica* lipase B in hydrophilic solvents (unpublished doctoral dissertation). Aalborg University, Denmark.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L.,

Jones, J.L. and Griffin, P.M. (2011) Foodborne illness acquired in the United States-major pathogens. *Emerg Infect Dis* **17(1)**, 7-15.

Seiler, D.A.L. and Russell, N.J. (1991) Ethanol as food preservative. *Food Preservatives* 153-171.

Shearer, A. C., Dunne, P., Sikes, A. and Hoover, D.G. (2000) Bacterial spore inhibition and inactivation in foods by pressure, chemical preservatives and mild heat. *J Food Protection* **63**, 1503-1510.

Smith, A., Nobmann, P., Henehan, G., Bourke, P. and Dunne, J. (2008) Synthesis and antimicrobial evaluation of carbohydrate and polyhydroxylated non-carbohydrate fatty acid ester and ether derivatives. *Carbohydrate Res* **343**, 2557–2566.

U.S. Code of Federal Regulations, Title 21, volume 3. (21CFR172.859). Sucrose fatty acid ester. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=172.859 (Revised April 1, 2013.)

Wagh, A., Shen, S., Shen, F.A., Miller, C.D. and Walsh, M.K. (2012) Effect of lactose monolaurate on pathogenic and nonpathogenic bacteria. *Appl Environ Microbiol* **78(9)**, 3465-3668.

Wagh, A. (2013) Characterization of lactose monolaurate for its antimicrobial and emulsification properties and its effect on crystallization behavior of anhydrous milk fat (doctoral dissertation). Utah State University.

Walsh, M.K., Bombyk, R.A., Wagh, A., Bingham, A. and Berreau, L. (2009) Synthesis of lactose monolaurate as influenced by various lipases and solvents. *J Molecular Catalysis B: Enzymatic* **60 (3-4)**, 171-177.

Wang, Y.J. (2004) Saccharides: modifications and applications. In *Chemical and Functional Properties of Food Saccharides*, ed. Tomasik, P. and Tomasik, T. pp. 35-46. New York: CRC Press.

Watanabe, T., Katayama, S., Matsubara, M., Honda, Y. and Kuwahara, M. (2000) Antibacterial carbohydrate monoesters suppressing cell growth of *Streptococcus mutans* in the presence of sucrose. *Current Microbiology* **41**, 210-213.

Xiao, D., Ye, R., Davidson, M., Hayes, D.G., Golden, D.A. and Zhong, Q. (2011) Sucrose monolaurate improves the efficacy of sodium hypochlorite against *Escherichia coli* O157:H7 on spinach. *Int J Food Microbiol* **145**, 64-6.

Yang C.M., Luedecke L.O. and Swanson B.G. (2003) Inhibition of microorganisms in salad dressing by sucrose and methylglucose fatty acid monoesters. *J Food Processing Preservation* **27**, 285-298.

Yu, H., Jing, H., Chen, Z., Zheng, H., Zhu, X., Wang, H., Wang, S., Liu, L., Zu, R., Luo, L., Xiang, N., Liu, H., Liu, X., Shu, Y., Lee, S.S., Chuang, S.K., Wang, Y., Xu, J., Yang, W., and the S. suis study groups. (2006) Human *Streptococcus suis* Outbreak, Sichuan, China. *Emerg Infect Dis* **12(6)**, 914-920.

APPENDICES

Appendix A: Antibacterial effects of LMM and LMD on different bacteria.

Table A.1: Antibacterial effect of LMM/DMSO on the growth of *B. cereus* in growth media.

Concentration (mg/ml)	Average log cfu/ml	Error
0.5	6.7	0.19
1	7.1	0.07
3	0	0
5	0	0

Error represents the standard deviation divided by the square root of the sample size.

Antibacterial effect of LMM/DMSO on the growth of *B. cereus* in growth media

Concentration (mg/ml)	1	3	5
p- value	< 0.0001	< 0.0001	< 0.0001

Table A.2: Antibacterial effect of LMD/DMSO on the growth of *B. cereus* in growth media

Concentration (mg/ml)	Average log cfu/ml	Error
1	8.72	0.09
3	0	0
5	0	0

Error represents the standard deviation divided by the square root of the sample size

Antibacterial effect of LMD/DMSO on the growth of *B. cereus* in growth media

Concentration (mg/ml)	1	3	5
p- value	0.092	<0.0001	<0.0001

Table A.3: Antibacterial effect of LMD/ETOH on the growth of *B. cereus* in growth media

Concentration (mg/ml)	Average log cfu/ml	Error
1	8.72	0.06
3	6.52	0.31
5	0	0

Error represents the standard deviation divided by the square root of the sample size

Antibacterial effect of LMD/ETOH on the growth of *B. cereus* in growth media

Concentration (mg/ml)	1	3	5
p- value	0.02	<0.0001	<0.0001

Table A.4: Antibacterial effect of LMM/DMSO on the growth of *M. KMS* in growth media.

Concentration (mg/ml)	Average log cfu/ml	Error
1	7.93	0.03
3	7.68	0.21
5	0	0

Error represents the standard deviation divided by the square root of the sample size

Antibacterial effect of LMM/DMSO on the growth of *M. KMS* in growth media.

Concentration (mg/ml)	1	3	5
p- value	0.71	0.32	< 0.0001

Table A.5: Antibacterial effect of LMD/DMSO on the growth of *M. KMS* in growth media.

Concentration (mg/ml)	Average log cfu/ml	Error
0.1	8.02	0.2
1	0	0
3	0	0
5	0	0

Error represents the standard deviation divided by the square root of the sample size

Antibacterial effect of LMD/DMSO on the growth of *M. KMS* in growth media.

Concentration (mg/ml)	0.1	1	3	5
p- value	0.99	<0.0001	<0.0001	<0.0001

Table A.5: Antibacterial effect of LMD/ETOH on the growth of *M. KMS* in growth media.

Concentration (mg/ml)	Average log cfu/ml	Error
1	7.64	0.12
3	7.67	0.28
5	0	0

Error represents the standard deviation divided by the square root of the sample size

Antibacterial effect of LMD/ETOH on the growth of *M. KMS* in growth media

Concenration (mg/ml)	1	3
p-value	0.47	0.62

Table A.6: Antibacterial effect of LMM/DMSO on the growth of *S. suis* in growth media.

Concentration (mg/ml)	Average log cfu/ml	Error
1	7.89	0.35
3	5.1	0.07
5	0	0

Error represents the standard deviation divided by the square root of the sample size

Antibacterial effect of LMM/DMSO on the growth of *S. suis* in growth media.

Concentration (mg/ml)	1	3	5
p- value	0.14	0.013	<0.0001

Table A.7: Antibacterial effect of LMD/DMSO on the growth of *S. suis* in growth media.

Concentration (mg/ml)	Average log cfu/ml	Error
1	8.58	0.08
3	0	0
5	0	0

Error represents the standard deviation divided by the square root of the sample size

Antibacterial effect of LMD/DMSO on the growth of *S. suis* in growth media.

Concentration (mg/ml)	1	3	5
p-value	0.02	<0.0001	<0.0001

Table A.8: Antibacterial effect of LMD/ETOH on the growth of *S. suis* in growth media.

Concentration (mg/ml)	Average log cfu/ml	Error
1	8.58	0.08
3	5.13	0.13
5	0	0

Error represents the standard deviation divided by the square root of the sample size

Antibacterial effect of LMD/ETOH on the growth of *S. suis* in growth media

Concentration (mg/ml)	1	3	5
p-value	0.01	0.005	<0.0001

Table A.9: Antibacterial effect of LMM/DMSO on the growth of *L. monocytogenes* in growth media.

Concentration (mg/ml)	Average log cfu/ml	Error
1	8.35	0.09
3	8.76	0.03
5	8.04	0.33

Error represents the standard deviation divided by the square root of the sample size

Antibacterial effect of LMM/DMSO on the growth of *L. monocytogenes* in growth media.

Concentration (mg/ml)	1	3	5
p-value	0.01	0.203	0.05

Table A.10: Antibacterial effect of LMD/DMSO on the growth of *L. monocytogenes* in growth media.

Concentration (mg/ml)	Average log cfu/ml	Error
1	8.62	0.30
3	9	0.09
5	7.8	0.24

Error represents the standard deviation divided by the square root of the sample size

Antibacterial effect of LMD/DMSO on the growth of *L. monocytogenes* in growth media

Concentration (mg/ml)	1	3	5
p-value	0.67	0.03	0.37

Table A.11: Antibacterial effect of LMD/ETOH on the growth of *L. monocytogenes* in growth media.

Concentration (mg/ml)	Average log cfu/ml	Error
1	8.62	0.30
3	0	0
5	0	0

Error represents the standard deviation divided by the square root of the sample size

Antibacterial effect of LMD/ETOH on the growth of *L. monocytogenes* in growth media

Concentration (mg/ml)	1	3	5
p-value	0.67	<0.0001	<0.0001

Table A.12: Antibacterial effect of LMM/DMSO on the growth of *E. faecalis* in growth media.

Concentration (mg/ml)	Average log cfu/ml	Error
1	8.99	0.02
3	9	0.02
5	8.75	0.05

Error represents the standard deviation divided by the square root of the sample size

Antibacterial effect of LMM/DMSO on the growth of *E. faecalis* in growth media.

Concentration (mg/ml)	1	3	5
p-value	0.01	0.001	0.09

Table A.13: Antibacterial effect of LMD/DMSO on the growth of *E. faecalis* in growth media

Concentration (mg/ml)	Average log cfu/ml	Error
1	9.36	0.05
3	8.74	0.21
5	7.73	0.08

Error represents the standard deviation divided by the square root of the sample size

Antibacterial effect of LMD/DMSO on the growth of *E. faecalis* in growth media

Concentration (mg/ml)	1	3	5
p-value	0.59	0.12	0.001

Table A.14: Antibacterial effect of LMD/ETOH on the growth of *E. faecalis* in growth media.

Concentration (mg/ml)	Average log cfu/ml	Error
1	9.36	0.05
3	8.74	0.21
5	0	0

Error represents the standard deviation divided by the square root of the sample size

Antibacterial effect of LMD/ETOH on the growth of *E. faecalis* in growth media

Concentration (mg/ml)	1	3	5
p-value	0.58	0.11	<0.0001

Table A. 15: Antibacterial effect of LMM/DMSO on the growth of *E. coli* O157:H7 in growth media.

Concentration (mg/ml)	Average log cfu/ml	Error
1	8.97	0.04
3	8.95	0.03
5	7.91	0

Error represents the standard deviation divided by the square root of the sample size

Antibacterial effect of LMM/DMSO on the growth of *E. coli* O157:H7 in growth media.

Concentration (mg/ml)	1	3	5
p-value	0.03	0.07	0.70

Table A. 16: Antibacterial effect of LMD/DMSO on the growth of *E. coli* O157:H7 in growth media.

Concentration (mg/ml)	Average log cfu/ml	Error
1	9.16	0.04
3	9.11	0.05
5	8.15	0.07

Error represents the standard deviation divided by the square root of the sample size

Antibacterial effect of LMD/DMSO on the growth of *E. coli* O157:H7 in growth media.

Concentration (mg/ml)	1	3	5
p-value	0.36	0.92	0.52

Table A. 17: Antibacterial effect of LMD/ETOH on the growth of *E. coli* O157:H7 in growth media.

Concentration (mg/ml)	Average log cfu/ml	Error
1	9.29	0.03
3	8.83	0.11
5	7.1	0.35

Error represents the standard deviation divided by the square root of the sample size

Antibacterial effect of LMD/ETOH on the growth of *E. coli* O157:H7 in growth media.

Concentration (mg/ml)	1	3	5
p-value	0.01	0.04	0.07

Table A.18: Antibacterial effect of LMM/DMSO on the growth of *S. mutans* in growth media.

Concentration (mg/ml)	Average log cfu/ml	Error
1	9.12	0.09
3	9.14	0.06
5	9.11	0.05

Error represents the standard deviation divided by the square root of the sample size

Antibacterial effect of LMM/DMSO on the growth of *S. mutans* in growth media.

Concentration (mg/ml)	1	3	5
p-value	0.93	0.37	0.06

Table A.18: Antibacterial effect of LMD/DMSO on the growth of *S. mutans* in growth media.

Concentration (mg/ml)	Average log cfu/ml	Error
1	8.47	0.07
3	8.25	0.28
5	8.15	0.13

Error represents the standard deviation divided by the square root of the sample size

Antibacterial effect of LMD/DMSO on the growth of *S. mutans* in growth media.

Concentration (mg/ml)	1	3	5
p-value	0.02	0.02	0.001

Table A.19: Antibacterial effect of LMD/ETOH on the growth of *S. mutans* in growth media.

Concentration (mg/ml)	Average log cfu/ml	Error
1	9.09	0.05
3	9.11	0.10
5	8.84	0.21

Error represents the standard deviation divided by the square root of the sample size

Antibacterial effect of LMD/ETOH on the growth of *S. mutans* in growth media.

Concentration (mg/ml)	1	3	5
p-value	0.004	0.37	0.63

Appendix B: Statistics for droplet size diameter measurements

Table B.1: Mean droplet diameter with respect to time (day 0 to day 4)

Time	N Obs	N	Mean	Std Dev	Minimum	Maximum
0	18	18	1.0639444	0.2672678	0.7080000	1.4880000
1	18	18	1.0401111	0.3726291	0.6310000	1.9260000
2	18	18	1.0382778	0.2197996	0.6270000	1.3720000
3	18	18	1.0282222	0.3425466	0.7000000	1.6670000
4	18	18	1.1263889	0.3701312	0.7680000	1.9390000

Table B.2: Mean droplet diameter with respect to type of emulsifier (1= Sisterna and 2= LMM)

Type	N Obs	N	Mean	Std Dev	Minimum	Maximum
1	45	45	1.2222444	0.3464483	0.6310000	1.9390000
2	45	45	0.8965333	0.1627235	0.6270000	1.2380000

Table B.3: Mean droplet diameter with respect to concentration of emulsifier

Conc	N Obs	N	Mean	Std Dev	Minimum	Maximum
0.1	30	30	1.0952667	0.2702284	0.6270000	1.4880000
0.25	30	30	1.1818000	0.4044626	0.7240000	1.9390000
0.5	30	30	0.9011000	0.1588331	0.7400000	1.3720000

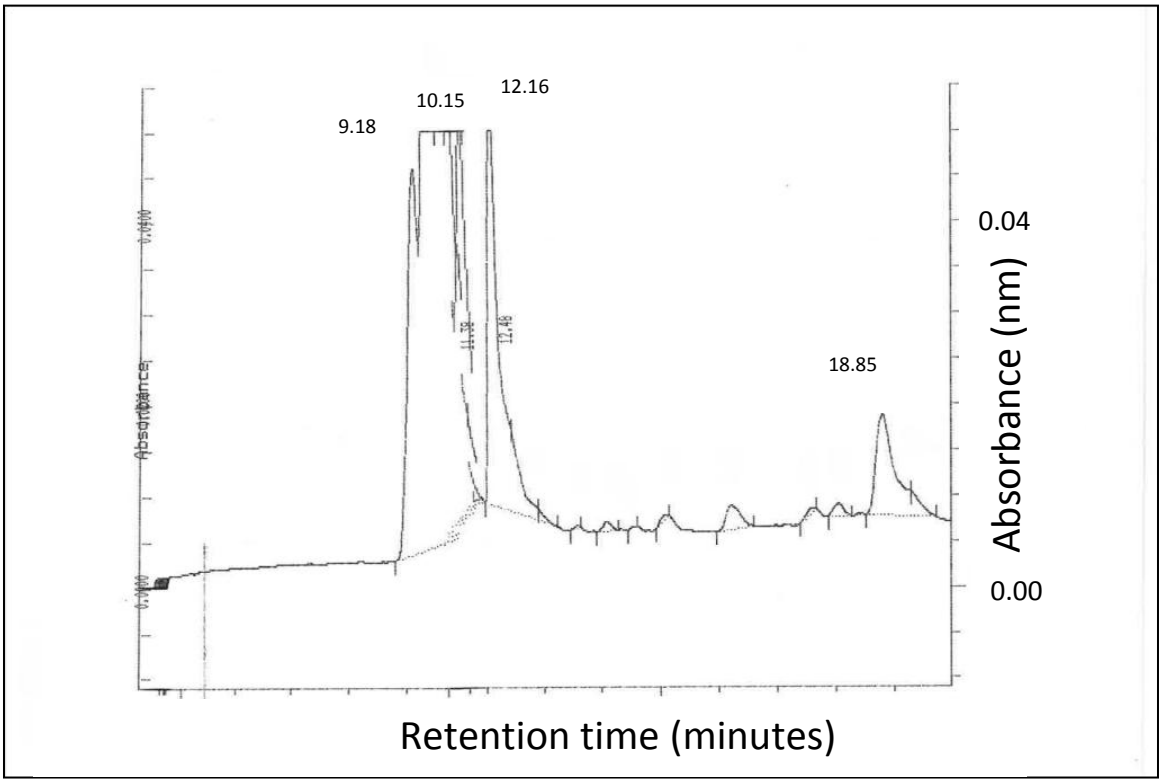
Table B.4: Mean droplet diameter of each type of emulsifier with respect to each concentration

Type	Concentration	N Obs	N	Mean	Std Dev	Minimum	Maximum
1	0.1	15	15	1.2566667	0.2260561	0.6310000	1.4880000
	0.25	15	15	1.4758000	0.3668793	0.8470000	1.9390000
	0.5	15	15	0.9342667	0.1803249	0.7420000	1.3720000
2	0.1	15	15	0.9338667	0.2105706	0.6270000	1.2380000
	0.25	15	15	0.8878000	0.1380017	0.7240000	1.1610000
	0.5	15	15	0.8679333	0.1318470	0.7400000	1.2160000

Table B.5: Mean droplet diameter of each type of emulsifier with respect to time

Type	Time	N Obs	N	Mean	Std Dev	Minimum	Maximum
1	0	9	9	1.1706667	0.3232576	0.7420000	1.4880000
	1	9	9	1.1743333	0.4774649	0.6310000	1.9260000
	2	9	9	1.1638889	0.1753478	0.8470000	1.3720000
	3	9	9	1.2814444	0.3121138	0.9280000	1.6670000
	4	9	9	1.3208889	0.4185918	0.7930000	1.9390000
2	0	9	9	0.9572222	0.1472105	0.7080000	1.1910000
	1	9	9	0.9058889	0.1629528	0.7370000	1.2380000
	2	9	9	0.9126667	0.1908271	0.6270000	1.2160000
	3	9	9	0.7750000	0.0874943	0.7000000	0.9980000
	4	9	9	0.9318889	0.1754469	0.7680000	1.2200000

Appendix C: Chromatogram profile of sucrose monolaurate



Peak Number	Retention Time	Component Name	Concentration mg / mL	Normalized Concentration	Peak Area	Peak Height	Base Code	Response Factor	Rel. Ret Time	Area Percent	Height Percent
1	9.182		0.0000	0.000	9.16439	0.04229	BCV	0.0000	0.0000	3.277	4.011
2	9.492		0.0000	0.000	32.64908	0.13981	VCS	0.0000	0.0000	11.673	13.261
3	9.600		0.0000	0.000	19.23787	0.11799	SNV	0.0000	0.0000	6.880	11.191
4	10.153		0.0000	0.000	179.64299	0.54319	VCS	0.0000	0.0000	64.246	51.518
5	10.717		0.0000	0.000	2.27291	0.02845	SNV	0.0000	0.0000	0.813	2.700
6	10.983		0.0000	0.000	6.53672	0.04613	VNS	0.0000	0.0000	2.337	4.380
7	11.047		0.0000	0.000	11.61972	0.04813	SCB	0.0000	0.0000	4.156	4.570
8	11.383		0.0000	0.000	1.60184	0.01057	SNB	0.0000	0.0000	0.573	1.002
9	12.165		0.0000	0.000	9.33862	0.04775	BCS	0.0000	0.0000	3.340	4.527
10	12.483		0.0000	0.000	1.61672	0.00898	SNS	0.0000	0.0000	0.578	0.851
11	12.942		0.0000	0.000	0.12488	0.00124	SNB	0.0000	0.0000	0.044	0.118
12	13.620		0.0000	0.000	0.01789	0.00019	BCB	0.0000	0.0000	0.007	0.018
13	14.118		0.0000	0.000	0.16587	0.00099	BCB	0.0000	0.0000	0.059	0.094
14	14.626		0.0000	0.000	0.01960	0.00007	BCB	0.0000	0.0000	0.004	0.007
15	15.153		0.0000	0.000	0.08147	0.00031	BCB	0.0000	0.0000	0.029	0.030
16	16.247		0.0000	0.000	0.74083	0.00265	BCB	0.0000	0.0000	0.265	0.251
17	17.664		0.0000	0.000	0.08818	0.00032	BCB	0.0000	0.0000	0.032	0.030
18	18.077		0.0000	0.000	0.39876	0.00142	BCB	0.0000	0.0000	0.143	0.135
19	18.848		0.0000	0.000	4.00630	0.01111	BCS	0.0000	0.0000	1.433	1.054
20	19.333		0.0000	0.000	0.42258	0.00266	SNB	0.0000	0.0000	0.151	0.252
TOTALS			0.0000	0.000	279.62021	1.05435				100.000	100.000