Title: Growth inhibitory properties of lactose fatty acid esters

Seung-Min Lee[a], Guneev Sandhu[b], Marie K. Walsh[a]*

[a]Department of Nutrition, Dietetics, and Food Sciences, Utah State University, 8700 Old Main Hill, Logan, Utah, 84322-4415, USA, choco1628@gmail.com

[b]Gunev Sandhu: Kerry Ingredients, 160 Terminal Ave., Clark, New Jersey, 07066 USA, guneevsandhu@gmail.com

[*]Corresponding author: Marie K. Walsh, Ph.D., Professor, Department of Nutrition, Dietetics, and Food Sciences, Utah State University, 8700 Old Main Hill, Logan, 84322-8700 Utah, USA.
Email: marie.walsh@usu.edu;
Phone: 435-797-2177; Fax: 435-979-2379

Abstract

Sugar esters are biodegradable, nonionic surfactants which have microbial inhibitory properties. The influence of the fatty acid chain length on the microbial inhibitory properties of lactose esters was investigated in this study. Specifically, lactose monooctanoate (LMO), lactose monodecanoate (LMD), lactose monolaurate (LML) and lactose monomyristate (LMM) were synthesized and dissolved in both dimethyl sulfoxide (DMSO) and ethanol. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were determined in growth media. LML was the most effective ester, exhibiting MIC values of <0.05 to <5 mg/ml for each Gram-positive bacteria tested (*Bacillus cereus, Mycobacterium KMS, Streptococcus suis,*

Listeria monocytogenes, *Enterococcus faecalis*, and *Streptococcus mutans*) and MBC values of <3.0 to <5 mg/ml for *B. cereus, M. KMS, S. suis, and L. monocytogenes*. LMD showed MIC and MBC values of <1 to <5 mg/ml for *B. cereus, M. KMS, S. suis, L. monocytogenes,* and *E. faecalis*, with greater inhibition when dissolved in ethanol. LMM showed MIC and MBC values of <1 to <5 mg/ml for *B. cereus*, *M. KMS,* and *S. suis*. LMO was the least effective showing a MBC value of <5 mg/ml for only *B. cereus,* though MIC values for *S. suis* and *L. monocytogenes* were observed when dissolved in DMSO. *B cereus* and *S. suis* were the most susceptible to the lactose esters tested, while *S. mutans* and *E. faecalis* were the most resilient and no esters were effective on *Escherichia coli O157:H7*. This research showed that lactose esters esterified with decanoic and lauric acids exhibited greater microbial inhibitory properties than lactose esters of octanoate and myristate against Gram-positive bacteria.

Key words: Lactose fatty acid esters, microbial inhibition, Listeria monocytogenes, Lactose monolaurate

Abbreviations: dimethyl sulfoxide (DMSO), ethanol (ETOH), MIC (minimum inhibitory concentration), MBC (minimum bactericidal concentration), LMO (lactose monooctanoate), LMD (lactose monodecanoate), LML (lactose monolaurate), LMM (lactose monomyristate)

1. Introduction

Sugar esters are nonionic surfactants used in a variety of applications in the food, pharmaceutical, and personal care industries. The microbial inhibitory activity of sugar esters has been studied. Although it has been shown that sugar esters inhibit bacterial growth, there is a lack of consensus as to which bacteria are most susceptible. While some studies have shown

48 inhibitory effects of Gram-negative bacteria (Ferrer et al., 2005; Habulin et al., 2008; Zhang et
49 al., 2014; Smith et al., 2008), others have shown inhibition of only Gram-positive bacteria
50 (Wagh *et al*. 2012; Piao *et al*. 2006). Studies have shown that esters containing laurate were
51 inhibitory against both Gram-positive and Gram-negative bacteria (Smith et al., 2008; Nobmann
52 et al., 2009; Zhang et al., 2014). A study on the microbial inhibitory activity of lactose
53 monolaurate showed low minimum inhibitory concentrations (MIC) and minimum bactericidal
54 concentrations (MBC) for *Listeria monocytogenes* and *Mycobacterium* sp. strain KMS, and no
55 inhibitory activity against Gram-negative bacteria (Wagh et al., 2012).
56 The nature and number of fatty acid chains esterified to sugars can be variable, yielding a
57 broad range of hydrophilic-lipophilic balances and microbial inhibitory activities (Szuts et al.
58 2012). Previous research showed that fatty acid derivatives such as monolaurin are highly
59 inhibitory and more inhibitory than lauric acid (Smith et al., 2008; Nobmann et al., 2009).
60 Others have reported that sugar monoesters of decanoic, myristic and palmitic acids were
61 microbial inhibitory (Piao et al., 2006; Habulin et al., 2008; Zhang et al., 2014). There was one
62 study investigating the microbial inhibition of sugar octanoate esters which showed no inhibitory
63 effects (Zhang et al., 2014).
64 Of the carbohydrate fatty acid esters previously investigated, sucrose esters have been the
65 most thoroughly studied (Nobmann et al., 2009). Other oligosaccharide esters of laurate,
66 including maltose, fructose and galactose have been synthesized and have generally been shown
67 to be very effective microbial inhibitory agents (Nobeman et al., 2009; Watanabe et al., 2000;
68 Devulapalle et al., 2004; Habulin et al., 2008), whereas hexose laurate did not suppress microbial
69 growth significantly (Watanabe et al., 2000).

While many studies examine the microbial inhibition of sugar esters in terms of MIC values, few studies have determined the MBC values of sugar esters. In this study we synthesized novel lactose esters including lactose monooctanoate (LMO), lactose monodecanoate (LMD) and lactose monomyristate (LMM). The microbial inhibitory properties of these esters (MIC and MBC) in microbial growth media, and the previously synthesized ester, lactose monolaurate (LML) (Wagh et al., 2012) were determined against Gram-positive (*Bacillus cereus, Mycobacterium KMS, Streptococcus suis, Listeria monocytogenes, Enterococcus faecalis* and *Streptococcus mutans*) bacteria and the Gram-negative bacteria, *Escherichia coli* O157:H7. Furthermore, we also determined the MIC and MBC values of the esters dissolved in two solvents, DMSO and ethanol. This allowed us to ascertain the role of the solvents in the microbial inhibitory activity.

2. Materials and Methods

2.1 Bacterial strains

Bacterial strains used are listed in Table 1. *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* 89/1591 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).

2.1 Materials and equipment

Materials and equipment included a high-performance liquid chromatography (HPLC) (Beckman System Gold 125 Solvent Module, Onterio, Canada) equipped with Luna 5µm C18 100Å (250 mm x 4.6 mm, Phenomenex, Torrance, CA, USA) and an evaporative light scattering detector (Agilent Technologies, Santa Clara, CA USA), incubator shaker, spectrophotometer (Beckman, USA), 48 microtitre well plates (Becton Diskinson, NJ, USA), brain-heart infusion (BHI) media, Lauria-Bertani (LB) media, granulated agar (BD, New Jersey, USA), lactose (Proliant, Iowa, USA), vinyl laurate**,** vinyl myristate, vinyl decanoate, vinyl octanoate (TCI, Portland OR, USA), lipase TM2 (immobilized from *Thermomyces lanuginose*), Tween 80, Whatman glass microfiber filters, molecular sieves (3A), 2-methyl-2-butanol (2M2B) (dried using 10% 3A molecular sieves), dimethyl sulfoxide (DMSO) (Sigma Aldrich, MO, USA), ethanol, and acetonitrile (HPLC grade, Thermo Fisher, PA, USA).

2.2 Lactose ester synthesis and purification

Enzymatic synthesis of LML was performed according to Walsh et al., (2009). Synthesis of LMO was conducted using lactose, vinyl octanoate, molecular sieves and immobilized lipase enzyme TM2. For a 60 mL reaction in 2M2B, 3 g of lactose, 6 g of dried molecular sieves, 1.7 mL of vinyl octanoate (lactose to fatty acid ratio of 1: 2.1) and 1.8 g TM2 were combined. The reactions were assembled in a 100 mL glass bottle and incubated at 60°C and 90 rpm for 2 days. The amount of LMO synthesized was determined using HPLC with the evaporative light scattering detector set at 60°C with a nitrogen gas pressure of 3.55 bar. There was a gradient from 10% acetonitrile-water (40:60, v/v) to 100% acetonitrile-water (95:5, v/v) as the mobile

phase. Synthesis of LMM and LMD was done as described above for LMO using the same molar ratios of lactose to fatty acid (vinyl myristate or vinyl decanoate).

For ester purification, the 2M2B reaction was filtered through a Whatman glass microfiber filter then dried in a hood for 24 h. The dry solids were suspended in 60 % ethanol, 40% water (60 ℃) and placed in a separatory funnel. The lower aqueous layer was drained into a beaker and dried in a hood for 24 h. After completely drying, the product powder was suspended in acetone, and then centrifuged for 15 min at room temperature at 2,000 x g and the supernatant analyzed via HPLC for the presence of di- tri- or higher saccharides. The acetone extraction was repeated until only the monoester was present in the pellet.

2.3 Microbial inhibitory studies

Stock solutions of LMO (60 mg/ml) and LMD (25 mg/ml) were prepared in 30% ethanol:water. Stock solutions of LML (60 mg/ml) were prepared in 50% ethanol:water and 100% DMSO. Stock solutions of LMO and LMD (60 mg/ml) were prepared in 100% DMSO. LMM was not soluble in 60% ethanol:water hence a stock (60 mg/ml) was prepared in 100% DMSO. Controls were 30% ethanol:water, 50% ethanol:water and 100% DMSO. Ester stock solutions were diluted into growth media to give final ethanol concentrations ranging from 0.5 to 10% and final DMSO concentrations ranging from 2 to 8%. All seven stocks of esters and controls were tested on the bacteria listed in Table 1.

Analysis of microbial inhibitory activities of LMO was performed by making a 5 strain cocktail of *L. monocytogenes* including C1-056, J1-177, N1-277, N3-013, and R2-499. The individual 5 stocks were stored at -80℃, and each individual freezer stock (20 µl) was added to 15 ml of BHI media. The *Listeria* strains were grown at 37 ℃ and 200 rpm for 24 h. Aliquots (2

ml) from each strain were combined in a test tube to develop the 5-strain stock cocktail. Aliquots, 315 µl, of the stock cocktail were grown in BHI media (12 ml), and incubated with shaking at 37 ℃ for 4 h. Aliquots of the 5-strain stock cocktail were kept at -80°C.

Stock solutions of the other bacteria were maintained at -80 ℃ before use. Aliquots of bacterial stock solutions (300 µl) were grown in 15 ml media at 37 ℃, 200 rpm for 24 h. Aliquots of the overnight growths (300 µl) were added to 12 ml media and grown again at 37 ℃, 200 rpm for 4 h before use. The growing cultures were monitored by optical density measurements at 660 nm (OD_{600}) and diluted with fresh media to reach an OD_{600} of 0.2 which was approximately 1 x 10^8 cfu/ml. An aliquot of the culture, 100 µl, was mixed with 10 ml fresh media containing 0.1% Tween 80. The ester stock ester solutions were added to each well for final concentrations of 0.05, 0.1, 0.5, 1, 3, and/or 5 mg/ml and each well contained a total of 0.5 ml. Controls contained the same concentration of ethanol or DMSO as the treatments. Each treatment and control was performed in triplicate and replicated three times. 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. All controls and treatments were plated on appropriate agar and incubated at 37°C for 24 hrs to obtain plate counts. 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 as determined by plate counts. Similarly, the MBC of each compound for each organism was reported as the minimum concentration of ester at which there was no cell growth as determined by plate counts.

3. Results

3.1 Minimum inhibitory concentrations (MIC) of lactose esters

In our earlier work, we showed that the novel lactose ester, LML (in 50% ethanol:water) was antimicrobial towards *L. monocytogenes* and *M. KMS*, but had no activity against Gram-negative bacteria (Wagh et al., 2012). In this study, additional lactose esters, LMO, LMD, and LMM were synthesized, and along with LML, were dissolved in both ethanol and DMSO, and tested for microbial inhibitory activity against Gram-positive bacteria and *E. coli O157:H7*. The control samples contained the same concentration of solvent as the treatments.

The MIC values of the lactose esters against various Gram-positive bacteria are listed in Table 2. LML was found to be the most effective microbial inhibitory ester since it showed MIC values (<0.05 to <5 mg/ml) for each Gram-positive bacteria tested in each solvent. On average, there were lower MIC values with LML/ETOH for *M. KMS, L. monocytogenes* and *E. faecalis*. The MIC for LML/DMSO with *E. faecalis* was 5 mg/ml, which was the highest MIC value for LML among the bacteria tested.

The MIC values of LMD/DMSO ranged from <1 to <3 mg/ml for *B. cereus*, *M. KMS* and *S. suis*. The MIC for LMD/DMSO for *L. monocytogenes, E. faecalis* and *S. mutans* was above 5 mg/ml. The MIC values for LMD/ETOH ranged from <3 to <5 mg/ml with no MIC values for *S. mutans*. Ethanol itself was inhibitory, specifically with *M. KMS* which showed no cells in the control or treatment with 5 mg/ml LMD/ETOH (corresponding to 10% ethanol), therefore, no MIC could be determined. LMD/ETOH inhibited the growth of *L. monocytogenes* and *E. faecalis* while LMD/DMSO showed no inhibitory effects on these bacteria.

LMM in DMSO showed inhibitory activity against *B. cereus*, *M. KMS* and *S. suis* with MIC values between <1 mg/ml and <5 mg/ml. However, the MIC values for LMM with *L. monocytogenes*, *E. faecalis* and *S. mutans* were >5 mg/ml.

LMO/ETOH showed no inhibitory effect at concentrations up to 5 mg/ml but LMO/DMSO was inhibitory to *B. cereus, S. suis* and *L. monocytogenes*. *S. suis* and *L. monocytogenes* were more sensitive with MIC values <3 mg/ml than *B. cereus* with an MIC value <5 mg/ml. No ester dissolved in either DMSO or ethanol showed microbial inhibitory activity against the Gram-negative bacteria tested (*E. coli* O157:H7).

3.2 Minimum bactericidal concentrations (MBC) of lactose esters

The MBC of the lactose esters are reported in Table 3 as well as the log reductions in the treatments as compared to the controls. No esters showed bactericidal activity against *S. mutans*. Out of the 4 compounds tested, LML was the only lactose ester to exert a bactericidal effect against *B. cereus, M. KMS, S. suis* and *L. monocytogenes* in both solvents used. The MBC values of LML/DMSO were <1 mg/ml for *B. cereus, M. KMS*, and *S. suis*. MBC concentrations of LML were lower in DMSO compared to ethanol for *B. cereus* and *S. suis.*

In tests against the Gram-positive bacteria, LMD/ETOH showed broad antimicrobial activity against *B. cereus, S. suis* and *L. monocytogenens* and *E. faecalis* with MBC values between <3 mg/ml and <5 mg/ml. However, LMD/DMSO was not shown to be bactericidal to *L. monocytogenes* or *E. faecalis* at concentrations up to 5 mg/ml. Furthermore, bactericidal activity of ethanol was shown against *M. KMS*, with no cells growing in the control or treatment at 10% ethanol as stated earlier for the MIC values. LMM/DMSO was effective against *B. cereus*, *M. KMS* and *S. suis* with MBC values between <3 and <5 mg/ml.

LMO/ETOH showed no bactericidal effects up to concentrations of 5 mg/ml whereas LMO/DMSO was only shown to have bactericidal activity against *B. cereus* at <5 mg/ml. DMSO was itself inhibitory towards *S. suis* with no growth in the treatment of controls with

LMO/DMSO containing 8% DMSO, therefore no MBC could be determined. *S. mutans* and *E. faecalis* were observed to be the most resilient among the bacteria tested and *B. cereus* was the most susceptible. Only LMD/ETOH was observed to be bactericidal against *E. faecalis*.

4. Discussion

Carbohydrate fatty acid derivatives are biodegradable, nontoxic and non-skin irritant surfactants with microbial inhibitory activity (Szuts et al., 2012). The microbial inhibitory properties of these derivatives are increasingly of interest and many of these compounds have been shown to inhibit Gram-positive rather than Gram-negative bacteria (Piao et al., 2006; Wagh et al., 2012). This study evaluated both microbial inhibitory and bactericidal properties of lactose esters. LML was shown to be the most effective lactose ester in preventing microbial growth, yielding the lowest MIC values in the range of <0.05 mg/ml to <5 mg/ml (0.095 mM to <9.53 mM) against each Gram-positive bacteria tested. Moreover *B. cereus* and *S. suis* appeared to be the most susceptible with MIC values obtained for each ester tested, and the lowest MIC value was obtained with LML/ETOH and *M. KMS* (<0.05 mg/ml or <0.095 mM). With regards to previous studies of bacterial inhibition with lactose esters, LML/ETOH showed inhibitory activity against *L. monocytogenes* at concentrations of 0.1 mg/ml (0.19 mM) (Wagh et al., 2012). Similar microbial inhibitory effects of LML were observed in another study in which LML/ETOH inhibited the growth of *L. monocytogenes* in milk, low fat yogurt and cheese at <5 mg/ml (Chen *et al*. 2013).

It is known that the identity of the sugar group attached to the ester plays a role in modulating the antimicrobial activity [3, 7]. The antimicrobial effect of sugar esters has traditionally be measured and reported as MIC values, with no MBC values given. Smith et al.,

(2008) and Nobmann et al., (2009) reported MIC values in the range of 0.04 mM to 0.31 mM for lauric methyl D-glucopyranoside and lauric ester of methyl α-D-mannopyranoside with *S. aureus* and *Listeria* strains. Watanabe et al. (2000) also showed inactivation of *S. mutans* by both galactose laurate and fructose laurate, with MIC values of 0.05 mg/ml and 0.2 mg/ml respectively, whereas hexose laurate did not suppress microbial growth. In a similar study, inhibitory effects of the sugar esters 6'-O-lauroylmaltose, 6'-O-lauroylsucrose, and 6''-O-lauroylmaltotriose were observed against *Streptococcus sobrinus*, with MIC values of 0.1 mg/ml (Devulapalle et al., 2004). Therefore, laurate sugar esters have previously been shown to be microbial inhibitory against Gram-positive bacteria.

The importance of the fatty acid was investigated in this study using octanoatic, decanoic, lauric, and myristic acids esterified to lactose. LMM and LMD were effective in controlling the growth of *B. cereus, M. KMS* and *S. suis*. Previous research showed that erythritol and xylitol monomyristoyl suppressed *Bacillus* growth with MIC values between 6.3 μg/ml and 12.5 μg/ml (Piao et al., 2006), which are lower than reported here. As for short chain esters, Zhang et al., (2014) reported that sucrose and glucose octanoate had no inhibitory effect against *S. aureus* and *E.coli* H7:O157. In contrast, we showed LMO/DMSO to have microbial inhibitory activity against *B. cereus, S. suis* and *L. monocytogenes* with MIC values ranging from 3 mg/ml to 5 mg/ml respectively.

Zhang et al., (2014) reported that sucrose and glucose monodecanoate showed inhibitory effects against *S. aureus* at 4 mg/ml and 3 mg/ml, respectively. In a similar study, Smith et al., (2008) and Nobman et al., (2009) reported that a glucose fatty acid ether containing decanoic acid showed the greatest activity against *S. aureus* and *Listeria* at concentrations of 0.04 mM but was effective against *E. coli* at 20 mM. In this study, we showed that LMD had MIC values for

253 all bacteria tested except *S. mutans*, although the MIC values were solvent dependent for *M.*
254 *KMS, L. monocytogenes* and *E. faecalis*.

255 Our previous research (Wagh et al., 2012) showed that LML was not inhibitory to the
256 Gram-negative bacteria, *E. coli O157:H7, Salmonella enterica* or *Klebsiella pneumonia* and this
257 study showed that the other esters (LMO, LMD and LMM) were not inhibitory to *E. coli*
258 O157:H7 (data not shown). On the other hand, there are a limited number of studies showing
259 microbial inhibitory properties of sugar esters against Gram-negative bacteria. Ferrer et al.,
260 (2005) and Habulin et al., (2008) both reported limited inhibition of *E. coli* by sucrose
261 monolaurate with MIC values of 4 mg/ml and 6.25 mg/ml respectively. Zhang et al., 2014)
262 showed that methyl α-D-glucopyranoside monolaurate was effective in inhibiting the growth of
263 both *S. aureus* and *E. coli* O157:H7 at a concentration of 0.188 mg/ml.

264 Compared to the amount of literature on the microbial inhibitory properties of sugar
265 esters, there is very little information about the effects of the solvent used. Previous studies on
266 microbial inhibitory activities of sugar esters involved dissolving sugar esters into an ethanol
267 solution (Smith et al., 2008; Nobmann et al., 2009; Wagh et al., 2012; Chen et al., 2013) or
268 DMSO (Ferrier et al., 2005) before diluting into growth media. Others have added esters directly
269 into growth media (Devulapalle et al., 2004; Piao et al., 2006). All of the esters used in the
270 current study were soluble in a 50% ethanol solution except LMM; therefore, we only tested
271 LMM in DMSO. Previous studies with LML showed that final ethanol concentrations greater
272 then 7.5% were microbial inhibitory towards *L. monocytogenes* (Chen et al., 2013). In this study
273 we found that 10% ethanol was antimicrobial to *M. KMS* and 8% DMSO was
274 antimicrobial/inhibitory to *S. suis.* The effect of the solvent on the cell growth can be observed
275 by the log reductions in Table 3, specifically for *S. suis* with LMM/DMSO and LMO/DMSO.

In general, the MIC values of the LML/ETOH treatments were lower than the LML/DMSO treatments suggesting compounding stress of both LML and ethanol lead to growth inhibition as suggested by Chen et al., (2013). Similar results are seen with LMD/ETOH, where MIC values were obtained for *L. monocytogenes* and *E. faecalis*, but not with LMD/DMSO. Conversely, the MBC values of LML/DMSO were lower or equal to the LML/ETOH values. Therefore, the effect of ethanol on the MBC values is not understood.

5. Conclusions

The results suggest that the chain length of the fatty acid ester significantly influences the microbial inhibitory and bactericidal activity of lactose esters towards Gram-positive bacteria. Lactose esters containing decanoate and laurate were more microbial inhibitory than esters containing octanoate and myristate. No esters inhibited the growth of the Gram-negative bacteria *E. coli O157:H7*. The solvent used to dissolve the esters influenced the microbial inhibitory activity for some bacteria. Ethanol (>7.5%) and DMSO (<8%) inhibited the growth of *L. monocytogenes* and *S. suis* respectively. Additional research on the microbial inhibitory activity of these esters in food systems without the need to prior dissolve in either ethanol or DMSO is needed.

Acknowledgement

This project was funded by the Utah Agricultural Experiment Station, Utah State University, and approved as journal paper number 8798.

References

Chen, Y., Nummer, B.A., Walsh, M.K. 2013. Antilisterial activity of lactose monolaurate in milk, drinkable yogurt and cottage cheese. Ltt. Appl. Microbiol. 58: 156-162. DOI: 10.1111/lam.12169.

Devulapalle, K.S., de Segura, A.G., Ferrer, A., Alcalde, M.G., Mooser, G., Plou, F.J. 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., Lopez-Cortez, N.L., Reyes-Duarte, D.R., Christensen, M., Dopa-Patino, J.L., Ballestros, 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. doi:10.1016/j.enzmictec.2004.02.009

Habulin, M., Sabeder, S., Knez, Z. 2008. Enzymatic synthesis of sugar fatty acid esters in organic solvent and in supercritical carbon dioxide and their antimicrobial activity. J. Supercritical Fluids 45: 338-345. doi:10.1016/j.supflu.2008.01.002

Nobmann, P., Smith, A., Dunne, J., Henehan, G., Bourke, P. 2009. The anitmicrobial efficacy and structure activity relationship of novel carbohydrate fatty acid derivatives against *Listeria* spp. and food spoilage microorganisms. Int. J. Food Microbiol. 128(3): 440-445. doi: 10.1016/j.ijfoodmicro.2008.10.008

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

Smith, A., Nobmann, P., Henehan, G., Bourke, P., Dunne, J. 2008. Synthesis and antimicrobial evaluation of carbohydrate and polyhydroxylated non-carbohydrate fatty acid ester and ether derivatives. Carbohydrate Res. 343: 2557-2566. doi: 10.1016/j.carres.2008.07.012

321 Szűts, A., Szabó-Révész, P. 2012. Sucrose esters as natural surfactants in drug delivery systems.
322 Int. J. Pharm. 433: 1-9. doi: 10.1016/j.ijpharm.2012.04.076
323 Wagh, A., Shen, S., Shen, F.A., Miller, C.D., Walsh, M.K. 2012. Effect of lactose monolaurate
324 on pathogenic and nonpathogenic bacteria. Appl Environ Microbiol. 78(9): 3465-3668. doi:
325 10.1128/AEM.07701-11
326 Walsh, M.K., Bombyk, R.A., Wagh, A., Bingham, A., Berreau, L. 2009. Synthesis of lactose
327 monolaurate as influenced by various lipases and solvents. J Molecular Catalysis B:
328 Enzymatic. 60 (3-4): 171-177. doi:10.1016/j.molcatb.2009.05.003
329 Watanabe, T., Katayama, S., Matsubara, M., Honda, Y., Kuwahara, M. 2000. Antibacterial
330 carbohydrate monoesters suppressing cell growth of *Streptococcus mutans* in the presence of
331 sucrose. Current Microb. 41: 210-213.
332 Zhang, X., Wei, W., Cao, X., Feng, F. 2014. Characterization of enzymatically prepared sugar
333 medium-chain fatty acid monoesters. J. Sci Food Agric. 95(8): 1631-7. doi:
334 10.1002/jsfa.6863
335
336
337
338
339
340
341
342
343

Table 1. Microorganisms and growth media used in this study

Microorganism	ATCC no./serovar	Gram reaction[a]	Growth medium
Bacillus cereus	13061	+	BHI
Mycobacterium sp. strain KMS	NA	+	LB
Streptococcus suis	89/1591	+	BHI
Listeria monocytogenes	EGDe	+	BHI
Listeria monocytogenes	FSL J1-177	+	BHI
Listeria monocytogenes	FSL N3-013	+	BHI
Listeria monocytogenes	FSL R2-499	+	BHI
Listeria monocytogenes	FSL N1-227	+	BHI
Enterococcus faecalis	V538	+	BHI
Streptococcus mutans	FSL R2-499	+	BHI
Escherichia coli O157:H7	EDL 931	-	LB

[a] +, positive; -, negative

NA = not available

Table 2. Minimum inhibitory concentrations of lactose esters as both mg/ml and mM concentrations. Esters were tested at concentrations up to 5 mg/ml.

	LMO DMSO	LMD DMSO	LMD ETOH	LML DMSO	LML ETOH	LMM DMSO
B. cereus	<5 mg/ml <10.7 mM	<3 mg/ml <6.0 mM	<3 mg/ml <6.0 mM	<1 mg/ml <1.9 mM	<1 mg/ml <1.9 mM	<1 mg/ml <1.8 mM
M. KMS	No	<1 mg/ml <2.0 mM	X[1]	<1 mg/ml <1.9 mM	<0.05 mg/ml[2] <0.095 mM	<5 mg/ml <9.0 mM
S. suis	<3 mg/ml <6.4 mM	<3 mg/ml <6.0 mM	<5 mg/ml <10.1 mM	<1 mg/ml <1.9 mM	<1 mg/ml <1.9 mM	<3 mg/ml <5.4 mM
L. monocytogenes	<3 mg/ml <6.4 mM	No	<3 mg/ml <6.0 mM	<3 mg/ml <5.7 mM	<0.1 mg/ml[2] <0.19 mM	No
E. faecalis	No	No	<5 mg/ml <10.1 mM	<5 mg/ml <9.5 mM	<1 mg/ml <1.9 mM	No
S. mutans	No	No	No	<1 mg/ml <1.9 mM	<3 mg/ml <5.7 mM	No

X[1] = no growth in treatment or control at 5 mg/ml

[2]Data obtained from Wagh et al., 2012.

No = No growth inhibition

Table 3. Minimum bactericidal concentration of lactose esters as both mg/ml and mM concentrations. Esters were tested at concentrations up to 5 mg/ml. The log reductions of the treatment samples compared to the controls are given as log values.

	LMO DMSO	LMD DMSO	LMD ETOH	LML DMSO	LML ETOH	LMM DMSO
B. cereus	<5 mg/ml <10.7 mM 7 log	<3 mg/ml <6.0 mM 9 log	<5 mg/ml <10.1 mM 7 log	<1 mg/ml <1.9 mM 7 log	<5 mg/ml >9.5 mM 8 log	<3 mg/ml <5.4 mM 8 log
M. KMS	No	<1 mg/ml <2.0 mM 8 log	X[1]	<1 mg/ml <1.9 mM 7 log	<1 mg/ml[2] <1.9 mM 4 log	<5 mg/ml <9.0 mM 8 log
S. suis	X[1]	<3 mg/ml <6.0 mM 7 log	<5 mg/ml <10.1 5 log	<1 mg/ml <1.9 mM 7 log	<5 mg/ml <9.5 mM 8 log	<5 mg/ml <9.0 mM 2 log
L. monocytogenes	No	No	<3 mg/ml <6.0 mM 6 log	<5 mg/ml <9.5 mM 8 log	<5 mg/ml[2] <1.9 mM 5 log	No
E. faecalis	No	No	<5 mg/ml <10.1 mM 4 log	No	No	No
S. mutans	No	No	No	No	No	No

X[1] = no growth in treatment or control at 5 mg/ml

[2]Data obtained from Wagh et al., 2012.

No = No MBC value