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Original Research Article

Antimicrobial Activity of Alternative Surfactants on Foodborne Pathogenic Bacteria

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ABSTRACT

This research aims to investigate the antimicrobial activity of selected surfactants that are derived from natural substances to use as a replacement for synthetic surfactants. Antimicrobial activities of the selected surfactants, including capryl glucoside (CA), coco glucoside (CG), and decyl glucoside (DG), were determined by the broth dilution method. Foodborne pathogenic bacteria, including *Escherichia coli* TISTR 780, *Salmonella* Typhimurium TISTR 292, *Listeria monocytogenes* Scott A, and *Staphylococcus aureus* TISTR 1466 were tested. The minimum inhibitory concentration (MIC) of tested alternative surfactants for Gram-positive bacteria and Gram-negative bacteria ranged between 0.003 - 0.2 and 5 - >10% (w/w), respectively. The minimum bactericidal concentration (MBC) of those bacteria ranged between 0.005 – 2 and 5 - >10% (w/w), respectively. Among tested surfactants, CG showed the strongest antimicrobial activity against *L. monocytogenes* (MIC=0.003% and MBC=0.005%), followed by *S. aureus* (MIC=0.09% and MBC=1%) and *E. coli* (MIC=7% and MBC=8%), respectively. However, there was no antibacterial effect on *S.* Typhimurium at the maximum concentration tested of 10%. The optimum pH condition for antibacterial activity was investigated *via* the time-kill assay. The destruction curves of *L. monocytogenes* against CG in different pH conditions, including 0.1M phosphate buffer saline (PBS, pH 7.2), 0.1M PBS (pH 5.6), and citrate buffer (pH 5.6), were plotted in comparison to the commercial fruit and vegetable cleaners used as a benchmark. The results showed that 0.5% CG in 0.1M PBS (pH 7.2) could reduce *L. monocytogenes* by 5 Log (~99.999% reduction) within 3 sec. After the exposure time of 5 min, the 0.5% CG in citrate buffer (pH 5.6) could reduce the number of *L. monocytogenes* and *S. aureus* by 4 Log (~99.99% reduction) and 3 Log (~99.9% reduction), respectively. Moreover, 0.5% CG in 0.1M PBS (pH 5.6) could reduce the number of *L. monocytogenes* by 3 Log (~99.9% reduction). All CG treatments could reduce the number of *E. coli* by 1 Log (~90% reduction). At the same time, the commercial cleaner could reduce the number of *L. monocytogenes*, *E. coli*, and *S. aureus* by 0.89 - 1.39 Log (~81% - ~90% reduction). This research demonstrated the antimicrobial efficacy of CG, which has the potential to be used as an alternative antimicrobial surfactant and can be applied to cleaners and sanitizers for household products.

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INTRODUCTION

Food sanitizer and food contact surface sanitizer, including hypochlorous acid, hydrogen peroxide, and quaternary ammonium compounds (QACs), are widely used in the food industry and household products. These sanitizers are high-efficacy disinfectants. However, they irritate the skin and respiratory system when in contact with prolonged exposure (Schmidt, 1997; Juszkiewicz et al., 2019). Nowadays, Europe countries have been concerned about QACs due to their health impact (Fischer et al., 2011; Osimitz and Droege, 2021). Thus, cleaning and sanitizing agents derived from natural substances have been attractive to use as alternative surfactants because they can be re-cyclable, bioabsorbable, and safe for use (De et al., 2015).

Nonionic surfactants do not have an electrical charge on the hydrophilic head group and hydrophobic tail. The primary role of nonionic surfactants is for use as emulsifying, foaming, and wetting agents (Xiang et al., 2019). They are low price, harmless, degradable, and eco-friendly (Zhao and Wan, 2007). Alkyl polyglycosides (APG) are sugar-based nonionic surfactants derived from natural substances, including vegetable oils and starch, which were synthesized in 1892 (Geetha and Tyagi, 2012). The structure of APG is comprised of a hydrophobic alkyl chain and a saccharide unit. Moreover, APG has been called a green surfactant, which is qualified by Generally Recognized As Safe (GRAS). They can be widely applied in many industries, such as foods, detergents, cosmetics, and pharmaceuticals (Aguirre et al., 2014; Geetha and Tyagi, 2012; Van Ginkel, 2007). Some researchers reported that APG had antimicrobial efficacy on some tested bacteria (El-Sukkary et al., 2009)

Therefore, this research aimed to investigate the antimicrobial activity of alternative surfactants on foodborne pathogens through a time-kill assay for use as alternative cleaners and sanitizers for household products.

MATERIALS AND METHODS

Microbial preparation

Escherichia coli TISTR 780, *Salmonella* Typhimurium TISTR 292, and *Staphylococcus aureus* TISTR 1466 were obtained from Thailand Institute of Scientific and Technological Research (TISTR), Pathum Thani, Thailand. *Listeria monocytogenes* Scott A was received from the Microbial Food Safety Laboratory, Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand.

All tested microorganisms were grown in tryptic soy broth (TSB; Merck, Darmstadt, Germany) except for *L. monocytogenes* cultured in TSB containing 0.6% yeast extract (YE; Difco, Sparks, USA). All cultures were subcultured twice, then incubated at 37°C for 24 h and 18 h before use.

Stock solution preparation

Alternative surfactants, namely capryl glucoside (CA) (Before & After Corporation Co., Ltd. Bangkok, Thailand), coco glucoside (CG) (Chanjao Longevity Co., Ltd. Bangkok, Thailand), and decyl glucoside (DG) (RR Cosmetics & Food Ingredients Co., Ltd. Bangkok, Thailand) were prepared at the desirable concentrations by dissolving in sterile deionized water (DI). Citrate buffer at pH 5.6, 0.1M PBS pH 5.6, and 0.1M PBS pH 7.2 were prepared. pH was adjusted with 1N HCl or 0.1N HCl, then sterilized at 121°C for 15 min before use. The pH of tested surfactants was measured by pH meter (Model Lab 850, Schott SI Analytics, Germany) according to AOAC 943.02 (Araujo et al., 2016). Two commercial cleaners, including commercial A and commercial B, were used as a benchmark and prepared according to the instructions of the products. Active compounds of the commercial A and B were alkyl polyglycoside 0.003% and 0.12%, respectively.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC and MBC were determined by the broth dilution method. A double-strength of Mueller-Hinton broth (MHB; Merck, Darmstadt, Germany) containing 5 Log CFU/mL of tested microbial was mixed with a surfactant solution to obtain the actual concentration of tested surfactant solution range from 0.01% to 20%. After that, a mixture was incubated at 37°C for 24 h, and enumeration of microbial survivors by spread plate technique on TSA for *E. coli*, *S.* Typhimurium, and *S. aureus* and TSAYE for *L. monocytogenes*. MIC is defined as the lowest concentration of an antimicrobial agent which inhibits visible microbial growth for 24 h. MBC is the lowest concentration of antimicrobial agents, which reduces 3 Log CFU/mL or 99.9% of the initial microbial load within 24h (Batpho et al., 2017).

Time-kill assay

Time-kill assay evaluated the response of tested bacterial cells against antimicrobial surfactants. The selected antimicrobial surfactant was tested with microbial cocktails in different conditions, including citrate buffer (pH 5.6), 0.1M PBS (pH 5.6), and 0.1M PBS (pH 7.2). The 18-h cell suspensions of *E. coli*, *L. monocytogenes*, and *S. aureus* were harvested by centrifuging at 4500 g for 10 min and washed twice with 0.1M PBS, pH 7.2. Microbial cocktails were prepared by mixing three strains of the harvested cells and diluting in 0.1M PBS to obtain about 5 Log CFU/mL. A buffer solution without antimicrobial agents was used as a control. The survival numbers of the pathogens were enumerated. One mL of sample was transferred into 9 mL of neutralizing buffer (0.5% sodium thiosulfate + 0.85% sodium chloride) (Rahman et al., 2012), then serially diluted and spread on selective media, including Modified Oxford (Sigma-Aldrich, USA) for *L. monocytogenes*, MacConkey (Merck, Darmstadt, Germany) for E. coli and mannitol salt agar (MSA; Difco, Sparks, USA) for *S. aureus*. Plates were incubated at 35℃ for 48 h. Two commercial cleaners were used as a benchmark and were prepared following the instruction of the product, and sterile tap water was used as a control. The experiments were run in two replications.

Statistical analysis

All data were shown as the mean with standard deviation. Mean values were compared using analysis of variance (ANOVA) at a significance level of 95% by the Statistical Package for Social Sciences (SPSS version 26.0) software.

RESULTS AND DISCUSSION

pH measurement

pH values of all tested surfactants indicated strong alkalinity ranging from 11.31-11.65. These surfactants with an alkaline cleaner property can dissolve fats, oils, grease, and other deposits that are protein based. However, highly alkaline products may cause corrosion and should not be touched with bare skin. Whereas the pH of commercial cleaners is fairly close to neutral pH and powerful enough for general cleaning tasks. Therefore, the suitable pH condition of the selected surfactants was also investigated in the latter experiment.

Table 1. pH of tested surfactants and two commercial cleaners

Tested Surfactants	pH
Capryl glucoside (CA)	11.65±0.00[a]
Coco glucoside (CG)	11.36±0.01[b]
Decyl glucoside (DG)	11.31±0.00[b]
Commercial cleaner A	7.73±0.07[d]
Commercial cleaner B	8.58±0.03[c]

The data represent mean values ± standard deviation of triplicate measurements. a-d means within the same column followed by different letters were significantly different ($p \leq 0.05$)

Antimicrobial activity

The antimicrobial activity of the surfactants was expressed in terms of MIC and MBC, as shown in Table 2. The results showed that CA, CG and DG had antibacterial activity against Gram-positive and Gram-negative bacteria. However, Gram-positive bacteria were more sensitive than Gram-negative bacteria. The MIC and MBC of Gram-positive bacteria were ranged between 0.003 to 0.2% (w/w) and 0.005 to 2% (w/w), respectively. While, Gram-negative bacteria had MIC and MBC, ranging between 5 to >10% (w/w) and 5 to >10% (w/w), respectively. Compared to all tested microorganisms, *L. monocytogenes* was the most sensitive strain due to its cell wall structure, and *S.* Typhimurium was the most resistant to antimicrobial surfactants. Gram-positive bacteria are more susceptible to antimicrobial agents due to a simple cell wall structure. These comprise only thick peptidoglycan. Gram-negative bacteria are more complex, consisting of lipopolysaccharides and phospholipid bilayer, covering thin peptidoglycan (Salton and Kim, 1996; Falk, 2019). Moreover, antimicrobial nonionic surfactants have affected Gram-positive bacteria by disrupting the cytoplasmic membrane. In contrast, Gram-negative bacteria show more tolerance due to the outer membrane (Moore, 1997).

Among the tested surfactants, CG showed the most potent antimicrobial surfactant against *L. monocytogenes*. However, CG was not efficacy against *S.* Typhimurium. Moreover, CA showed the most power against Gram-negative bacteria, both *E. coli* and *S.* Typhimurium. Thus, the test surfactant's effectiveness depended on the target microorganisms.

Time-kill assay

The maximum concentration of CG at 0.5% and 5 min of contact time was used in this study according to Notification of the Ministry of Public Health (No. 412) B.E. 2562 Issued by virtue of the Food Act B.E. 2522 Re: Cleaning Agent or Sanitizer for Food Product (FDA, 2019), and FDA Announcement on Specification of Alkyl Polyglycoside (FDA, 2020)

Table 2. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of capryl glucoside (CA), coco glucoside (CG), and decyl glucoside (DG).

Tested microbial	MIC (%w/w)			MBC (%w/w)		
	CA	CG	DG	CA	CG	DG
Gram-negative bacteria						
E. coli	5	7	6	5	8	7
S. Typhimurium	6	>10	6	7	>10	7
Gram-positive bacteria						
L. monocytogenes	0.01	0.003	0.005	0.05	0.005	0.007
S. aureus	0.2	0.09	0.09	2	1	1

The data represent mean values of triplicate measurements
CA = capryl glucoside, CG = coco glucoside, DG = decyl glucoside.

In the time-kill assay section, CG was selected to investigate the response of *L. monocytogenes, S. aureus,* and *E. coli* due to its potent antibacterial activity based on the MIC and MBC values. CG, at a maximum allowance of 0.5%, was added to different buffer solutions, including citrate buffer pH 5.6, 0.1M PBS pH 5.6, and 0.1M PBS pH 7.2, to explore the optimum pH condition. Survivors were evaluated for no longer than 5 min after the exposure time. The buffer solutions without 0.5% CG were used as a negative control. The commercial cleaners launched in the market were used as a benchmark. The results showed that 0.5% CG had potent antibacterial activity against *L. monocytogenes* (Figure 1A).

The strongest antilisterial activity was presented in 0.1M PBS pH 7.2, which decreased the cell numbers by 5 Logs (~99.999% reduction) within 3 sec (0.0005 min), followed by citrate pH 5.6 and 0.1M PBS pH 5.6 that reduced by *ca* 4 Logs or ~99.99% reduction within 5 min, respectively. Moreover, 0.5% CG in citrate buffer pH 5.6 reduced *S. aureus* numbers by 3 Logs (~99.9% reduction) and *E. coli* numbers by 1.3 Logs (~90% reduction) within 5 min (Figure 1B and 1C). In contrast, the antibacterial activities of commercial cleaners were not different from tap water, reducing bacterial cells by *ca* 0.8-1 Log CFU (less than 90% reduction) within 5 min (Figure 1D-1F and Table 3). In commercial treatments, our results agree with previous studies, which found that commercial veggie wash was the least effective against foodborne pathogenic bacteria (Fishburn et al., 2012).

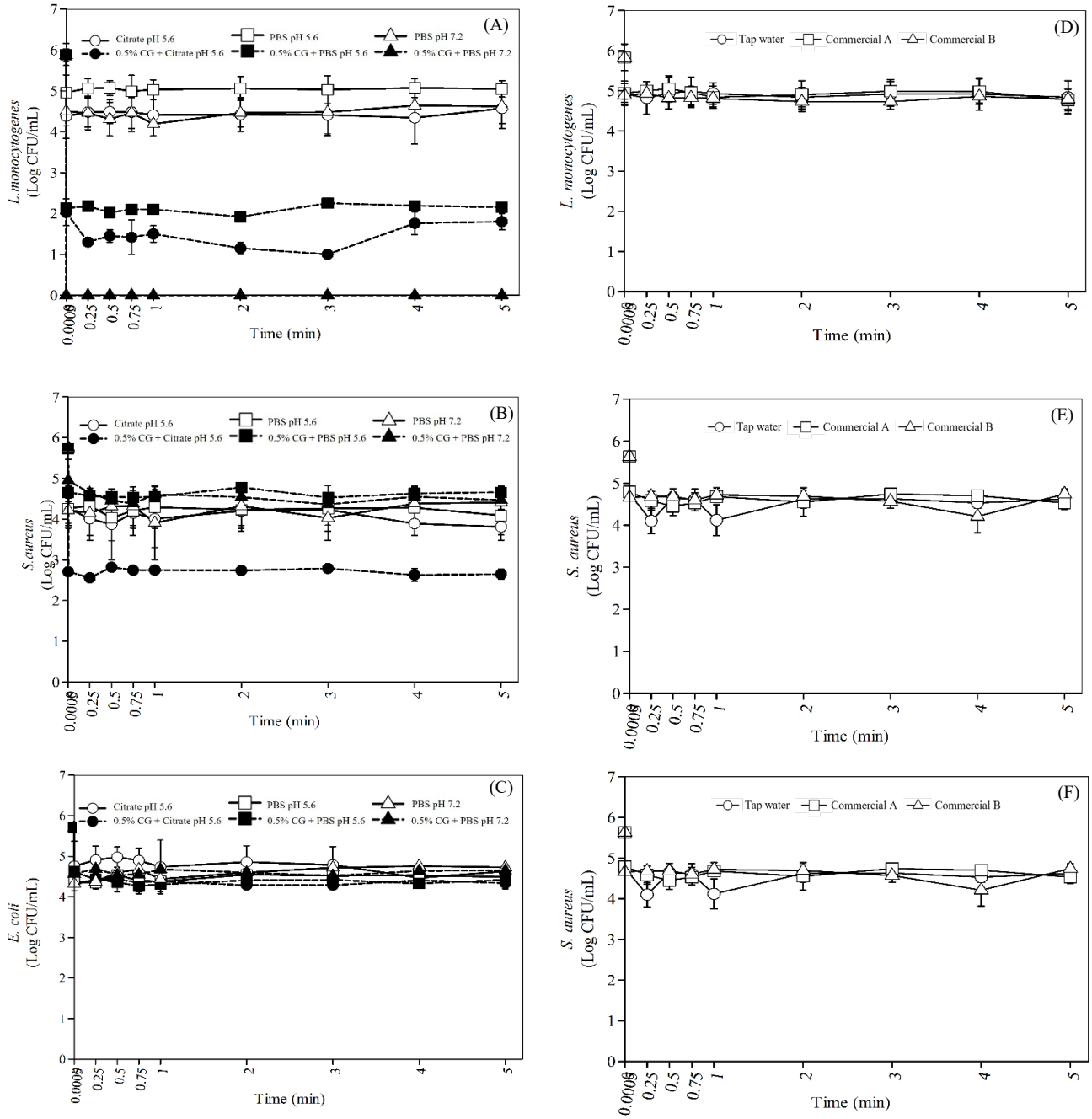

Figure 1. The destruction curves of 0.5% CG against *L. monocytogenes* (A and D), *S. aureus* (B and E) *and E. coli* (C and F) in different buffer solutions (A-C), including citrate (pH 5.6), 0.1M PBS (pH 5.6) and 0.1M PBS (pH 7.2), and commercial cleaners (D-F) within 5 min.

Considering the different pH values, pH 5.6 and pH 7.2, adjusted by the same buffer solution of 0.1M PBS and exact concentration of CG at 0.5%, the antibacterial activity of CG against Gram-positive bacterScia, namely *L. monocytogenes* and *S. aureus*, at higher pH (pH 7.2) (Log reduction ranges from 1.29 to 5.87) was more substantial than the lower pH (pH 5.6) (Log reduction ranges from 1.06 to 3.74). This phenomenon can be described as when the pH values of 0.1M PBS decreased, the zeta potential value of surfactant in the buffer solution might be negatively charged (Malhotra and Coupland, 2004). In general, the charge on the bacteria cell surface was negative (Gottenbos et al., 2001). Therefore, there was an electrostatic repulsion between the negative charge of the antimicrobial agent and the tested microbial, causing less antibacterial activity.

Comparing the type of buffer solution at the same pH of 5.6 and the same concentration of CG at 0.5%, the antibacterial activities of CG in citrate buffer (Log reduction ranges from 1.37 to 4.07) had a broader spectrum than those in 0.1M PBS (Log reduction ranges from 1.06 to 3.74). Thus, 0.5% CG in citric buffer pH 5.6 had the most potent antibacterial activity and was superior to commercial cleaners.

Table 3. Log reduction of pathogenic bacteria after being treated with 0.5% CG in different pH conditions, commercial cleaners and tap water for 5 min.

Tested microorganisms	0.5% CG			Commercial A	Commercial B	Tap water
	0.1M PBS pH 7.2	0.1M PBS pH 5.6	Citrate buffer pH 5.6			
L. monocytogenes	5.87 (3 sec)	3.74	4.07	0.99	1.04	0.99
S. aureus	1.29	1.06	3.07	1.01	0.89	1.00
E. coli	1.06	1.29	1.37	0.90	1.39	1.01

CONCLUSIONS

Coco glucoside is a surfactant that showed strong antimicrobial efficacy against foodborne pathogens. Adding 0.5% CG in the 0.1M PBS pH 7.2 had the most potent antibacterial activity against *L. monocytogenes* within 3 sec. Furthermore, 0.5% CG in the citric buffer pH 5.6 showed a broad spectrum of antibacterial activity superior to commercial cleaners. Therefore, CG in a suitable pH and buffer solution can be applied as an alternative antimicrobial surfactant for cleaner and sanitizer in household and industrial products to control microbial food safety.

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