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PREPARATION AND INVESTIGATION OF HIGH EFFICIENCY ANTIBACTERIAL LIQUID DISHWASHING DETERGENT WITH JAMTS SALTS

A high-efficiency liquid dishwashing detergent was prepared by using jamts salt as an antibacterial agent. The surface cleaning and antibacterial property of the detergent resolved its unifunctionality problem. The antibacterial activity of the detergent was demonstrated through an agar diffusion assay against *Micrococcus luteus, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis and Enterococcus durans*. Results showed that the prepared detergent was highly effective against all tested microorganisms. The results of chemical accelerated tests indicated that the detergent would be effective for at least 6 months. The antibacterial property and detergency performance of the high-efficiency antibacterial liquid dishwashing detergent were compared with those of a commercial antibacterial detergent containing 8–10 % Sodium lauryl sulfate. The detergency performance of the high-efficiency detergent reached was superior to that of the commercial antibacterial detergent.

Key words: *Jamts sals, Lemon essential oil, Dishwashing detergent, Antibacterial property, Detergency performance.*

Introduction

Detergents are commonly used in domestic and industrial cleaning. They can be classified as hard surface cleaners, laundry detergents, and dishwashing detergents in accordance with their applications [1]. Although food production technology has been modernized, food safety issues continue to receive increasing attention because of the ubiquity of bacteria, fungi, and other pathogenic microorganisms [2; 3]. Contaminated kitchen surfaces or hands can cause microbial infections because pathogenic microorganisms can adhere to hard surfaces or skin [4]. Surface conditions that promote bacterial growth include water, nutrients, and beneficial temperatures. Bacteria can multiply and form biofilms as microbial communities adhere to one another in extracellular polymeric matrixes [5]. Tableware is prone to contamination and may transfer bacteria to food because it is constantly in contact with the environment, which contains a wide range of bacterial species. Some studies showed that the prevalence of pathogenic bacteria is slightly higher in the homes of users of non-biocides products than that in the homes of users of biocide products but that detergent cleaning can effectively reduce the amount of bacteria on the surfaces of tableware [6; 7; 8]. Several types of detergents are commercially available. For example, a commercial antibacterial detergent containing 8–10 % Sodium lauryl sulfate which is classified as irritating to the eyes and skin [9] was used as a comparison in these experiments. Triclosan, another commonly used antimicrobial, may be toxic [10]. In addition, triclosan resistance can develop into cross-resistance to other antimicrobials [11].

It has been found that detergents produced through the fermentation of natural peels have an antibacterial effect. Given the concerns regarding the adverse health effects of synthetic food preservatives, the requirement for antibacterial agents has increased [13]. It was reported that essential oils have bacteriostatic or bactericidal activity against pathogens and can thus be used as natural antibacterial agents [14]. Lemon essential oil (LEO) is volatile oil extracted from lemon. Its main antibacterial ingredient is citric acid. It is a kind of light yellow oily liquid, which has the advantages of aromaticity, safety, non-poison, high-efficiency sterilization, and no compatibility contraindication [15]. Lemon, which is the most active ingredient in phenolic volatile oils and the most important LEO ingredient, can increase cell membrane fluidity and permeability by compressing the fatty acid chain of phospholipids; this action causes ions in the cytoplasm to flow out of the cell and results in cell death [16]. [17] showed that LEO is an effective antibacterial additive to hand-washing and surface-cleaning detergent solutions and is a potential alternative to triclosan and chloroxylenol.

Mixtures of surfactants are used to improve the application properties of dishwashing liquids and laundry detergents [18]. Environmentally friendly surfactants are often selected to prepare detergents in consideration of economic and environmental factors. The material, application, aging deformation, corrosion resistance, and safety of detergents should be also considered. Cationic surfactants are usually not used in detergents because anions are attached on greasy and soiled surfaces. Anionic surfactants reduce the cleaning power of detergents by precipitating divalent ions from detergents in water with high hardness [19]. Consequently, the addition of zwitterionic and/or nonionic surfactants to anionic surfactants is preferred. Amphoteric surfactants, such as betaines, which possess excellent detergency on oily residues and biodegradability, are used frequently in household detergents [20]. The cleaning of betaines with excellent temperature stability is much better when the nonionic surfactant is used with the amphoteric and can thicken the composition without using a thickener. Consequently, the mixture of cocamidopropyl betaine (CAB-35) and alkyl polyglycoside (APG), with lower skin and eye irritation, was preferred for dishwashing detergents and worked better with oil stain than other detergents.

Although the antibacterial properties of LEO have been extensively studied and exploited, LEO requires further development before it can be considered as a commercial product. The detergency of surfactants toward oily residues has been studied by several research groups [21]. Nevertheless, information on the antibacterial properties of detergents remains limited. Other products contain sodium lauryl sulfate (SLS), alkyl polyglycoside (APG), cocamidopropyl betaine (CAB), Jamts salts (JTS), LEO, and distilled water. In this study, experiments were conducted to characterize the antibacterial activity of an Jamts salt and LEO system added to a prepared detergent and to investigate the detergency, antibacterial property, and shelf life of the prepared detergent.

Experimental

Microorganisms and Materials

Micrococcus luteus, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis and Enterococcus durans were used as test strains. Stock cultures were stored –20 °C and resuscitated through inoculation in 10 mL of nutrient broth medium (10 g L−1 peptone, 5 g L−1 beef extract, and 5 g L−1 NaCl) at 37 °C for 18–24 h. Nutrient agar purchased from Probic Chemical Industries Co., Ltd (Guangzhou, China) Co., Ltd (Beijing, China) was used for the subculture of bacteria. Pharmaceutical grade LEO was supplied by Huamei Natural Plant Oil Refinery (Jiangxi Province, China) and Shuden mountain of Jamts salt was supplied by Davsan brand LLC (Ulaanbaatar, Mongolia) used as an antimicrobial ingredient. Phenoxyethanol ($C_8H_{10}O_2$), glycerin and distilled water were of analytical grade and purchased from Probic Chemical Industries Co., Ltd (Guangzhou, China). Surfactants comprising amphoteric surfactant, coco-betaine (30 wt %), Sodium Cocoyl Gluconate (SCG, 87 wt %) [27] and cocomidopropyl betaine (CAB, 35 wt %) are one of the most important ingredients in this liquid detergent, which were supplied by Probic Chemical Industries Co., Ltd (Guangzhou, China) Co., Ltd (Guangzhou, China).

Preparation of High-Efficiency Antibacterial Liquid Dishwashing Detergent

Coco-betaine (30 wt%) was slowly dissolved in distilled water (59.2 wt %) at 75 °C and 2400 r/min. Then, the SCG, CAB, Glycerin and LEO (8 wt %), Jamts salts (5 wt %), mixture was added to the coco-betaine solution. The pH of the mixture was adjusted with Jamts salt (3 wt %).

Determination of the pH of the Prepared Detergent

pH was measured by a pH transducer (Mettler Toledo Instruments (Shanghai) Co., Ltd) at 25^0C, 45^0C, room temperature.

Determination of Antibacterial Activity

The antibacterial activity was tested against the Gram-positive bacterial strains *M. luteus, S. aureus, S. epidermidis, E. faecalis, and E. durans* and the Gram-negative strain *E. coli* using the agar diffusion method. One hundred microlitres of test microorganisms (10^6 colony-forming units (CFU)/mL), which were grown in nutrient broth media for 18 h, were spread over the surface of a meat peptone agar medium in Petri dishes. Agar well (8-mm diameter), which were loaded with the JTS-5 (jamts salt 8 % and LEO 0,8 % (500 µg/disc), and commercial antibacterial detergent containing 8–10 % Sodium lauryl sulfate (500 µg/disc), were soaked on the agar well of the Petri dishes. The Petri dishes were incubated at 37°C for 24 h; then, the diameters of the inhibition zones were measured in millimetres. Kanamycin (10 µg/disc for *S. aureus, E. coli, M. luteus, S. epidermidis*, 100 µg/disc for *E. faecalis and E.durans*) was used as a standard antibiotic.

Antibacterial activity was determined by measuring the inhibition zone diameter (IZD, in mm) produced by the samples against microorganisms [23].

Determination of Bacterial Count for the Prepared Detergent

Further decimal dilutions were prepared with sterile saline solution as follows: 0, 5/50, and 1/100. Next, 5 mL of diluted samples (positive control for sterile saline) was uniformly smeared onto plates. Nutrient agar was poured into plates containing the samples through the pour plate technique. Plates were incubated at 37 °C for 24 h prior to colony enumeration. All experiments were conducted three times.

Determination of Valid Time for the Prepared Detergent

Samples were sealed and placed in a constant temperature and humidity incubator at 24 °C for 3 months. The appearance of the sample was observed. The agar diffusion assay (described in section

"Determination of Antibacterial Activity through the agar diffusion assay") was carried out before and after placement. Evaluation standards showed that the decline rate of sterilization was less than or equal to 10 % after 3 months at 18 °C and the sample can be effective for 1 year. The decline rate of sterilization β was calculated on the basis of the IZD before and after placement and is expressed by the following equation:

$$\beta = \frac{Xo - Xt}{Xo} x100\%$$

where X_0 and X_t represent the IZD before and after placement, respectively.

Results and Discussion

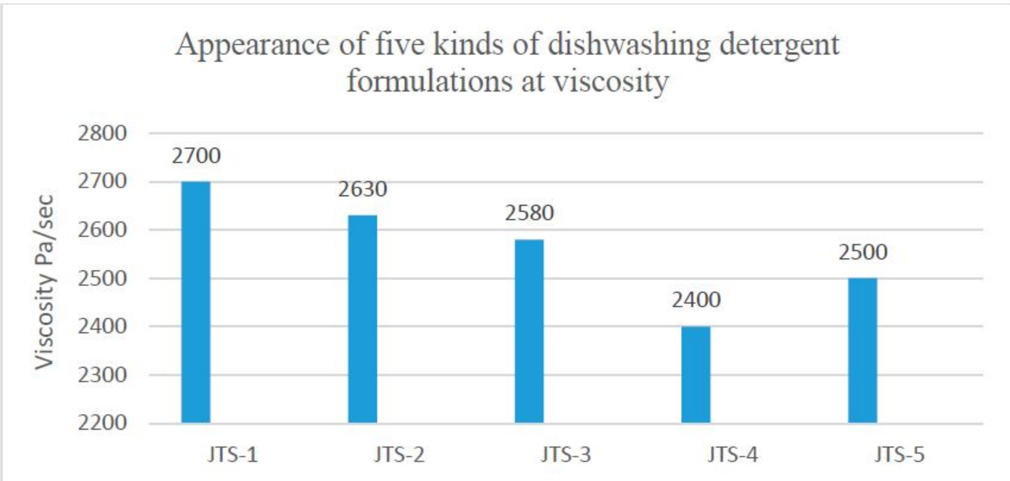
Detergency Performance Analysis of Oily Soil from Glasses

Table 1 shows five kinds of dishwashing detergent formulations. Graphic 1 presents the effects of five kinds of dishwashing detergent formulations at different viscosity.

Table 1

Dishwashing antibacterial detergent formulations

Formulations	CB (wt%)	SCG (wt%)	CAB (wt%)	Jamts (wt%)	Gly (wt%)	Phen (wt%)	LEO (wt%)	Distilled water
JTS-1	8	9	8	1	3.5	0.8	0.8	68.9
JTS-2	7	9	7	2	3	0.8	0.8	70
JTS-3	6	9	6	4	2.5	0.8	0.8	70
JTS-4	5	9	5	6	2	0.8	0.8	71
JTS-5	5.1	9	5.1	8	1.5	0.8	0.8	69.7



Graphic. 1 Appearance of five kinds of dishwashing detergent formulations at viscosity

As shown in Graphic 1 the higher the surfactant, the higher the viscosity, or more than 25 00 Pa/sec. The JTS-5 sample has a suitable viscosity of 2 500 Pa/sec.

pH of the Prepared Detergent

The pH of the 1/100 dilution of JTS-5 was 6,52, which met the pH requirement of 4,0–10,5. Antibacterial Activity Analysis for Jamts salt and LEO and most microorganisms including *M. luteus, E. coli, S. aureus, S. epidermidis, E. faecalis and E. durans* were used in the antibacterial activity experiment [24; 25].

Table 2

Antibacterial activity of JTS-5 (jamts salt 8 % and LEO 0,8 %) of other commercial dishwashing liquid

Antibacterial dishwashing liquid	Bacteria	JTS-5 (jamts salt 8% and LEO 0.8% /New prepared/	JTS-5 (jamts salt 8% and LEO 0.8% /after 6 months /	Km /control/	Other commercial dishwashing liquid. /with SLS/	Km /control/
Antibacterial activity (Inhibition zone, mm)	*E. coli*	17	14.15	-	15.85	15.5
	S. aureus	25.15	24.5	21.75	22.15	17.3
	M.luteus	24.16	31	12.65	22.7	17.5
	E.faecalis	22.7	22.65	12.55	19	11.1
	S.epidermis	21	20.5	13.1	16.3	10
	E.durans	21.5	20.5	17.45	18.65	13

The results for the antibacterial activity of JTS-5 and other dishwashing liquid in the market in Table 3. That JTS-5 and other commercial dishwashing liquid in the market exhibit broad-spectrum bactericidal performance. The JTS-5 of IZD values of E. coli 17 mm and S. aureus 25,15 mm, M.luteus 24,16mm, E.faecalis 22,7mm, S.epidermis 21 mm, E.duras 21,5 mm respectively, when exposed to detergent containing jamts salt 8 % and 0,8 % LEO. That is, E. coli, S. aureus, M.luteus, E.faecalis, S.epidermis, E.duras was more resistant than S. aureus JTS-5 (jamts salt 8 % and LEO 0,8 %) possessed strong antibacterial activity, especially against S. aureus. Specifically, the IZD of S. aureus was 24,5 mm under JTS-5 (jamts salt 8 % and LEO 0,8 %). Compared to commercial dishwashing liquid with a sample of other antibacterial activity commercial dishwashing liquid (SLS), which measured at 22.15 mm for was S. aureus. Accordingly, this formulation was highly effective against the tested bacteria while being safe for the human body.

Values are mean ±SD of triplicate analysis; 5 mm is the size of agar well IZD the diameter of inhibition zone.

Prepared Dishwasher Liquid Analysis of shelf time for Antibacterial

Figure 2 shows the efect of diferent times on bacterial survival. In the experiments conducted with the bacterial counts the count of *M.luteus* was increased by nearly 6 per and *E.durans* bacteria was increased by approximately 2 per. In this Figure shows the bar graph of the bacterial counts, showing no signifcant diferences, but bacteria still exhibited *M.luteus* was 6 months later than antibacterial activity is increased.

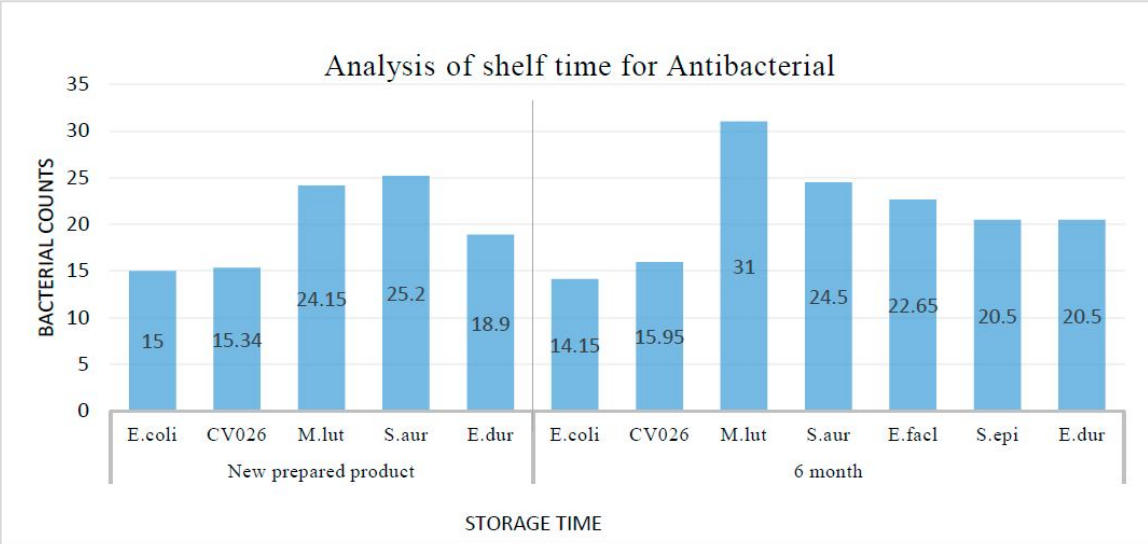


Figure 2. The efect of diferent times on bacterial survival

Shelf life Analysis of the Prepared Detergent

The valid time for the prepared detergent was determined by the microbiological assay which was the effect of killing bacteria for the sample JTS-5 before and 6 months after placement.

The bacteriostatic test results of JTS-5 are given in Table 3. After 6 months, the inhibitory effects of JTS-5 on *E. coli and S. aureus, M.luteus, E.faecalis, S.epidermis, E.durans* were weakened by less than 20 %. From here germicidal efficacy would be valid for at least 1 year.

The stability test results of JTS-5 are also presented in Table 3. These results show that the detergents are highly stable. Furthermore, JTS-5 was more effective against Gram-positive bacteria than Gram-negative bacteria before and after 6 months of incubation under certain conditions.

Table 3

Antibacterial activities of the prepared detergent before and 6 months after placement

Storage time (Months)	Inhibition zone (mm)					
	E. coli	*S. aureus*	*M. luteus*	*E. faecalis*	*S.epidermis*	*E.durans*
0	15	25.2	24.15	22.9	21	18.9
6	14.15	24.5	31	22.65	20.5	20.5

Conclusions

A high-efficiency antibacterial liquid dishwashing detergent with jamts salt that resolved the unifunctionality problem of commercially available detergents was prepared in this work. The detergency performance, antibacterial activity and shelf life of the prepared detergent were determined. The detergency performance of JTS-5 reached and was superior to that of a commercial antibacterial detergent in the market, which had a detergency performance. They showed antibacterial activities against *M. luteus, E. coli, S. aureus, S. epidermidis, E. faecalis and E. durans* but the prepared detergent was more effective against tested bacteria and safer for the human body. The high-efficiency antibacterial property of the prepared detergent is illustrated in Figure 2, which clearly indicates the differences between the sensitivities of *M. luteus, E. coli, S. aureus, S. epidermidis, E. faecalis and E. durans* to the detergent. Under normal circumstances tests revealed the detergents would retain their antibacterial activities for at least 6 months. The results suggest that the JTS-5 detergent is more effective against Gram-positive bacteria over a 6 months.

Given that the components of JTS-5 are environmentally friendly, non-toxic, and inexpensive, it could be used as an alternative for commercial detergents. This work was mainly performed using laboratory control strains.

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ПОЛУЧЕНИЕ И ИССЛЕДОВАНИЕ ВЫСОКОЭФФЕКТИВНОГО АНТИБАКТЕРИАЛЬНОГО ЖИДКОГО СРЕДСТВА ДЛЯ МЫТЬЯ ПОСУДЫ С ПОВАРЕННОЙ СОЛЬЮ

Высокоэффективное жидкое средство для мытья посуды было приготовлено с использованием поваренной соли в качестве антибактериального средства. Очистка поверхности и антибактериальные свойства моющего средства решили проблему его многофункциональности. Антибактериальная активность детергента была продемонстрирована с помощью диффузионного анализа в агаре в отношении *Micrococcus luteus, Escherichia coli, золотистого стафилококка, эпидермального стафилококка, Enterococcus faecalis и Enterococcus durans*. Результаты показали, что приготовленное моющее средство было высокоэффективным против всех протестированных микроорганизмов. Результаты химических ускоренных испытаний показали, что моющее средство будет эффективным не менее 6 месяцев. Антибактериальные свойства и моющие свойства высокоэффективного антибактериального жидкого средства для мытья посуды сравнивались с таковыми у коммерческого антибактериального моющего средства, содержащего 8–10 % лаурилсульфата натрия. Моющие свойства высокоэффективного моющего средства превосходили показатели коммерческого антибактериального моющего средства.

Ключевые слова: *поваренная соль, эфирное масло лимона, средство для мытья посуды, антибактериальные свойства, моющие свойства.*