CHAPTER 5

Bioactive Properties of Sugar Fatty Acid Esters

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Abstract: Sugar fatty acid esters are biodegradable and biocompatible nonionic biobased surfactants or emulsifiers, obtained from abundant renewable resources. They have a dozen of applications in food, cosmetic and pharmaceutical industries. In this book chapter, we briefly reviewed the enzymatic synthesis of sugar fatty acid esters in solvent-free system and their bioactive characteristics, including antimicrobial activity, anti-tumor activity and anti-insect activity. In addition, we compared the antimicrobial and antitumor properties of sugar fatty acid esters synthesized from enzyme with commercial sugar fatty acid ester produced and purified from a chemical reaction.

Keywords: Anti-insect activity, Antimicrobial activity, Antitumor or anticancer activity, Sugar fatty acid esters.

BACKGROUND

Sugar-fatty acid esters, nonionic biobased surfactants, are synthesized from renewable resources such as saccharides (*e.g.*, fructose and sucrose) and fatty acids (*e.g.*, oleic, lauric and palmitic acids). Fig. (**1**) depicts the reaction scheme for sucrose oleate synthesis, catalyzed by the enzyme lipase. Sugar esters have a broad spectrum of applications in food, cosmetics and pharmaceutical industries because of their low toxicity and irritability, biodegradability, and biocompatibility. Their main use is as emulsifiers, due to their amphiphilicity.

As useful functional additives, emulsifiers are widely employed in food processing for the improvement of the stability of multiphase systems in food products. They enable two distinct and immiscible phases to form a stable quasi-homogeneous solution that remains stable for a significantly long time. In addition, emulsifiers modify and improve the physical properties of the continuous phase in food products, promoting their employment as dispersing

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agents, foamers, and stabilizers [1]. The relative proportion of hydrophilic and lipophilic behavior of surfactants and emulsifiers is often expressed as the hydrophilic-lipophilic balance, or HLB. The HLB value ranges from 0 to 20, with low numbers (<9) indicative of lipophilic behavior and high numbers (>11) representing hydrophilicity. The HLB of sugar esters can be tailored through controlling the number of fatty acyl groups per molecule and the length of the fatty acyl chain to cover almost the entire HLB range.

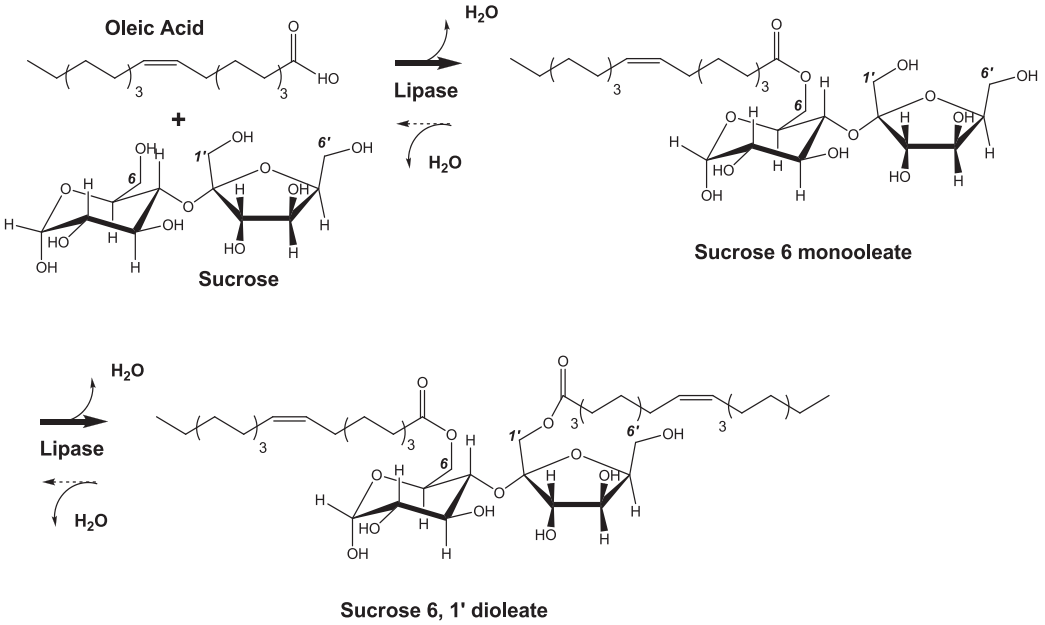

Fig. (1). Lipase catalyzed synthesis of sucrose oleate.

Food-based products that contain sugar esters include baked goods, fruit coatings, and confectionery foods [2, 3]. The effects of sugar esters on the nucleation, growth, and crystallization behavior of high-melting milk fat fraction-sunflower oil blends, have been evaluated for products such as chocolate and confectionaries [2]. Sugar esters are also used in coffee creamers, liqueurs, fruit drinks and whippable toppings [4 - 6]. The employment of sugar esters as additives for drug formulations and delivery has also been investigated. Sucrose stearate was employed as surfactant for nanoemulsions employed in transdermal drug delivery, to replace lecithin, which has a high tendency towards self-aggregation and is prone to chemical degradation [7]. In another study, sugar ester nano-vesicles were employed to encapsulate the antioxidant enzyme catalase for wound healing [8]. In addition, the effect of sucrose esters on transdermal permeation of lidocaine and ketoprofen was examined [9]. The investigators found that sugar

esters facilitate skin permeability and drug absorption [9]. The possession of antitumor properties and antimicrobial activity (discussed in the next section) has furthered the use of sugar esters in pharmaceutical products. Sucrose esters are also used in cosmetics and personal care products including oral and dental care [10, 11].

Chemical Synthesis of Sugar Esters

Sugar esters are commonly produced by chemical methods under extreme conditions, for instance high temperature and pressure, and often in the presence of alkaline or acid catalysts [12, 13], leading to safety issues, environmental concerns and undesired byproducts. For example, the synthesis of sorbitan-fatty acid esters using the conventional chemical method consists of a two-stage process, including dehydration of sorbitol in the presence of the acid catalyst (*e.g.* NaH_2PO_3) at 150-200°C, followed by alkali (*e.g.*, Na_2CO_3-)-catalyzed esterification with fatty acids at 200-250°C [1]. In addition, it was reported that base- (K_2CO_3-) catalyzed transesterification sucrose esters were performed in dimethyl formamide (DMF), an expensive and bioincompatible solvent, at 90 °C with fatty acid methyl ester, serving as acyl donor [14].

Enzymatic Synthesis of Sugar Esters

In contrast, biocatalytic synthesis (*e.g.* using lipases) has received great attention owing to improved sustainability of the reaction: near-ambient pressure and temperature (resulting in lower energy consumption and carbon dioxide emissions), the absence of alkaline or acidic catalysts (leading to lower amounts of waste products), and a narrow product distribution, because mono- and diesters are selectively synthesized *via* the primary hydroxyl groups of the saccharide acyl acceptor.

However, significant barriers hamper the application of immobilized thermophilic lipases for use in industrial production of sugar esters. The major issue is the poor miscibility of polar and non-polar substrates, leading to slow reaction rates. Several different methods have been used to overcome this hurdle. Among them, the utilization of polar organic solvent or their mixtures is considered as the most common approach. Common solvents employed for this purpose include *tert*-butyl or *tert*-amyl alcohol, methyl ethyl ketone, acetone, acetonitrile; or their mixtures with very polar solvents, such as dimethylsulfoxide (DMSO) [8, 15 - 19]. If such systems are operated correctly, partially solubilized acyl acceptor can be employed; and, the reaction medium's composition and temperature can be tuned to selectively precipitate out the monoester product [17 - 21]. In addition to

the use of polar organic solvent, the employment of ionic liquids [22] and pressurized solvent systems near or above their critical points (*e.g.*, supercritical CO_2/solvent mixtures) [23, 24] have shown enhanced rates of enzymatic synthesis. However, these solvent systems present several disadvantages, including the high cost of solvents and/or their recovery or disposal, reduction of process safety, the loss of the enzymatic activity, and the environmental impact of solvent utilization.

Recently, "green" processing for the lipase-catalyzed synthesis of saccharide-fatty acid esters has been developed by our group, employing a small amount of organic solvent with low toxicity. In detail, stirred batch experiments were performed at 55°C with immobilized lipases and the fed-batch delivery of acyl acceptor (fructose or sucrose) in the presence or absence of *tert*-butanol (t-BuOH). Yield of 80-93% were achieved for immobilized *Rhizomucor miehei* lipase-(RML-) catalyzed esterification of oleic acid and saccharide (fructose or sucrose) using near-stoichiometric amounts of substrates in batch mode for ~1 week, with product enriched in monoester [25, 26]. t-BuOH, a tertiary alcohol, was used only during the initial period for the improvement of the miscibility of acyl donor and acceptor, and completely evaporated away within 12-24 h, typically equivalent to ~25% conversion. The sugar ester products improved the miscibility of the substrates, saccharide and oleic acid, the latter also serving as solvent.

During the next phase of research in our group, bioreactor systems containing packed-bed or stirred-tank bioreactors (PBBR and STBR, respectively) and a packed "desorption" column of saccharide crystals mixed with silica gel for delivery of saccharide, operated under solvent-free conditions and undergoing continuous recirculation, were employed. Conversions of 80-85% were achieved [25]. However, reaction rates were several-fold lower than that of the batch mode due to low saccharide concentrations inherent to the bioreactor systems [27].

To increase the saccharide concentration, hence the reaction rate, in the bioreactor systems, metastable suspensions of saccharide crystals (10-200 μm) dispersed in solvent-free mixtures of fatty acyl substrate + sugar ester product were employed. The suspensions were formed by stirring the solid-phase saccharide and the liquid-phase media for several minutes, followed by centrifugation to remove excessively large particles [28]. The saccharide-enriched solvent-free media, containing 0.5-3.0 wt% sugar, was continuously recirculated through a PBBR-based closed-loop bioreactor system until the saccharide was consumed by the reaction. Subsequently, the solvent-free media was isolated from the bioreactor system, and treated with additional saccharide and subjected to the protocol

described above to form metastable suspensions. The employment of suspensions improved the reaction rate and yield: ~88% conversion of fructose and oleic acid into fructose-oleic acid ester, or FOE (92 wt% monoester and 8 wt% diester) was achieved within 6 days, starting with a reaction medium consisting of 75 wt% oleic acid and 25 wt% FOE. Free evaporation served as the means of removal of the co-product water up to achievement of ~60% conversion, after which a more stringent means of water removal was introduced into the reactor system, to lower the water content. Different methods for water removal were assessed: a molecular sieve packed column, N_2 bubbling, vacuum pressure, or a combination of the latter two). The optimal performance was achieved when initiating N_2 + vacuum (2.16 $mg_{H2O}h^{-1}$) upon reaching 60% ester, to lower the liquid-phase water concentration to ~0.40 wt% [29]. When employing the optimal conditions, 92.6 wt% of FOE was obtained within 132 h, yielding a productivity of 0.297 $mmol_{Ester}h^{-1}g_{\ lipase}^{\ -1}$ [29].

The effect of the different acyl donors (oleic, caprylic, lauric and myristic acids) and acceptors (fructose, sucrose, glucose and xylose) on lipase catalyzed synthesis of saccharide-fatty acid esters were performed using solvent-free suspensions of saccharide crystals (50-200 μm) in a mixture of acyl donor/fructose oleate (90 wt% monoester and 10 wt% diester) at a ratio of 75/25 w/w initially [30]. A nearly linear relationship between initial saccharide concentration and initial rate of reaction and final ester concentration was reported, independent of the acyl donor or acceptor. The reaction rate and yield achieved in STBRs were slightly lower than those of PBBR [30]. Suspensions with the highest saccharide concentration correspond to saccharide crystals with the smallest average size, since large-sized crystals were sedimented out during the formation of the suspensions. The highest yield was achieved using fructose and oleic acid as substrates (92.3 wt% FOE, consisting of 92 wt% monoester) in a packed-bed bioreactor (PBBR). The activity of RML was completely retained during four successive runs for solvent-free FOE synthesis, during a 22 day reaction period [30].

To prevent the need of reforming the solvent-free saccharide suspensions offline (after consumption of the acyl acceptor), a novel bioreactor system was developed that allowed for *in situ* formation of suspensions [31]. Metastable suspensions were formed through continuous stirring in the reservoir, with saccharide added slowly and continuously to the reservoir. The suspension-based medium was transport *via* a peristaltic pump through an in-line filter of 180 μm nominal size to remove larger suspensions. This system produced 84 wt % ester (90% of which was monoester), and a productivity of 0.195 $mmol_{Ester}\ h^{-1}\ g_{RML}^{\ -1}$ [31]. The

resultant technical grade product can potentially be used directly, without further purification. In addition, a mathematical model based on mass balances and a zeroth-order kinetic model was successfully developed to predict the concentration of substrates (oleic acid and saccharide) in the reservoir during the time course of reaction [31].

In recent research, high-speed homogenization, high-intensity ultrasound, and their combination were assessed for the particle size reduction of sucrose crystals to enhance solvent-free lipase-catalyzed synthesis of sucrose oleate at 65 °C [32]. The combination of homogenization and ultrasound significantly decreased the particle size of suspended sucrose crystals from 88 to 18 μm, and allowed for a smaller initial sugar ester concentration, 10 wt% [32]. The suspension-based medium was charged to a stirred tank bioreactor that also contained RML or immobilized *Candida antarctica* lipase B (CALB), the latter of which was pre-incubated in oleic acid prior to use, to enhance its activity. Under optimal conditions, 83.3 wt % ester was achieved within 8 days [32].

Antimicrobial Activity

Sucrose esters are commercially used in Japan in canned beverages for inhibiting the growth of spore forming bacteria as antibacterial agents [28]. As emulsifiers, the presence of the sugar fatty acid ester may affect cell membranes at a low concentration, leading to a change in the permeability of cell membranes [33], thereby resulting in metabolic inhibition, growth arrest or cell lysis [34]. Table **1** summarizes the effects of antimicrobial activities of different sugar fatty acid esters on different microorganisms.

Table 1. Antimicrobial activities of different sugar fatty acid esters [1].

Sugar Ester	Microorganism Name	Minimum Inhibitory Concentration (g/L)	Inhibition (%)	Reference
Fructose dilaurate	*Bacillus cereus*	9.375	41.9± 2.5	[35]
Lactose monocaprylate	*Escherichia coli*	N/A	>50%	[36]
Lactose monocaprylate	*Staphylococcus aureus*	>4	N/A	[36]
Lactose monocaprate	*Escherichia coli*	N/A	30%-50%	[36]
Lactose monocaprate	*Staphylococcus aureus*	4	N/A	[36]
Lactose monolaurate	*Candida albicans*	N/A	30%-50%	[36]
Lactose monolaurate	*Enterococcus faecalis*	1	N/A	[37]
Lactose monolaurate	*Escherichia coli*	N/A	30%-50%	[36]

(Table 1) contd.....

Sugar Ester	Microorganism Name	Minimum Inhibitory Concentration (g/L)	Inhibition (%)	Reference
Lactose monolaurate	*Staphylococcus aureus*	0.5	N/A	[36]
Lactose monolaurate	*Streptococcus suis*	0.1	N/A	[37]
Lactose monolaurate	*Listeria monocytogene*	0.1	N/A	[37]
Maltose monocaprylate	*Escherichia coli*	N/A	>50%	[36]
Maltose monocaprylate	*Staphylococcus aureus*	>4	N/A	[36]
Maltose monocaprate	*Escherichia coli*	N/A	>50%	[36]
Maltose monocaprate	*Staphylococcus aureus*	4	N/A	[36]
Maltose monolaurate	*Candida albicans*	N/A	30%-50%	[36]
Maltose monolaurate [2]	*Bacillus sp.*	0.8	93	[15]
Maltose monolaurate [2]	*Bacillus stearothermophilus*	2	44	[15]
Maltose monolaurate [2]	*Erwinia carotovora*	4	8.0	[15]
Maltose monolaurate	*Escherichia coli*	N/A	30%-50%	[36]
Maltose monolaurate [2]	*Lactobacillus plantarum*	4	68	[15]
Maltose monolaurate [2]	*Pichia jadinii*	4	6.0	[15]
Maltose monolaurate	*Staphylococcus aureus*	0.25	N/A	[36]
Maltose monolaurate [2]	*Pseudomonas fluorescens*	4	1.9	[15]
Maltose monolaurate [2]	*Zygosaccharomyce s rouxii*	2	8.0	[15]
Maltose monopalmitate [2]	*Bacillus sp.*	2	94	[15]
Maltose monopalmitate [2]	*Bacillus stearothermophilus*	2	31	[15]
Maltose monopalmitate [2]	*Lactobacillus plantarum*	2	11	[15]
Maltose monopalmitate [2]	*Pichia jadinii*	2	1.6	[15]
Maltose monopalmitate [2]	*Staphylococcus aureus*	2	2.3	[15]
Maltose monopalmitate [2]	*Zygosaccharomyce s rouxii*	1	3.5	[15]
Sucrose monocaprylate	*Escherichia coli*	N/A	>50%	[36]
Sucrose monocaprylate	*Staphylococcus aureus*	>4	N/A	[36]
Sucrose monocaprate	*Escherichia coli*	N/A	>50%	[36]
Sucrose monocaprate	*Staphylococcus aureus*	4	N/A	[36]
Sucrose monolaurate [2]	*Bacillus sp.*	4	95	[15]
Sucrose monolaurate	*Bacillus cereus*	9.375	93.4 ± 2	[35]
Sucrose monolaurate	*Candida albicans*	N/A	30%-50%	[36]
Sucrose monolaurate	*Enterococcus faecalis*	1	N/A	[37]
Sucrose monolaurate	*Escherichia coli*	N/A	30%-50%	[36]
Sucrose monolaurate [2]	*Escherichia coli*	4	26	[15]
Sucrose monolaurate [2]	*Erwinia carotovora*	4	22	[15]

(Table 1) contd.....

Sugar Ester	Microorganism Name	Minimum Inhibitory Concentration (g/L)	Inhibition (%)	Reference
Sucrose monolaurate [2]	*Lactobacillus plantarum*	4	69	[15]
Sucrose monolaurate	*Listeria monocytogene*	0.1	N/A	[37]
Sucrose monolaurate [2]	*Pichia jadinii*	4	5.0	[15]
Sucrose monolaurate [2]	*Pseudomonas fluorescens*	4	2.6	[15]
Sucrose monolaurate	*Staphylococcus aureus*	0.25	N/A	[36]
Sucrose monolaurate	*Streptococcus suis*	0.01	N/A	[37]

[1] N/A: not available; [2] 6'–O-acyl ester

Habulin *et al.* studied the antimicrobial activities of sucrose monolaurate. They found that the sugar ester at a concentration of 9.4 mg/mL successfully inhibited the growth of *Bacillus cereus* [35]. Ferrer *et al.* evaluated the performance of sugar esters prepared *via* biocatalysis on the growth in liquid medium of various microorganisms (Gram-positive, Gram-negative and yeasts). 6-O-lauroylsucrose and 6'-O-lauroylmaltose at concentrations of 0.8 mg/mL yielded 90% inhibition of the growth of *Bacillus* sp., 69% inhibition of *Lactobacillus plantarum*, and 26% inhibition of *Escherichia coli* at 4 mg/mL. Sucrose dilaurates and 6-O-lauroylglucose have no antimicrobial activity, probably because of their low aqueous solubility or the presence of cytoderm lipopolysaccharides and membrane lipids, which may form complexes with the sugar esters and prevent their accumulation in the transport cell membrane [36, 38, 39]. In addition, carbohydrate esters failed to significantly inhibit the growth of yeasts (*Zygosaccharomyces rouxii* and *Pichia jadinii*) [15]. Wagh *et al.* examined the antimicrobial activities of sucrose monolaurate and lactose monolaurate. When treated with these two monoesters, Gram-positive bacteria were more susceptible to inhibition than Gram-negative bacteria. The minimal bactericidal concentrations of lactose monolaurate ranged from 5 to 9.5 mM for *Listeria monocytogenes* isolates and 0.2 to 2 mM for *Mycobacterium* isolates [37]. The morphological properties of *Listeria monocytogenes* N3-013 (Fig. **2**) and *Mycobacterium species*. strain JLS in the presence of lactose monolaurate were recorded by SEM (Fig. **3**). *Listeria* cells possess normal rod shape with some cocci (Fig. **2A**). It was observed that the sugar ester induced a change in the morphology for *Listeria,* from rods to cocci (Fig. **2B**) occurred. Fig. (**3**) indicates the cell surface of *Mycobacterium* species strain JLS became rough upon the addition of lactose monolaurate, compared to that of the control (Fig. **3A**).

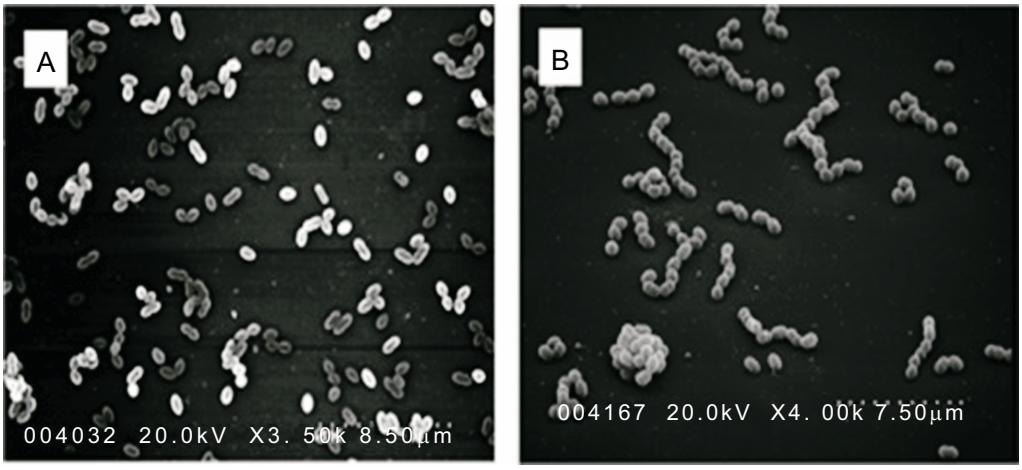

Fig. (2). SEM images of *Listeria monocytogenes* N3-013 under (**A**) control conditions (magnification, ×3,500) and (**B**) after treatment with 1 mg/ml lactose monolaurate for 24 h (magnification, ×4,000) [37].

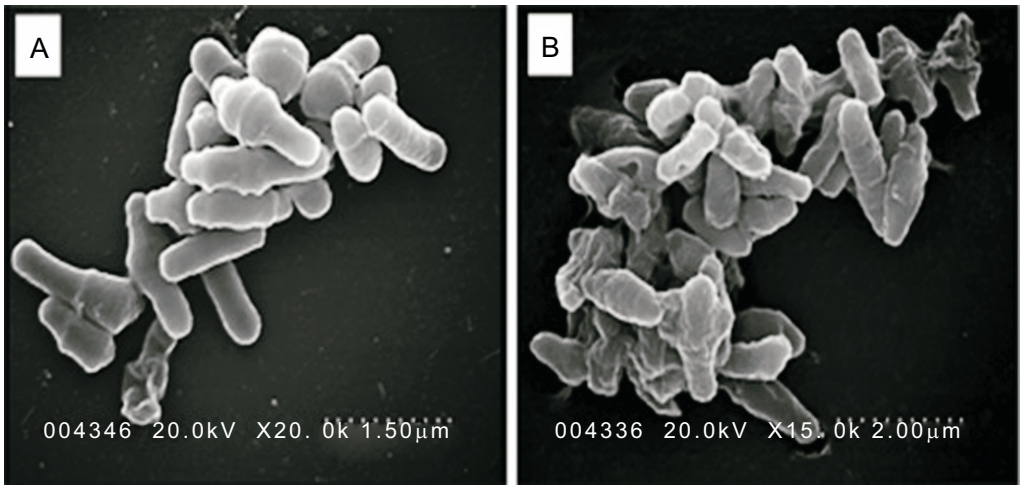

Fig. (3). SEM images of *Mycobacterium sp.* strain JLS under (**A**) control conditions and (**B**) after treatment with 1 mg/mL of lactose monolaurate for 24 h. The cell surface after sugar ester treatment was wrinkled compared to that of the control [37].

Zhang *et al.* investigated the effects of disaccharide core (sucrose, maltose, lactose), length of the fatty acid (caprylic, capric and lauric acid), degree of substitution (monoester and diester) and anomeric configuration (α- and β-ester) on antimicrobial properties were assessed for three common pathogens, *Staphylococcus aureus* (Gram-positive bacterium), *E. coli* O157:H7 (Gram-negative bacterium) and *Candida albicans* (yeast). The results illustrate that all of the monoesters were more effective against *S. aureus* compared to *E. coli* [36]. In addition, the length of fatty acid chain had a significant impact on antibacterial

activity. Among the medium chain fatty acid monoester tested in this study, the lauroyl monoesters exhibited the best antimicrobial performance against *S. aureus*. The order of antimicrobial performance corresponding to the carbon chain length was listed as follows: $C_8<C_{10}<C_{12}$, suggesting that the HLB values are highly correlated with the inhibition of microorganism growth. This result verifies that sugar ester may permeate the surface of the bacteria *via* acyl moieties, and interfere with a series of physiological functions [36].

Piao *et al.* reported that monodecanoyl, monolauroyl, monomyristoyl, monopalmitoyl erythritol and xylitol esters displayed strong inhibitory effect with xylitol monolaurate against *B. cereus*; in contrast, many sugar esters in this study failed to inactivate *E. coli* [40]. However, sucrose monolaurate mixed with sodium hypochlorite significantly inhibited *E. coli* O157:H7 on spinach at a concentration of 10 mg/mL [41].

Sugar esters prepared *via* enzymatic transesterification were evaluated for their inhibition of two glucosyltransferases of great homology from *Streptococcus sobrinus* and *Leuconostoc mesenteroides* NRRL B-512F. The results demonstrated that 6-O-lauroylsucrose, 6'-O-lauroylmaltose and 6"-O-lauroy lmaltotriose at 100 μg/mL showed complete inhibition of the growth of *S. sobrinus* in agar plates. As a result, these nontoxic sugar fatty acid esters are very promising for inclusion in oral-hygiene products aimed at disrupting plaque formation and preventing caries [42]. Fig. (**4**) depicts the absence of bacterial colonies in the Petri dishes in the presence of 100, 200 and 400 μg/mL of 6"-O-lauroylmaltotriose and a large amount of bacterial colonies occurred in the control in the absence of sugar fatty acid esters.

Yang *et al.* demonstrated that sucrose and methylglucose esters with medium and long chain fatty acids suppressed the growth of two microorganisms involved in the spoilage of salad dressings, *Zygosaccharomyces bailii* and *Lactobacillus fructivorans* [43]. Sucrose monoesters of myristic and palmitic acid were the most effective inhibitors. For methylglucose monoesters, medium chain fatty acid derivatives (lauric and myristic acid esters) were more active. Sucrose and methylglucose oleate exhibited only slight antimicrobial activity. For a given acyl donor, sucrose monoesters were usually more effective than methylglucose esters. In salad dressing, 1% sucrose monoesters containing sucrose lauric, myristic, or palmitic fatty acid esters significantly ($P < 0.05$) inhibited the growth of *Z. bailii* and *L. fructivorans*, which are comparable to the performance of 0.1% sodium benzoate, a common preservative. The storage experiments indicate that the growth of *Z. bailii* was nearly 100% inhibited by sucrose laurate, myristate and

palmitate after 9 days of salad dressing storage [43].

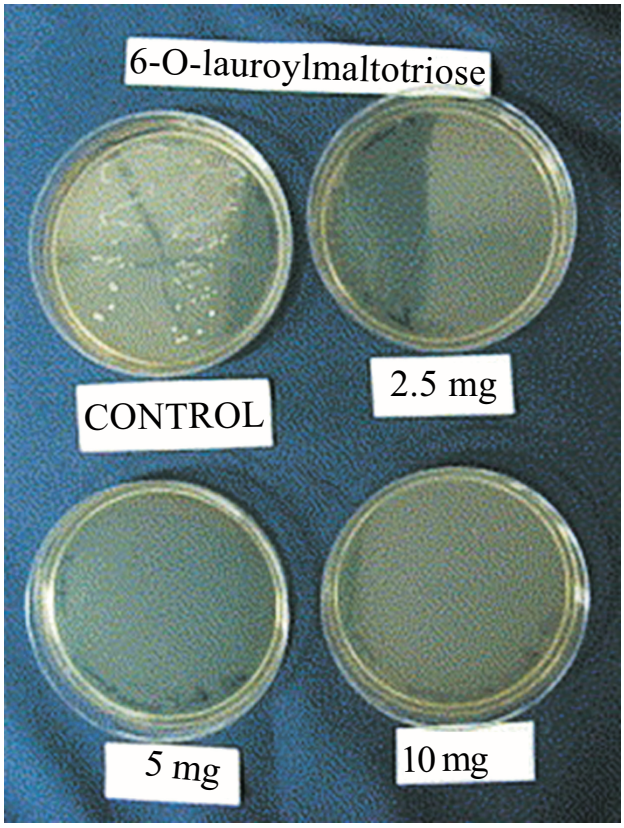

Fig. (4). Cell growth of *Staphylococcus sobrinus* in the presence of 100, 200 and 400 μg/mL of 6″-O-lau roylmaltotriose in agar plate. The values given in the figure correspond to 25 mL of medium [42].

Nobmann and others indicated that the lauryl alcohol ethers of methyl α -D-glucopyranoside and lauric acid esters of methyl α-D-mannopyranoside can prevent the growth of *L. monocytogenes* with a minimum inhibitory concentration (MIC) value of 0.04 mM. In addition, they revealed that the antimicrobial activity is highly related to the carbohydrate moiety of the derivatives and that the chemical bond type also had a significant impact on antimicrobial efficacy [44]. Japanese scientists found that galactose and fructose laurates showed the highest growth-inhibitory effect on *Streptococcus mutans*. In contrast, hexose laurates displayed no antibacterial activity, suggesting that the type of the hydroxyl group in carbohydrate moiety could affect the antibacterial activity [45].

In summary, sugar esters possess antimicrobial activity against many Gram-positive and Gram-negative microorganisms, the extent of which is highly associated with the type of sugar, number and type of fatty acid esterified along

with the degree of esterification. In addition, sugar esters can suppress the growth of yeasts.

Antitumor Activity

The antitumor (cytotoxic) activity of 6”-O-lauroylmaltotriose and 6”-O-palmit oylmaltotriose against two human cancer cell lines, Hep-G2 and HeLa was assessed. The latter sugar ester produced 50% inhibition (IC_{50}) values of 2.3 μM (1.7 μg/mL) for Hep-G2 and 3.6 μM (2.7 μg/mL) for HeLa cells, while the former had no significant inhibitory effect (Fig. **5**) [46]. Japanese researchers evaluated disaccharide esters, including 6,6'- and 4,4'-di-O-alkanoyl-α−,α-trehalose diesters and 6,6'-diamino-6,6'-dideoxy-N,N'-dialkanoyl- α−,α-trehaloses, n-dodecyl-β-D-maltoside, and 6,6'-diamide-trehaloses, to prevent release of the tumor necrosis factor-alpha (TNF-alpha), which is a proinflammatory cytokine playing an essential role in various pathological states. The results demonstrate that 6,6'-di-O-octanoyl-α−,α-trehalose and n-dodecyl-β-D-maltoside inhibited tumor cell growth on mouse skin initiated with DMBA (7, 12-dimethylbenzanthracene). The tumor growth in tumor-bearing mice during a 15 week treatment period was decreased from 60.0 to 13.3 by trehalose monooctanoate and to 46.7 when treated with n-dodecyl-β-D-maltoside. Moreover, the former sugar ester suppressed TNF-alpha gene expression [47]. Nishikawa *et al.* reported that sugar fatty acid ester with long fatty acid chains, for instance, stearate, palmitate, and myristate esters, have more significant anti-tumor effect than esters with short fatty acid chains such as laurate and caprylate esters. In addition, monoesters have a better performance on the inhibition of tumor cell growth than di-esters and tri-esters. Moreover, trehalose diesters with the carbon chain lengths from 8 to 12 also suppressed tumor necrosis [48].

Kato *et al.* found that monolaurin and sucrose monolaurate showed strong inhibition activity on Ascitic tumor cells of *Ehrlich carcinoma*. In contrast, sorbitan laurate and polyoxyethylene sorbitan monolaurate (Tween 20) did not possess antitumor activity. Furthermore, the sucrose esters of fatty acids and the propylene glycol ester of myristic acid were the most effective against the tumor tested. It is proposed that each lipid or sugar might have specific affinity to the tumor cells depending on the type of cell and subsequently attack the cell, resulting in the presence of antitumor activity [49].

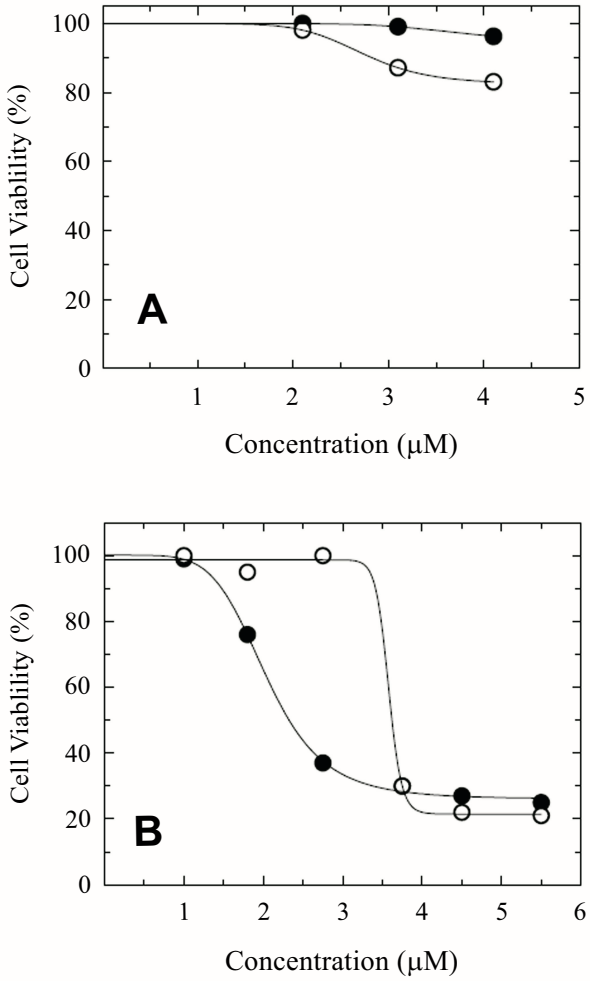

Fig. (5). Cell viability of several cellular models after exposure to maltotriose esters. (**A**) 6”-O-lauroy lmaltotriose and (**B**) 6”-O-palmitoylmaltotriose. Hep-G2 (●) and HeLa (○) cell lines were exposed to monoester 24 h, and cell viability was measured by the MTT test after a further 24 h treatment [46].

Anti-insect Activity

Several reports of anti-insect activity for sugar esters have been reported in the literature (Table **2**). In the early 1990s, sucrose esters were used as the primary insecticidal compounds to inhibit glandular trichomes [50] and green peach aphid [51]. The potent insecticidal activities of sugar fatty acid esters rendered them as a new class of natural insecticides that could be potentially exploited for commercial use in future. However, there is no information reported regarding the relationship between the chemical structures of sugar fatty acid esters and insecticidal activity. There are two hypotheses for the effectiveness of sugar esters as anti-insect agents. The first is that sugar esters function as surfactants that

dewax and remove the protective coatings of insects. The insect would suffer from dehydration and/or the microbial attack. The second hypothesis is that sugar esters interfere with the metabolism of the insect.

Table 2. Anti-insect activities of sucrose esters.

Sucrose Ester	Insect Name	Concentration (mg/ml)	Mortality rate (%)	Reference
Mixture [1]	Greenhouse whitefly, *Trialeurodes vaporariorum Westwood*	0.1	69.39 ± 3.22	[50]
	Sweetpotato whitefly, *Bemisia tabaci Gennadius*	0.1	94.92 ± 5.62	[50]
Mixture [2]	Green peach aphid, *Myzus persicae Sulzer*	0.1	~70	[51]
Sucrose octanoate[3]	Tobacco hornworm, *Manduca sexta Johannson*	2.4	41.7±10.1	[52]
	Tobacco aphid, *Myzus nicotianae Blackman*	2.4	98.5±1.1	[52]
	spotted spider mite, *Tetranychus urticae Koch*	2.4	100.5±0.0	[52]
Mixture [1]	Pear psylla, *Cacopsylla pyricola Foerster*	1.0	99.3±1.6	[53]

[1] Isolated from Leaf-surface extracts of *Nicotiana gossei* consisting mostly of *3,*6-di-O-acyl-6'-O-acetyl sucroses, and 3,6-di-O-acyl-1',6'-di-*O-acetyl sucrose*, with acyl groups consisting of 5-methylhexanoic and 5-methyheptanoic acidsl ; [2] isolated from exudate of type B trichomes from *Solanum berthaultii* leaflets, consisting mostly of 3',3,4,6-tetra-O-acyl sucroses, with acyl groups consisting of 2-methyl-propanoic (45%), 2-methylbutyric (28%), 8-methylnonanoic (23%), capric (2%), and lauric (2%) acids; [3] > 60% monoester.

Sucrose octanoate, sorbitol octanoate, sorbitol decanoate, sorbitol caproate, xylitol octanoate, xylitol decanoate and xylitol dodecanoate were synthesized and their insecticidal properties were evaluated against a range of arthropod pests. Dosage-mortality studies of sugar fatty acid esters at low concentrations of 80-160 ppm were conducted on pear psylla (*Cacopsylla pyricola Foerster*) for pear substrates, tobacco aphid (*Myzus nicotianae Blackman*) and tobacco hornworm (*Manduca sexta*) for tobacco substrates, and twospotted spider mite (*Tetranychus urticae Koch*) for apple substrates. The anti-insect activities of sugar esters were comparable to insecticidal soap (M-Pede, Dow AgroSciences L.L.C., San Diego, CA). The insecticidal activities of sugar fatty acid esters varied depending on their solubility in water and the emulsion stability. A direct relationship between the molecular structure of the sugar esters and anti-insect activity could not be determined. Sucrose octanoate with high content of monoester displayed the highest activity against the range of arthropod pests at low concentrations of 1200-2400 ppm. All of the sugar esters inhibited *T. urticae* at a low

concentrations, 400 ppm [52]. In another study, sucrose esters composed of C_6-C_{12} fatty acids exhibited desirable insecticidal properties against many soft-bodied arthropod pests. It was reported that sucrose octanoate has the highest activity against a range of arthropod species. The insecticidal activity of the synthetic sucrose octanoate was evaluated at a concentration of 4 and 8 mg/mL^{-1}, resulting in 72.5% of reduction in first-instar larvae of *Lymantria dispar* after 36 hours and an 80% decrease in *Aphis glycines* after being treated for 5 days [54]. The potent insecticidal activity of natural sucrose esters (sucrose octanoate and sorbitol octanoate) against persistent and damaging whiteflies has been demonstrated [52].

Chortyk and co-workers concluded that di- and tri-esters of sucrose and short (< C12) acyl chains were effective as insecticides to sweet potato whiteflies [55]. The cited work indicated that the eight hydroxyl groups of sucrose could theoretically be esterified with fatty acids, forming 8 different sucrose monoester, 28 diester and 56 triester isomers. In fact, it is very difficult to compare the performances of pure monoesters and diesters on insecticidal activities [55]. In addition, it was revealed that the insecticidal performances of sugar esters with the different chain lengths were highly associated with the insect species. Furthermore, sugar esters were successfully used as anti-insect agents against pear psylla [53], tobacco hornworms [56], and two-spotted spider mites [50]. Therefore, since the sugar fatty acid esters are nontoxic to humans and animals, and are also biodegradable, they appear to be good insecticide candidates.

Bioactive Properties of Enzymatically Prepared *versus* Commercially Available Sugar Esters

We compared the antimicrobial and antitumor properties of technical grade sucrose and fructose oleates (> 90% pure) synthesized *via* biocatalysis to a commercially available sucrose laurate [57]. Antimicrobial activity was measured by protocol described in the literature, which involved measurement of turbidity (absorbance at 600 nm) [46]. For better solubility and dispersion of sugar esters, 0.1% Tween-80 was added into the growth media. It was observed that 0.1% Tween-80 by itself had no marked effect on cell growth when compared to controls (data not shown). For evaluating the antitumor activities of sugar fatty acid esters, viability and cell density were determined by the trypan blue dye exclusion test [58]. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was conducted based on the well-established method in literature [59] for the evaluation of the *Ehrlich ascites* tumor cell viability.

Antimicrobial and Antitumor Activity

As represented in Fig. (**6**), all sugar esters facilitated growth inhibition of gram-positive foodborne pathogens [57]. Fructose oleate displayed higher antimicrobial activity than those of sucrose oleate and sucrose laurate against *L. plantarum*, *Pediococcus pentosaceus*, and *B. subtilis*. However, the sucrose esters had a stronger impact on inhibiting the growth of *S. aureus* than fructose oleate. In addition, all sugar esters prevented the growth of *P. pentosaceus* and *B. subtilis*. In contrast, when the concentration >0.5 mg/ml, no significant improvement on microbial inhibition of *L. plantarum* was observed.

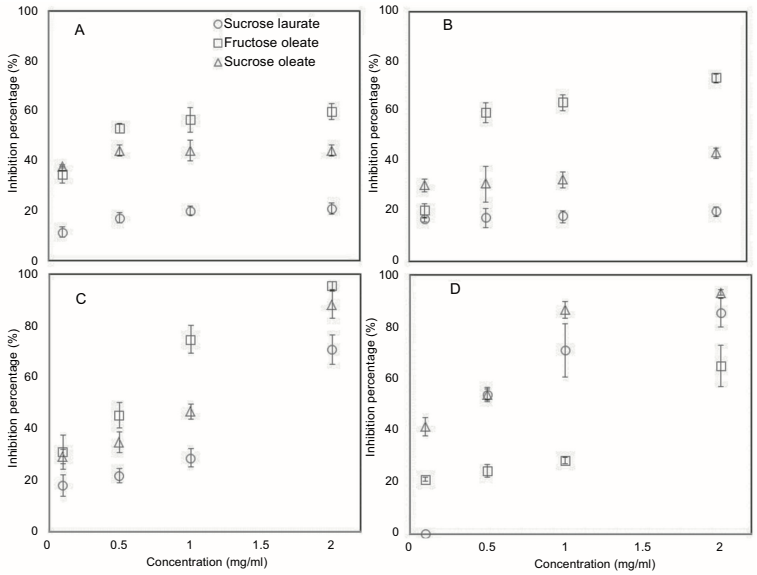

Fig. (6). The antimicrobial properties of sugar fatty acid esters against (**A**): *Listeria plantarum*, (**B**) *Pediococcus Pentosaceus*; (**C**) *Bacillus subtilis*, and (**D**) *Staphylococcus aureus* [57]. Reproduced with permission from MDPI AG.

As plotted in Fig. (**7**), all of the sugar fatty acid esters can prevent the growth of *Ehrlich ascites* tumor cells in a dose-dependent manner [57]. Compared to the fructose oleate, sucrose esters displayed superior antitumor activities. Of note, there was no significant difference between the enzymatically prepared sucrose ester and the commercial sucrose laurate product at the identical concentration, even though the former was not subjected to downstream purification. In addition, the antitumor activities of the sugar esters increased with the increase of the concentration for all three esters.

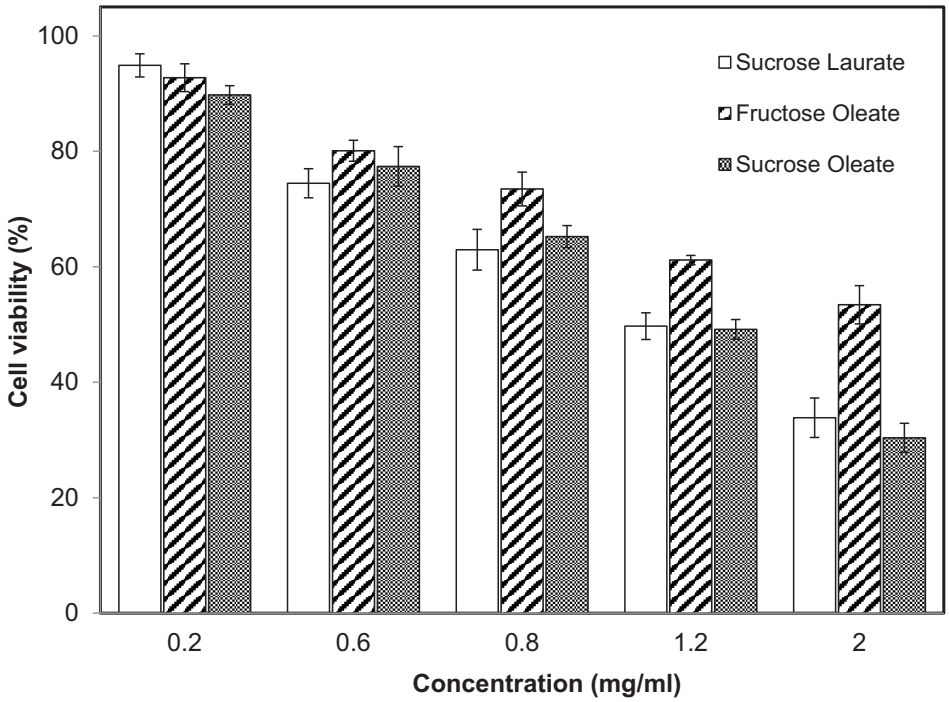

Fig. (7). Antitumor activity of sugar esters [57]. Reproduced with permission from MDPI AG.

CONCLUSION

Sugar-fatty acid esters are an important class of non-ionic, synthetic, environment friendly biobased surfactants or emulsifiers used in the pharmaceutical, cosmetic and food industry. Sugar esters possess a broad range of bioactive activities, including antimicrobial, antitumor and anti-insect activities. Their bioactive properties are associated with the type of sugar, and the number and type of fatty acid esterified to the sugar. In addition, the antimicrobial and antitumor activity of sugar esters prepared using lipases were evaluated, and determined to be comparable to a commercial sucrose laurate product that was prepared chemically. Hence, sugar esters are promising bioactive compounds for a wide range of applications.

CONFLICT OF INTEREST

The authors confirm that the authors have no conflict of interest to declare for this publication.

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