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CHARACTERIZATION AND APPLICATION OF SUCROESTERS AS ANTIMICROBIAL AGENTS

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ABSTRACT

Sucrose esters are nontoxic and biodegradable compounds with a large scale application in food industry as emulsifiers and solubilisator in cosmetics, agriculture and pharmaceuticals. Sucrose esters composed of C6 – C12 fatty acids have desirable insecticidal properties against many soft-bodied arthropod pests. Sucrose esters with saturated fatty acids (C10 to C18) and 10-unsaturated undecylenic acid have been synthesized. The obtained sucrose esters were characterized by FT-IR spectroscopy. The antimicrobial activity of some esters of sucrose has been investigated. Sucrolaurate was characterized with well-pronounced antimicrobial activity against fungi *Aspergillus* and *Penicillium*, Gram-positive bacteria: *Bacillus subtilis* and *Staphylococcus aureus*. Unsaturated undecylenic ester of sucrose showed also very well antimicrobial activity against *Candida albicans,* exhibited inhibition activity against Gram-positive bacteria: *Bacillus subtilis* and *Bacillus cereus,* and Gram-negative *E. coli*, as also inhibited growth of *Pseudomonas aeruginose*, against which undecylenic acid was inactive. On the base of this investigation sucroundecylenic and sucrolaurate ester could be used as potential antimicrobial agent for protecting plants and foods against some microorganisms.

Key words: *sucroesters, FT-IR, antimicrobial activity*

INTRODUCTION

Sugar esters synthesized from renewable sources such as fatty acids and sucrose constitute an interesting group of nonionic surfactants. They have broad application in food industry, in cosmetics as detergents, oral-care products, and medical supplies, and also in agriculture as insecticides [1, 7, 14]. Sucrose esters have very wide HLB, excellent physical properties and surface activity. Sucroesters were investigated to possessed emulsion stabilization activity, as sucrose laurate and sucrose palmitate have the capacity to stabilized O/W emulsions [10].

Apart from their emulsifing properties, they are completely biodegradable, harmless to the environment, non-toxic, skin-compatible, odorless and tasteless [1,7]. When they are ingested, they are hydrolyzed to form normal food products [12]. The use of these compounds in medicine and food may also due also to their antimicrobial and antitumor activity. In general Gram-positive were more susceptible than Gram-negative bacteria. These esters reorganize the cellular membrane altering its permeability [2, 6].

The preparation of esters of sucrose and saturated or slightly unsaturated fatty acids or mixtures of higher polyenolic acids has been published. It is known that with an increase in the degree of unsaturation of the hydrocarbon moiety the solubility of esters of sucrose rises, which is important for medicinal purpose. These compounds are more active because of the high biological activity of the free polyunsaturated fatty acid [3]. For the undecylenoyl sugar esters (1`-O-(10-undecylenoyl) sucrose) obtained by enzyme catalyzed reactions have been reported to possess superior surface tension activity and biodegradability, and can be utilized as anti-fungal, anti-bacterial and anti-viral activities in cosmetic, medicine and food [13].

Sucrose esters are naturally produced in the glandular secretions of leaf hairs of Nicotiana plants [7, 14]. For industrial scale needs they can be synthesized using either chemical or biological catalysts [2, 7, 8]. The chemical synthesis is usually base-catalyzed with alkali metals (sodium or potassium) hydroxides, carbonates and alkoxies at high temperature. Esterification of sucrose can be performed by reacting with acyl chlorides or anhydrides but more promising are chemical or biochemical methods based on ester interchange of methyl, cyanomethyl or ethyl esters of higher fatty acid esters with sucrose or their acetates under atmospheric, reduced pressure or in an inert gas atmosphere (nitrogen). Usually esterification process is carried out in solvent DMF and DMSO as reaction media. [6, 8, 15].

Enzyme-catalyzed processes for esterification of sucrose are more selective than chemical and conducted in mild conditions [2], but the purified enzymes is needed.

The aims of our current research are chemical synthesis and characterization of sucrose esters with different length of fatty acid chain (C10 to C16) and their application as antimicrobial agents. One of the main tasks of our study is to investigate the effect of obtained sucroesters on the growth of several microorganisms involved in food spoilage and poisoning (*Bacillus* sp.) and in a diversity of disease (*E. coli*, *St. aureus, Candida albicans*).

MATERIAL AND METHODS

Esterification process of sucrose with fatty acid methyl esters (C10 to C18) was conducted in molar ratio 2:1, in a dry two-neck round bottom flask with a thermometer and a reflux on a heating magnetic stirrer. The esterification process was carried out with 0.3 eq. NaOMe used as catalyst for 3 hours at 70°C under nitrogen atmosphere. The reaction mixture was neutralized and then crystalized with water. For synthesis of sucroundecylenic ester the reaction mixture were dissolved in 10% NaCl and washed twice with n-BuOH to obtained the ester. The solvent was removed by vacuum evaporation yielded as yellow-colored oil, which was recrystallized with ethanol-water (1:1/v/v).

The resulting sucrose esters were characterized by melting point and FT-IR spectroscopy. Melting point of sucrose esters were measured on a melting point apparatus BŰCHI 510 in capillary glass tube. The FT-IR was recorded in KBr pellets on a Nicolet FT-IR Avatar Nicolet Termo Science spectrometer in the range 4.000 – 400 cm^{-1} and absorption was reported in wavenumbers (cm^{-1}).

The antimicrobial effect of the synthesized sucrose laurate against bacteria *Bacillus subtilis, Staphylococcus aureus, Listeria monocytogenes, E.coli* and fungi *Aspergillus* and *Penicillium* was evaluated by agar diffusion method. The number of mold spores in 1 ml spore suspension was determined by counting in a chamber of Thoma [10].

For determination of antibacterial and antifungal activities of other sucroesters micro dilution method was employed as recommended by National Committee for Clinical Laboratory Standards [11] with some modifications. Microorganisms strains tested in this study were bacteria, namely *Escherichia coli* NBIMCC 858, *Bacillus subtilis* NBIMCC 1711, *Bacillus cereus* NBIMCC 1085, *Pseudomonas aeruginosa* NBIMCC 3590, *Pseudomanas fluorescens* NBIMCC 1442 and strain of fungus *Candida albicans* NBIMCC 72. The analysis of antimicrobial activity of tested substances were performed using different liquid media according of the microorganism: for *E. coli* – Luria Bertani Broth Miller (Himedia), for *Candia albicans* – GPM (Merck), and for Ps. *aeruginosa,Ps. fluorescens, B. subtilis*, *B. cereus*, Nutrient broth (Merck). Tested esters and 10-undecylenic acid were dissolved in 2% DMSO. The inoculated plates were incubated for 24 hours at 35ºC and at 25ºC for bacterial and fungi, respectively. After incubation the absorbance was read at 620 nm against controls (2% DMSO). The percent inhibition was calculated by equation (1). Each test was performed in triplicate and the results were presented as mean±SD.

$$Inhibtion\,(\%) = \frac{A_{620}(control) - A_{620}(sample)}{A_{620}(kontrol)} \times 100 \qquad (1)$$

RESULTS

A variety of fatty acid esters of sucrose were synthesized by transesterification reaction in absence of solvent only with presence of catalyst NaOMe (Figure 1). All of the obtained sucrose esters-caprinate, laurate, palmitate and stearate present white powder without smell with mealting points 98 – 102 °C, 85 – 87°C, 75°C, 62°C respectively. Sucroundecylenate was in liquid state at room temperature and a yellow-solid at 7°C.

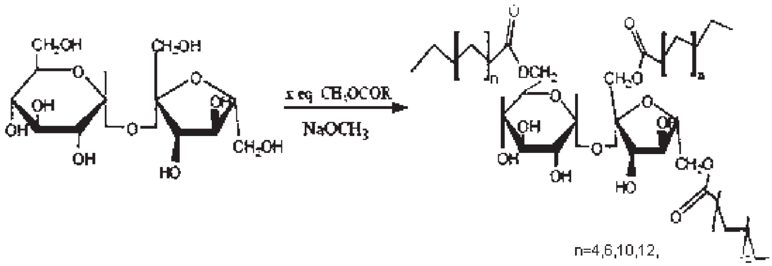

Figure 1. *Esterification of sucrose with fatty acid methyl esters (FAME)*

The obtained IR spectrum of sucrose esters indicated the decrease in width of the characteristic band for free hydroxyl group (O ─ H stretch of free hydroxyl in sucrose) at 3330 cm^{-1}. The new band around 1742 cm^{-1} characteristic of carbonyl ester functions, and the intensification on the CH_2 signals around 2920 cm^{-1} pointed out the presence of alkyl group was observed. The stretch at around 1745 cm^{-1} (C ─ O stretch of ester) meant that the esterification of sucrose has been successful. Apparently, 1728 and 995 cm^{-1} (glycoside bond stretch of sucrose) showed that the products were sucrose esters.

In FT-IR spectrum of sucroundecylenate can be observed that the bands at 3300cm^{-1} diminished, because of esterification of OH groups and new stretch vibrations at 3080 cm^{-1}, due to end C = C bonds and carbonyl ester stretch vibration at 1745 cm^{-1} appeared (Figure 2).

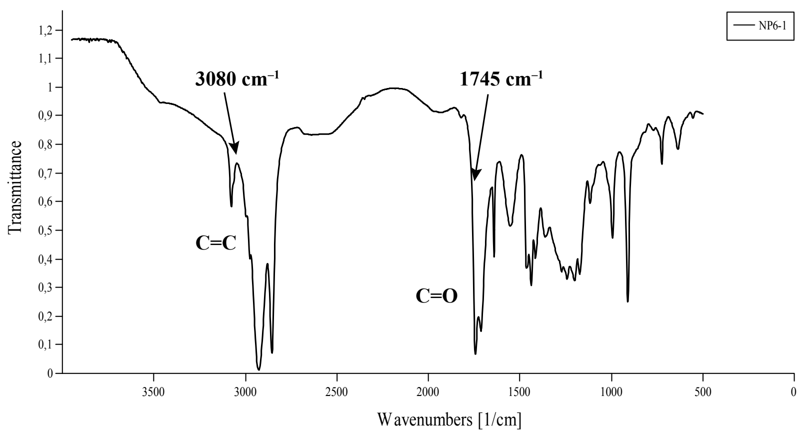

Figure 2. *FT-IR spectrum of the synthetic undecylenic acid sugar ester*

Sucrolaurate with concentration 0,1% inhibited the growth of bacteria *Listeria monocytogenes*, *B. subtilis* and *St. aureus*, and fungi *Penicillium* sp. *Aspergillus* sp., but was inactive to Gram-negative *E. coli.* Our results for inactivity of sucrose laurate against *E.coli* were in accordance with the reports of Kabara and Hatchcox [4, 5]. The concentration was used to test the ester, but for further studies, the high concentration could be applied for better results of its antimicrobial activity.

Table 1. *Antimicrobial activity of sucrolaurinate (0.1% water solution)*

	Microorganisms					
Cfu, ml	*B. subtilis*	*E. coli*	*St. aureus*	*Listeria monocytogenes*	*Penicillium sp.*	*Aspergillus sp.*
	$1,3.10^{9}$	$1,8.10^{8}$	$1,2.10^{10}$	$9,2.10^{10}$	2.10^{5}	1.10^{5}
D, mm	15*	–	11	17	8**	9**

*Diameter of zone with inhibition growth, mm, *after 24 h for bactiria,*
*** after 48 h for fungi*

Tested sucrose, sucropalmitate and sucrostearate in concentration 1 mg/mL were inactive against *Candida albicans* and Gram-negative bacteria. However, in this concentration sucroundecylenic ester showed very good antimicrobial properties. Sucroundecylenic fatty acid ester inhibited not only the growth of Gram-possitive bacteria (*B. cereus* and *B. subtilis*), but also act as antimicrobial agent against Gram-negative (*E. coli* and *Ps. aerogenosa*). This sucroester possesses well-pronounced antifungal activity. Sucroundecylenic ester shows stronger inhibition activity against *Candida albicans* than undecylenic acid (Table 2).

Table 2. *Screening of antimicrobial properties of sucroundecylenate and undecylenic acid.*

Compound/ Microorganism	Inhibition*, %					
	Bacillus cereus	*Bacillus subtilis*	*Ps. fluorescences*	*Ps. aeruginosa*	*E coli*	*Candida albicans*
Sucroundecylenate	75,1 ± 0,3	59,2 ± 0,9	–	12,5 ± 4,9	17 ± 4,9	86,6 ± 1,9
Undecylenic acid	62,8 ± 3,1	58,9 ± 3,6	–	–	15 ± 2,7	68,1 ± 3,9

**Concentration of all tested compounds was 1 mg/mL*

We observed that undecylenic acid at concentration 1 mg/mL not inhibited growth of Gram negative *Ps. aeruginosa.* This fact was in the accordance with statement of Kabara [5]. The highest antimicrobial activity sucroundecylenate showed against *Candida albicans* 86%. The chemically synthesized by us sucroundecylenic ester is new alternative as active agent with well-pronounced biological activity. It could be applied as new antimicrobial agent in food, cosmetics or medicine.

CONCLUSION

Sucrose esters with saturated fatty acids (C10 to C18) and 10-unsaturated undecylenic acid C11 were synthesized and characterized by FT-IR. Antimicrobial activity of sucrolaurinate and sucroundecylenate ester were investigated. The antimicrobial activity of sucrolaurinate is impressive, as this sucroester inhibited growth of Gram-positive, bacteria (*B. subtilis*), *St. aureus*, *Listeria monocytogenes* and fungi *Aspergillus* and *Penicillum*. The chemically synthesized sucroundecylenic ester is more active than undecylenic acid. In comparison between them this unsaturated sucrose ester exhibited better antimicrobial activity against Gram-positive bacteria, inhibition activity against yeast *Candida albicans* and Gram-negative *E. coli*, even it also inhibits growth of *Pseudomonas aeruginosa*, against which undecylenic acid is inactive. Sucroundecylenic ester is a proper candidate as a novel antimicrobial agent for future application in plants prevention, cosmetics and medicine for curing disease.

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