Ultrasound-Assisted Synthesis of Sucrose and Fructooligosaccharides Esters as Bio-Plasticizers

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Received October 30, 2015; Accepted January 04, 2016

ABSTRACT: This article presents the results obtained by the ultrasound-assisted synthesis of sucrose and fructooligosaccharides (FOS) esters with fatty acids (palmitic and 10-undecylenic), the identification of the products and the study of their possible application as plasticizers in plastics processing. Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectroscopy were used to identify the synthesized carbohydrate esters. The plasticizing effect of the sucrose palmitate applied in polyvinyl chloride (PVC) was evaluated by the glass transition temperatures; the decrease of the T_g with the increase of the ester content could be accepted as confirmation of the esters' plasticizing effect.

The antimicrobial activity of the synthesized sucrose and FOS esters was evaluated; the results allowed us to conclude that the 10-undecynoyl esters of sucrose and fructooligosaccharides could be successfully applied as potential antimicrobial agents, as well. The sucrose palmitate plasticizing effect was evaluated by determining the glass transition temperatures of thin polyvinyl chloride films on the basis of differential scanning calorimetry (DSC) and changes of mechanical properties. The results showed a decrease of the glass transition temperature with the increase of the ester content, which confirmed the plasticizing effect of the obtained esters.

KEYWORDS: Sucrose esters, fructooligosaccharides esters, ultrasound-assisted, transesterification, "green" plasticizer, antimicrobial activity

1 INTRODUCTION

Plasticizers are substances that reduce the hardness and increase the plastic properties (elasticity and flexibility) of polymers. Many of the commonly used plasticizers are with limited use in practice due to environmental problems or their toxic effect on people; for example, phthalates, usually used as plasticizers for PVC, are classified as toxic to the human reproductive system. Studies in this area have been aimed at replacing these plasticizers with harmless substances or "green chemistry" products [1–3]. Carbohydrate esters and polyol esters were successfully applied as plasticizers to cellulose esters, PVC and polylactide [4]. Polyol-based ester plasticizers obtained from wood flour were successfully synthesized and experimentally applied as plasticizers to polylactide [5]. Recently synthesized renewable oligoesterisosorbide plasticizers also demonstrate good plasticizing efficiency for PVC [6]. Three glucose hexanoate esters and sucrooctaacetate were synthesized and studied by Yin and coauthors; the results indicated the large potential of the obtained esters as "green" PVC plasticizers [7].

The conventional Fischer-Speier esterification is based on the high temperature esterification of fatty acids with alcohols in the presence of inorganic catalysts. The processes are nonselective, consume large amounts of energy and in some cases are not environmentally friendly. The current situation requires the development of efficient methods in terms of energy consumption and environmental safety. According to Huang *et al.*, transesterification of sucrose with fatty acids can be successfully accelerated by ultrasonic irradiation. They reported good yield (above 73%) of sucrose monoesters with n-acyl chains of 8, 12, 14, and 16 carbon atoms with a high percentage of obtained monoesters [8]. However, the ultrasound-assisted synthesis of sucrose and FOS esters with 10-undecylenic acid are still not well studied and there is a lack of scientific information about it.

The aim of this study was to synthesize sucrose and fructooligosaccharides esters by ultrasound-assisted transesterification, to identify and characterize the obtained products, and to study their possible application as "green" plasticizer and antimicrobial agents.

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DOI: 10.7569/JRM.2015.634125

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Synthesis of Esters

The following reagents were used to synthesize the studied esters: n-butanol, methanol, n-hexane, dimethyl sulfoxide (DMSO) (Panreac); palmitic acid (Reachim); anhydrous Na_2SO_4 (Riedel-de Haën); K_2CO_3, NaCl, acetone, HCl, ethyl acetate (Chimtex); tetrahydrofuran (Panreac); fructooligosaccharides Frutafit CLR (degree of polymerization DP = 7–9, Rosendaal). Sodium methoxide (CH_3ONa) was prepared as previously described by Rogge and Stevens [9].

2.1.2 Sample Preparation

Amorphous polyvinylchloride (PVC) (EMKA Ltd.) was used to study the possible application of synthesized esters—in particular, sucrose palmitate as a plasticizer. The PVC was used as obtained by the supplier. The samples were prepared by dissolving the polymer in THF at 40 °C for 2 h.

2.2 Methods

2.2.1 Synthesis of Esters

The ultrasound-assisted synthesis was conducted in an ultrasonic bath (generator power 100 W, frequency 44 kHz) according to the methodologies presented below. The syntheses were performed in a heterogeneous medium, where the cavitation bubbles that were formed in the solution of alcohol and methyl esters of fatty acid esters stroke suspended sucrose or inulin molecules.

***Synthesis of sucrose esters*:** In an Erlenmeyer flask of 100 cm^3 were placed 50 cm^3 methanol and methyl palmitate and sucrose in a molar ratio of 1:1. Then 0,5 mol catalyst K_2CO_3 was added. The mixture was sonicated at room temperature for 2 h. After the end of the reaction the solvent was distilled off. The residue was dissolved in 25% NaCl/n-butanol 1:1 and the mixture was separated by liquid-liquid extraction. Three times extraction with 20 ml n-butanol was performed. The combined extracts were dried with anhydrous Na_2SO_4 and the solvent was distilled off. The reaction conditions such as temperature, molar ratio "ester/sucrose," type and amount of the catalyst, were defined in our previous studies [10]. Sucrose-10-undecylenate were synthesized with ultrasound irradiation in DMSO, molar ratio 1:3 (sucrose: methyl-10-undecylenate), 0,3 eq CH_3ONa as catalyst was previously described by us [11, 12].

***Synthesis of fructooligosaccharides esters*:** The calculated amount of Frutafit CLR was weighed in a three-neck round-bottom flask, then connected to a reflux and placed in the ultrasonic bath. CH_3ONa (0,3 eq. based on anhydrous fructose equivalents) units was added to FOS and the temperature was raised to 70 °C. After 10 min sonification, 0,1 equiv methyl palmitate (methyl 10-undecylenate) was added slowly. The sample was sonicated at 70 °C for 60 min under nitrogen atmosphere. After that the reaction mixture was allowed to stay at room temperature for 12 h [13]. For the fructooligosaccharide palmitate ester the following isolation procedure was applied. The reaction mixture was neutralized with 0,1 N HCl to pH 6–7, then poured into 300 cm^3 acetone under vigorous stirring. The obtained FOS esters were allowed to cool at 4 °C, then were filtered under vacuum and washed with acetone once again. The resultant white powder was dried at 50 °C under vacuum. To obtain FOS 10-undecylenate ester the reaction mixture was pretreated as in the above-mentioned procedure for sucrose esters.

2.2.2 Identification of Esters

In order to exclude possible changes in the chemical structure due to the ultrasound effect, Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy (^1H and ^{13}C NMR) were applied to identify the obtained products.

***Identification by FTIR spectroscopy*:** FTIR spectra of the synthesized esters were recorded on a Nicolet Avatar spectrometer (Thermo Scientific, USA, ZnSe crystal, resolution 4 cm^{-1}), on KBr pellets, in the frequency range of 4000–500 cm^{-1}.

***Identification by ^1H and ^{13}C NMR spectroscopy*:** The ^1H and ^{13}C NMR spectra were recorded on a Bruker spectrometer at a frequency of 500 MHz in $CDCl_3$ (sucrose palmitate) and 151 MHz in CD_3OD (FOS palmitate) with a tetramethylsilane (TMS) as standard.

2.2.3 Study of Plasticizing and Antimicrobial Effect

2.2.3.1 Evaluation of the Plasticizing Effect of Esters

To study the possible application of the sucrose palmitate as polymer plasticizer, thin PVC films (thickness 0.22–0.32 mm) containing different amounts of ester were used. The plasticizing effect was evaluated on the basis of the glass transition temperature (T_g) of the samples, determined by differential scanning calorimetry (DSC) [14].

***Samples preparation*:** The PVC films were prepared with 0, 10, 20, 40 and 60 wt% of sucrose palmitate as a plasticizer. The specified amount of PVC was dissolved in 100 cm^3 THF in the water bath at 40 °C

until the complete dissolution of the polymer. Then the above-mentioned amount of sucrose palmitate was added and the mixture was stirred by a magnetic stirrer for 2 h until complete homogenization. The solutions were poured into glass Petri dishes and dried at room temperature in a vacuum oven for 7 days.

***Differential scanning calorimetry*:** The thermal properties of the PVC films were studied by a DSC 204 F1 Phoenix (NETZSCH Gerätebau GmbH) calorimeter using Al pans (2,5–4,2 g of sample), at heating/cooling rate of 10 K/min, in the following sequence: heating from 20 to 200 °C (first scan); cooling in liquid nitrogen from 200 to −50 °C; second heating from −50 to 200 °C (second scan). The measurements were carried out in argon at a flow rate of 20 cm^3/min. The value of T_g for each sample was determined in the second scan as the inflection point on the thermogram by Proteus software.

***Tensile testing*:** Tensile testing of all films was performed by a Lloyd LS1 apparatus (Lloyd Instruments, AMETEK, Inc.) equipped with pneumatic grips and NEXYGENPlus materials testing software. The crosshead speed was 100 mm/min. Nine to twelve rectangular specimens of size 5 × 100 mm and thickness 0,22–0,33 mm were tested and the average value was calculated for each PVC film.

***Evaluation of the antimicrobial activity*:** The antimicrobial activity of the sucrose and fructooligosaccharides palmitate and 10-undecylenate esters was determined by the microdilution method [11] against microorganisms *Escherichia coli* NBIMCC 858, *Bacillus subtilis* NBIMCC 1711, *Bacillus cereus* NBIMCC 1085, *Pseudomonas aeruginosa* NBIMCC 3590, *Pseudomonas fluorescens* NBIMCC 1442 and a strain of fungus *Candida albicans* NBIMCC 72. The tested compounds were dissolved in 2% DMSO. The inoculated plates were incubated for 24 hours at 35 °C and at 25 °C for bacteria and fungi, respectively. The absorbance was read at 620 nm against control (2% DMSO).

3 RESULTS AND DISCUSSION

3.1 Identification of the Synthesized Esters by FTIR

The successful ultrasound-assisted synthesis of carbohydrate esters was proved by the results from FTIR (Figure 1). The obtained IR spectra of sucrose and fructooligosaccharides (palmitate and 10-undecylenate) esters indicated a decrease in the width of the characteristic band for the free hydroxyl group (O–H stretch of free hydroxyl) at 3330 cm^{-1}. A new band around 1742 cm^{-1}, characteristic of carbonyl ester strechings, was observed. The intensification of the CH_2 signals around 2920 cm^{-1} indicated the presence of an alkyl group. The stretch at around 1745 cm^{-1} (C–O stretch of ester) meant that the esterification of sucrose and fructooligosaccharides was successfully performed. Apparently, the bands at 1728 and 995 cm^{-1} (glycoside bond stretch) confirmed that the obtained products were sucrose and fructooligosaccharides esters. In the FTIR spectra of sucrose and fructooligosaccharides 10-undecylenate ester the bands at 3300 cm^{-1} diminished because of the esterification of OH groups. New stretch vibrations at 3080 cm^{-1} due to terminal H-C= bonds and carbonyl ester stretch vibration at 1745 cm^{-1} appeared. The stability of the double bond was observed due to the presence of C=C stretches at 1643 cm^{-1}, and =C-H bends at 917 cm^{-1} (Figure 1b).

3.2 Identification of the Esters by ^1H and ^{13}C NMR Spectroscopy

In the ^1H NMR spectra of the sucrose palmitate the characteristic chemical shifts for carbohydrates in the range of 3,39 ~ 5,38 ppm (protons of glucose part) and 3,58 ~ 4,39 ppm (protons of fructose residue) were observed. The methyl group of palmitic acid was shown as a triplet at 0,87 ~ 0,89 ppm, while the methylene groups were in the 1,17 ~ 2,35 ppm range. In the ^{13}C NMR spectrum the carbonyl carbon atom band was observed at 174.89 ~ 175.12 ppm, and the carbon atoms of the carbohydrate moiety were found in the range of 61,32 ~ 103,88 ppm. The data obtained by NMR confirmed that the result of the ultrasound-assisted transesterification process was a mixture of sucrose monopalmitate, which was in good agreement with the results reported by Huang *et al.* [8]. The possible structure is presented in Figure 2a.

The esterification of FOS was also confirmed by ^1H and ^{13}C NMR spectroscopy as FOS palmitate was dissolved in CD_3OD. The following shifts were observed on the ^{13}C NMR spectrum of the FOS palmitate: δ 179,1 (C=O), 103,7 (Cquat on 2 position); 82,2 (CH on 5 position); 78 (CH on position 3); 74,6 (CH on position 4); 62,2 (CH_2 on position 1); 33,7 (CH_2), 31,8 (CH_2); 29,49; 29,39; 29,36; 29,28; 29,16; 29,07; 28,96; 28,78; 28,76; 26,41; 24,63; 22,88; 22,33; 13,16; 13,05 (CH_3). In comparison with the NMR spectra of fructooligosaccharides, new shifts typical for CH_2 from the palmitoyl chain appeared below 60,1 ppm. The obtained results were in accordance with the spectra of the inulin esters synthesized with CH_3ONa in DMF and DMSO [15]. The presence of shifts at 178–174 ppm (C=O) confirmed the successful esterification of the fructooligosaccharides. These data were in agreement with the reported shifts at 175,5–175,1 ppm (C=O) for 6-O-palmitoylsucrose and 6′-O-palmitoylsucrose synthesized by ultrasonic irradiation [8], and polyvinyl inulin adipate esters, as well [9]. The possible structure is presented in Figure 2b.

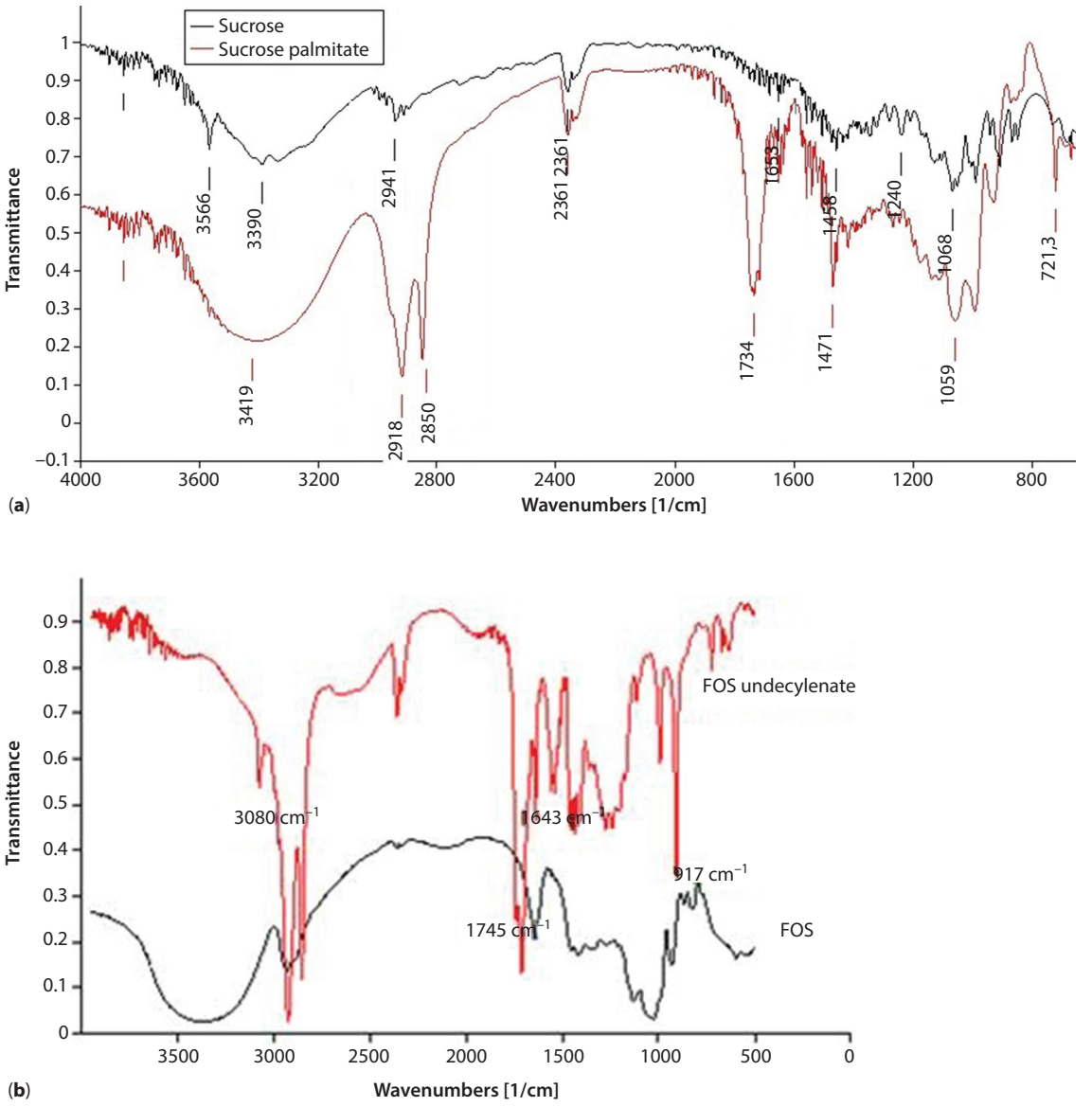

Figure 1 FTIR spectra of sucrose palmitate (**a**) and fructooligosaccharides 10-undecylenate (**b**) esters.

3.3 Evaluation of the Plasticizing Effect

3.3.1 Thermal Properties

According to Wypych [14], the mechanism of plasticizing is based on the increased mobility of the macromolecules and supramolecular structures due to the distribution of the plasticizer on the macromolecules' surface. A parameter related to the mobility of the structural elements is the glass transition temperatures, T_g, of the polymer—therefore it could be used to evaluate the plasticizing effect.

The results obtained by DSC registered a single T_g on the thermogram of the studied samples. The value of T_g depended on the content of sucrose palmitate; with the increasing of the ester's content the T_g shifts toward the lower temperatures (Figure 3). Upon increasing the content of the sucrose palmitate a well-expressed reduction in the T_g was observed, while at 40 and 60% the difference was minimal.

3.3.2 Mechanical Properties

Blending the PVC with a plasticizer should lower the stress and modulus of elasticity (E-modulus). Besides the glass transition temperature, the decreasing of the modulus also proves the plasticizing effect of the esters, as this increases the flexibility of the PVC. A graphical interpretation of the results obtained for the modulus and the stress at break of the studied PVC depending on the ester content was presented in

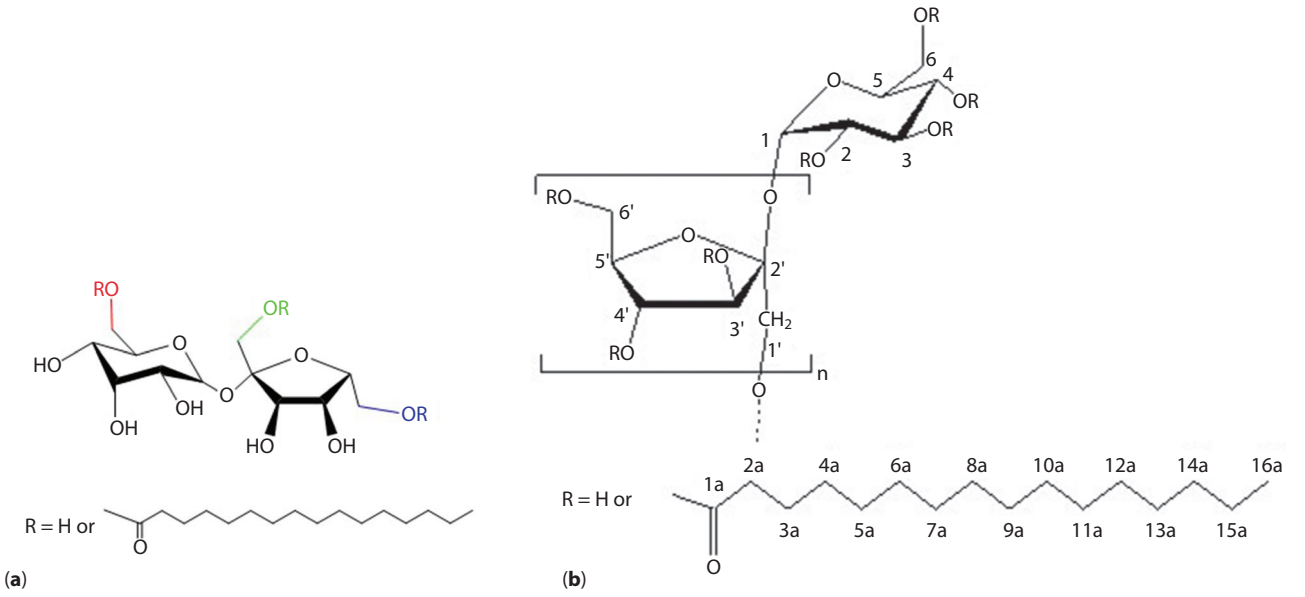

Figure 2 Possible structure of the synthesized esters: (**a**) sucrose palmitate, (**b**) FOS palmitate esters.

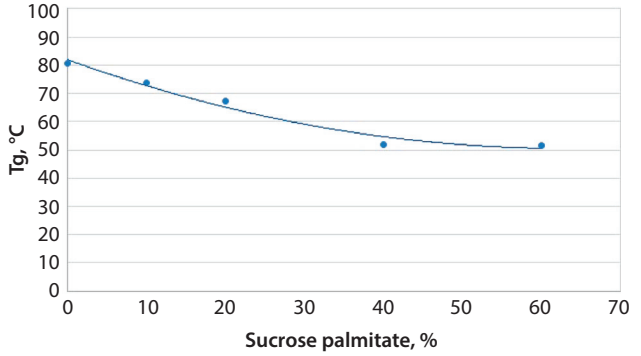

Figure 3 Changing of glass transition temperature, T_g, depending on the sucrose palmitate content.

Figure 4. The results indicated that both modulus of elasticity and stress at break decreased with increasing the amount of the plasticizer (Figure 4).

3.4 Antibacterial and Antifungal Activities

The results from antimicrobial activity tests was summarized in Table 1.

The saturated palmitate esters of sucrose and fructooligosaccharides did not show any antimicrobial activity. This finding was in accordance with the previous reports on the sucrose and glycerol esters [16, 17]. From the results obtained, 10-undecylenic acid at a concentration of 1 mg/cm^3 did not inhibit growth of Gram-negative *Ps. aeruginosa*. This fact was in accordance with the statement of Kabara and Marshall [18]. The methyl-10-undecylenate possessed lower antimicrobial activity than the 10-undecylenic acid and its carbohydrate esters. In addition, the inactivity of the methyl esters of most fatty acids against some microbial strains was previously reported by Kabara and Marshall [18]. However, the unsaturated 10-undecylenic esters of the sucrose and fructooligosaccharides in a concentration of 1 mg/cm^3 demonstrated significant antimicrobial activity against *Candida albicans*, as the percentage of inhibition was 94 and 84%, respectively. These sucrose esters were more active than the 10-undecylenic acid well-known for its strong antibacterial and antifungal activity [19]. Moreover, in this study the antimicrobial activity of the fructooligosaccharides esters was demonstrated for the first time. It was clearly shown that the sucrose and fructooligosaccharides 10-undecylenate ester inhibited the growth of Gram-positive bacteria *Bacillus subtilis* and *Bacillus cereus* and Gram-negative *E. coli*. Only the sucrose-10-undecylenic ester inhibited the growth of *Pseudomonas aeruginosa*, against which 10-undecylenic acid was inactive.

The synthesized unsaturated esters of the sucrose and fructooligosaccharides could be applied as potential antimicrobial agents. They could be successfully used not only in the preservation of food, but also in medicine and cosmetics for preparation of antifungal ointment for curing diseases caused by *Mycobacterium smegmatis* and *Candida albicans*. Therefore, besides being used as plasticizers in plastics processing they could also be potentially used in packaging as an additive, providing the surface layer with antibacterial and antifungal properties.

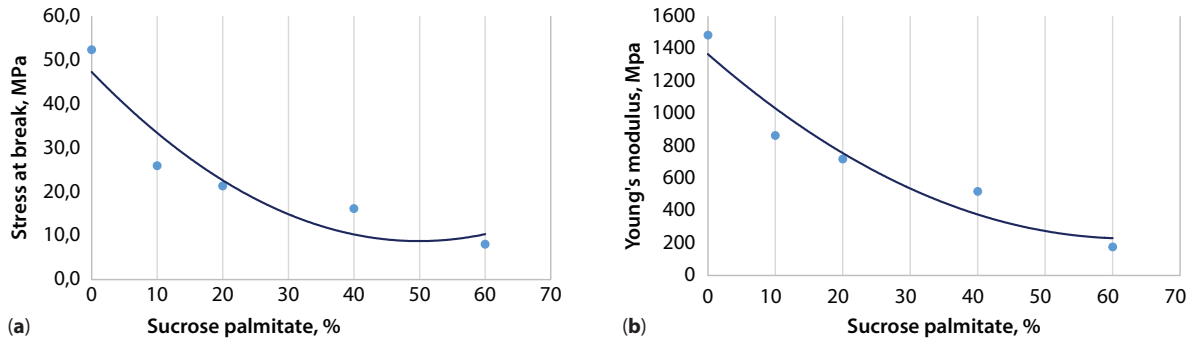

Figure 4 Mechanical properties of PVC films containing sucrose palmitate: (**a**) stress at break, (**b**) modulus of elasticity.

Table 1 Antimicrobial properties of sucrose and fructooligosaccharides 10-undecylenate esters and 10-undecylenic acid.

Test compounds	Inhibition*, %					
	Bacillus cereus	*Bacillus subtilis*	*Ps. fluorescens*	*Ps. aeruginosa*	*E. coli*	*Candida albicans*
Sucrose-10-undecylenate	75,1 ± 0,3	59,2 ± 0,9	–	12,5 ± 4,9	17 ± 4,9	86,6 ± 1,9
Fructooligosaccharide-10-undecylenate DE = 0,08	24,1 ± 2,2	23,0 ± 1,6	–	–	4,2 ± 1,0	94,1 ± 0,2
Methyl-10-undecylenate	15,2 ± 3,1	17,1 ± 2,3	–	–	9,5 ± 3,4	22,1 ± 4,5
10-Undecylenic acid	62,8 ± 3,1	58,9 ± 3,6	–	–	15 ± 2,7	68,1 ± 3,9

* The concentration of all tested compounds was 1 mg/cm^3.

4 CONCLUSIONS

Based on the obtained experimental results and their analysis above, it could be concluded that ultrasound-assisted esterification leaded to the synthesis of the expected sucrose esters and fructooligosaccharides, which was confirmed by the results from the FTIR and NMR analyses.

The sucrose palmitate esters demonstrated a promising plasticizing effect with respect to PVC; this conclusion was supported by the decreasing values of T_g and modulus of elasticity when increasing the ester content. As they possess good thermal stability (180 °C) [18], they could be successfully applied as a "green" plasticizer in PVC processing. Additional experimental work should be performed to clarify the behavior of the esters in real plastic processing when applied as bio-plasticizers, partly or completely replacing the conventional ones.

The unsaturated sucrose and fructooligosccharides esters showed antibacterial and antifungal activity. They could be applied as potential antimicrobial agents and be successfully used not only in the preservation of food, but also in medicine and cosmetics.

ACKNOWLEDGMENTS

The authors would like to extend their gratitude for the support of Nevena Petkova and Nikola Burdjiev from St. Klimet Ohridski University, Sofia, for the NMR spectra, Prof. Atanas Pavlov and Ivan Ivanov for the antimicrobial activity, and Assoc. Prof. Maria Marudova for the differential scanning calorimetry.

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