"Green" Synthesis of Sucrose Octaacetate and Characterization of Its Physicochemical Properties and Antimicrobial Activity*

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Sucrose octaacetate (octa-*O*-acetylsucrose) has been synthesized by esterification of sucrose with acetic anhydride using ultrasound-assisted irradiation. This sucrose ester is a white, water-insoluble substance with a bitter taste. The FT-IR and NMR spectra confirmed acetylation and revealed the hydrophobic incorporation in sucrose molecule. Furthermore, the foamability, foam stability, emulsification and antimicrobial properties of octa-*O*-acetylsucrose were evaluated. Foams and 50 % (oil/water) model emulsions were prepared with 2 % (w/w) octa-*O*-acetylsucrose. The obtained results demonstrate the formation of emulsions and foams with high stability (50–70 %). The antimicrobial activity of octa-*O*-acetylsucrose was evaluated against seventeen microorganisms (Gram-positive and Gram-negative bacteria, yeasts, and fungi). Octa-*O*-acetylsucrose inhibited the growth of fungi *Penicillium* sp., *Rhizopus* sp. and *Fusarium moniliforme* at 5 mg cm^{-3}, and yeasts *Candida albicans* at 1 mg cm^{-3}. Inhibition against Gram-positive and Gram-negative bacteria was not observed. The obtained results demonstrate the potential applications of octa-*O*-acetylsucrose as a foaming agent, oil-in-water emulsion stabilizer, and antifungal substance in pharmaceutical and cosmetic preparations.

Keywords:
octa-*O*-acetylsucrose, ultrasound-assisted synthesis, foam, antimicrobial properties

Introduction

Sucrose fatty acid esters have been the cause of increased interest as odorless, nontoxic, and biodegradable nonionic surfactants with a large scale application in the food industry as emulsifiers, anti-fungal and anti-bacterial agents[1–5], edible coating reagents[6], solubilizator and detergents in cosmetics and pharmaceuticals[7], insecticides in agriculture[7–9] and as bio-plasticizers in engineering[5–7]. In addition, sugar esters are produced from cheap and available renewable raw materials[8,9]. Furthermore, acetylated esters of sucrose are naturally found and derived from several plant species of *Nicotiana* and *Petunia*[10,11].

Sucrose octaacetate is used in many pesticide products and insecticides because of its inert and safety status. It has also been approved by the US Food and Drug Administration as both a direct and indirect food additive, and as a nail-biting and thumb-sucking deterrent in over-the-counter drug products. Sucrose octaacetate may be directly added to food as synthetic flavoring substance, adhesive, packaging material, and adjuvant. Its commercial uses include its addition to lacquers and plastics[7]. This wide application of sucrose acetates requires effective and environmentally friendly methods for their production. The novel approach to the acetylation of sucrose includes lipase-catalyzed esterification of partially acetylated sucrose for the production of biodegradable and biocompatible emulsifiers[12]. Some reports have demonstrated the acetylation of various mono- and disaccharides with Ac_2O–NaOAc under microwave and conventional conditions[13–15]. Some encouraging results have been published in relation to the acetylation of lactose by ultrasound-assisted irradiation[14] and in the modification of some long chain fatty acids sucrose esters[4,5,16]. So far, no detailed results have been published regarding the acetylation of sucrose using the

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environmentally friendly approach of cavitation caused by ultrasonic waves. Moreover, relevant information on the physicochemical properties of sucrose octatacetate is not available in comparison with sucrose esters with long fatty acid chains. Therefore, the aim of the present research is to accelerate the eco-friendly synthesis of sucrose octaacetate by using a "green" method – ultrasound-assisted esterification, and to evaluate its foaming and emulsifying properties for its potential application in agriculture, pharmacy, and cosmetics.

Materials and methods

Reagents and materials

D-(+)-Sucrose octaacetate (98 % purity) was purchased from Sigma-Aldrich and used as received. All other reagents and solvents were of analytical grade.

Synthesis of octa-*O*-acetyl-sucrose

Sucrose octaacetate was synthesized by the reaction of 10.0 g (0.029 mol) sucrose with 30 cm^3 (0.27 mol) acetic anhydride in a two-neck round-bottom flask with 3.0 g (0.036 mol) sodium acetate as a catalyst under the following conditions:

– direct heating (over a hot plate, 800 W) upon boiling for 60 min (conventional synthesis);

– ultrasonic irradiation in a VWR ultrasonic bath (VWR, power 30 W, 45 kHz) for 30 min (US synthesis).

A tube containing anhydrous calcium chloride was fixed on the top of the reflux and a digital thermometer was placed in the flask for temperature monitoring. The reaction mixture was then poured into a 200 cm^3 water-ice mixture, stirred vigorously, and left at 18 °C overnight. Octa-*O*-acetyl-sucrose was precipitated in an excess of cold water as a white solid, filtered, and then washed again with cold water. The acetyl ester was recrystallized from 95 % ethanol and re-precipitated with water, and then dried in a vacuum-oven to constant weight. The sucrose octaacetate was characterized by physicochemical methods.

Methods for characterization

Melting point and water activity Sample melting point was determined on an electro-thermal melting point apparatus BŰCHI 510 (Germany) in a capillary glass tube. Here, water activity (a_w) was measured with a water activity meter (AquaLab Pre, Labcell Ltd., UK).

Thin layer chromatography (TLC) The TLC analysis was performed on silica gel Kieselgel 60 F_{254} plates (Merck, Germany) with toluene/ethyl acetate/methanol/water 10:5:4.5:0.2 (v/v/v/v) as an eluent. Spots were detected by spraying the plates with 10 % (v/v) H_2SO_4 in methanol, and visualized by heating in an oven at 120 °C for 5 min[9].

FT-IR spectroscopy The infrared spectra of the samples were recorded on a Nicolet FT-IR Avatar Nicolet (Thermo Science, USA) spectrometer using KBr pellets, and the absorption was reported in wavenumbers (cm^{-1}) in the frequency range of 4000–400 cm^{-1}. Each spectrum was recorded after 120 scans.

NMR analysis Similarly, the ^1H and ^{13}C NMR spectra of the sucrose octaacetate were recorded on a Bruker Advance III 500 MHz spectrometer using $CDCl_3$ as a solvent. ^{13}C NMR spectrum was recorded operating at 151 MHz. All chemical shifts were reported in ppm with reference to TMS.

Hydrophilic-lipophilic balance (HLB) calculation Sucrose octaacetate HLB values were calculated by Griffin`s methods[17].

Foamability and foaming stability Foamability and foaming stability of the sucrose octaacetate were evaluated by the previously described method[2] with slight modifications[18]. In brief, the aqueous dispersion of sucrose octaacetate 0.2 g dm^{-3} was placed in 50 cm^3 in stoppered graduated cylinders and the height of each solution (H_0, cm) was measured. The solution was then shaken for 1 min, and the foam height (H_2, cm) and the total height (H_1, cm) were determined immediately. Following a stay for 1, 5, 10, 15, 20, 25, 30 min and 60 min, the foam height (H_3, cm) was recorded at 25 °C. All the experiments were performed in triplicate. Foamability and foaming stability were calculated using the following equations:

$$\text{Foamability (\%)} = [(H_1 - H_0)/H_0] \cdot 100 \quad (1)$$

$$\text{Foaming stability (\%)} = (H_3/H_2) \cdot 100 \quad (2)$$

Characterization of 50/50 O/W model emulsions with sucrose octaacetate Twenty-milliliter solution (2.0 wt %) was homogenized with 20 cm^3 sunflower oil for 5 min at 1000 rpm on a homogenizer (Ultra Turrax IKA T18 Basic, Germany). Emulsion stability of the prepared 50/50 oil-in-water (O/W) emulsions was evaluated by centrifugation and determination of separated phases. Ten cm^3 of each emulsion were placed in graduated centrifuge tubes, and centrifuged at 314 rad s^{-1} for 15 min[19]. Emulsion stability (S) was defined by the formula (3):

$$S\ (\%) = [(V_o - V)/V_o] \cdot 100 \quad (3)$$

where: V_o –volume of the emulsion cm^3; V –volume of the separated oil phase, cm^3 [18].

It was also evaluated by a temperature test. Five cm^3 of each emulsion was placed in test tubes, which were stored at four different temperatures: –18 °C (frozen); 4 °C (refrigerator temperature); 25 °C (room temperature), and 50 °C (water bath or thermostat) for 24 hours and 48 hours[19].

Turbidity measurement of the emulsion stability The dispersion of the emulsions was evaluated by spectrophotometric measuring of turbidity (TU, %) at a wavelength of 540 nm (Camspec-M 107 spectrophotometer, UK)[18].

Antimicrobial activity Antimicrobial activity tests were performed under the following conditions:

– *Test microorganisms.* Three Gram-positive bacteria (*Bacillus subtilis* ATCC6633, *Bacillus subtilis* 46/H1, *Bacillus methylotrophicus* BM47), three Gram-negative bacteria (*Escherichia coli* ATCC8739, *Salmonella* sp. – clinical isolate, *Salmonella abony*), yeasts (*Candida albicans* – clinical isolate, *Candida tropicalis, Saccharomyces cerevisiae*), and fungi (*Aspergillus niger*, *Aspergillus awamori, Aspergillus oryzae, Fusarium oxysporum, Fusarium moniliforme, Beauveria bassiana, Penicillium* sp., *Rhizopus* sp.) from the collection of the Department of Microbiology at the University of Food Technologies, Plovdiv, Bulgaria, were selected for the antimicrobial test. The concentration of the viable cells and spores in the suspensions for inoculation was adjusted to $1.0 \cdot 10^5$ CFU cm^{-3} (for fungal spores) and $1.0 \cdot 10^9$ CFU cm^{-3} (for bacterial and yeast cells).

– *Culture media.* LBG-agar medium (10 g tryptone, 5 g yeast extract, 10 g NaCl and 10 g glucose and 15 g agar per 1 dm^3 of deionized water) with pH 7.5 for the cultivation of Gram-positive, Gram-negative and yeasts was used. Malt extract agar medium (20 g malt extract, 20 g dextrose, 6 g peptone and 15 g agar per 1 dm^3 of deionized water) with pH 5.5 for the cultivation of the fungi was used. Both agar media were autoclaved for 20 min at 121 °C[20].

– *Antimicrobial assay.* Aqueous methanol (80 %) was used as a solvent to prepare desired solutions (1, 5 and 10 mg cm^{-3}) of octa-*O*-acetylsucrose US (synthesized by ultrasound-assisted irradiation). For determination of antimicrobial activity, the disc diffusion method in LBG-agar medium was implemented with slight modification[15].

The melted LBG-agar medium was poured into Petri dishes (d = 10 cm) and after its hardening, the Petri dishes were inoculated with suspensions of the test microorganisms[21]. Sterile paper discs with a diameter of 6 mm were then placed on the surface of the agar medium, and 6 μL from octa-*O*-acetylsucrose solutions in two replicates were pipetted on the discs. As controls, 80 % methanol and the antibiotic Nystatin (40 μg cm^{-3}) were used.

Inoculated Petri dishes were cultivated for 24 hours at 37 °C for *Escherichia coli, Salmonella* sp., *Salmonella abony, Candida albicans* and 30 °C for *Candida tropicalis, Saccharomyces cerevisiae, Bacillus subtilis*, *Bacillus methylotrophicus*, *Aspergillus niger, Aspergillus awamori, Aspergillus oryzae, Fusarium oxysporum, Fusarium moniliforme, Beauveria bassiana*, *Penicillium* sp. and *Rhizopus* sp. Antimicrobial activity was determined by measuring the diameter of the inhibition zones around the discs[20,21].

Results and discussion

Synthesis and characterization of sucrose acetates

The octa-*O*-acetyl-sucrose was obtained after the esterification of the sucrose with an excess of acetic anhydride with sodium acetate as a catalyst. Ultrasound-assisted acetylation of sucrose is illustrated in Fig. 1, and as a result of this process, the eight free hydroxyl groups are esterified to acetyl esters.

Octa-*O*-acetyl-sucrose presented white odorless powder with a bitter taste, soluble in acetone, DMSO, 95 % ethanol, methanol and insoluble in water. Therefore, its hydrophobic character was due to the substitution of free OH groups with acetyl residues. The ultrasound-assisted acetylation of sucrose reduces the time for synthesis to 30 min, and

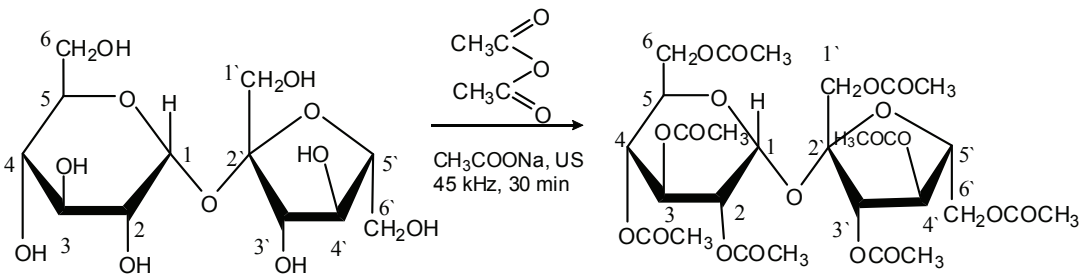

Fig. 1 – *Ultrasonic synthesis of sucrose acetates*

Table 1 – *Characterization of sucrose and sucrose acetates*

Sample	Yield, %	Melting point, ºC	a_w (mean ± SD)
Sucrose	–	186–188	0.780 ± 0.002
Octa-*O*-acetylsucrose (Sigma)	–	81–83	0.406 ± 0.002
Octa-*O*-acetylsucrose conventional	62	82–84	0.408 ± 0.002
Octa-*O*-acetylsucrose US	78	82–83	0.405 ± 0.003

energy, as the reaction was conducted at a lower temperature and 30 W power in comparison to the conventional heating for esterification. Higher yield was obtained by ultrasound-assisted esterification of sucrose with acetic anhydride at a mild temperature of 45 °C (Table 1).

The octa-*O*-acetylsucrose obtained under boiling temperature appeared as white solids with dark-yellow hues and caramel-like odor even after the recrystallization with ethanol. Because of degradation products formed during the high temperature of the esterification process, the yield of sucrose octaacetate was lower than the ultrasound-assisted synthesis (Table 1). An additional separation was needed because lower esters as mono-, di- and triacetate of sucrose were formed under ultrasonic irradiation (Fig. 1). These esters transformed the white solids in a semi-liquid substance at room temperature. The water activity of the synthesized esters was lower than the initial sucrose. The a_w values of the sucrose octaacetate were in the range of 0.390 to 0.406, which were in accordance with our previous reports on alkylated carbohydrates[5].

Octa-*O*-acetylsucrose (78 % yield) is a crystalline solid, mp. 87–88 °C (reported[11,15] mp. 88–89 ºC). R_f = 0.9 (toluene/ethyl acetate/methanol/water = 10:5:4.5:0.2). FT-IR (KBr): 1751, 1736 cm^{-1} (C=O).

^{1}H NMR (500 MHz, $CDCl_3$): 5.61 (1H, d, J = 3.5 Hz, H-1 from glucose residue), 5.38 (1H, d, J = 5.5Hz,–H1`), 5.36 (1H, d, $J$ = 5.7 Hz, H-3′), 5.31, 5.30, 5.29 (1H, t, $J$ = 5.8 Hz, H-4′), 5.02 (1H, t, $J$ = 11.0 Hz, H-4), 4.94 (1H, dd, $J$ = 10.0 and 3.8 Hz, H6`), 4.29–4.21, 4.19, 4.17, 4.16, 4.15, 4.13, 4.06 (8H, 2×m, H-5, H-6, H-1′, H-5′ and H-6), 2.12 (3H, s, –$OCOCH_3$), 2.04 (6H, s), 2.03 (3H, s), 2.02 (6H, s) (from 2.04–2.02 total 15H, s, 5×–$OCOCH_3$), 1.96 (3H, s, –$OCOCH_3$), 1.91 (3H, s, –$OCOCH_3$) ppm.

^{13}C NMR (126 MHz, $CDCl_3$) 170.72, 170.50, 170.13, 170.11, 170.05, 169.91, 169.68, 169.53 (8×CH_3CO), 104.00 (C-2`), 89.9 (C-1), 79.11(C-5′), 75.68, 74.97, 70.27, 69.62, 68.49, 68.19 (C-2, C-3, C-4, C-5, C-3′, C-4′), 63.65, 62.87, 61.74, (C-6, C-6′, C-1′), 20.72–20.56 (8× CH_3CO) ppm.

Sucrose and its octaacetate esters were dissolved in 95 % ethanol and analyzed by thin-layer chromatography (TLC). The spots of synthesized esters (3) coincided with R_f values of commercial standard octa-*O*-acetylsucrose (Sigma) (2), whereas R_f values obtained for all sucrose octaacetate were found to be 0.9, therefore, it was possible to consider that the desired sugar esters had been obtained.

Furthermore, the sucrose-*O*-acetyl esters were analyzed by infrared spectroscopy (Fig. 2), and the obtained spectra showed strong new bands at 1751 cm^{-1} attributed to stretching of C=O group, 1383 cm^{-1} ($\delta_{C–H}$) and 1236 cm^{-1} ($\nu_{C–O}$) were due to the presence of ester bonds of acetyl residues linked to sucrose moiety. Moreover, the strong bands at 3392 cm^{-1} in the sucrose spectrum, characteristic for the OH stretching vibration, disappeared in the sucrose octaacetate spectrum as a result of a successful substitution. Additionally, the presence of bands at 910 cm^{-1}

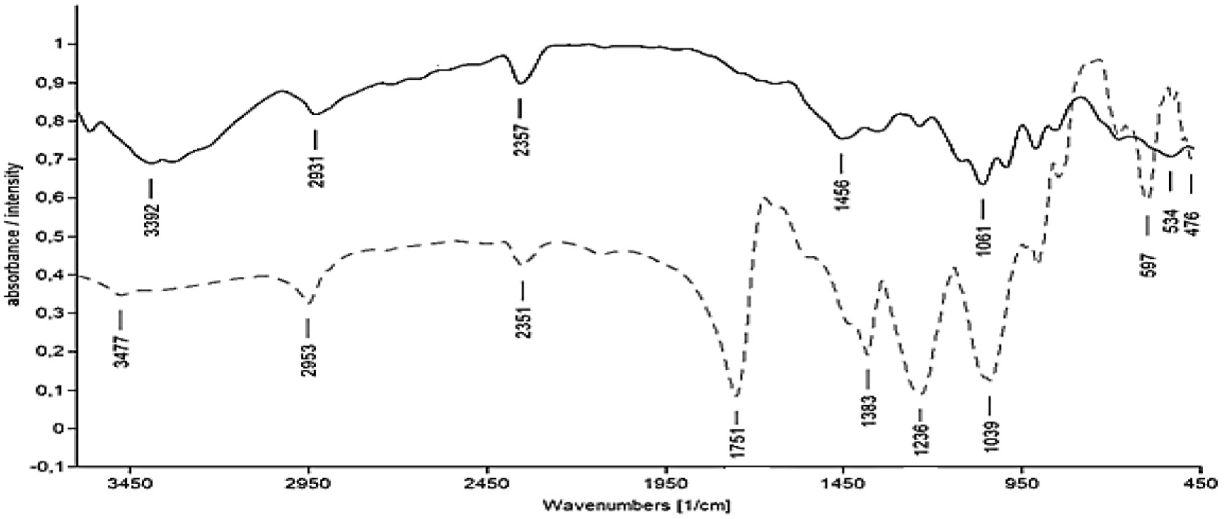

Fig. 2 – *FT-IR spectra of sucrose (–) and octa-O-acetylsucrose (– –) synthesized by ultrasonic irradiation*

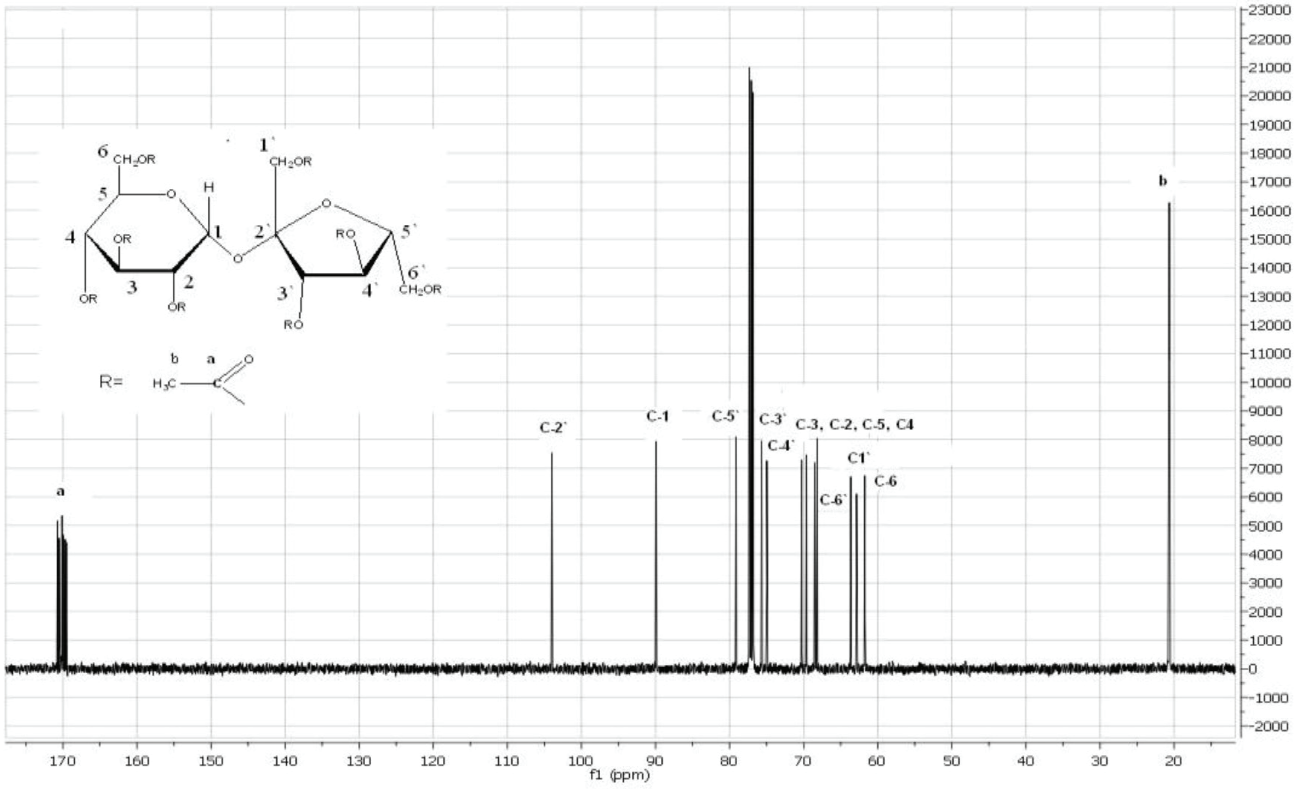

Fig. 3 – *^{13}C NMR spectrum (126 MHz, $CDCl_3$) of octa-O-acetylsucrose synthesized by ultrasound-assisted irradiation*

in both spectra proved that the resulting ester contained α-D-Glc*p* residue in chain; bond stretching of sucrose.

In the ^{1}H NMR spectrum, twenty-four protons resonated at δ 2.12 (3H, s), 2.04 (6H, s), 2.03 (3H, s), 2.02 (6H, s), 1.96 (3H, s) and 1.91 (3H, s) corresponding to the eight acetyloxy groups. The structure of sucrose residue could also be observed with 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). An isolated doublet around 5.3 ppm is due to anomeric proton from glucose unit (1H-Clc*p*). The structure of 1′,2,3,3′,4,4′,6,6′-octa-*O*-acetylsucrose was additionally confirmed by the ^{13}C NMR spectrum (Fig. 3), where eight chemical shifts, characteristic for acetyl carbonyl, had clearly appeared at 170.72, 170.50, 170.13, 170.11, 170.05, 169.91, 169.68 and 169.53 ppm. In addition, all the methyl carbons from the acetyl residue appeared at 20.5 ppm (8×CO**C**H$_3$). Carbon atoms of the sucrose moiety were found in the range of 61.32 ~ 103.88 ppm. Data obtained on the NMR spectra of the sucrose octaacetate were in accordance with the previous report on the chemically enzymatic and naturally isolated, chemically and enzymatically modified sucrose octaacetates[11,12,15]. Therefore, the esters synthesized by conventional and ultrasound-assisted methods corresponded to the octa-*O*-acetylsucrose.

Foaming and emulsifying properties

On the basis of the calculated HLB values of the octa-*O*-acetylsucrose (HLB 7–8), it was decided to check their foaming and emulsifying properties.

Foaming properties

Foaming properties of octa-*O*-acetylsucrose (standard and synthesized by ultrasound-assisted esterification) were evaluated by the foaming capacity (FC) and foam stability (FS). These two parameters were important for the evaluation of forming foams[22]. Changes in the foaming properties of the sucrose octaacetate in the initial and final state after 60 min are presented (Fig. 4).

Foaming stability was influenced by the standing time. It was shown that foam stability of octa-*O*-acetylsucrose slightly decreased during standing time from 1 min to 60 min. Likewise, it was clearly observed that foam height slowly began to decrease immediately after foaming formation. Foam capacity of octa-*O*-acetylsucrose could be measured at a concentration of 0.2 g dm^{-3} for only 30 min, which did not exceed 52–55 %. No significant differences were observed between standard and ultrasound-assisted synthesized sucrose octaacetate. Foam stability of the *O*-acetyl sucrose was close to the previous report on the lactose lauryl esters[2]. The sucrose octaacetate was the perfect candi-

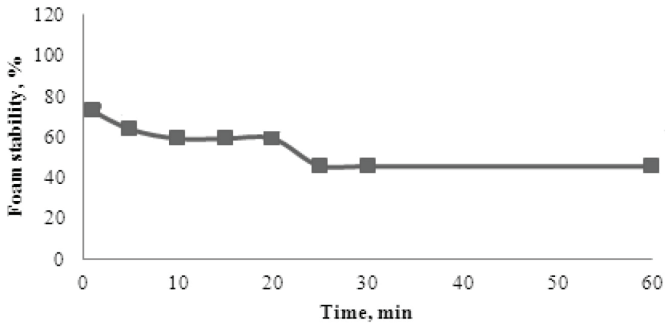

Fig. 4 – *Foam stability of octa-O-acetylsucrose from ultrasound-assisted synthesis*

date for an ideal foam-forming agent because it is non-toxic and able to produce abundant, thick, and stable foam at a low concentration of 0.2 g dm^{-3}.

The emulsifying properties of the sucrose octaacetates were studied in model systems with 2.0 % (wt) relative to the aqueous medium and 50 % oil phase of the emulsion of the oil/water type. From the obtained results, it was found that both sucrose octaacetates were characterized with closed dispersity 54–60 % (Table 2). The most stable O/W emulsions were formed with 2 % synthetic sucrose octaacetates.

The impact of thermal processing and freezing on the emulsion stability was also evaluated (Table 3). All prepared emulsions were unstable at –18 °C and 50 °C for 24 h, respectively. More stable were emulsions stored at 4 °C followed by 20 °C. More detailed studies are required for the emulsion stability of the sucrose acetate.

Table 2 – *Emulsion stability (in % separated phase) of 50/50 O/W model emulsion*

Ester concentration, 2.0 % (wt) of oil phase	Separated phase, % ± 0.1			*T*, %
	Oil	Water	Emulsion	
Octa-*O*-acetylsucrose – standard (Sigma)	65.7	32.3	0.0	54.1
Octa-*O*-acetylsucrose – US	26.1	69.6	4.3	60.0

Table 3 – *Temperature test for emulsion stability (%) of 50/50 O/W emulsions prepared with 2.0 % (wt) octa-O-acetyl-sucrose*

Substance	Temperature, °C	Water	O/W Emulsion	Oil
Octa-*O*-acetylsucrose – standard (Sigma-Aldrich)	–18	30	0	70
	4	32	62	6
	25	32	60	8
	50	38	0	62
Octa-*O*-acetylsucrose – US	–18	38	52	10
	4	28	68	4
	25	10	84	6
	50	41	20	39

Antimicrobial properties of octa-O-acetylsucrose

Many studies deal with antimicrobial activity of sucrose esters with fatty acids. In general, monoesters were more active than diesters, but in some cases, polyesters also demonstrate good activity[2,3,5,21]. To the best of our knowledge, the octaacetate esters of sucrose are little investigated for antimicrobial activity. In this study, we investigate the effect of octaacetyl ester of sucrose against the growth of seventeen microorganisms (Gram-positive and Gram-negative bacteria, yeasts, and fungi) with the results being summarized in Table 4.

The antimicrobial studies revealed that octa-*O*-acetylsucrose was more active against fungi and yeasts (Table 4). Applied solvent (80 % CH_3OH) did not cause antimicrobial activity. It was clearly observed that sucrose acetate esters inhibited the mycelial growth of fungi *Aspergillus oryzac*, *Penicillium* sp., *Rhizopus* sp. and *Fusarium moniliforme* and yeast *Candida albicans* at a concentration of 1, 5 and 10 mg cm^{-3}. Octa-*O*-acetylsucrose showed moderate antimicrobial activity against *Penicillium* sp. and *Rhizopus* sp. comparable with the activity of control Nystatin. In contrast, the growth of the fungi *Aspergillus niger, Aspergillus awamori, Fusarium oxysporum* and *Beauveria bassiana* remained unaffected. This is the first report about screening of the antimicrobial activity of octa-*O*-acetylsucrose against fungi *Beauveria bassiana, Penicillium sp., Rhizopus sp.* and *Fusarium moniliforme*, yeasts *Candida tropicalis, Candida albicans, Saccharomyces cerevisiae,* and bacteria (*Salmonella abony* and *Bacillus methylotrophicu*s). The substance was active against yeasts *Candida albicans* at concentration 1 and 5 mg cm^{-3}. No inhibition against Gram-positive and Gram-negative bacteria was detected. Matin *et al.*[15] also reported that octa-*O*-acetylsucrose in concentration 50 μg (dw) cm^{-3} did not inhibit the growth of *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Aspergillus niger, Fusarium equiseti* in concentration 100 μg (dw) cm^{-3}, respectively. Similarly to sucrose myristate and sucrose palmitate, glycerol acetates[23,24], octa-*O*-acetylsucrose did not inhibit the growth of food born pathogen *B. subtilis.* Another important finding is that octa-*O*-acetylsucrose stimulates the growth of *Bacillus methylotrophicus*, therefore, this substance is a substrate for its growth. *Bacillus methylotrophicus* bacteria are with a key role in control of corn stalk rot caused by *Fusarium graminearum*[25]. Numerous specialized methylotrophs have been described, including a great diversity of methanotrophs – most of them are methane con-

Table 4 – *Antimicrobial activity of octa-O-acetylsucrose expressed as diameter of zones of inhibition in mm (d_{disc} = 6 mm)*

Test microorganisms	Diameter of inhibition zones, mm									
	Octa-*O*-acetylsucrose						Controls			
	1 mg cm^{-3}		5 mg cm^{-3}		10 mg cm^{-3}		80 % CH_3OH		Nystatin	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Aspergillus niger	-	-	-	-	-	-	-	-	-	-
Aspergillus awamori	-	-	-	-	-	-	-	-	-	-
Aspergillus oryzae	8*	-	8	-	8	-	-	-	8	-
Fusarium oxysporum	-	-	-	-	-	-	-	-	-	-
Fusarium moniliforme	8	-	9*	9	9	9	-	-	-	-
Beauveria bassiana	-	-	-	-	-	-	-	-	-	-
Penicillium sp.	8	-	10**	10	8	-	-	-	8	-
Rhizopus sp.	-	-	8	-	8	-	-	-	9	-
Candida tropicalis	-	-	-	-	-	-	-	-	-	-
Candida albicans	8	8	8	8	-	-	-	-	-	-
Saccharomyces cerevisiae	-	-	-	-	-	-	-	-	-	-
Salmonella sp.	-	-	-	-	-	-	-	-	N/A	N/A
Salmonella abony	-	-	-	-	-	-	-	-	N/A	N/A
Escherichia coli ATCC8739	-	-	-	-	-	-	-	-	N/A	N/A
Bacillus subtilis ATCC6633	-	-	-	-	-	-	-	-	N/A	N/A
Bacillus subtilis 46/H1	-	-	-	-	-	-	-	-	N/A	N/A
Bacillus methylotrophicus BM47	-	+	+	-	-	-	-	-	N/A	N/A

Legend: *low antimicrobial activity, ** moderate antimicrobial activity; "-" no inhibition, "+" – stimulation of growth, N/A – not applied

sumers and some are able to grow on multicarbon compounds[26]. Moreover, *Bacillus methylotrophicus* BM47 was reported to possess high antifungal activity against phytopathogens *Fusarium oxysporum* and *Aspergillus flavus*[27]. The stimulating activity of octa-*O*-acetylsucrose over *Bacillus methylotrophicus* BM47 could be successfully used in agriculture for plant protection against fungal phytopathogens. Influence of structure on the antimicrobial activity of octa-*O*-acetylsucrose was studied. It was confirmed that short acetyl moiety of sucrose esters is unable to affect the growth of Gram-positive and Gram-negative bacteria in comparison with sucrose laurinate and undecylate ester that possess strong antimicrobial activity[3,5,23,24,28].

Because of its antifungal activity, proved by the experiments, octa-*O*-acetylsucrose could be successfully applied in agriculture for development of new formula in prospective crop and plant protection, for example, environmentally friendly fungicide based on sucrose esters. The promising foaming and emulsifying ability of octa-*O*-acetylsucrose offers an adequate solution to problems related to treatment and adhesion over the root or leaf surfaces. Additional investigation may be required concerning this field of application, and it could be the object of further experimental work.

Conclusions

A "green" method for synthesis of the octa-*O*-acetylsucrose by ultrasound-assisted esterification in the absence of a solvent by using acetic anhydride and sodium acetate as a catalyst has been demonstrated. The structure of the resulting ester has been elucidated by FT-IR and NMR spectroscopy, and a study of sucrose octaacetate as a surface active agent with HLB 9–10 was carried out. The obtained results revealed promising foamability, foaming stability of octa-*O*-acetylsucrose, and its use as antifungal substance against *Penicillium sp*. These properties of octa-*O*-acetylsucrose and its ability to stabilize oil-in-water emulsions could find potential application in beverages, drug delivery matrix, agriculture, and cosmetic products.

References

1. *Megahed, M.,* Preparation of sucrose fatty acid esters as food emulsifiers and evaluation of their surface active and emulsion properties, Grasas y Aceites **50** (1999) 280. doi: https://doi.org/10.3989/gya.1999.v50.i4.668
2. *Zhang, X., Song, F., Taxipalati, M., Wei, W., Feng, F.*, Comparative study of surface-active properties and antimicrobial activities of disaccharide monoesters, PLoS ONE **9** (2014) 1. doi: https://doi.org/10.1371/journal.pone.0114845
3. *Matev, G., Petkova, N., Vrancheva R., Ivanov, I., Pavlov, A., Denev, P.*, Synthesis, characterization and application of sucroesters as antimicrobial agents, University of Plovdiv "Paisii Hilendarski" Bulgaria, Scientific papers **39** (5) (2014) 17.
4. *Petkova, N., Vassilev, D., Grudeva, R., Koleva, M., Denev, P.,* Ultrasound-assisted synthesis of undecylenoyl sucrose esters, International Scientific Conference "Unitech 2015" 20 – 21 November 2015 Gabrovo, University Publishing House "V. Aprilov" – Gabrovo, 2015, 517.
5. *Vassilev, D., Petkova, N., Koleva, M., Denev, P.,* Ultrasound-assisted synthesis of sucrose and fructooligosaccharides esters as bio-plasticizers, J. Renew. Mater. **4** (2016) 24. doi: https://doi.org/10.7569/JRM.2015.634125
6. *Yamauchi, N., Tokuhara, Y., Ohyama, Y., Shigyo, M.,* Inhibitory effect of sucrose laurate esters on degreening in *Citrus nagato-yuzukichi* fruit during storage, Postharv. Biol. Technol. **47** (2008) 333. doi: https://doi.org/10.1016/j.postharvbio.2007.08.003
7. CFR: 40 CFR 180.910, Inert reassessment: sucrose octaacetate (CAS reg. no. 126-14-7). Office of Prevention, Pesticides, And Toxic Substances, United States Environmental Protection Agency Washington, D.C. 20460, (2005) 1.
8. *Li, Sh., Song, Z., Liu, Zh., Bai, S.,* Characterization and insecticidal activity of sucrose octanoates, Agron. Sustain. Dev. **28** (2008) 239. doi: https://doi.org/10.1051/agro:2007037
9. *Song, Zi-j., Shu-jun, L., Chen, X., Li-mei, L., Song, Zh.*, Synthesis of insecticidal sucrose esters, For. Stud. China **8** (2006) 26. doi: https://doi.org/10.1007/s11632-006-0019-2
10. *Chortyk, O., Stanley, J., Quincy, T.,* Characterisation of insecticidal sugar esters of Petunia, J. Agric. Food Chem. **45** (1997) 270. doi: https://doi.org/10.1021/jf960322f
11. *Singh, A., Singh, A., Begum, A., Sahai, M.,* Two acyl sucroses from *Petunia nyctaginiflora*, Phytoch. **63** (2003) 485. doi: https://doi.org/10.1016/S0031-9422(03)00162-6
12. *Steverink-de Zoete, M., Kneepkens, M., de Waard, P., van Oosterom, M., Slaghek, T., Gotlieb, K.,* Enzymatic synthesis and NMR studies of acylated sucrose acetates, Green Chem. **1** (1999) 153. doi: https://doi.org/10.1039/a901381d
13. *Patil, B., Babu, V.,* A rapid and convenient synthesis of α and β forms of acetylated derivatives of sugars under microwave irradiation, Ind. J. Chem., Section B: Organ. Chem. Med. Chem. **43B** (2004) 1288.
14. *Peng, X., Yang, J. Y., Kováč, P.,* Observations on the preparation of β-lactose octaacetate, J. Carbohyd. Chem. **31** (2012) 711. doi: https://doi.org/10.1080/07328303.2012.739230
15. *Matin, M., Bhuiyan, M., Afrin, A., Debnath, D.*, Comparative antimicrobial activities of some monosaccharide and disaccharide acetates, J. Sci. Res. **5** (2013) 515. doi: https://doi.org/10.3329/jsr.v5i3.15695
16. *Huang, D., Jiang, X.*, Improved synthesis of sucrose fatty acid monoesters under ultrasonic irradiation, Ultrason. Sonochem. **17** (2010) 352. doi: https://doi.org/10.1016/j.ultsonch.2009.08.009
17. *Griffin, W. C.,* Classification of surface-active agents by "HLB", J. Soc. Cosmet. Chem. **1** (1949) 311.
18. *Murdzheva, D., Vasileva, Iv., Petkova, N., Ivanov, I., Todorova, M., Denev, P.,* Physicochemical properties of new alginic acids derivatives, Indust. Technol. **3** (2016) 59.
19. *Vlaseva, R., Ivanova, M., Petkova, N., Denev P., Stoyanova A., Schreiner, M.*, Obtaining stable food emulsions between milk and corn oil, Bulg. J. Agric. Sci. **20** (2014) 1085.
20. *Tumbarski, Y., Petkova, N., Ivanov I.,* Polyphenolic content, antioxidant activity and antimicrobial properties of leaf extracts from dandelion (*Taraxacum officinale*), Indust. Technol. **3** (1) (2016) 141.
21. *Petkova, N., Gencheva, G., Tumbarski, Y., Vasslev, D., Koleva, M., Denev, P.,* Ultrasound-assisted synthesis of inulin esters with potential antimicrobial activity, Unitech-2016, International Scientific Conference, 18 – 19 November 2016, Gabrovo, **I**, 2016, 408.
22. *Ferreira, M., Benringer, R., Jost, R.,* Instrumental method for characterizing protein foams, J. Food Sci. **60** (1995) 90. doi: https://doi.org/10.1111/j.1365-2621.1995.tb05613.x
23. *Conley, A., Kabara, J.,* Antimicrobial action of esters of polyhydric alcohols, Antimicrob. Agents Chemother. **4** (5) (1973) 501. doi: https://doi.org/10.1128/AAC.4.5.501
24. *Kabara, J., Marshall, D.*, Medium-chain fatty acids and esters, in *Davidson, M. P., Sofos, J. N., Branen, A. L.* (Eds.), Antimicrobials in Food, 3 ed., CRC Press, Taylor & Francis Group, New York, 2005, pp 328–336.
25. *Li, Y., Geng, X., Ji, P., Pan, Ch., Wei, Sh.,* Isolation and evaluation of a Bacillus methylotrophicus strain for control of corn stalk rot, Biocontrol. Sci. Technol. 26 (2016) 727. doi: https://doi.org/10.1080/09583157.2016.1144047
26. *Madhaiyan, M., Poonguzhali, S., Kwon, S., Sa, T. M., Bacillus methylotrophicus* sp. nov., a methanolutilizing, plant-growth-promoting bacterium isolated from rice rhizosphere soil, Int. J. Syst. Evol. Microbiol. **60** (2010) 2490. doi: https://doi.org/10.1099/ijs.0.015487-0
27. *Tumbarski, Y., Petkov, E., Denkova Z.*, Study on the influence of the cultural conditions and the composition of the culture medium on the antimicrobial activity of *Bacillus methylotrophicus* BM 47 against some fungal phytopathogens, J. Glob. Biosci. **4** (8) (2015) 2990.
28. *Ferrer, M., Soliveri, J., Plou, F., Lopez-Cortes, N., Reyes-Duarte, D., Christensen, M., Copa-Patiño, J., Ballesteros, A.,* Synthesis of sugar esters in solvent mixtures by lipases from *Thermomyces lanuginosus* and *Candida antarctica* B and their antimicrobial properties, Enzyme Microb. Technol. **36** (4) (2005) 391. doi: https://doi.org/10.1016/j.enzmictec.2004.02.009