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Article

Physicochemical Characterization and Antimicrobial Properties of Inulin Acetate Obtained by Microwave-Assisted Synthesis

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Abstract: Microwave-assisted irradiation was performed for esterification of chicory inulin with high degree of polymerization with acetic anhydride without a solvent only with a catalyst. The resulting esters were characterized by melting point, hydrophilic-lipophilic balance, thin-layer chromatography, ultraviolet spectroscopy, Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) spectroscopy. Inulin acetate demonstrated a high degree of acetylation (2.5–3.0) and presented a white, water-insoluble substance with bitter taste. The FTIR and NMR spectra confirmed esterification and demonstrated the incorporation of hydrophobic residue to the water soluble inulin backbone. Swelling capacity, water holding, oil-holding capacities, the foamability, foam stability and emulsifying properties were also evaluated. Inulin acetate showed promising foam stability 52% for 60 min and formed stable emulsions at concentration 0.2 g/L with 50 and 80% oil phases. Its water holding capacity was lower than the oil holding capacity. In addition to this, for the first time, the antimicrobial potential of inulin acetate was tested against seventeen microorganisms (Gram-positive and Gram-negative bacteria, yeasts and fungi). Inulin acetate (10 mg/ml) inhibited the growth of *Bacillus cereus*, *Escherichia coli* ATCC 8739, *Salmonella abony*, *Candida albicans* and *Penicillium* sp. However, inulin acetate demonstrated antimicrobial activity at concentration 1 mg/ml against *Listeria monocytogenes* 863, *Escherichia coli* 3398, *Candida albicans* 8673, *Fusarium oxysporum* and *Aspergillus niger*. The current study demonstrated the applications of "green" synthesized inulin acetate as a foaming agent, oil-in-water emulsion stabilizer and antimicrobial substance in pharmaceutical, agricultural and cosmetic preparations.

Keywords: Inulin acetates; microwave irradiation; FTIR and NMR spectroscopy; foams; emulsions; antimicrobial activity

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1 Introduction

Inulin is a natural polydisperse storage polysaccharide that belongs to fructan family. It consists mainly of 2–60 fructose units in linear chains with $\beta(2\leftrightarrow1)$ glycosidic linkages and typically linked to a terminal α-glucose unit [1]. Inulin has no E-number, but it is approved by the Food and Drug Administration (FDA) as Generally Recognized As Safe (GRAS) [2]. The molecular weight of inulin, physicochemical properties and its industrial application depend on the degree of polymerization (DP). In addition, due to its biocompatibility, water solubility, and non-toxicity, prebiotic and immunostimulating activity, it found application in foods and pharmaceuticals [1,3–5].

In the recent decade the application of inulin esters in pharmacy and medicine constantly increases [3–7]. Among them, inulin acetate (InAc) gain more attention as a novel biodegradable, biocompatible and ecofriendly material [6–9]. It was successfully used as an encapsulating agent for the formation of microparticles with gallic acids [10], alpha-tocopherol [11], dexpanthenol, a water-soluble model drug (*E*,*E*)-bis(amidinobenzylidene)cycloheptanone [(*E*,*E*)-BABCH] as serine inhibitor [3], chlorhexidine and chymotrips [4] and indomethacin [5]. It found application as plastizer and an additive in Poly (Vinyl Chloride) [12]. Moreover, InAc application as a drug delivery carrier targeting colon and vaccine adjuvant found enormous application [13–17]. In addition, InAc also activates the innate immune system [17] and demonstrated antimicrobial potential [18]. In our earlier research, the influence of the length of inulin chain and the acetyl residues on the growth of different microorganisms was demonstrated [18].

The biological activity and application of InAc as the biocompatible biopolymer in drugs requires the ecofrendly approach for their synthesis. Most of the methods for their synthesis include the toxic solvents as N,N-dimethylformamide and pyridine at room temperature [3–5,8,9,15,16]. Efficient acetylation of mono- and disaccharides by microwave irradiation without solvent only with catalysts was demonstrated [19,20]. Moreover, the accelerated microwave irradiation for the synthesis of inulin acetate without solvent, but only with sodium acetate was successfully performed by Petkova et al. [21], Vassilev et al. [12] for inulin from Jerusalem artichoke and chicory. Therefore, the investigation and searching of the appropriate accelerated accetylation following the principle of "green" chemistry deserve attention.

To the best of our knowledge, there are no enough scientific data for the antimicrobial activity of long chain inulin acetates with a high degree of esterification and their application for cosmetic and pharmaceutical purposes. In addition, the information about solubility, foamability and emulsifying properties of inulin acetate are still absent. Therefore, the aim of the current study was to obtain inulin acetates using microwave irradiation, as a "green" method for synthesis, to elucidate the structure of resulting esters by spectral methods and to evaluate their physicochemical and antimicrobial properties.

2 Materials and Methods

2.1 Materials

Long-chain chicory inulin Frutafit TEX Frutafit® TEX (Sensus, the Netherlands) with an average DP 22 was used for esterification. Acetic anhydride (Sigma Aldrich, USA) was used as an acetylating agent. Anhydrous sodium acetate (Merck, Germany) was used as catalyst. 95% (v/v) ethanol (Merck, Germany) was used for inulin acetate recrystallization. All other reagents and solvents were of an analytical grade scale and they were used without further pretreatment.

2.2 Methods

2.2.1 Synthesis of Inulin Acetate

Long chain chicory inulin (16 g) was vigorously stirred with 9.6 g anhydrous sodium acetate and 96 ml acetic anhydride (Fig. 1) in a round bottom flask. The molar ratio inulin:sodium acetate:acetic anhydride was 1:10:1. The sample was connected to the reflux with fixed on top anhydrous calcium chloride for limitation the excess of moisture. Microwave irradiation was performed in a microwave system (Daewoo KOR, power

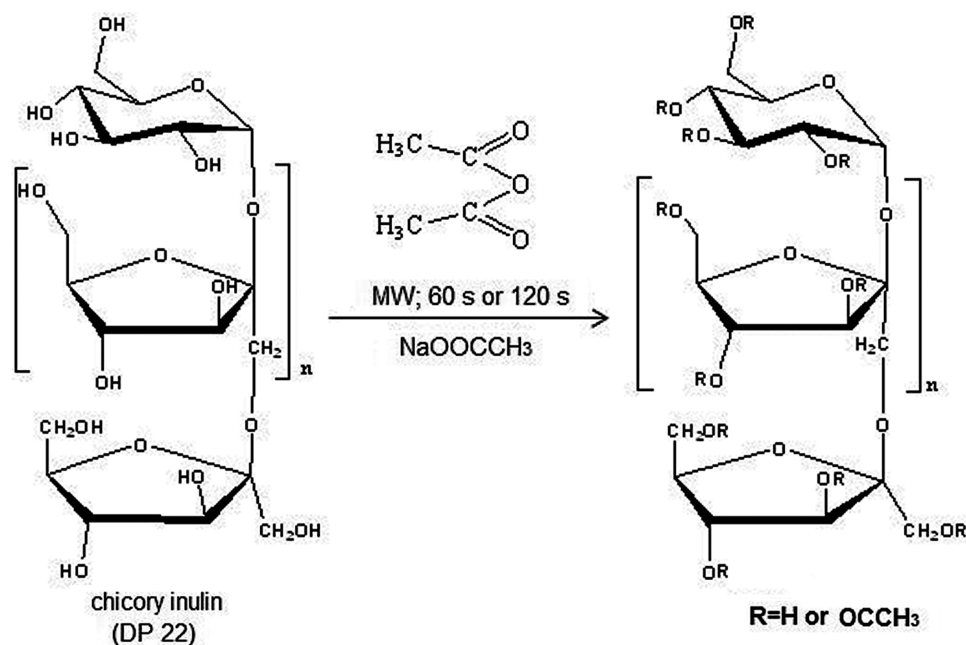

Figure 1: Microwave-assisted esterification of chicory inulin with acetic anhydride

700 W and 2450 MHz frequency) for 60 and 120 seconds, respectively. Then the reaction mixture was poured into a 200 ml water-ice mixture, stirred vigorously and left at –18°C overnight. Inulin acetates were precipitated in an excess of cold water as a white solid, filtered and then washed with cold water. The precipitated inulin acetate was re-crystallized by 95% (v/v) ethanol and re-precipitated with water, and then was dried in a vacuum-oven. Additional synthesis was performed with different molar ratio between inulin:sodium acetate:acetic anhydride (1:6:2) for irradiation 60 s.

2.2.2 Characterization

Melting point of initial inulin and their acetates were measured on a melting point apparatus Kofler. Hydrophilic-lipophilic balance (HLB) was calculated by the Griffin's method [22]. The thin layer chromatography 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 a mobile phase. Ethanol solution of sucrose octaacetate (octa-*O*-acetylsucrose) and water solution of inulin, both with concentration 10 mg/ml were used as standards. The spots were detected after 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 [23].

2.2.3 UV Spectroscopy

Inulin acetates (2 mg/ml) were dissolved in the solvents with different polarity increasing in the following order: chloroform, methanol, ethanol, acetonitrile, dimethylformamide, dimethyl sulphoxide. The UV spectra was recorded on UV-30 SCAN spectrophotometer in the wavelength range from 190 to 400 nm.

2.2.4 FTIR Spectroscopy

The FT-IR spectra of the samples (2 mg) were collected on VERTEX 70v FTIR Spectrometer (Brucker, Germany) in pellets with KBr (Honeywell/Fluka FT-IR grade > 99%). The spectra were recorded over a wavenumber range of 4000–400 cm^{-1} at 132 scans with a spectral resolution of 2 cm^{-1}. The absorption was reported in wavenumbers (cm^{-1}). The obtained FTIR spectra were used for calculation of the degree of acetylation of InAc as relative intensity of IR bands at 1745 cm^{-1} was divided to C=O/1020 cm^{-1} [16].

2.2.5 NMR Spectroscopy

^{1}H and ^{13}C NMR spectra were recorded using a Bruker AVIII 500 MHz spectrometer operating at a frequency of 500 MHz and 126 MHz, respectively. Inulin acetate (25 mg/0.6 ml) was dissolved in $CDCl_3$. The chemical shifts (δ) were expressed in ppm.

2.2.6 Phyicochemical Properties

Water-Holding and Oil-Holding Capacity

The water-holding and oil-holding capacities of InAc were determined in duplicate, as previously described [24]. Briefly, inulin ester (100 mg) was put into preweighed 50 ml polypropylene centrifuge tubes. Then 10 ml deionized water or sunflower oil was added, respectively. The tubes were closed and vigorously shaken. After 24 h at 20°C the samples were centrifuged at 3500 rpm for 15 min. The excess of water and oil was removed. The tubes were then weighed and dried at 105°C to constant weight.

Foam Ability and Foam Stability

Foam ability (FA) and foaming stability (FS) of inulin acetates were evaluated by the previously described method [25]. In brief, the 20 ml aqueous dispersion of inulin acetates in three different concentrations (0.05, 0.1 and 0.2 g/L) was placed in 50 ml in stoppered graduated cylinders and the height of each solution (H_0, cm) was measured. The solution was vigorously shaken for 1 min, and the foam height (H_2, cm) and the total height (H_1, cm) were determined immediately. The foam height (H_3, cm) at 1, 5, 10, 20, 30 min and 60 min was recorded at 25°C. All the experiments were performed in duplicate. Reproducibility of the results was expressed as mean ± 10%. Foam ability and foaming stability were calculated using the following equations:

$$FA(\%) = [(H_1 - H_0)/H0] \times 100 \quad (1)$$

$$FS(\%) = (H_3/H_2) \times 100 \quad (2)$$

Emulsion Properties

Three concentrations of inulin acetate (0.05, 0.1 and 0.2 g/L) were used for the preparation of three types of emulsions with 20, 50 and 80% oil phase. Sunflower oil (Biser, Bulgaria) was purchased from the local market. Inulin acetates were dispersed in the oil phase by stirring on a magnetic stirrer at 45°C for 10 min. The water solution was homogenized with inulin esters and sunflower oil for 5 min at 1000 rpm on a homogenizer (Ultra Turrax IKA T18 Basic, Germany). The emulsion stability of the prepared emulsions with inulin acetate was evaluated by centrifugation at 3000 × g (Hettich EBA 20, Germany) for 20 min. The height of the emulsified layer was measured and determination of separated phases was performed. The emulsifying ability was calculated as a ratio of the height of the emulsified layer and the height of the total content of the tube and presented in percentages (%) [20]. Emulsion stability at different temperature was also evaluated. Five ml of each emulsion was placed in a graduated test tube, and it was 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 [26].

2.2.7 Antimicrobial Activity

Test Microorganisms

Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Bacillus subtilis* 46/H1, *Bacillus cereus Listeria monocytogenes* 8632, *Staphylococus aureus* 745), Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Escherichia coli* 3398, *Salmonella typhy* 745, *Salmonella abony*), yeasts (*Candida albicans*—clinical isolate, *Candida albicans* 8673, *Candida tropicalis, Saccharomyces cerevisiae*) and fungi (*Aspergillus niger, Fusarium oxysporum, Beauveria bassiana, Penicillium* sp.) were selected to be used in the

antimicrobial assay. Only *Escherichia coli* 3398, *Staphylococus aureus* 745, *Listeria monocytogenes* ATCC 8632 were obtained from the Collection of the Department of General and Applied Microbiology, Sofia University. The other microorganisms were from the collection of the Department of Microbiology at the University of Food Technologies, Plovdiv, Bulgaria. The concentration of the viable cells and spores in the suspensions for inoculation was adjusted to 1.0×10^5 cfu/ml (for fungal spores) and 1.0×10^9 cfu/ml (for bacterial and yeast cells).

Culture Media

LBG agar (Luria-Bertani glucose 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) was used for the cultivation of Gram-positive, Gram-negative and yeasts, as well as for the agar-well diffusion assay. For these two purposes 50 g of LBG-agar were dissolved in 1 L of deionized water, the final pH was adjusted to 7.5. *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 before use.

Antimicrobial Assay

The antimicrobial activity was determined by the standard agar well diffusion method in LBG agar. The test bacteria were cultured on the LBG agar at 37°C for 24 h, while the test fungi were grown on malt extract agar at 30°C for 7 days or until sporulation. The inocula of test microorganisms were prepared by homogenization of a small amount of biomass in 5 mL of sterile 0.5% NaCl. The concentrations of the viable bacterial cells and fungal spores in the inocula were determined using a Thoma's counting chamber. Their final concentrations were adjusted to 1.0×10^8 cfu/ml of bacterial cells and 1.0×10^5 cfu/ml for fungal spores, then inoculated in preliminarily melted and tempered at 45–48°C LBG agar media. The inoculated media were transferred in quantity of 20 ml in sterile Petri dishes (d = 10 cm) and allowed to solidify. Then six wells (d = 6 mm) per dish were cut. The samples of the esters were pipetted in quantity of 60 µL into the agar wells.

Aqueous methanol (80%) was used as a solvent to prepare different concentration (1, 5, 10, 25 and 50 mg/ml) of inulin acetates. Methanol (80%), the antibiotics Nystatin (40 µg/ml), Ampicillin (10 µg/ml), Chlornitromicin (250 µg/ml) and Biseptol (400 µg/ml) were used as controls. The inoculated Petri dishes were incubated at 37°C (for test bacteria/yeasts) and at 30°C (for *B. subtilis* 46/H1, and fungi). The antimicrobial activity was determined by measuring the diameter of the inhibition zones around the wells at the 24th and 48th hours of incubation. Sensitive were the microorganisms with inhibition zones of 18 mm or more; moderately sensitive—with inhibition zones from 12 to 18 mm; resistant—with inhibition zones up to 12 mm or completely missing [18].

3 Results

3.1 Synthesis of Inulin Acetate

The results from the synthesis and characterization of inulin acetate by microwave-assisted irradiation were summarized (Tab. 1). All resulting InAc presented white powder substances, slightly bitter, water insoluble, but soluble in organic solvent as methanol, ethanol, acetone, chloroform, DMSO, DMF. The highest degree of acetylation (3.0) was obtained only for 60 s irradiation, but the yield was the lowest one. The variation of molar ratio did not affect significantly the DA.

Inulin acetate synthesized by microwave-irradiation only for 120 s gave the highest yield (62% yield). It is a crystalline white solid (mp. 79–82°C). The reported melting point values were near to reported previously in the range of 87–92°C data by Wu et al. [4], Poulain et al. [3] and Petkova et al. [21]. Significant decrease in the values of melting points was observed after the incorporation of acetyl groups in inulin chain. In comparison, initial inulin has a high melting point 178°C. The substitution of OH groups with acetyl moieties reduced more than 2 times the meling point of inulin esters in the comparison

Table 1: Yields and characterization of InAc obtained under different conditions

Sample	Conditions	Reaction time, s	Molar ratio[a]	Yield, %	Melting point, °C	DA[b]	HLB
InAc 1	MW[c]	60	1:10:1	12	79–82	3.0	7.9
InAc 2	MW	60	1:6:2	22	75–83	2.8	11.4
InAc 3	MW	120	1:10:1	62	79–82	2.5	11.8
InAc 4 [12]	conventional synthesis	60 min under reflux	1:10:1	78	76–79	2.9	6.9

Note: [a]Molar ratio inulin:sodium acetate:acetic anhydride, [b]Degree of acetylation, [c]microwave irradiation.

with initial inulin. Inulin acetate was dissolved in 95% ethanol and analyzed by thin-layer chromatography (TLC). The spots of corresponding different synthesized esters were characterized with R*f* values 0.9 (toluene/ethyl acetate/methanol/water 10:5:4.5:0.2 (v/v/v/v)). In comparison with the classical synthesis, accelerating microwave irradiation inulin acetate was obtained with similar characteristics, but for shorter time only for 2 min.

Moreover, the other advantages of this study are the significant reduction of acetylation time and the ignoration of an application of DMF as solvent for esterification. In addition, microwave-assisted esterification of inulin with acetic anhydride in a presence of anhydrous sodium acetate results inulin acetates with a degree of acetylation in the range from 2.5 to 2.9 and these esters were characterized as highly acetylated (Tab. 1). These values are comparable with some data for synthesis of inulin acetate with DMF. For example, We et al. [4] reported that fast acetylation in DMF occurred in the first hour, followed by a slower reaction; about 65% acetylation was completed within the initial 1.5 h. Jain et al. [5] reported for the synthesis of inulin acetates with DA 1.618 and 54% conversion of hydroxyl groups, respectively for 24 h at 40°C for 24 h with DMF. Other study demonstrated synthesis of inulin acetate with DA 2.1 by microwave-assisted esterification of inulin form Jerusalem artichoke [21]. Acetylated inulin with the degree of substitution of 2.4, corresponding to a conversion of the hydroxyl groups of 80% in DMF for 24 h was obtained by Waltz et al. [6]. However, in our study, inulin acetate with good yield was obtained by microwave-assisted acetylation only for 120 s without solvent only with catalyst with 83% conversion of hydroxyl groups.

The calculated HLB values of inulin acetate was 7.9 and was near to calculate HLB values of the octa-*O*-acetylsucrose (HLB 7–8) [20]. Therefore, inulin acetate present amphyphilic molecule, water dispersible, with potential use as wetting and spreading agents and w/o emulsifier.

3.2 UV-VIS Spectroscopy

The characteristic UV-VIS spectra of inulin acetates dissolved in different solvents were presented (Fig. 2). In addition, the absorption properties of inulin esters in different polarity solvents were summarized in Tab. 2.

From the obtaining results of the absorption properties of inulin acetate was found that in the proton donating solvent methanol and ethanol absorption maximum detected at 200.5 nm (Fig. 2C). Shoulders were observed at 221 and 216 nm in methanol and ethanol, respectively. Moreover, in methanol it was observed low intensity broad band in UV spectra at 275 nm (Tab. 2). However, only in acetonitrile spectra inulin acetate demonstrated two absorbtion maxima at 191.5 and 206.5 nm. In chloroform spectra of inulin acetate the maximum absorbtion band was found at 238 nm and a shoulder at 280 nm (Fig. 2A). In other proton accepting solvents (DMF and DMSO) the absorption bands were in the range from 257 to

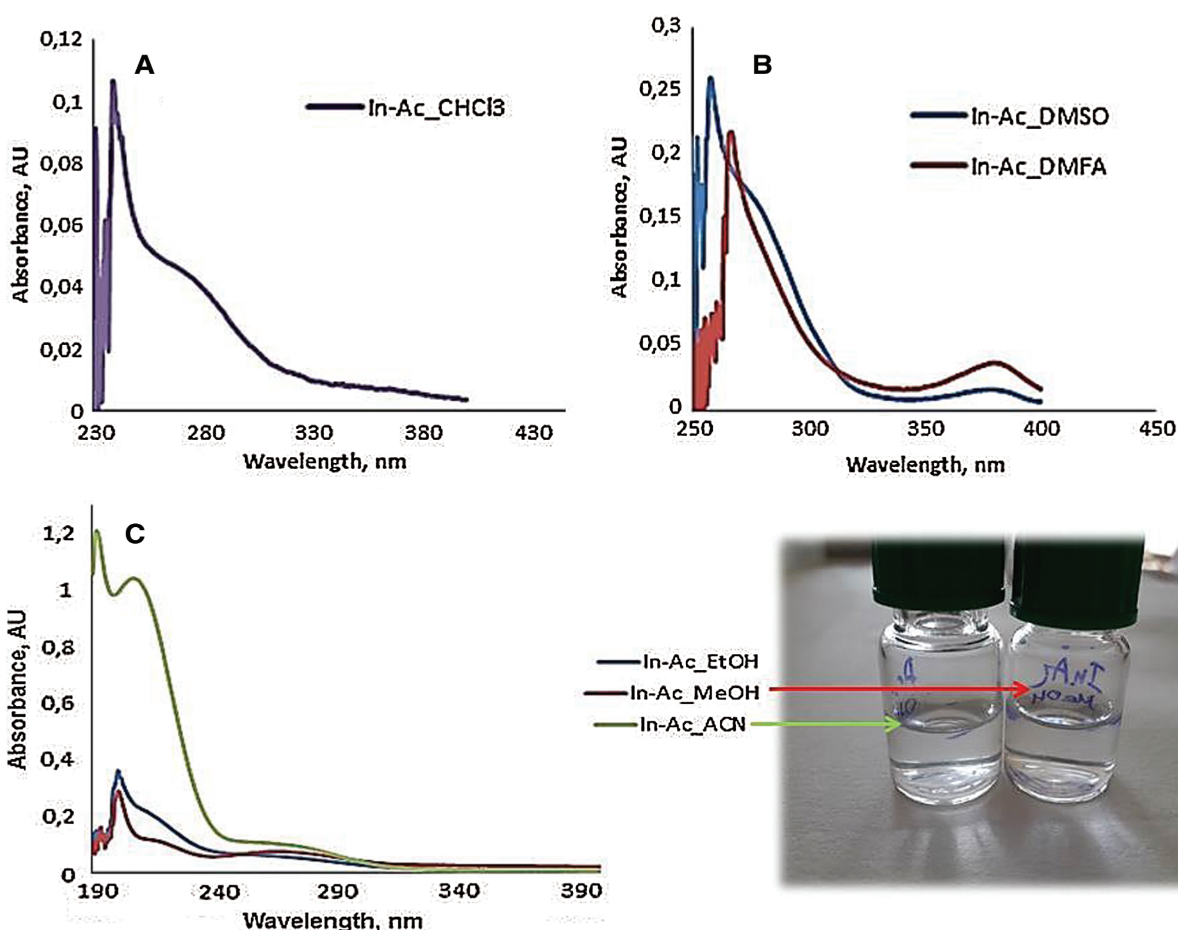

Figure 2: UV-VIS spectra of inulin acetates dissolved in different solvent (A) $CHCl_3$ (B) DMSO and DMF and (C) ethanol, methanol and acetonitrile

266 nm. In DMSO was observed band at 266 nm. This band at DMF as a solvent was a shift at around 10 nm to lower wavelengths in the comparison with DMSO spectra (Fig. 2B).

In general, inulin acetates in all of investigated solvents gave clear transparent solutions with higher absorption ability in comparison with previous reports for sucrooctaacetates [29]. Contrary to their reports our data demonstrated high values and the possibility to be investigated by UV spectroscopy. In addition, this is the first report of the absorbtion properties of inulin acetate in the different polarity solvents.

3.3 FTIR Spectroscopy

FT-IR spectroscopy was used for identification of functional groups of chicory inulin and microwave-assisted inulin acetates. The incorporation of acetyl residues in inulin chain was confirmed by FTIR spectra (Fig. 3).

The broad band at 3476 cm^{-1} typical for the stretching vibrations of OH groups in inulin significantly decreased after esterification. This could be explained with the disappearance of intermolecular hydrogen bonds and substitution of OH with acetyl (–$COCH_3$) residues. In the FTIR spectrum of inulin acetates a strong new band at 1749 cm^{-1} appeared that was assigned with stretching vibration of C=O groups from ester (Fig. 3). New bands appeared at 1373 cm^{-1} due to bending of C–H from CH_3 and –C–O stretching

Table 2: The absorbtion properties of inulin acetate in different polarity solvents

Solvent	Properties	Polarity parameter ΔF [27]	Polarity index [28]	λ_{max}, nm	Molar absorptivity, $L.mol^{-1}.cm^{-1}$
Chloroforme	Proton accepting solvent	0.1458	2.7	238 280 shoulders	0.106 0.043
Methanol	Proton donating solvent	0.3110	5.1	200.5 221 shoulder 275 broad band	0.289 0.101 0.073
Ethanol	Proton donating solvent	0.1541	5.2	200.5 216 shoulder	0.362 0.198
Acetonitrile	Proton accepting solvent	0.3054	5.8	191.5 206.5 270 broad band	1.209 1.042 0.101
DMF	Proton accepting solvent	0.2745	6.4	266 379.5	0.218 0.037
DMSO	Proton accepting solvent	0.2634	7.2	257.5 278 shoulder 380 broad band	0.261 0.135 0.037

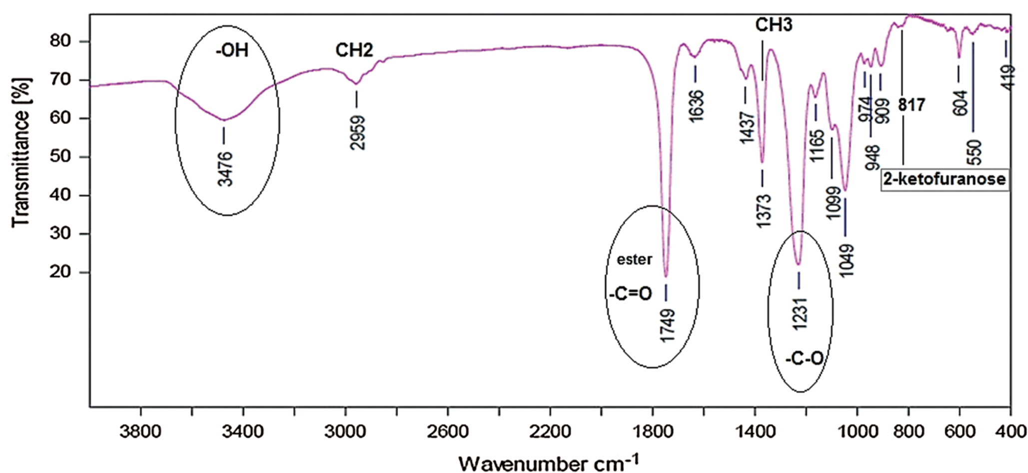

Figure 3: FTIR spectrum of inulin acetates (DA = 2.5) after microwave–assisted synthesis

vibrations at 1236 cm^{-1} that correspond to the acetyl (–$COCH_3$) groups in the inulin chain. Moreover, the presence of characteristic bands at 817 cm^{-1} proved that resulting ester contained *β*-D-fructose residues linked by 1→2 glycoside bonds. In addition FTIR spectrum of inulin acetate contained other typical bands of inulin at 2969, 1437 and 1049 cm^{-1} respectively. All these findings confirmed the successful acetylation of inulin. The reported FTIR bands for inulin acetates were in accordance with some previous reports for FTIR spectra of the acetylated inulin from agave, chicory, dahlia and Jerusalem artichoke [2,3,6,5,12,15,16,18,21].

3.4 NMR Spectroscopy

The structure of inulin acetates was additionally confirmed by the ^{1}H and ^{13}C NMR spectra.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (s, 8H), 5.78–5.52 (m, 63H), 5.52–5.34 (m, 68H), 5.15 (d, J = 44.9 Hz, 9H), 5.01 (dd, J = 129.6, 34.5 Hz, 18H), 4.39 (d, J = 8.7 Hz, 64H), 4.36–4.09 (m, 160H), 3.94–3.68 (m, 118H), 3.68–3.59 (m, 8H), 2.38–2.03 (m, 628H), 2.03–1.63 (m, 32H). 13C NMR (126 MHz; CDCl3): δ 170.67, 170.09, 169.78, 103.73 (**C2f**) 77.69, 77.30, 77.05, 76.79, 75.81, 75.51, 63.84, 62.69, 20.79, 20.66 and 20.43 ppm.

^{1}H NMR spectrum of inulin acetate showed chemical shifts typical for a methyl side chain of acetyl groups (CO**CH_3**) that appeared at ~2 ppm. The degree of acetylation of inulin acetate was estimated from its ^{1}H-NMR spectrum by using relative change in the ratio of the integrals of resonance peaks at ~2 ppm corresponding to methyl protons of acetate (acetylation), and these at 3.5–5.5 ppm corresponding to protons of the fructose skeleton (native inulin), respectively. Waltz et al. [6] also reported that in acetylated inulin appeared the signals of the inulin-backbone at δ = 5.55 ppm−5.08 ppm (t/m, 3H, methine) and δ = 4.27 ppm−3.61 ppm (d/m, 4H, methylene). The number of acetyl groups per fructose unit of the microwave-synthesized inulin acetate was calculated to be 2.5 that corresponded to 83% acetylation. This inulin acetate ester was characterized as a highly acetylated one. These results are close to these ones reported by other authors [2,3,5,21].

The structure of inulin acetates was additionally confirmed by the ^{13}C NMR spectrum (Fig. 4). Three chemical shifts characteristic for acetyl carbonyl was clearly appeared at δ 169.76, 170.03 and 170.67 ppm. All the methyl carbons from the acetyl residue appeared at δ 20.5 (3 × $COCH_3$). The chemical shifts relevant to carbon atoms of the inulin moiety (glucose and fructose residues) were found in the range of 62.70 to 103.73 ppm. In anomeric region was found a shifts typical for C2f that was involved in the linkage β-(2↔1)-D-fructofyranosyl-fructose. Similar chemical shifts were reported for inulin acetates synthesized in DMF by stirring for 24 h [2–6,8] and for inulin acetates obtained by conventional synthesis only with catalyst without solvent media [18].

3.5 Functional Properties

3.5.1 Water and Oil Holding Capacity

Water and oil holding capacity bring about functional properties, enhanced flavor and sensory properties, enhanced the application of modified carbohydrates into cosmetic or food products.

To the best of our knowledge the functional properties of inulin acetates were not investigated. The first step was to evaluate the potential of inulin acetate to absorb water and oil. Its water and oil holding capacities were presented (Fig. 5). The esterification of inulin brings about the increasment of the oil holding capacity of modified polymer. The oil holding capacity of inulin acetate was higher than their WHC and reached 3.9 g oil/g sample. In comparison, unmodified commercial chicory inulin (DP 22) showed the water-holding capacity 1.6 g water/g sample, and OHC 3.5 g oil/g sample [30]. In our case, the presence of acetyl residues in the inulin chain decreased approximately four times and the WHC reached below 0.4 g water/g sample (Fig. 5).

This could be explained with the fully substitution of hydrophilic OH groups in inulin backbone with acetyl residues and decreased water solubility of inulin acetate. The OHC of inulin acetate brings about the application of this modified biopolymer as a mouthfeel enhancer in pharmaceutical and food formulations and drug design.

3.5.2 Foaming Properties

Therefore, the evaluation of foamabily and foam stability of long-chain in inulin acetates deserves attention. The results for foaming properties of inulin acetate were shown (Fig. 6). The foaming

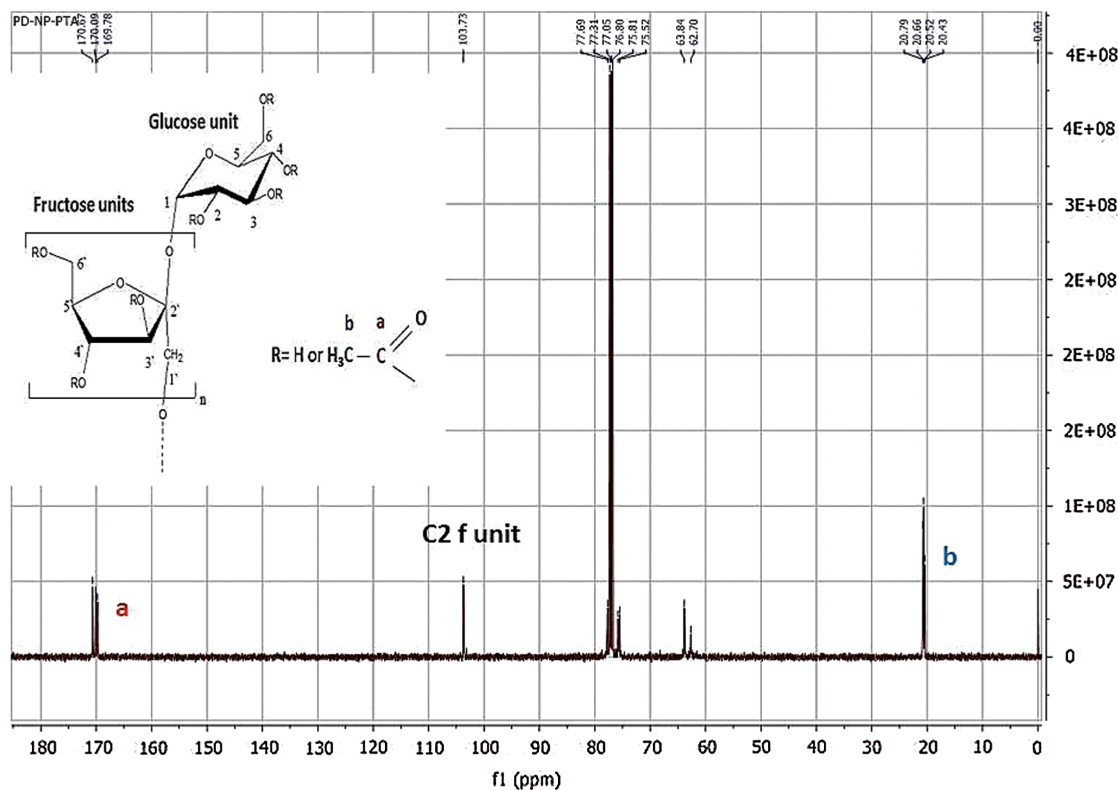

Figure 4: ^{13}C NMR spectrum (126 MHz, $CDCl_3$) of inulin acetate (DA = 2.5) synthesized by microwave-assisted irradiation

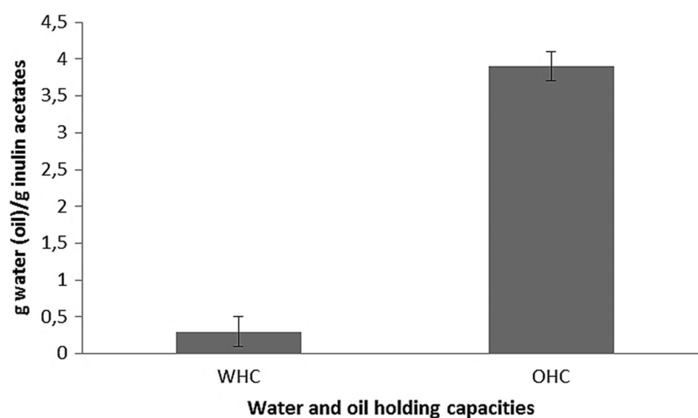

Figure 5: Water and oil holding capacities of inulin acetates (DA = 2.5)

properties of sucrose ester were investigated [20,25]. The foaming properties of modified oligofructoses long chained fatty acid ester (lauric and palmitic) were demonstrated [31]. However, any data for the potential of inulin acetates as foaming agent were not found.

As clearly observed, the foam ability values increased with increasing the concentration of inulin acetates (Fig. 6A). Foam stability over time was assessed by measuring the foam volume from 1 to

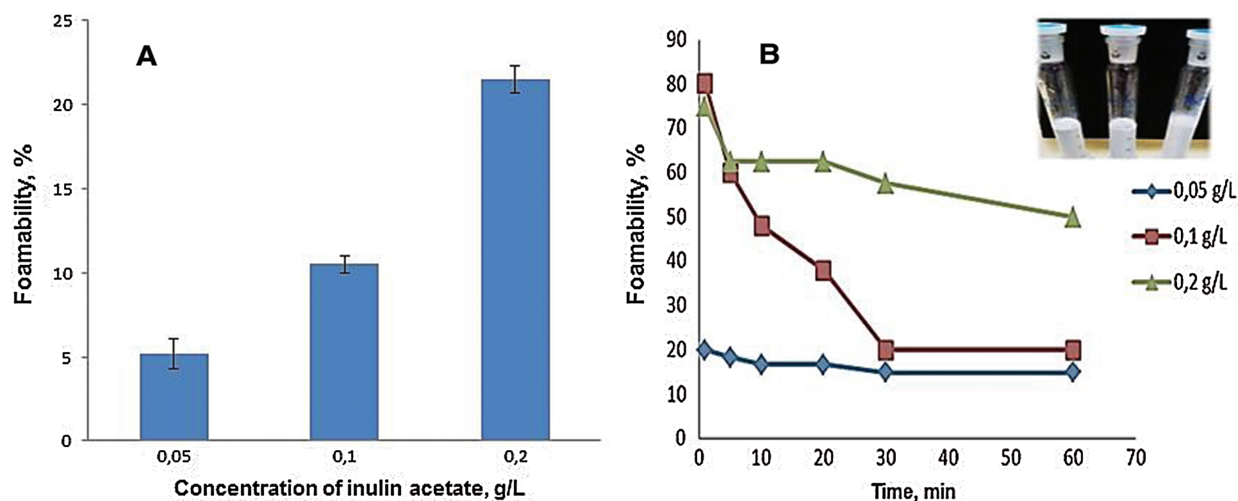

Figure 6: Foaming ability (A) and foaming stability (B) of inulin acetates (DA = 2.5) at different concentrations for 60 min at 25°C

60 min (Fig. 6B). The foam stability is characterized by the volume of the entrapped air, still remaining in the foam after a certain period of time. For foams obtained with 0.2 and 0.1 g/L inulin acetate the FS decreased by 20% for the first 5 min, while for lower concentration (0.05 g/L) remained stable until 20 min. Inulin acetate in the concentration 0.2 g/L formed the most stable foam with FS 52% for 60 min. In comparison, foam stability of octa-*O*-acetylsucrose at a concentration of 0.2 g/L for only 30 min, which did not exceed 52–55% [20]. In comparison similar concentration were used to test the foaming properties of sucrose and oligofructose laurinate and palmitate [20,25,31]. Therefore, the promising foaming properties in higher concentration of inulin acetates demonstrated their future possible application in detergents for cosmetic and medicinal properties.

3.5.3 Emulsifying Properties

The emulsifying properties of inulin acetates were evaluated in the model systems prepared by the concentration of ester (0.05, 0.1 and 0.2 g/L) and oil phase 20, 50 and 80% respectively (Tab. 3). The most stable emulsions were formed with inulin acetate in concentration 0.2 g/and oil phase 50 and 80% oil phase (Tab. 3). The similar trend for better emulsion stability in the high concentrations was observed in our earlier results for octa-*O*-acetylsucrose in 50/50 o/w emulsions [20]. However, inulin acetates demonstrated better emulsifying properties in the comparison with octa-*O*-acetylsucrose.

The influence of thermal processing and freezing on the emulsion stability was also evaluated (Tab. 4). The most stable at –18°C, 4, 25 and 50°C for 24 h were 80% emulsions prepared with inulin acetate in the concentration 0.2 g/L, followed by 50% emulsions with 0.05 g/L inulin ester. More stable were emulsions stored at 4°C, 25°C and 50°C. However, more detailed studies are required for the emulsion stability of inulin acetate.

3.6 Antimicrobial Activity

To the best of our knowledge, the inulin acetate was slightly investigated for antimicrobial activity in the comparison with sucrose esters. In our previous investigation, conventional esterified inulin with different DP were tested and demonstrated slight antifungal activity [18]. In the current research, for the first time the antimicrobial activity of long-chained inulin acetates with different DA was tested against seventeen microorganisms (Gram-positive and Gram-negative bacteria, yeasts and fungi) and the data were

Table 3: Emulsion stability (in % separated phase) prepared with different concentration inulin acetates (DA = 2.5) and oil phases

Inulin acetate concentration, g/L	Separated phase, %			
	Oil	Water	Emulsion	Sediment
20% oil phase				
0.05	20	68	4	0
0.1	16	72	12	0
0.2	20	70	9	1
50% oil phase				
0.05	5	30	55	0
0.1	5	27	68	0
0.2	**3**	**17**	**80**	0
80% oil phase				
0.05	34	16	50	0
0.1	8	22	60	10
0.2	**8**	**16**	**66**	10

Table 4: Temperature test for emulsion stability (%) of different emulsions prepared with inulin acetates

Inulin acetates, g/L	t, °C	20% oil phase			50% oil phase			80% oil phase		
		Oil	Water	Emulsion	Oil	Water	Emulsion	Oil	Water	Emulsion
0,05	−18	80	20	0	1	1	**98**	6	0	**94**
	4	0	24	**96**	0	0	**100**	6	30	64
	25	32	68	0	1	29	**70**	14	0	**86**
	50	16	28	56	15	1	**84**	16	60	24
0,1	−18	4	56	30	0	0	**100**	6	0	**94**
	4	0	56	44	50	20	30	4	0	**96**
	25	24	76	0	0	45	**55**	20	60	20
	50	8	70	24	20	0	**80**	10	0	**90**
0,2	−18	40	44	16	44	0	56	0	0	**100**
	4	0	36	**64**	60	0	40	4	0	**96**
	25	14	40	6	2	71	27	10	0	**90**
	50	20	60	20	30	0	**70**	10	0	**90**

summarized in Tabs. 5 and 6. Inulin acetates with DA 2.5 and 3.0 at concentration 1 mg/ml showed moderate antimicrobial activity against food born pathogenes *Listeria monocytogenes* ATCC 8632 and *Staphylococus aureus* 745 in the low concentrations. Its activity is comparable with the control antibiotic Biseptol (Tab. 5). However, inulin acetates did not inhibit the growth of bacteria *Bacillus subtilis* 46/H1, *Bacillus subtilis*

ATCC 6633, *Salmonella typhy* 745, yeasts (*Sacch. cerevisiae, Candida tropicalis*) and fungus *Beauveria bassiana*.

In addition, the short-chain inulin acetates (DP 7–9) showed the highest antifungal activity in the concentration of 5 mg/ml against the fungus *Beauveria bassiana* [18]. However, long chain chicory inulin acetate (DP 22) with DA 2.6 and 3.0 did not inhibit the growth of *Beauveria bassiana* (Tab. 5). Inulin esters in the concentration 1 mg/ml demonstrated low antimicrobial activity against fungi, especially *Fusarium oxysporum* and *Aspergillus niger*. In comparison, octa-*O*-acetylsucrose inhibited the growth of fungi *Penicillium* sp., *Rhizopus* sp. and *Fusarium moniliforme* at 5 mg/ml, and the yeast *Candida albicans* at 1 mg/ml [20]. In our case, inulin acetate (DA = 2.5) inhibited the growth of fungus

Table 5: Antimicrobial activity of inulin acetates in concentration 1 mg/ml expressed as diameter of zones of inhibition in mm (d_{disc} = 6 mm)

Test-microorganism	Inhibition zones, mm					
	Inulin acetate		Controls			
	DA = 3.0	DA = 2.5	Nystatin 40 µg/mL	Ampicillin, 10 µg/mL	Chlornitromycin, 250 µg/ml	Biseptol, 400 µg/ml
Gram positive						
Bacillus subtilis 46/H1	–	–	N/A	20	N/A	N/A
Bacillus subtilis ATCC 6633	–	–	N/A	N/A	N/A	12
Listeria monocytogenes ATCC 8632	14**	12*	N/A	N/A	N/A	11
Staphylococus aureus 745	–	14**	N/A	N/A	N/A	13
Gram negative						
E.coli ATCC 8739	–	–	N/A	14	N/A	N/A
E.coli 3398	11*	–	N/A	N/A	N/A	16
Salmonella typhy 745	–	–	N/A	N/A	N/A	14
Salmonella abony	–	–	N/A	–	N/A	N/A
Yeasts						
Sacch. cerevisiae	–	–	–	N/A	N/A	N/A
Candida tropicalis	–	–	–	N/A	N/A	N/A
Candida albicans	–	–	–	N/A	N/A	N/A
Candida albicans 8673	21***	16**	N/A	N/A	19	N/A
Fungi						
Aspergillus niger	8*	8	12*	N/A	N/A	N/A
Penicillium sp.	–	–	8	N/A	N/A	N/A
Beauveria bassiana	–	–	–	N/A	N/A	N/A
Fusarium oxysporum	8	8	–	N/A	N/A	N/A

Note: *low antimicrobial activity up to 12 mm, **moderate antimicrobial activity (12–18 cm); ***strong antimicrobial effect (>18 cm) "–" no inhibition, N/A–not applied.

Table 6: Antimicrobial activity of inulin acetates (DA = 2.5) expressed as diameter of zones of inhibition in mm (disc = 6 mm)

Test-microorganism	Concentration of inulin acetate, mg/ml					Controls		
	1	5	10	25	50	80% CH_3OH	Nystatin 40 µg/mL	Ampicillin 10 µg/mL
Bacillus subtilis 46/H1	-	-	-	-	-	-	N/A	**20**
Bacillus cereus	-	-	**8***	**9**	**9**	-	N/A	**22**
E. coli ATCC 8739	-	-	**8***	**8***	**12****	-	N/A	**14**
Salmonella abony	–	–	**7***	**8**	**10**	-	**N/A**	-
Candida albicans	–	–	**8***	**9**	**11**	-	-	**N/A**
Penicillium sp.	–	–	**10***	**11**	**18*****	-	**8**	**N/A**

Note: *low antimicrobial activity up to 12 mm, ** moderate antimicrobial activity (12–18 cm); *** strong antimicrobial effect (>18 cm) "–" no inhibition, N/A – not applied.

Penicillium sp. in higher concentration (from 10 to 50 mg/ml), as the moderate antifungal activity was observed at concentration 50 mg/ml (Tab. 6).

The degree of acetylation of inulin esters influenced the antimicrobial activity of inulin acetates. Inulin aceate with DA = 3.0 demonstrated the highest antimicrobial activity. The strongest antimicrobial effect of inulin ester (DA = 3.0) was demonstrated against *Candida albicans* 8673 (Tab. 5) in the low concentration 1 mg/ml. In addition, only inulin acetate (DA = 3) inhibited the growth of Gram negative bacteria (*E.coli* 3398). In comparison with inulin acetates with different DP in concentration 1 mg/ml that did not demonstrate inhibition against Gram-positive and Gram-negative bacteria [18], in this study inulin acetates demonstrated better antibacterial activity (Tabs. 5 and 6).

Moreover, the dependence of the concentration of inulin esters were tested (Tab. 6). Inulin acetate (from 10 mg/ml to 50 mg/ml) inhibited the growth of *Bacillus cereus, Escherichia coli* ATCC 8739, *Salmonella abony, Candida albicans* and *Penicillium* sp. The highest antimicrobial activity was observed at concentration 50 mg/ml. However, inulin acetate demonstrated antimicrobial activity at concentration 1 mg/ml against *Listeria monocytogenes* 863, *Escherichia coli* 3398, *Candida albicans* 8673, *Fusarium oxysporum* and *Aspergillus niger* (Tab. 5).

This is the first detailed evaluation of the antimicrobial potential of inulin acetates. The other general observation is that similarly to sucrose octaacetates, sucrose myristate and sucrose palmitate and glycerol acetates [20,25], inulin acetates did not inhibit the growth of food born pathogen *B. subtilis*. The acetyl residue attached to the inulin backbone may affect the growth of Gram-negative bacteria in comparison with sucrose laurinate and inulin and sucrose undecylate esters that possess strong antimicrobial activity [12,32].

The current research was the first reported that evaluated long chain chicory inulin acetates. Because of the demonstrated antibacterial and antifungal activity, proved by the experiments, inulin acetates could be successfully applied in cosmetics, medicine and agriculture. Due to its better antinfungal activity against *Aspergillus niger*, *Fusarium oxysporum* and *Penicillium* sp. inulin acetates could be used for development of new formula in plant protection and ecofriendly fungicide. The promising foaming and emulsifying ability of inulin acetates offers an adequate solution to problems related to treatment and adhesion over the root or leaf surfaces. These functional properties in combination with antimicrobial activity could be successfully used also for application of inulin acetates in pharmaceutical preparation and food conservation.

4 Conclusion

The current study demonstrated the efficiency of microwave irradiation for quick and effective synthesis of inulin acetate (DA = 2.5) only with catalyst sodium acetate and acetic anhydride in the absence of solvent media only for 120 s. On the basis of the calculated HLB values of inulin acetates, the evaluation of their functional properties as oil-holding capacity, water holding capacity, their foaming and emulsifying ability presented interest for their potential application in pharmacy and cosmetics. The antimicrobial activity of inulin acetates reveals a new aspect of their potential application against some plant and food born pathogens. "Green" synthesized inulin acetate were evaluated as a foaming agent, oil-in-water emulsion stabilizer and antimicrobial substance in pharmaceutical, agricultural and cosmetic preparations.

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