



## Rapid Communication

## Emulsifying properties of sugar-based surfactants prepared by chemoenzymatic synthesis

Sara Sangiorgio<sup>a</sup>, Eleonora Pargoletti<sup>a</sup>, Marco Rabuffetti<sup>a</sup>, Marina Simona Robescu<sup>b</sup>, Riccardo Semproli<sup>b</sup>, Daniela Ubiali<sup>b</sup>, Giuseppe Cappelletti<sup>a,\*</sup>, Giovanna Speranza<sup>a,\*</sup><sup>a</sup> Department of Chemistry, Università degli Studi di Milano, via Golgi 19, 20133 Milan, Italy<sup>b</sup> Department of Drug Sciences, University of Pavia, Viale Taramelli 12, 27100 Pavia, Italy

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## ABSTRACT

Sugar Fatty Acid Esters (SFAEs) are a class of non-ionic surfactants that can be synthesized from inexpensive natural resources. Depending on carbon chain length and nature of the sugar head group, SFAEs cover a wide range of hydrophilic-lipophilic balance (HLB) values, which result in tunable tenside properties and in turn relevant for a wide variety of industrial applications. Three sugar-based surfactants (6-*O*-lauroyl-, 6-*O*-palmitoyl- and 6-*O*-stearoyl-1-*O*-butyl glucopyranosides) have been prepared by a lipase-catalyzed esterification of isomeric mixture of *n*-butyl glucosides. Specifically, their interfacial features together with W/O emulsifying properties and stability over time have been finely evaluated (interfacial tension (IFT) values, W/O emulsion turbidity and water droplet size distribution), resulting 6-*O*-palmitoyl- and 6-*O*-stearoyl-1-*O*-butyl glucopyranosides as the most efficient ones.

## 1. Introduction

Surfactants (or surface-active agents) are amphiphilic compounds widely used in almost every industrial field because of their ability to reduce surface and interfacial tensions, thus stabilizing dispersed systems [1]. In recent years, due to their high worldwide consumption volumes, surfactant residues are becoming an environmental risk because they are continuously discharged into treated and untreated wastewaters, thus entering in several environmental compartments, *i.e.*, surface and marine waters, sediments and sludge-amended soils, *etc.* [2]. Environmental concerns about effects of conventional tensides, mainly derived from petroleum precursors, have increased the market demand for more benign compounds [3].

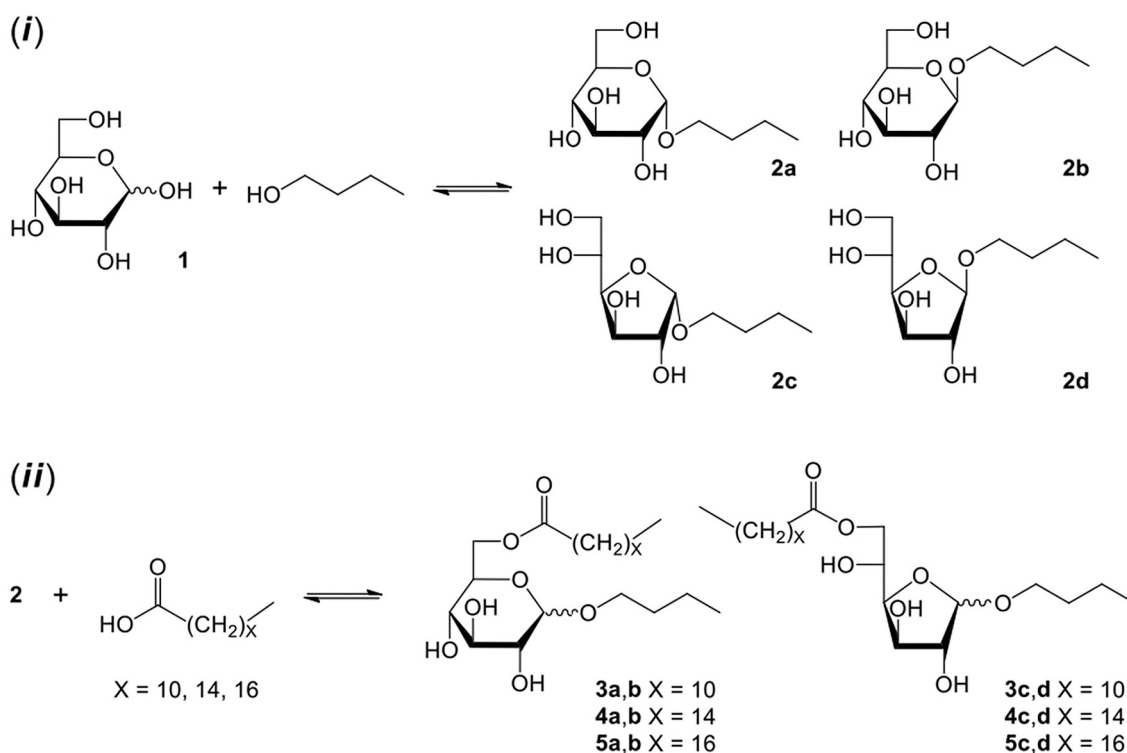
Sugar fatty acid esters (SFAEs) are non-ionic surfactants that show excellent surface and interfacial tension reduction capability, along with low toxicity and easy biodegradability. For these reasons, they are extensively used as emulsifiers in many market sectors, *i.e.* cosmetic, food and pharmaceutical industry and they are becoming important commodity chemicals [4]. Moreover, these biosurfactants can be produced from renewable resources, *i.e.* fatty acids derived from vegetable oils and animal fats as well as sugars coming from industrial waste and biomasses [5]. Furthermore, according to the nature of the hydrophilic

sugar head group and the hydrophobic carbon chain length and type, as well as the degree of esterification, SFAEs can be tuned with various hydrophilic-lipophilic balance (HLB). The resulting unique physico-chemical properties, displaced by this family of surfactants, translate into a wide range of possible applications, *i.e.* O/W or W/O emulsifiers, solubilizing agents, lubricants, penetrating enhancers and pore forming agents [6]. Today, the only sugar-based surfactants available on the market, used as emulsifiers, are sorbitan esters and ethoxylated sorbitan esters, known under the trade names of Span® and Tween®, as well as sucrose esters and alkyl polyglycosides [4].

The current industrial syntheses of SFAEs still rely on harsh reaction conditions (*i.e.*, high temperature and alkaline catalysts), which result, in most cases, in complex mixtures of products with different degrees of esterification and different acylation positions, as well as mixtures of undesired by-products, deriving from side reactions, such as sugar dehydration/caramelization [7]. Alternatively, the esterification reaction, which is crucial for the SFAEs synthesis, can be catalyzed by enzymes, specifically by lipases [8]. Because of the striking different polarity of the SFAE components, a key issue in the enzymatic synthesis is the selection of the proper solvent and/or co-solvent able to solubilize both the sugar and the fatty acid moieties without deactivating the enzyme. Polar aprotic solvents, solvents mixtures or hindered alcohols/

\* Corresponding authors at: Department of Chemistry, University of Milan, via Golgi 19, 20133 Milan, Italy.

E-mail addresses: [giuseppe.cappelletti@unimi.it](mailto:giuseppe.cappelletti@unimi.it) (G. Cappelletti), [giovanna.speranza@unimi.it](mailto:giovanna.speranza@unimi.it) (G. Speranza).



**Fig. 1.** i) D-(+)-glucose (1) derivatization with *n*-BuOH catalyzed by Amberlyst® 15. ii) Novozym® 435-catalyzed esterification of *n*-butyl-*O*-glucosides (2) with lauric, palmitic, and stearic acid in molten state.

ketones (*i.e.*, *N,N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), solution of DMSO/*tert*-butanol (*t*-BuOH); 9:1, 2-methyl-2-butanol (2M2B), methyl ethyl ketone (MEK)) have been reported [7,9]. However, to design a green process, solvents should generally be avoided, especially if hazardous to people and environment. Moreover, solvent can dramatically affect enzyme stability, thus the use of immobilized enzymes is generally preferred. The commercially available Novozym® 435 (Novozymes A/S, Denmark), a lipase type B from *Candida antarctica* (CALB) immobilized on Lewatit VP OC 1600 (Lanxess, Germany) polymethacrylate carrier, is the most used biocatalyst for SFAEs synthesis. However, in most reaction conditions reported so far, yields are generally poor [9].

A proper modification of the sugar moiety, namely its conversion into a less polar derivative, followed by solvent-free esterification could be an effective approach to overcome the above-mentioned drawbacks. Moreover, obeying to the Twelve Principles of the Green Chemistry [10], the sugar derivatization should be preferably performed by using safe, nontoxic, and environmentally friendly reagents, solvents, and catalysts under mild conditions. Among the various strategies to reduce the polarity of a sugar moiety, two reactions can be considered, *i.e.* acetalization and Fischer glycosidation. The former approach consists in the generation of sugar acetals: it is known that, in the presence of an acid catalyst, aldehydes and ketones react with suitably arranged diols of saccharide derivatives to give cyclic acetals [11]. These protected sugars can be enzymatically esterified, but the acetal groups have to be selectively hydrolyzed to restore the polar head of the surfactant. This experimental approach was not pursued because i) protection/deprotection steps have been demonstrated not to be economically feasible for the industrial production of tensides [12] and ii) glucose monoesters have been found to have poor surfactant properties [13].

Instead, the second strategy is based on the synthesis of glycosides, namely saccharide derivatives in which the sugar is bonded to a non-sugar moiety (aglycone) *via* a glycosidic bond. In the case of Fischer glycosidation, the reaction is performed in the presence of an alcohol

and an acid catalyst, thus affording *O*-glycosides as products [11]. These sugar derivatives show an improved solubility in molten fatty acids with respect to unprotected sugars and the aglycone moiety of the glycoside esters does not need to be removed, thus being included in the final surfactant structure.

Although several studies on the enzymatic synthesis of glycoside esters have been carried out [12,14], to the best of our knowledge, only few data on the physicochemical performances of these compounds have been reported so far. Thus, the aim of this study is the enzymatic synthesis of some glycoside esters, namely 6-*O*-lauroyl-, 6-*O*-palmitoyl- and 6-*O*-stearoyl-1-*O*-butyl glucopyranosides, and the assessment of their surface properties, in particular the emulsifying capabilities.

## 2. Experimental

### 2.1. Materials

All solvents and reagents were purchased from Merck Life Science (Milano, Italy) and were used without further purification. Electrospray ionization mass spectra (ESI-MS) were recorded on a Thermo Finnigan LCQ Advantage spectrometer (Hemel Hempstead, Hertfordshire, UK). NMR experiments were performed at 298 K on 400 MHz Bruker NMR spectrometer (Bruker Corporation, Billerica, MA, USA) equipped with a z-gradient coil probe. Chemical shifts ( $\delta$ ) are given in parts per million (ppm) and were referenced to the solvent signal (DMSO-*d*<sub>6</sub> and deuterium oxide (D<sub>2</sub>O),  $\delta_{\text{H}}$  2.50 ppm,  $\delta_{\text{C}}$  39.52 ppm and  $\delta_{\text{H}}$  4.79 ppm from TMS, respectively). The fatty acid profile of commercial sunflower oil (see Supplementary data) was determined by gas chromatography/mass spectrometry (GC/MS) analysis, after base-catalyzed transmethylation, using the protocol FIL-IDF 182:1999, reported elsewhere [15].

### 2.2. Synthesis of *n*-butyl-*O*-glucoside isomeric mixture

D-(+)-Glucose (1) was suspended in dry *n*-butanol (*n*-BuOH) (final

concentration 0.1 M) in the presence of the strongly acidic cation exchange resin Amberlyst® 15 (10%, w/w) and 3 Å molecular sieves (25%, w/w), at 120 °C (Fig. 1 (i)). After 2.5 h, the reaction was stopped by filtration of the solid catalyst, the alcohol was removed under reduced pressure and the reaction mixture was submitted to flash chromatography (dichloromethane (DCM)/methanol (MeOH); 9:1) to give *n*-butyl-*O*-glucosides (**2**) as a viscous syrup (Y = 91%). The isomeric mixture of glucosides (**2**) was characterized by TLC, ESI-MS and NMR analysis (See Supplementary data). NMR signals were identified by comparison with data reported in the literature [16]. D-(+)-Glucose, Amberlyst® 15 and molecular sieves were dried at 90 °C overnight prior to use.

### 2.3. Synthesis of 6-*O*-lauroyl-, 6-*O*-palmitoyl and 6-*O*-stearoyl-1-*O*-butyl glucopyranosides

Isomeric mixture of *n*-butyl-*O*-glucosides (**2**), fatty acids (lauric, palmitic and stearic acids) in molar ratio 1:1 and Novozym® 435 (10%, w/w) were mixed together and charged into a round-bottom flask. The mixture was heated to 80 °C while rotating the flask by means of a glass oven B-585 Kugelrohr. After fatty acids melted, the reactions were performed under reduced pressure (30 mmHg) (Fig. 1 (ii)). After 8 h, reaction mixtures were taken up in ethyl acetate (EtOAc) and the immobilized enzyme was removed by filtration. Then, the esters were extracted in EtOAc (2 times) from 1 M NaOH, the organic phases were collected, dried over Na<sub>2</sub>SO<sub>4</sub> and the solvent was removed under reduced pressure. The most abundant products were isolated by flash chromatography (*n*-hexane/EtOAc; 2:8), thus affording three SFAEs: 6-*O*-lauroyl-1-*O*-butyl glucopyranosides (**3a,b**) (Y = 29%), 6-*O*-palmitoyl-1-*O*-butyl glucopyranosides (**4a,b**) (Y = 56%), 6-*O*-stearoyl-1-*O*-butyl glucopyranosides (**5a,b**) (Y = 65%), which were fully characterized by TLC, ESI-MS and NMR analyses (See Supplementary data, Paragraphs 1 and 2).

### 2.4. Solubility measurements

To assess the solubility of the products, 6-*O*-lauroyl- (**3a,b**), 6-*O*-palmitoyl- (**4a,b**) and 6-*O*-stearoyl- (**5a,b**) 1-*O*-butyl glucopyranosides (5 mg) were added to different solvents (2 mL, see Table S1 in Supplementary data, Paragraph 4) and further mixed using a hand-operated laboratory piston-type homogenizer (at 3000 rpm, 30 s; Vortex Mixer, VELP Scientifica, Usmate, Italy). Some of them were also submitted to a heating step (at 50 °C for 30 min) to improve the product solubility (see Table S2 in Supplementary data).

### 2.5. Interfacial tension (IFT) measurements and emulsifying properties

The sunflower oil/water interfacial tension (IFT) values were measured at (25 ± 1) °C by means of Gibertini tensiometer following the Du Noüy ring method and by varying the amount (in the range between 0.004 and 0.350%wt in oil) of 6-*O*-lauroyl- (**3a,b**), 6-*O*-palmitoyl- (**4a,b**) and 6-*O*-stearoyl- (**5a,b**) 1-*O*-butyl glucopyranosides. Prior to tensiometric measurements, several parameters were introduced in the relative software to set up the method, such as liquid density, platinum ring and wire radii, required by the Harkins-Jordan correction. Data were reported as average values on three different replicates.

Water (milli-Q) in sunflower oil (W/O) emulsions were prepared using a Thermo Fisher Q700 sonicator equipped with a 3 mm-titanium alloy microtip. The operative conditions contemplate a frequency of 20 kHz in pulsed mode (3 s on and 3 s off) at 50% amplitude for 45 s. Preliminary tests regarding the W/O emulsion formation were carried out to select the best conditions: two different concentrations (4.5 and 6.0 mM solubilized at 80 °C) of 6-*O*-palmitoyl-1-*O*-butyl glucopyranoside (as model) and three phase volumes ( $\Phi = 0.16, 0.14$  and  $0.13$ ) were adopted. Once optimized the experimental conditions (4.5 mM,  $\Phi = 0.13$ ), stability tests were performed also with 6-*O*-lauroyl- and 6-*O*-

stearoyl-1-*O*-butyl glucopyranosides by means of confocal microscopy and turbidimetric measurements over time (from 1 h to 1 month).

Droplets size distribution of both fresh and aged emulsions was evaluated by processing (by ImageJ software) the images (droplets number up to 150) acquired by Nikon A1 laser scanning confocal microscope (LSCM) working in oil immersion (NA1.4) equipped with a 60× objective. Before each analysis, emulsions were stained with Rhodamine B (a dye soluble only in the water phase) and samplings in the central part of each vial (where the emulsions showed whitish color typical of well-emulsified systems) were made. Images were acquired with an excitation wavelength of 561 nm and emitted signal was detected between 770 and 620 nm.

To evaluate the emulsions stability within 72 h, turbidimetric method [17] was adopted by following the absorbance value (Shimadzu UV-Vis spectrophotometer UV-2600) of the prepared samples at a fixed wavelength, in a 1 cm path length optical cell. Since the investigated systems showed a decreasing absorbance trend in the 400–700 nm range, a wavelength of 550 nm was chosen. Turbidity ( $\tau$ ) was calculated according to the equation reported elsewhere [17], together with the turbidity ratio (R) which is a useful, rapid and simple method to evaluate emulsions stability against sedimentation. R is defined as the ratio of turbidity at high and low wavelengths (in the present case 700 and 450 nm,  $R = \tau_{700} / \tau_{450}$ ; see in the Supplementary data Paragraph 3). Then, the slope of turbidity ratio over time was calculated within 50 min. No samples dilution was performed according to Bai et al. [18].

## 3. Results and discussion

### 3.1. Green credentials of SFAEs

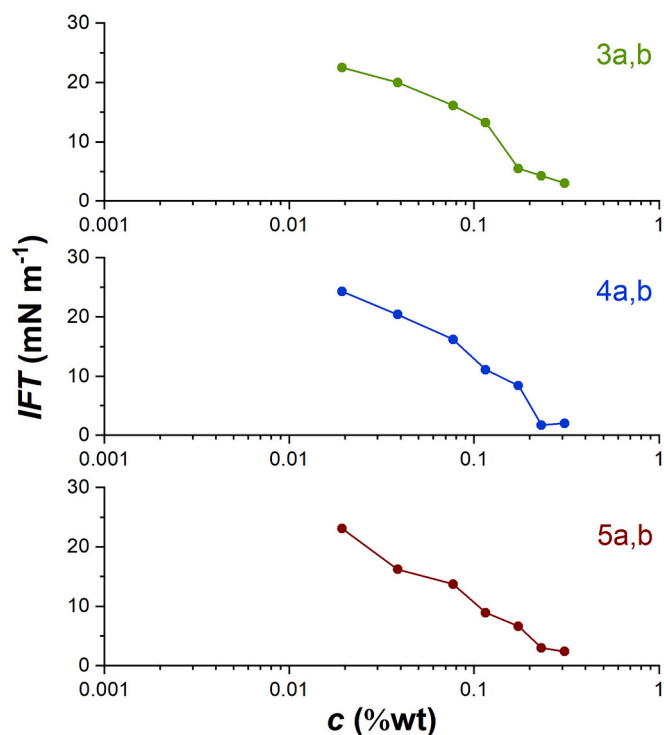
The building blocks used to synthesize the tensides here reported fulfil some of the Green Chemistry principles. In particular, glucose, which constitutes the sugar-based polar head of the surfactant, is readily available from starch and lignocellulose biomass [19], in compliance with the paradigm of Circular Economy.

*n*-Butyl glucoside was selected as sugar derivative because it seemed to be a protected sugar apolar enough to be soluble in molten fatty acids. In addition, according to Prat and co-workers [20], *n*-BuOH is classified as a green and recommended solvent because it meets criteria of safety, occupational health, environment, quality and risk of impurities, as well as general industrial constraints, *i.e.*, boiling point, freezing temperature, density, recyclability, and cost. Moreover, it is the shortest chain naturally occurring alcohol immiscible with water, thus favoring its removal from the reaction mixture. Amberlyst® 15, selected as acid catalyst for the Fischer glycosidation, is a commercially available macroreticular catalyst, prepared from cross-linking of styrene with divinyl benzene followed by sulfonation and it finds application as heterogeneous catalyst in a broad range of non-aqueous organic syntheses, even at industrial scale [21].

Finally, saturated fatty acids (lauric, palmitic and stearic acids) used as acyl donors in the enzymatic esterification are the main components of vegetable oils, which are historically and currently one of the most important renewable feedstocks of the chemical industry [22].

### 3.2. Synthesis of *n*-butyl glucoside esters and their characterization

The Fischer glycosidation, carried out with *n*-BuOH as a solvent in the presence of Amberlyst® 15 as an acid catalyst, led to an isomeric mixture of *n*-butyl-*O*-glucosides (**2**) with different ring sizes and configuration of the anomeric carbon (Fig. 1 (i)). It is known that the proportion of the various glycosidic forms ( $\alpha$ -,  $\beta$ -pyranosides, **2a**, **2b** and  $\alpha$ -,  $\beta$ -furanosides, **2c**, **2d**) present in the equilibrium mixtures at the completion of the reaction depends upon the relative thermodynamic stabilities of the isomers, which are governed by solvent, temperature, type of the sugar and chain length of the alcohol [11]. In our hands, the relative ratio between the glycoside isomers, namely **2a/2b/2c/2d**,



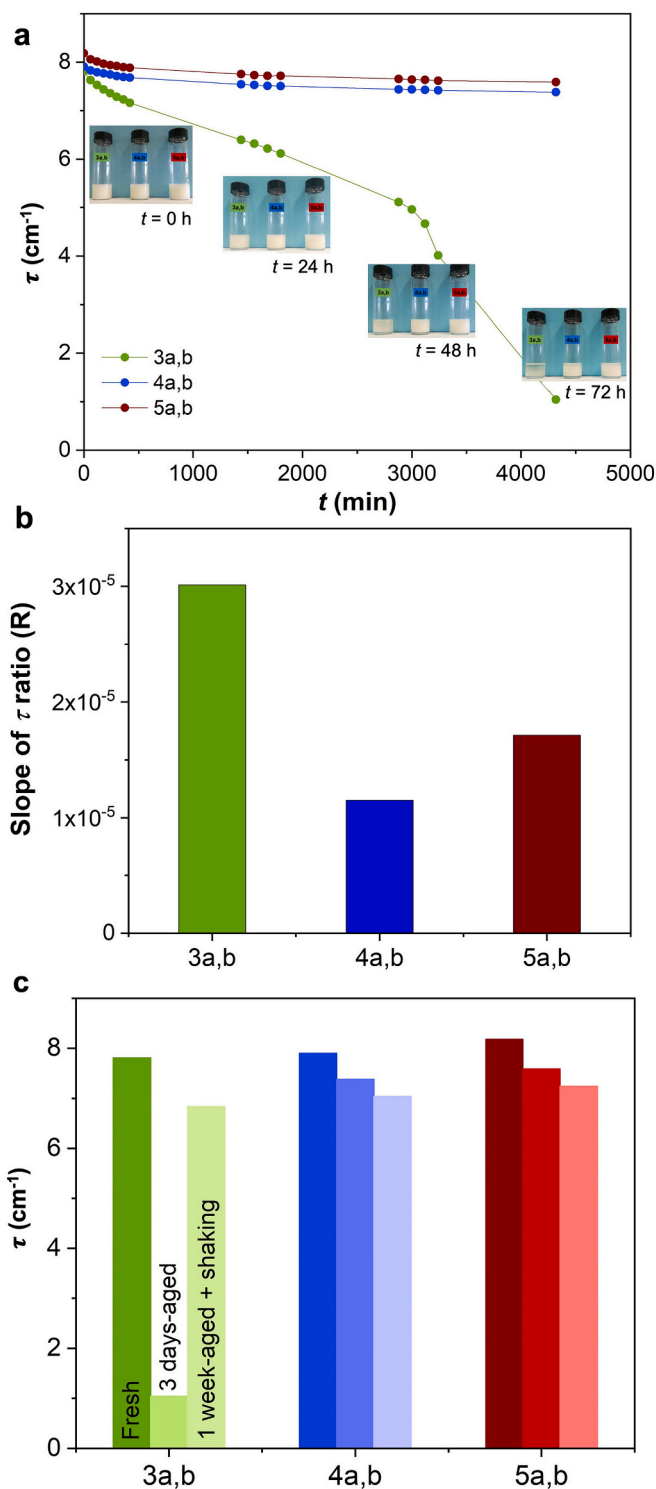
**Fig. 2.** Comparison of sunflower oil/water interfacial tension (IFT) data by varying the amount of 6-*O*-lauroyl- (**3a,b**), 6-*O*-palmitoyl- (**4a,b**) and 6-*O*-stearoyl- (**5a,b**) 1-*O*-butyl glucopyranosides in the range 0.004 and 0.350%wt in oil. Data were reported as average values on three different replicates at room temperature. The error bars are within the symbols and all below  $1 \text{ mN m}^{-1}$ .

resulted to be 46:38:6:10, by  $^1\text{H}$  NMR analysis in  $\text{D}_2\text{O}$  (see Supplementary data, **Fig. S6**). Such values were estimated as the ratio of the areas of anomeric proton signals of each isomer present in the reaction mixture.

This isomeric mixture of *n*-butyl-*O*-glucosides (**2**) was submitted to a Novozym® 435-catalyzed esterification with lauric, palmitic, and stearic acid in a solvent-free system, at  $80^\circ\text{C}$  and under reduced pressure (30 mmHg) (**Fig. 1 (ii)**). A glass oven B-585 Kugelrohr was used to carry out the reactions in place of magnetic stirring to avoid enzyme beads wrecking, thus favoring water removal and shifting the equilibrium towards the products formation.

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra, recorded in  $\text{DMSO-}d_6$ , of the reaction products (**3**, **4**, **5**) demonstrated that the enzymatic esterification is highly regioselective: only the acylation of the primary hydroxyl group was observed, with the  $\alpha$ -anomer prevailing over the  $\beta$ -one ( $\alpha/\beta$  ratio *ca.* 6:4).

Then, solubility tests in common polar, apolar and polar aprotic organic solvents were carried out (see Supplementary data). All the synthesized surfactants are soluble in almost all solvents tested (Table S1), except for water and alkanes (*n*-hexane and *n*-heptane). Particularly, in the latter cases, a slight increase of solubility occurred after a heating step at  $50^\circ\text{C}$  for 30 min (Table S2). These behaviours can be explained by using the Hansen parameters ( $\delta_D$ ,  $\delta_P$  and  $\delta_H$  for dispersive, polar and hydrogen bonding components, respectively) of the solvents (Table S1), representing a powerful tool to understand the solubility features. As it can be seen from the Table S1, water and alkanes, being at the extremes of the polarity scale, are unable to solubilize the prepared sugar-based derivatives; instead, solvents characterized by multiple components ( $\delta_D$ ,  $\delta_P$  and  $\delta_H$ ) allow the complete solubilization, as a result of their affinity towards the amphiphilic character of surfactants.



**Fig. 3.** a) Turbidity values ( $\tau$ ) of the prepared emulsions over time at fixed wavelength (550 nm). Insets: photos of the three samples at 0, 24, 48 and 72 h; b) slope of turbidity ratio ( $R$ ) values as a function of the adopted surfactant; c) turbidity values of fresh and 3 days-aged emulsions (at fixed wavelength of 550 nm) alongside with  $\tau$  data of the 1 week-aged samples subjected to a further agitation.

### 3.3. Interfacial features and emulsions stability against sedimentation

Sugar-based surfactants usually find application as emulsifiers in cosmetic, food, and pharmaceutical industries since they are odourless, tasteless and usually biodegradable, fulfilling physiological,

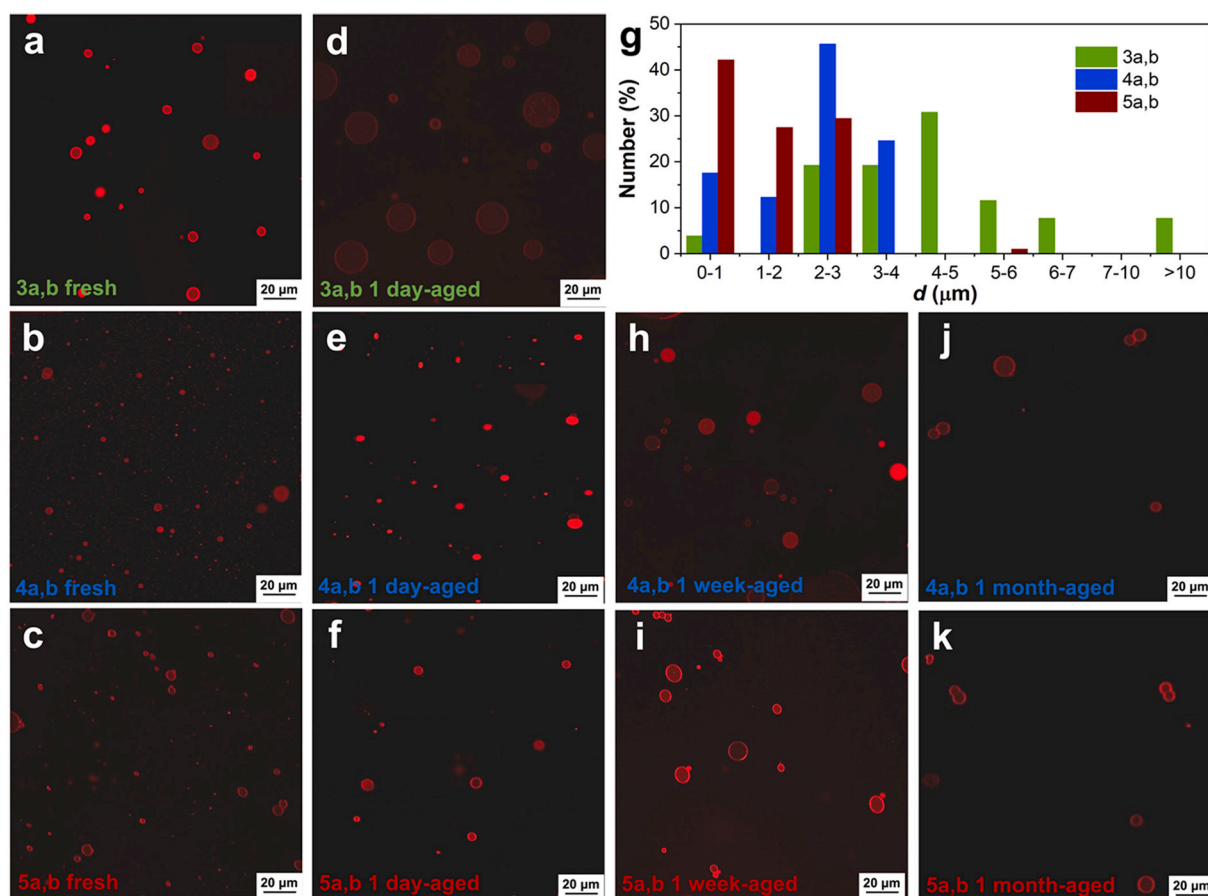


Fig. 4. LSCM images of *a-c*) freshly prepared, *d-f*) 1 day-aged, *h-i*) 1 week-aged and *j-k*) 1 month-aged 6-*O*-lauroyl- (**3a,b**), 6-*O*-palmitoyl- (**4a,b**) and 6-*O*-stearoyl- (**5a,b**) 1-*O*-butyl glucopyranosides emulsions; *g*) relative droplets size distribution (as number percentage) of the fresh samples.

dermatological and biological requirements.

To test the emulsifying properties of the three synthesized *n*-butyl glucopyranoside esters, IFT studies were performed according to the Du Noüy ring tensiometric method [23]. All the surfactants (Fig. 2) allowed the sunflower oil/water IFT reduction from  $26 \text{ mN m}^{-1}$  to a value lower than  $3 \text{ mN m}^{-1}$  at concentrations above 0.130%wt comparable to previously reported results with similar surfactants [18,24]. In order to corroborate these promising surface properties, emulsifying behaviour and stability over time were assessed by turbidity measurements [25] and microscopy images. Concerning the former, the change in turbidity over time is strictly related to the emulsion-breaking processes since  $\tau$  decreases at any wavelength with the increase of destabilization phenomena [17]. Fig. 3 (a) exhibits gradually decreasing curves in the case of 6-*O*-palmitoyl- (**4a,b**) and 6-*O*-stearoyl- (**5a,b**) 1-*O*-butyl glucopyranosides, whereas a sharp  $\tau$  reduction is appreciable for 6-*O*-lauryl-based (**3a,b**) surfactant. Notably, both **4a,b** and **5a,b** resulted sufficiently stable up to 72 h, whereas **3a,b** molecule caused the sedimentation of the water droplets already after 48 h. Furthermore, the magnitude variation of the emulsion instability was evaluated by determining the turbidity ratio (R) together with its slope within the first 50 min Fig. 3 (b). As reported by Song et al. [17], the less stable emulsion, the faster the turbidity ratio decrease; in the present case the slope was minimized adding **4a,b** and **5a,b** tensides, thus representing the most stable systems with respect to **3a,b** one. This is probably due to the shorter alkyl chain of the sugar derivatives thus limiting the affinity with the oil phase (characterized by traces of lauric acid [26], see Paragraph 5 in the Supplementary data), as also corroborated by both photos in insets of Fig. 3 (a) and confocal microscopy images (Fig. 4). Indeed, micrographs of the freshly prepared emulsions (Fig. 4 (a-c)) show very small droplets for all the three systems (1–20 μm) due to the ultrasound-assisted

emulsification technique [27]. However, the relative distribution is quite different: the **3a,b**-based one exhibits a maximum centred around 4–5 μm, conversely the other two systems are characterized by an average diameter lower than 3 μm (Fig. 4 (g)). In addition, the obtained formulations were evaluated over time and in accordance with the turbidity results, after an ageing of 1 day (Fig. 4 (d-f)), a faster destabilization can be appreciated only in the case of the 6-*O*-lauryl-1-*O*-butyl glucopyranosides presence (average  $d \geq 20 \mu\text{m}$ , Fig. 4 (d)). After 1 week, a five-fold droplets size increase can be noticed for both **4a,b** and **5a,b**-based systems (Fig. 4 (h,i)) whereas the third molecule (e.g., **3a,b**) leads to a faster concomitant droplets growth (by Ostwald ripening) and sedimentation. A further destabilization of **4a,b** and **5a,b**-based emulsions was achieved after 1 month, clearly observing the droplets aggregation into larger ones for **5a,b** and concomitantly a huge decrease of the droplets number (Fig. 4 (k)). On the contrary, the palmitoyl moiety seems to preserve the drops interlayer therefore resulting in a slightly greater stability against sedimentation (Fig. 4 (j)). Indeed, by analyzing the commercial sunflower oil composition through GC/MS analysis, a two-fold content of palmitic acid with respect to stearic acid is appreciable (See Supplementary data, Paragraph 5). Therefore, a moderately better stabilization of the emulsions generated in presence of **4a,b** with respect to **5a,b** could be due to the higher relative affinity of the palmitoyl derivative with the oil phase.

In order to confirm the previous outcomes, turbidimetric tests were carried out on the 1 week-aged emulsions after simple hand-agitation till the recovery of a milky system. Fig. 3 (c) shows the obtained results: all the adopted surfactants are able to partially restore the starting conditions, even if the **4a,b** and **5a,b** molecules seem to be the optimal ones.

#### 4. Conclusion

Herein, three sugar-based surfactants have been enzymatically synthesized in good yields and high purity by means a solvent-free system, starting from a modified sugar to enhance its solubility in molten fatty acids. The physico-chemical properties of these surfactants, in terms of sunflower oil/water interfacial tension and W/O emulsifying capability, have been deeply investigated. For all surfactants a IFT reduction of around  $20 \text{ mNm}^{-1}$  allows the stabilization of W/O emulsion prepared by ultrasound-assisted method, that guarantees a very fine droplet size distribution ( $\langle d \rangle$  around  $3 \mu\text{m}$ ). To the best of our knowledge, stability tests of the prepared glucoside esters-assisted emulsions, through turbidimetric data and confocal microscope images, have never been reported so far. Our results indicate that both 6-O-palmitoyl- (**4a,b**) and 6-O-stearoyl- (**5a,b**) 1-O-butyl glucopyranosides generate the most stable W/O emulsions, avoiding the sedimentation even after one month ageing.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.colcom.2022.100630>.

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