Graphene hetero-multilayer on layered platinum mineral Jacutingaite (Pt_2HgSe_3): Van der Waals heterostructures with novel optoelectronic and thermoelectric performances

Comparison of glycine-acyl surfactants prepared from black soldier fly fats, coconut oil and palm kernel oil.

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Short running title: Glycine-acyl surfactants from black soldier fly fat, cococnut-and palm kernel oil

ABSTRACT

Black soldier fly (*Hermetia illucens*) larvae are a new source of high quality bio-based materials that can be implemented for technical applications. Black soldier fly larvae can be bred in high numbers on small surfaces and on organic waste streams, making large scale industrial breeding possible. Fats from the black soldier fly are very rich in lauric acid and the fatty acid profile resembles that of palm kernel and coconut oil. Therefore, similar applications of black soldier fly fats to these plant-derived oils can be envisaged.

The aims of this work were (1) to use black soldier fly fat, palm kernel and coconut oil to synthesize a glycine-acyl surfactant by means of a Schotten-Baumann reaction; (2) to determine the yield and purity of the reaction products; and (3) to determine solubility, foaming capacity, surface tension and critical micelle concentration of the surfactants in comparison to a commercially-available glycine-coconut oil surfactant, Amilite GCS-11®.

The average yield of each reaction was satisfactory (70% or higher). The in-house synthesized surfactants had a fatty acid profile similar to the fatty acid profile of the initial fat/oil. All in-house synthesized surfactants showed similar properties, regardless of the source of the fat/oil, but they were slightly less performant regarding foaming capacity compared to the commercial surfactant.

It is concluded that black soldier fly fats are a proper alternative to coconut or palm kernel oil to prepare glycine-acyl surfactants.

Key words: black soldier fly fats, *Hermetia illucens*, glycine-acyl surfactant, surface tension, CMC, foaming

1. INTRODUCTION

The overexploitation of natural resources has an enormous impact on the environment, and therefore the development of novel products with a smaller impact and of no concern for human health is of crucial importance. The search for novel materials is not only driven by the decline in fossil resources that will eventually become limited, but also by the impact that human activities have on the planet e.g. in waste production, depletion of natural resources and destruction of natural ecosystems. The industry is increasingly using plant materials as renewable sources for oleochemical applications [1].

However, also when using plant materials, the impact on the environment is considerable [2], as the increase in demand for vegetable oils and biofuels contributes to tropical deforestation, habitat fragmentation and loss in biodiversity [3,4]. In addition, the use of valuable land for non-food/feed crops is a point of criticism.

Therefore, more research for alternative sources of biobased chemicals is needed, especially for the use of biomaterials for non-food/feed applications.

Another large impact of human activities on our planet is the production of food and resulting associated waste, such as organic food-waste streams [5,6]. If these wastes can be converted into profitable biomass, the ecological and economic burden of this waste can be reduced. Ideally, in a circular economy, this waste will be efficiently turned to useable, environmentally-friendly biomaterials with no risk for human or environmental health. Insects may contribute to the development of this circular economy and in fact they have already found initial implementation in the food and feed sectors [7].

Insects can also be used as a source for biomaterials such as fats, proteins and chitin, which may find diverse uses in (chemical) industry. By coupling the breeding of the insects to waste stream reduction [8], insects may become a sustainable alternative in the production of biomaterials (e.g. fats). A survey clearly stated that consumers are willing to try insect-based technical products, but depending on the product type there are barriers that need to be addressed [9]. In addition, also regulatory steps need to be taken into account before insects can be used in a circular economy setting.

Examples of the use of insects, can be found in the work that has been done on the black soldier fly (BSF, *Hermetia illucens*). BSFs are relatively easy to breed in large quantities on small surfaces. The larvae can be bred on organic waste streams [10] and the resulting fats are being explored for their potential use in biofuels [11,12]. In addition, fats of the BSF have also been used as partial butter replacement in bakery products [13]. Other species like the mealworm, crickets and locusts mainly find application in food and feed as these oils are rich in unsaturated fatty

acids (>60%) and may contain omega-3 fatty acids which indicate nutritional higher value [14]. However, to the best of our knowledge, only our lab reported on the implementation of insect fats/oils in technical products.

Recently, we have shown that fats extracted from locusts and crickets can be used in cosmetic hand cream formulations [15]. Fats of BSFs contain a large proportion of lauric acid and as a consequence it is not ideal to use in leave-on cosmetic applications because of its impact on skin integrity [16,15]. However, the BSF fatty acid profile resembles palm kernel and coconut oil and therefore BSF fats may be used in similar applications as these oils. As BSFs can be bred in massive quantities on small surfaces, the rearing of these insects may prove to be a very attractive alternative to palm and coconut plantations.

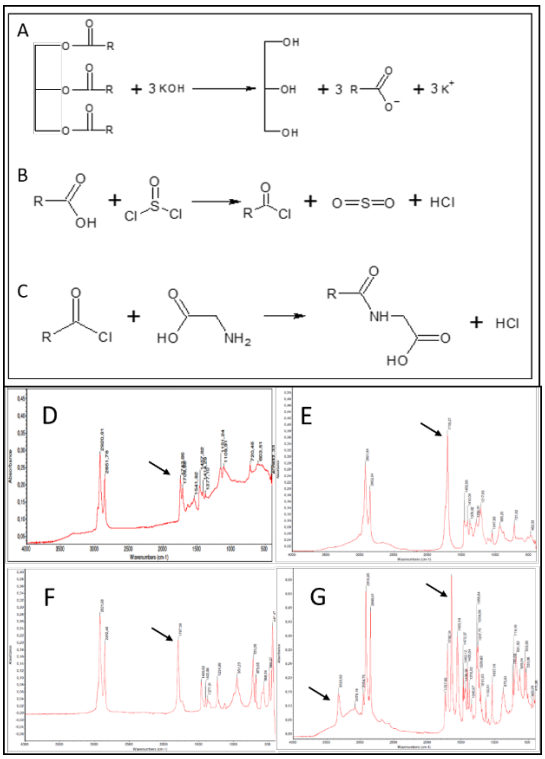
Amino acid based surfactants are gaining the interest of scientists as they are considered to be biodegradable and less toxic than traditional surfactants. They can be synthesized using renewable sources and their ease of degradability in harmless byproducts makes them safer for the environment [17]. Recently, N-acyl amino acid (Gly, Ala and Ser) surfactants have been prepared using a thermal amidation protocol using castor oil and cottonseed oil as sources for the apolar fatty acid chain [18, 19]. Given the use of coconut oil for the commercial preparation of Gly-surfactants (e.g. Amilite GCS-11®), we investigated the production of this type of amino acyl surfactant based on fats from the BSF.

In this paper, BSF fats, palm kernel oil (PKO) and coconut oil (CCO) were used to synthesize a glycine-acyl surfactant. The yield of the reactions, as well as several parameters of the synthesized surfactants were compared to Amilite GCS-11®, a commercially available, chemically similar glycine-coconut oil surfactant.

2. RESULTS AND DISCUSSION

2.1. Synthesis of surfactants

Figure **1.** Schotten-Baumann synthesis of amino acid - acyl surfactants (A-C) and FTIR spectra of products obtained during and after the synthesis (D-G).



Legend: Overview of the Schotten-Baumann synthesis procedure: hydrolysis of fat to fatty acids (A), formation of fatty acyl chlorides (B) and formation of glycine-acyl surfactant (C). Panel D-F show the FTIR profiles of intact fat (D), free fatty acids (E), fatty acyl chlorides (F) and glycine-acyl surfactants (G).

Several batches of Gly-based fatty acid surfactants were made, using palm kernel oil, coconut oil and black soldier fly fat. Three batches of BSF fats were used. One batch was derived from BSF pre-pupae that were obtained from our own breeding capacity at Thomas More and the 2 other batches were obtained from 1 of two commercial breeders. FTIR was used to evaluate the formation of the desired products in each of the 3 different synthesis steps (Fig. 1 D, E, F and G). Fig. 1D presents an image of intact fat with a characteristic ester-stretch at approximately 1740 cm^{-1}. Free fatty acids with a free carboxyl-stretch at approximately 1706 cm^{-1} are shown in Fig. 1E. Fig. 1F shows fatty acyl chlorides with a stretch at approximately 1790 cm^{-1}. The glycine-acyl surfactant with characteristic N-H stretch of the amide-group at approximately 3300 cm^{-1} and the C=O stretch of the amide at 1650 cm^{-1} is shown in Fig. 1G.

The yields of the fat hydrolysis to fatty acids (step A, figure 1) and of the coupling reaction of Gly to fatty acid chlorides (Figure 1, step C) of the reaction are presented in Tables 1 (step A) and 2 (step C). The exact yield of reaction B was not determined but is close to 100%.

The average yield over all fat hydrolysis reactions was 86.3% (+/- 10.2%). Individual fat hydrolysis reactions ranged from 70% up to > 95% (Table 1). An ANOVA analysis showed that there were significant differences in

the yield of the fat hydrolysis depending on the source (p = 0.0067). Post-hoc testing of the different fat sources indicated that the BSF-fats from source 1 had significantly less yield compared to BSF-fats from source 3 (p = 0.046) and from palm kernel oil (p = 0.046). Other fat sources gave similar high yields.

Table **1.** Average yield and standard deviation of fat hydrolysis in small scale (20 g fat) and upscaled reactions (5x and 10x). N= number of experiments/reactions.

Sample	**Scale**	**N**	**Fat hydrolysis (%)**	
			Ave	SD
BSF source 1	1x (20 g)	9	79*	8
BSF source 2	1x (20 g)	2	95	0.5
BSF source 3	1x (20 g)	2	97*	1
Coconut oil	1x (20 g)	4	90	10
Palm kernel oil	1x (20 g)	2	97*	0.3
BSF source 1	5x (100 g)	1	73	
Coconut oil	5x (100 g)	1	89	
BSF source 1	10x (200 g)	1	89	

Legend: An ANOVA analysis comparing all 1x scale reactions showed statistically significant differences (p = 0.0067). * individually statistically different groups.

The overall average yield of coupling glycine to the fatty acid chloride was 71.5% (+/- 11.5%) (Table 2), with individual reaction efficiencies ranging from 50% (initial attempts) up to approximately 90%. There was no statistical difference in reaction yields (1x reactions) based on the fatty acid chloride source. The yields of the 5x reaction sizes seemed larger but this was not statistically tested because only 1 (Coconut oil) or 2 (BSF source 1) reactions were run at the 5x scale.

Table **2.** Average yield and standard deviation (SD) of the coupling reaction of Gly to fatty acid chlorides at small scale (5 g fatty acid) and upscaled (5x and 10x) reactions (Number of reactions N=21 in total).

Sample	**Scale**	**N**	**Gly-coupled to FA**	
			Mean	**SD**
BSF source 1	1x (5 g FA)	7	67.1	7.1
BSF source 2	1x (5 g FA)	2	60.4	1.7
BSF source 3	1x (5 g FA)	3	77.4	11.8
Coconut oil	1x (5 g FA)	2	64.6	5.9
Palm kernel oil	1x (5 g FA)	3	73.6	16.8
Coconut oil	5x (25 g FA)	1	83.8	

BSF source 1	5x (25g FA)	2	83.4	2.6
BSF source 1	10x (50g FA)	1	77.6	
All		21	71.5	11.5

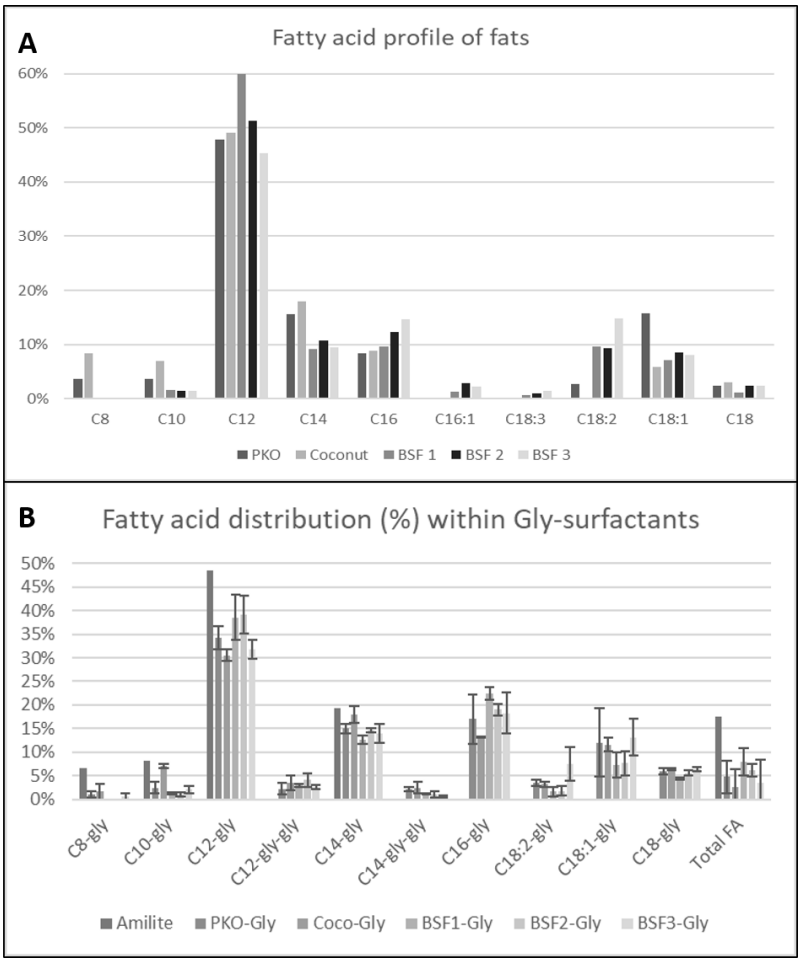
Legend: ANOVA analysis comparing the yields of the 1x reactions was not statistically significant

In conclusion, synthesis of glycine-acyl surfactants can be achieved efficiently using the Schotten-Baumann procedure in which a fatty acid chloride reacts with the amino group of glycine to form a surfactant. We observed that similar yields for this procedure are obtained, regardless the source of the fat (from BSF, coconut or palm kernel oil), with the exception of the fat-hydrolysis step. In that step, we observed a statistically significant lower yield for BSF fats from source 1. This difference is most likely related to the fat quality as fats from source 1 were stored for a longer period.

2.2. Surfactant and fatty acid profile

Fig. 2A shows the fatty acid profiles of the BSF fats (derived from 3 different sources), palm kernel oil and coconut oil (measured with GC-FID (N=1 of each fat/oil)). Fig. 2B shows the profiles of the corresponding glycine-surfactants (N=3 for each type of fat/oil; determined by LC-MS). MS detection was done in the negative mode and relative peak areas were used as a semi-quantitative measure as no standards were used for quantification. The general distribution of the fatty acid profile is roughly reflected in the distribution of the fatty acid chains of the surfactants. The surfactants are pure and contain little remaining free fatty acids (indicated in Fig. 2B as Total FA; typically <5 Area%). It is also noted that double Gly-bound C12 and C14 surfactants are formed as well. Some differences can be observed in the profiles of the initial oil samples and derived surfactant-mixtures. For all sources, Gly-C12 was produced less than expected and Gly-14, Gly-C16, Gly-C18 were overrepresented. Given the semi-quantitative determination of the surfactant profiles with LC-MS, no statistical test was performed to determine the significance of the observed differences between surfactant profile and initial fatty acid profile.

Figure **2.** (A) Fatty acid profile of BSF, coconut and palm kernel oil (N=1) and (B) fatty acid chain distribution in Gly-acyl surfactants (N=3) and of Amilite GCS-11®(N=1).



Legend: For the fats, only 1 sample was profiled (N=1). For the Gly-surfactants, the SD is based on 3 replicates (N=3), for Amilite GCS-11® only 1 sample was profiled (N=1)

The surfactant molecules show a fatty acid profile which on average reflects the overall fatty acid profile in the initial fat/oil source. However, there are differences in the profiles among individual samples, which may need to be taken into consideration if the products are commercially exploited. In comparison to the in-house synthesized surfactants, Amilite GCS-11® shows a less complex profile, containing a large fraction of C12-Gly (>48%), C14-Gly (19%), C10-Gly (8%) and C8-Gly (6%), but not of larger fatty acids coupled to Gly (no C16-Gly or C18-Gly). Also more than 15% free fatty acids are present in the Amilite-surfactant.

2.3. Solubility

Solubility data are presented in Table 3. Basically all in-house synthesized surfactants behave the same and are slightly less soluble compared to Amilite GCS-11®. Solubility was pH dependent and at pH 11 solubilities of 10

g/L were obtained. At 20 g/L the in-house synthesized surfactants were soluble after heating, but they were not stable and a precipitate was formed at room temperature, while Amilite GCS-11® remained dissolved.

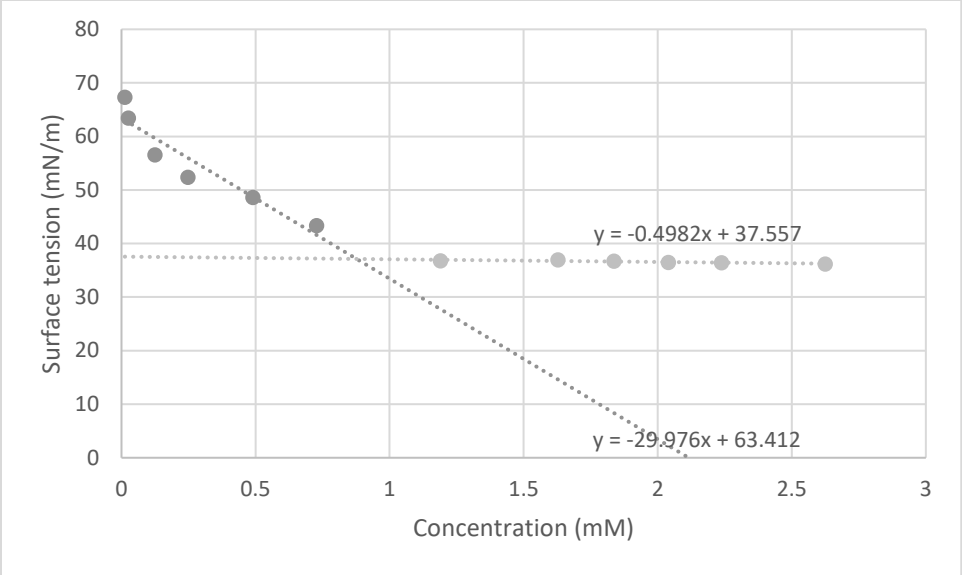
Table **3.** Solubility of the Gly-surfactants (N=1).

Surfactant	**pH 7**	**pH 9**	**pH 11**	**pH 13**
Amilite GCS-11®	< 1g/L	1 g/L	10 g/L	20 g/L after heating stable upon cooling to RT
BSF-Gly / CCO-Gly / PKO-Gly	< 1g/L	1 g/L	10 g/L	20 g/L after heating, but not stable upon cooling to RT

2.4. Surface tension reduction and CMC

Fig. 3 shows an example plot of the surface tension reduction as a function of the concentration of the surfactant solution. The surface tension of the solution dropped until the Critical Micelle Concentration (CMC) was reached. From this concentration onwards, the surface tension remained constant at a minimum value. The CMC was calculated based on the crossing point of the regression line drawn between the points below the CMC and the regression line drawn between the points where the surface tension remained constant.

Figure **3.** Example plot of the surface tension reduction in function of the amount of surfactant added.



The average CMC and the average minimal surface tension of the different surfactant batches are shown in Table 4. The average minimal surface tension (STmin) was very similar among the in-house synthesized surfactants and ranged from 35.9 up to 36.8 mN/m. These values are slightly higher than the value of 35.4 for Amilite GCS-11®.

An overall ANOVA indicated that the average STmin was significantly different among groups ($p = 0.025$). Comparing groups 2 by 2 using the Tukey HSD procedure indicated that only BSF 3_Gly was significantly different from Amilite GCS-11®. The average CMC-values ranged from 0.82 mM up to 1.2 mM, which were lower than the 3.14 mM observed for Amilite GCS-11®. ANOVA indicated that these CMC-values were significantly different among groups ($p = 0.0023$). The Tukey HSD procedure showed that the CMC of Amilite GCS-11® was significantly higher compared to all in-house synthesized surfactants (all p-values < 0.01) and that there were no statistically significant differences in CMC among any of the in-house synthesized surfactants.

Table **4.** CMC (mean and SD) and ST min (mean and SD) of surfactants. N= number of independent samples of surfactants that were used to determine the CMC and STmin

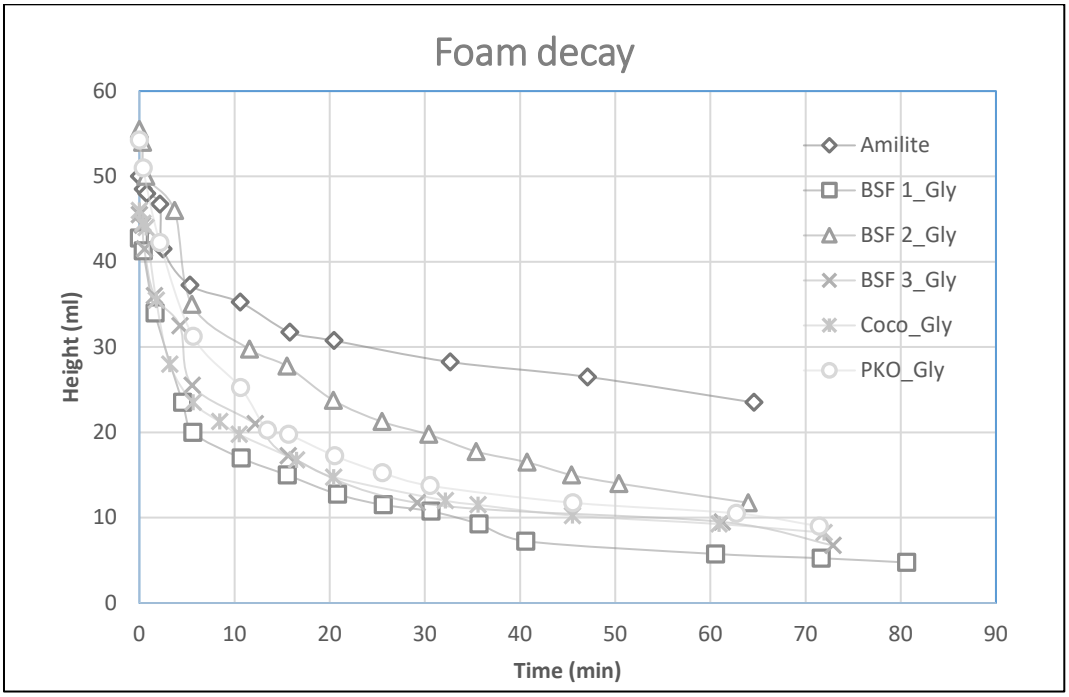
Sample	**ST min (mN/m)**		**CMC (mM)**	
	mean	**SD**	**mean**	**SD**
Amilite GCS-11® (N=2)	35.4	0.1	3.14	0.46
BSF 1_Gly (N=4)	36.5	0.3	1.15	0.26
BSF 2_Gly (N=3)	36.6	0,1	0.82	0.56
BSF 3_Gly (N=3)	36.9	0.4	1.20	0.16
PKO_Gly (N=3)	35.9	0.6	0.86	0.45
CCO_Gly (N=2)	36.6	0.1	1.08	0.09

Zhang et al. (2013) [18] also synthesized a glycine-cocoyl surfactant which had a minimal surface tension of 33 mN/m and a CMC of 0.21 mM. The differences between all these surfactants are relatively low and may be the result of small differences in composition between the commercial surfactant, the Zhang et al. surfactant and the in-house synthesized surfactants from this study. Moreover, the minimal surface tension and the CMC in Zhang et al. [18] were obtained by a Wilhelmy plate method, using a different tensiometer model, which may also explain the slightly different values. Liu et al. [19] also prepared different amino-acyl surfactants (glycine, alanine, serine) using castor and cottonseed oil. Whereas the CMC values were 1.09 mM and 0.856 mM for the glycine surfactant based on castor oil and cottonseed oil, the minimal surface tensions were 39 mN/m and 35 mN/m, respectively. This larger difference in CMC between the castor oil derived glycine-surfactant with the cottonseed oil and the glycine-surfactants based on BSF, palm kernel and coconut oil may be due to the unique composition of the castor oil, which contains > 90% ricinoleic acid (C18:1, an omega-9 unsaturated fatty acid containing a hydroxyl-group). Cottonseed, palm kernel, coconut and BSF fats/oils contain a larger variety of different chain-length fatty acids.

2.5. Foaming capacity

In Fig. 4 the initial foam height and the foam decay in function of time are shown for Amilite GCS-11®and for the in-house synthesized Gly-surfactants. Initial foam heights were similar for all surfactants; however, the foam decayed faster for the in-house synthesized surfactants than for Amilite GCS-11®. The differences in foam height between samples were evaluated at selected timepoints using ANOVA, followed by 1-to-1 comparisons correcting for multiple testing. There were no differences in foam heights at timepoints up to 15 min. At 15 min the ANOVA analysis indicated differences among the average foam heights between groups (p= 0.045). Although 1 to 1 analyses between groups were not significant (Tukey HSD, all p-values are > 0.05), borderline significant differences were observed among BSF source 2 and Amilite GCS 11® (p=0.056), and CCO and Amilite GCS-11® (p=0.062). At the 30 min timepoints, the ANOVA was highly significant (p= 0.0034) and individual comparisons indicated that the Amilite GCS-11® foams height was larger than the foam heights of the other groups (with the exception of BSF_S3 all foam heights were significantly different from Amilite GCS-11® with $p < 0.05$). Similar results were obtained at timepoint 60 and 90 min. When comparing in-house synthesized surfactants based on the BSF or on a plant-derived surfactant (coconut oil or palm kernel oil), no statistical differences were observed in foam height at the selected timepoints (all p-values > 0.05).

Figure **4.** Initial foam height and foam decay as a function of time.



Legend: Initial height (mL) and decay of the foams as a function of time (min). Each datapoint is the average of 3 independent measurements (N=3). Error bars are omitted for clarity of the figure.

The differences in the observed solubility (paragraph 2.3) and foaming properties between Amilite GCS-11® and the in-house prepared surfactants might be explained by the differences in the (fatty acid) profile between Amilite GCS-11® and the in-house synthesised surfactants, but also by the occurrence of some unidentified contaminants, which may be present in the in-house synthesized products. However, the fact that all in-house prepared surfactants behave similarly suggests that the type of the fat/oil that is used as starting material, is not the cause of variation, and that in-house prepared surfactants based on BSF fat behave equally well as in-house prepared surfactants based on coconut oil or palm kernel oil.

3. MATERIALS AND METHODS

3.1. Black Soldier Fly

Black soldier flies were obtained from 3 different insect breeding facilities (3 sources), 1 facility was our own breeding facility at the Thomas More campus and the two other sources from where BSF larvae were obtained were 2 commercial insect breeders (BESTICO B.V. and Protix). The exact source of each batch of BSF was anonymized in the text by labeling them BSF 1, 2 and 3.

Black soldier flies were bred at the Thomas More campus Geel in a greenhouse at average day temperature of 30 °C and relative humidity of 50-90 %. The breeding process is similar to the process described in [20]. Female flies deposit their eggs in cardboard structures. Eggs were harvested and placed shielded from light until they hatched. For this study, the larvae were cultivated on chicken feed. When they reached the pre-pupae stage, the pre-pupae migrated out of the chicken feed and were harvested. The pre-pupae were quickly frozen using a Coldline blast freezer and were subsequently dried by freeze-drying in a Büchi Lyovapor L-200. The dried material was stored until further processing. The BSF larvae that were obtained from the 2 insect breeders were processed similar to the pre-pupae.

3.2. Chemicals and fats

All chemicals, sodium hydroxide, ammonium acetate, acetonitrile, methanol, hexane, acetic acid, ethyl acetate, petroleum ether (40-65 °C), NaCl, thionylchloride, BF_3, 2,2,4-trimethylpentane, 2,6-di-tert-butyl-4-methylphenol used for the synthesis, purification and analytical procedures were bought from Sigma-Aldrich and VWR Chemicals (Belgium). The standards capric acid, lauric acid, myristic acid, palmitic acid, oleic acid and linoleic acid were purchased at Nu-Check Prep Inc (Minnesota, USA). The internal standards methyl-undecanoate and methyl-heptadecanoate were obtained at Sigma-Aldrich. Coconut and palm kernel oil were kindly provided by Eastman and Oleon, respectively. Amilite GCS-11® was kindly provided by Ajinomoto-Omnichem.

3.3. Extraction of BSF fats

The dried BSF-pre-pupae were grinded prior to the fat extraction. About 3 L of hexane was added to 1.5 kg of ground insect material. The suspension was stirred for 1 h at room temperature and was then filtered using an air press filter, separating the hexane/fat mixture from the remaining solid fraction (consisting mainly of chitin and proteins). The hexane was subsequently removed and recycled by evaporation in a rotavapor device.

3.4. Fatty acid profile determination with GC-FID

The fatty acid composition was determined by gas chromatography coupled to FID using a GC 2010 plus (Shimadzu Benelux b.v.). For the separation of the fatty acids, a HP5 column (30 m, 0.25 µm) of Agilent Technologies was used. The column temperature gradient started at 170 °C, increased with 3 °C/min until 230 °C was reached, which was kept for 15 min, Injector temperature was set at 250 °C.

For the standard solution, 25.0 mg of the standards capric acid, lauric acid, myristic acid, palmitic acid, oleic acid and linoleic acid was dissolved in 2.0 mL 2,2,4-trimethylpentane (HPLC quality) with 50 mg/mL 2,6-di-tert-butyl-4-methylphenol. As internal standard mix 500.0 mg methyl-undecanoate and 50.0 mg methyl-heptadecanoate were dissolved in 5.0 mL trimethylpentane with 50 mg/mL 2,6-di-tert-butyl-4-methylphenol.

The reference mix was made with 200 µL of the standard mix and 200 µL of the internal standard mix. This was dried under nitrogen.

Test samples contained 150 mg of fat, dissolved in 5.0 mL 2,2,4-trimethylpentane with 50 mg/mL 2,6-di-tert-butyl-4-methylphenol. An amount of 1.0 mL was mixed with 200 µL internal standard mix and dried under nitrogen.

1.5 mL 20 g/L NaOH in methanol was added to the samples and reference mix. These samples were heated in a heating bath at 100 °C. After cooling down, 2.0 mL BF_3 was added. This was again heated in a heating bath of 100 °C for 30 min. After cooling down, 1.0 mL 2,2,4-trimethylpentane was added and the samples were shaken for 30 sec. An amount of 3.0 mL of saturated NaCl was added and the samples were shaken for 15 sec. The 2,2,4-trimethylpentane fraction was transferred to a vial, washed with 1.0 mL H_2O, and the sample was dried with water-free sodiumsulphate. One µL of the sample was injected on the GC column in split mode.

3.5. Fourier transform infrared spectroscopy

Infrared spectra of the fats were acquired on a Thermo Scientific Nicolet iS5 FTIR spectrometer with a iD7 ATR single reflection diamond module. Each spectrum was the result of averaging 16 scans taken at a resolution of 4 cm^{-1}. All spectra were measured at 21 °C.

3.6. LC-MS analysis of surfactants

The composition of the surfactants (qualitative analysis) was determined by liquid chromatography (Waters 2695 separations module and Waters 996 Photodiode Array Detector) coupled to mass spectrometry (Waters micromass

ZQ) (LC-MS). A small amount of surfactant was dissolved in a mixture of butanol/acetic acid/ water (40/20/10) and filtered to remove remaining particles. The LC-MS was run at 30°C on a C18 3.5 µm column (4.6x100 mm XTerra), over a linear gradient ranging from 95% buffer A (2 mM ammonium acetate + 0.04% acetic acid) and 5% buffer B (2 mM ammonium acetate + 0.04% acetic acid in a mixture of water/acetonitrile (10/90) to 30% buffer A and 70% buffer B during 12 min and subsequently over a linear gradient (11 min) to 100% buffer B, at which it was kept for 5 minutes. A 1µL injection volume was used and mass spectrometry was performed in negative ionization mode with operating scanning of m/z 100-600, scan time of 1 sec with an inter-scan delay of 0.3 sec.

3.7. Surface tension and CMC

Surface tension measurements were done on an Attention Sigma Force Tensiometer 700 (Biolin Scientific) using a Du Nouy ring approach. A starting volume of 40 mL ultrapure water was used, from which sequentially a specific volume was subtracted, followed by the addition of the same volume of a 0.01 M glycine-surfactant solution. These steps were repeated, thereby gradually increasing the concentration of the Gly-surfactant in the solution. The decrease of the surface tension was monitored in function of the amount of glycine-surfactant added. The surface tension was then plotted against the surfactant concentration. From this plot the minimal surface tension and the CMC were determined. For each surfactant tested, the experiment was performed 3 times.

3.8. Solubility in function of pH

Solubility (at room temperature) was evaluated by attempting to dissolve specified amounts (1 g, 10 g, 20 g) of surfactant in water at different pH values. The substance was considered dissolved when a clear solution was obtained.

3.9. Foaming capacity

The foaming capacity of a 0.01 M solution of the surfactants was evaluated by pouring 30 mL of a 0.01 M surfactant solution in a 100 mL cylinder (*in triplo*). The cylinder was turned upside down 5x and the initial foam height (expressed in mL) and foam decay (expressed in mL) was measured as a function of the time.

3.10. Schotten-Baumann synthesis of glycine-acyl surfactants

Amino acid-acyl surfactants were synthesized by the Schotten-Baumann reaction [21]. In a first step, the fats were hydrolyzed to free fatty acids and glycerol (Fig. 1A). Twenty gram of fat (BSF, palm kernel oil or coconut oil) was dissolved in 100 mL ethanol (99%) in a 500 mL flask. An amount of 8.4 g KOH was added and the reaction mixture was heated at 50 °C for 1 h. A volume of 200 mL tap water (45 – 50 °C) was added to the mixture and the reaction mixture was extracted 3 times with 80 mL petroleum ether. The water phase, containing the fatty acid salts, was retained and the organic phase discarded. 1M HCl was added to the water phase in order to decrease the pH < 2. The protonated free fatty acids were extracted with petroleum ether (80 mL). The organic phase was dried with $MgSO_4$ and then filtered. The petroleum ether was removed by vacuum evaporation in a rotavapor.

The second step of the reaction involved the formation of fatty acid chlorides (Fig. 1B).
Approximately 5 g of fatty acids was weighted in a 100 mL round-bottom flask and 3 mL thionylchloride was added. The reaction mixture was gradually heated under reflux conditions using a water bath. A gas trap was used to capture the HCl and SO_2 vapors. The reaction was heated as long as gas formation was visible in the gas trap. The reaction was cooled down to room temperature.

The third step of the reaction involved the coupling of the fatty acid chlorides to glycine (Fig. 1C). Approximately 5 g glycine was dissolved in 25 mL water in a 2-neck 100 mL flask and the pH was adjusted to 9 with 4 M NaOH. A volume of 25 mL acetone was added and 5 mL of the fatty acid chlorides was gradually added to the reaction mixture. The pH was again adjusted to 9. Thirty minutes after adding all fatty acid chlorides, the pH was checked and adjusted again to 9 if necessary. The mixture was stirred at room temperature overnight. Hereafter the mixture was acidified with 2 M HCl to a pH < 2. A precipitate was formed, which was filtered with a Buchner filter. The surfactants were washed with 40 mL warm water (approximately 50 °C) during 10 min. The mixture was filtered again and the surfactants were stirred with 80 mL petroleum ether during 1 h. The mixture was filtered and the surfactant was air-dried. A white powder was obtained.

The reactions described above were also upscaled 5 and 10 times, by adjusting (5 or 10 times) the amounts of products and reaction volumes. Reaction times were kept the same.

Yields of the reactions were estimated as follows. The starting materials (BSF fats, coconut oil and palm kernel oil) for the syntheses have slight differences among batches. Even if black soldier flies are reared under standard

condicition on the same substrate, the extracted fats will have a slightly different composition. Therefore, average molecular weights (of fats, fatty acids and glycine-fatty acid surfactant molecules) were used for the calculation of expected weights under the assumption of 100% efficient reactions. Average molecular weights of fatty acids of 224, 213 and 230 g/mol were used for palm kernel, coconut and black soldier fly oil/fat, respectively The observed weights after the reactions were compared to the calculated expected weights to determine an approximate yield of the reaction.

3.11. Statistics

Statistical tests were performed in R (version 3.6.1; R Core team, 2015) in the RStudio interface (version 1.2.5001) [22, 23]. Efficiency of fat hydrolysis and surfactant syntheses were compared among groups using ANOVA, followed by post-hoc tests using the Tukey Honestly Significant Difference (HSD) procedure. P-values < 0.05 were considered significant.

The deviation from individual surfactants compared to their fatty acid content in the initial fat sample was not statistically tested due to the low number of replicates (N=3 per initial fat source) and the semi-quantitative determination of the surfactant-profile.

Degradation of foam height was evaluated at selected timepoints by ANOVA followed by post-hoc tests using Tukey HSD procedure.

CONCLUSION

As a proof of concept that insects can provide a durable source of biomaterials and may play a valuable role in a circular economy, we derived surfactants from BSF fats, which can be used for technical applications (e.g. cosmetics). Here we extracted fats from BSF, coconut oil and palm kernel oil and converted them to glycine-acyl surfactants. The surfactant molecules were subsequently characterized and compared with Amilite GCS-11®, a commercial available glycine-acyl surfactant based on coconut oil. It was shown that BSF-fats can indeed be used for the synthesis of glycine-acyl surfactants with similar performance to the commercial available Amilite GCS-11®.

According to the producer, Amilite GCS-11® is a mild and hypoallergenic surfactant with recommended use in facial wash and soap products. Based on the similar behaviour of the surfactants in our experiments, the surfactants based on BSF fats could have similar applications. However, several additional tests will need to be performed before BSF fats may be implemented for cosmetic applications. These tests include a toxicological assessment and

demonstration that the derived surfactants are safe for human use (e.g. showing no irritation potential). Especially if the BSF are reared on organic waste streams, an investigation of the potential presence of undesired contaminants such as, for example, pesticides and residual solvents, should be performed.

In order to implement BSF larvae at an industrial scale, many hurdles still need to be overcome, including changing legislation allowing to use BSF larvae and to rear them on organic waste streams, development of cost-effective and green processes to extract BSF materials and a drastic upscaling of capacity (e.g. stacking) while reducing the breeding costs. Only by solving issues in all these domains, the implementation of the BSF for technical applications may become a viable sector in a future circular economy.

CONFLICT OF INTEREST

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