Black Soldier Larvae Oil in cosmetic emulsions

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Abstract

Black soldier fly larvae oil (BSFLO) is rich in lauric acid, and its fatty acid profile is similar to that of palm kernel oil and coconut oil. Therefore, it can be considered an alternative to these vegetable oils. More than 80% of world palm oil yields come from Malaysia and Indonesia, which has caused severe deforestation of equatorial and tropical rainforests. BSFLO is produced by pressing and drying the larval phase of the life cycle of the Hermetia illucens (Black soldier fly) of the family Stratiomyidae. Fly larvae feed on biological waste, which they successfully process, so ecologically clean circular oil production is realized. Few attempts have been made to use insect oil to produce value-added products. Fats and oils are commonly used in cosmetics, which are a major component of skin care creams.

Our work aims at presenting exemplary formulations of a cosmetic product hand cream, containing purified black soldier fly larvae oil. We successfully applied a purification procedure consisting of five stages (degumming, dehumidification, neutralization, bleaching, and deodorization) for BSFLO to obtain a lighter and clearer oil suitable for inclusion in cosmetic products. NMR characterization of the oil before and after the purification showed no change in the fatty acid profile. Cosmetic cream formulations were prepared with purified and non-purified oil, and their properties were compared to formulations with palm kernel or coconut oil. Good quality products with purified BSFLO were obtained, resembling those with palm kernel or coconut oil.

Keywords: BSFLO, purification; hand cream; palm kernel; coconut oil

Introduction.

Nowadays, we are witnessing the destruction of valuable ecosystems worldwide. The deforestation of tropical forests due to palm oil production is a small part of the examples of the negative impact of human activities on nature. More than 80% of the world's palm oil production comes from Malaysia and Indonesia, which has caused severe deforestation in equatorial and tropical rainforests. In recent years, some efforts to protect the environment and its diversity have focused on including mass crops of insects as alternative sources of bioactive substances [1-4]. The larvae of the black soldier fly are of great interest because of their ability to process human and agricultural waste and, more precisely, to consume a wide range of organic materials. Larvae are also a sustainable source of protein and fat [4]. Most companies that currently breed the black fly soldier and its larvae produce high-quality protein used in animal feed, aquaculture, and fish feed [5,6]. There is also data on biodiesel production from fly larvae [7]. According to Muller et al. [4], in 2017, 222 active companies were raising black soldier flies and their larvae for protein and feed production.

The production of proteins and feed from the larvae of the black soldier fly usually takes place by drying the larvae under the sun or in ovens. As a by-product of this process, an oil is obtained, which is further mechanically squeezed from the dry larvae (pressing). For purification, the oil is filtered to separate coarse particles. BSFLO contains a high percentage of medium-chain fatty acids (C12 and C14). The content of unsaturated fatty acids is relatively low, approximately 21% [6]. Because of the high lauric acid content, the oil can be used as a raw material for detergent production for soaps and cleansing cosmetic products [8]. Although a full toxicological assessment, primarily due to the high content of lauric acid and the possible presence of unwanted contaminants such as residual pesticides and other active small molecules, is still required, one could also consider the oil a promising natural raw material for the production of leave-on products. Therefore, the development of BSFLO-based leave-on products is worthy of investigation.

Present work aimed to demonstrate the capabilities of the BSFLO to replace palm kernel and coconut oil in leave-on cosmetic formulations. As a first step, we performed the purification of the BSFLO to improve its physicochemical and sensory properties. Hand cream formulations were further prepared and characterized with different oils: purified and non-purified BSFLO, coconut, and palm kernel oils.

Materials and Methods.

Materials: The raw BSFLO was kindly provided by Nasekomo AD, Bulgaria. For the purification procedures, we used sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), dichloromethane (DCM), and charcoal. These chemicals were purchased from Merck (FOT, Bulgaria) and were of analytical grade. Deionized water (Elix purification system, Millipore) was used to prepare the solutions and during the purification procedure.

For the formulation of the cosmetic creams, we used different oils: Coconut oil (local supplier), Palm kernel oil (Biopark cosmetics), Olive oil (local supplier), Caprylic/Capric Triglycerides (IOI Oleochemical GmbH), and Shea butter (CSP Trade Ltd.). The emulsions were stabilized with a mixture of the emulsifiers Steareth 20 (Brij S20, Croda), Cetyl alcohol (Sigma-aldrich), and Stearic acid (Acros). We added several humectants in the aqueous phase: glycerin (CSP Trade Ltd.), propylene glycol (CSP Trade Ltd.), PEG-400 (Teocom Bulgaria), allantoin (Esperis Spa, Italy), and provitamin B5 (Mayam elements). Xanthan gum (Rheocare XGN, BASF SE) was added as a rheology modifier and methylparaben (Sigma-Aldrich) as a preservative. Vitamin E (Bio herbs) and a Perfume (Esperis Spa, Italy) were also added to have a composition close to that available on the market. Deionized water obtained from Elix purification system (Millipore) was used for the cosmetic prototypes.

Methods

BSFLO purification

Crude larvae oil was purified in five stages: degumming, dehumidification, neutralization, bleaching, and deodorization. The procedure for oil purification was adapted from Ref. [5]. Before the actual refining process, we centrifuged the BSFLO to remove the solids present in the original batch. This was performed at 5250 rpm for 20 minutes at 40 °C using Sigma 3K15 centrifuge.

Next, degumming & dehumidification are performed to remove phospholipids and slimy gums from the oil by treatment with water and acid. The process takes place in two steps: 1) Stirring with water to separate the water-soluble phospholipids: 7 ml of water were added to 100 g of crude oil, and the mixture thus obtained was stirred at app. 750 rpm at 70℃ for an hour. After that, the resulting mixture was centrifuged at 6000 rpm for 10 min to separate the oil. 2) Acid treatment of the oil: 0.5 g sulfuric acid (0.5%) was added to 90 g oil, and the mixture was stirred at 750 rpm at 70 ℃ for an hour. Afterward, centrifugation at 6000 rpm for 10 mins was applied.

Neutralization. The process of neutralization, also known as alkaline refining, was performed to remove free fatty acids from the oil. We added 0.2 g sodium hydroxide (10%)

to 70 g degummed oil and left the mixture to stir at 750 rpm at 80 °C for an hour. The obtained thus mixture was left overnight before the oil separation to settle the formed soaps well. The separated oil was additionally centrifuged for 15 mins at 6000 rpm before washing with water. Washing with hot water was applied until pH dropped to 7.

Bleaching was performed to remove color compounds, natural pigments, and oxidizing products. 2 g of charcoal was added to 40 g oil, the mixture was stirred on a magnetic stirrer for 30 minutes, and then centrifuged (6000 rpm, 15 min).

Deodorization was performed to remove volatile aldehydes and ketones, contributing to the unpleasant odor of the oil. 30 ml of bleached oil was dissolved in 50 ml of DCM and subjected to distillation to dryness under vacuum at 80 °C using a rotary evaporator Rotavapor R210. After removing DCM, the distillation continued with 60 ml of water at 60 °C for another 2 hours. The temperature was gradually increased to 80-90 °C until the water was evaporated entirely.

^{1}H-NMR spectra of the BSFL oil before and after applying the complete purification procedure were taken with a Bruker Avance III HD 500 spectrometer (BrukerBioSpin GmbH, Rheinstetten, Germany), operating at 500 MHz.

Cosmetic cream formulation

We have prepared four formulations of a hand cream containing different oils: crude and purified BSFL oil, as well as palm kernel and coconut oil, which are respectively named cream I, II, III and IV. The composition of the creams, given in Table 1, was chosen to be rich in humectants in order to have a composition closer to those of the current market products. The emulsifier's type and concentration was chosen to be the same as that of other similar formulations shown to have good emulsion stabilization [9].

The water and the oil phases were prepared by mild stirring and heating to 80 °C. Emulsification was performed after mixing the phases (100 g in total) utilizing an Ultra-Turrax homogenizer at 8000 rpm for 5 minutes at 75-80 °C. The perfume was added after cooling the formulations to 30 °C and additional homogenization at 8000 rpm for 30 seconds was applied. pH was adjusted to 5.5 at the end.

Table 1. Composition of hand cream formulations (it wt%) containing different medium-chain triglyceride oils: crude and purified BSFLO, coconut, and palm kernel oil.

Phase A – oil phase 25 wt %	I with crude BSFLO	II with purified BSFLO	III with coconut oil	IV with palm kernel oil
Crude BSFLO	7			
Purified BSFLO		7		
Coconut oil			7	
Palm kernel oil				7
Olive oil	4	4	4	4
Shea butter	2.5	2.5	2.5	2.5
Steareth 20	3	3	3	3
PEG -400	2	2	2	2
Caprylic/Capric Triglycerides	1	1	1	1
Cetyl alcohol	2	2	2	2
Stearic acid	3	3	3	3
Vitamin E	0.5	0.5	0.5	0.5
Phase B aqueous phase 75 wt %				
Glycerin	3	3	3	3
Xanthan gum	0.5	0.5	0.5	0.5
Propylene glycol	0.5	0.5	0.5	0.5
Distilled water	70.1	70.1	70.1	70.1
Allantoin	0.2	0.2	0.2	0.2
Pro vitamin B5	0.5	0.5	0.5	0.5
Methyl paraben	0.2	0.2	0.2	0.2
Phase C				
Perfume	q.s	q.s	q.s	q.s
Lactic Acid	q.s	q.s	q.s	q.s

The obtained emulsions were characterized by optical microscopy in order to compare the shape and size of the droplets, and the type of emulsions. Microscopic observations were performed in transmitted light with Axioplan microscope (Zeiss, Germany) having Epiplan objectives (10x, 20x, and 50x), and connected to CCD camera and a digital recorder. Emulsions were diluted with 5 mM SDS aqueous solution prior to observation.

Rheological characterization of the oils and the creams was performed with a rotational rheometer Bohlin Gemini (Malvern Instruments, UK) in cone and plate geometry (with element CP2/60) at 25 °C.

Emulsion stability was tested after tempering at 40 °C for 24 hours, freezing at -18 °C for 3 hours, tempering to 25 °C and centrifugation at 6000 rpm for 10 minutes. Extreme centrifugation was additionally applied at 15000 rpm for 20 minutes in duplicate.

Results

The BSFLO purification procedure resulted in more transparent, lighter in color, and almost odorless oil than the raw one (see Fig. 1). To determine possible changes in the oil composition, we compared the ^{1}H-NMR spectra and the viscosity of both initial and purified samples. Figure 2 shows the obtained ^{1}H-NMR spectra. Similarly to the results in Ref. [8], we have not observed any significant changes, i.e. the fatty acid content has remained the same. However, the viscosity of the purified oil was decreased (by ~ 10% at 40 °C). Note that the refined and the crude BSFLO are semi-solid at room temperature, so the viscosity was measured and compared at 40 °C, see Fig. 3. The thickness of the used palm kernel oil (solid-liquid at room temperature) and the coconut oil (semi-solid at room temperature) were also measured at 40 °C. The corresponding data is also shown in Fig. 3. The viscosity of the coconut oil practically coincided with that of the purified BSFLO. The viscosity of the palm kernel oil was slightly higher, but the other three oils had virtually identical flow curves and Newtonian behavior at a shear rate above 1s^{-1}. The values at the shear rate of 150 s^{-1} are as follows: 0.029 Pa.s for crude BSFLO, 0.027 Pa.s for purified BSFLO, 0.027 Pa.s for coconut oil, and 0.044 Pa.s for the palm kernel.

The prepared emulsions are shown in the containers (Fig. 4) and under the microscope (Fig. 5). As can be seen, no noticeable differences can be detected in the emulsion drop sizes. However, significant differences in the color were observed: the yellow color of the cream with crude BSFLO (Fig. 4A) and light yellow with the purified oil (Fig. 4B) in contrast to the white creams with coconut and palm kernel oil (Fig.4C and D respectively). Furthermore, the cream with the crude BSFLO had the specific smell of the oil, although the perfume added. The cream with the purified BSFLO was much pleasant in smell and comparable to these with coconut and palm kernel oil.

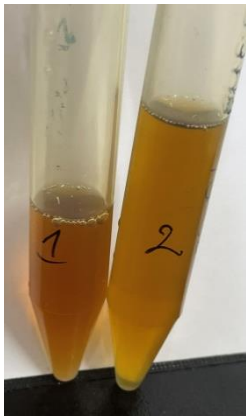


Figure 1. Test tube 1 – crude BSFLO; test tube 2 – purified BSFLO.

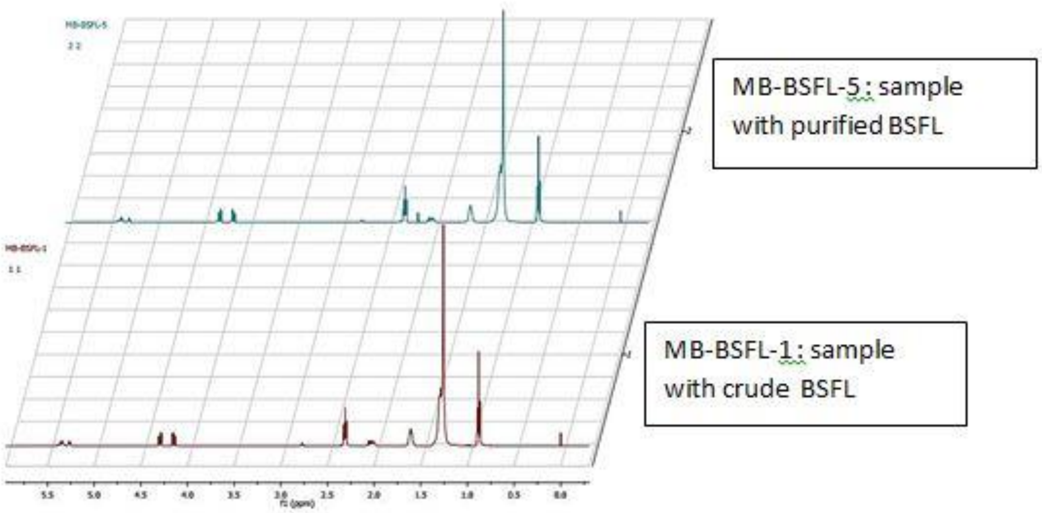


Figure 2. ^{1}H-NMR-spectra of crude and purified BSFLO.

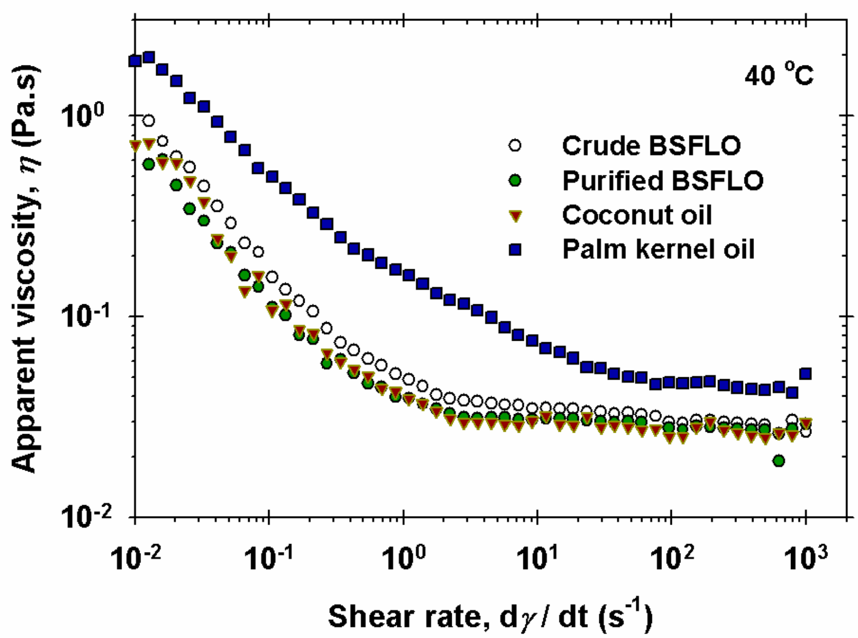


Figure 3. Comparison between the viscosities of crude and purified BSFL oil, coconut and palm kernel oils.

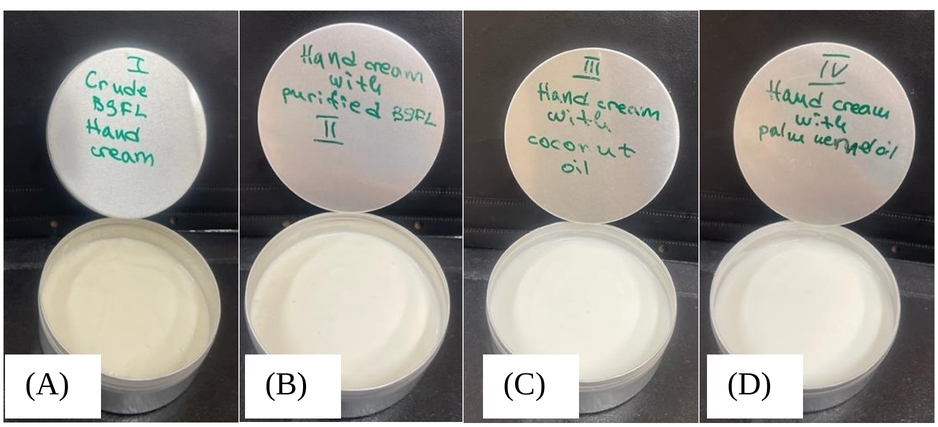


Figure 4. (A) Hand cream with crude BSFLO. (B) Hand cream with purified BSFLO. (C) Hand cream with coconut oil. (D) Hand cream with palm kernel oil.

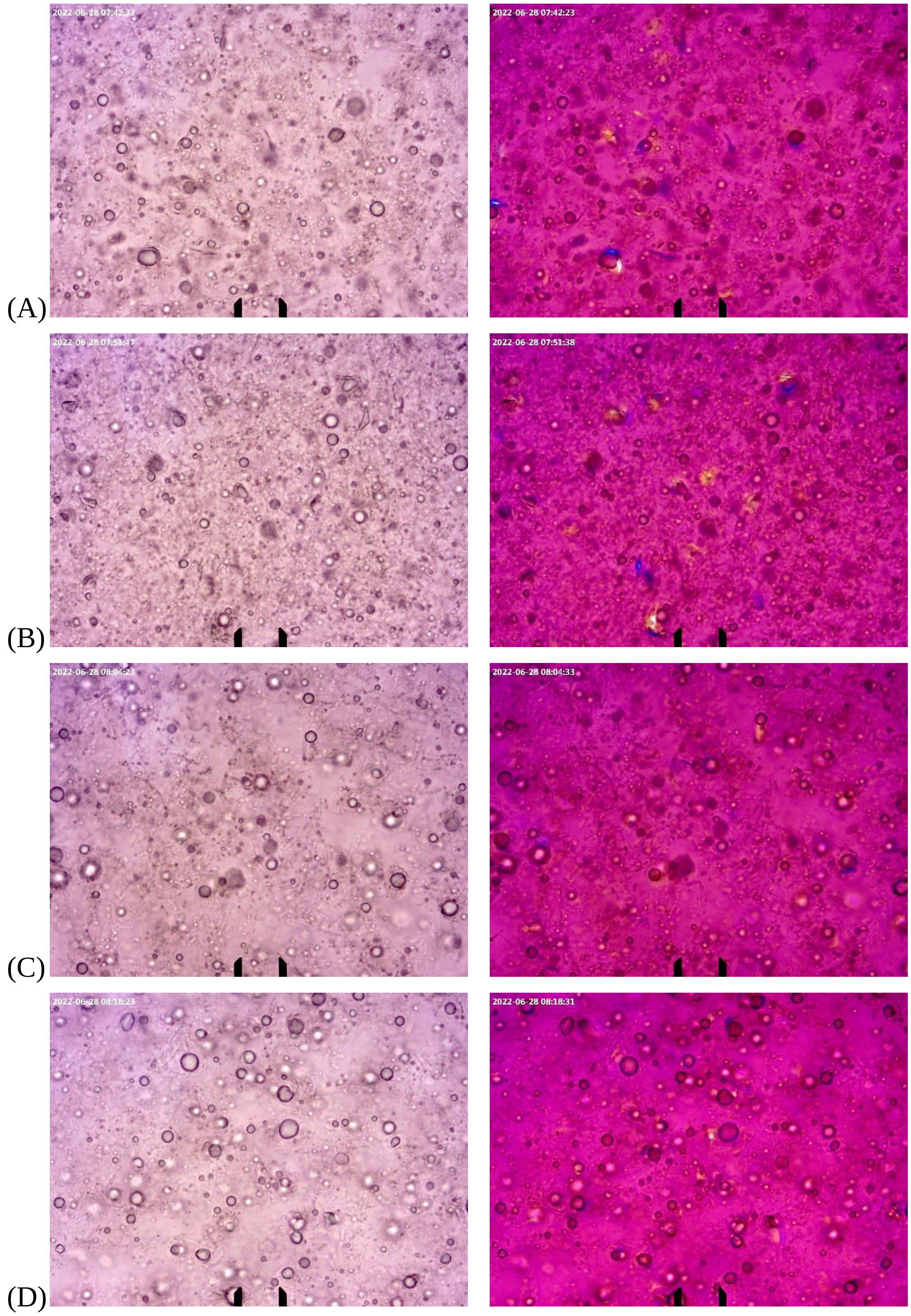


Figure 5. Microscopic pictures of hand cream formulations containing: (A) crude BSFLO; (B) purified BSFLO; (C) coconut oil and (D) palm kernel oil. The cream samples were diluted with 5 mM SDS before observations. On the left-hand side, the pictures are taken in transmitted light, while on the right-hand side in polarized. The scale bar is 20 μm.

The hand cream formulations were observed under the microscope in transmitted and polarized light. In Fig. 5, one can see that all emulsions looked similar. Oil droplets with spherical shapes were seen, and the size of the droplets in the different hand cream formulations was identical. The pictures in polarized light suggested the presence of crystalline structures in all emulsions, as evidenced by the yellow/blue color, see the right column in Fig. 5.

To characterize the rheological behavior of the prepared creams and to compare the effect of the use of BSFLO vs more commonly used oils (coconut and palm kernel oil), we performed two types of rheological experiments – (i) viscosity as a function of the shear rate characterization, and (ii) yield stress measurement. The results from the first type of experiment are shown in Fig. 6. As one can see, all hand cream formulations had almost the same viscosity values – around 667 ± 24 Pa.s at the shear rate 0.02 s^{-1}. The exception is the hand cream with added crude BSFLO – the viscosity of this emulsion was more than two times higher (almost 1500 Pa.s) than the other emulsions. This difference could hardly be attributed to the viscosity of the oil itself since the emulsion of the most viscous oil (palm kernel oil) was the same as that of the emulsions with purified BSFLO and coconut oil. The highest viscosity of the emulsion with crude BSFLO could be rather attributed to the solid admixture and lipids present in it before the purification.

The second type of performed rheological experiments were the yield stress measurements. In these experiments, one applies a shear stress (in a given interval of values), and the viscosity is measured. Before reaching the yield stress value of the sample, the measured viscosity has almost constant values (plateau region). Upon increasing the applied shear stress at a certain value, the viscosity drops, and the stress at which this drop happens is what we will set as yield stress of the hand creams. This type of measurement is one of the most commonly used tests for determining the yield stress, see Refs. [10-14]. The results obtained with the prepared emulsions are shown in Fig. 7. As it can be seen from the curves, the yield stress of the creams increased in the order: purified BSFLO < crude BSFLO < coconut oil < palm kernel oil, and the values were 9.1, 14.4, 25 and 27.4 Pa, respectively. Lowest yield stress of the purified BSFLO sample hints for very easy spreading, which could be well apprized by the customers.

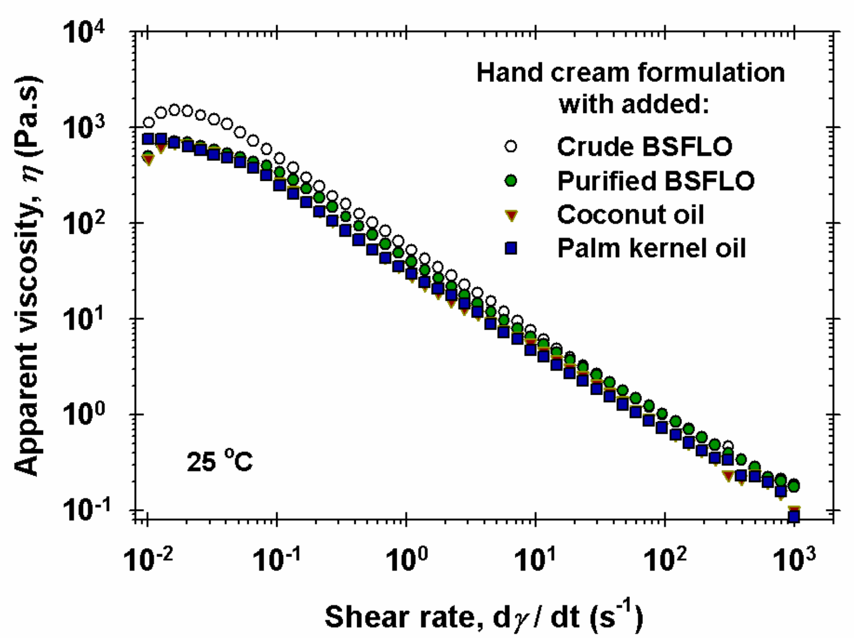


Figure 6. Viscosity of the hand cream formulations as a function of the shear rate.

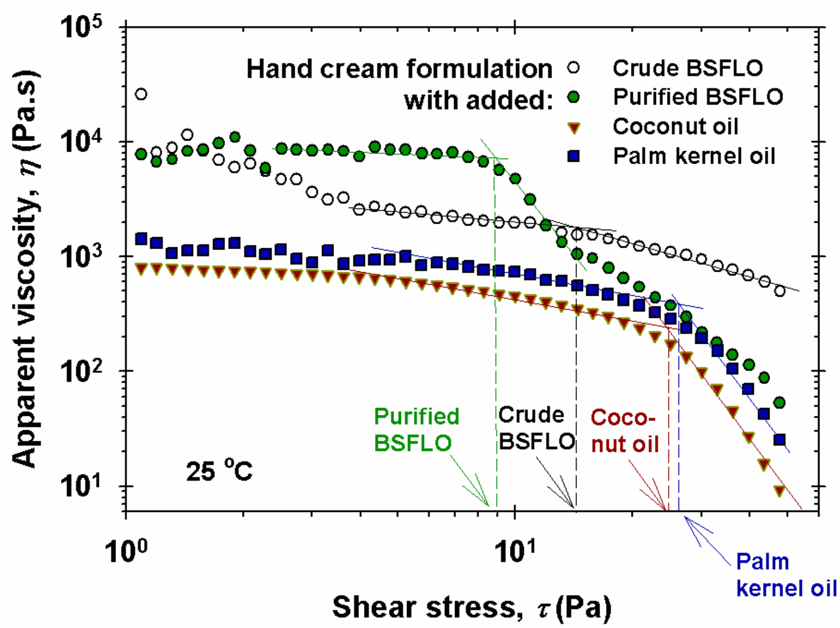


Figure 7. Viscosity vs shear stress plot for the yield stress determination of the hand cream formulations.

Small amounts of the hand cream formulations were placed in Eppendorf test tubes and subjected to stability tests using centrifugation. The samples were stored at 40 °C for 24 hours, followed by 3 hours at -18 °C, and then left to equilibrate at room temperature for 30 minutes. The centrifugation was done twice – once at speed of 6000 rpm for 10 minutes and after that (in more extreme conditions) at speed of 15000 rpm for 20 minutes. After each centrifugation, the samples were checked to see if there was any oil separation indicating for the emulsion's destruction. Such separated oil was not observed, and the emulsions were stable even after the more extreme centrifugation at 15000 rpm.

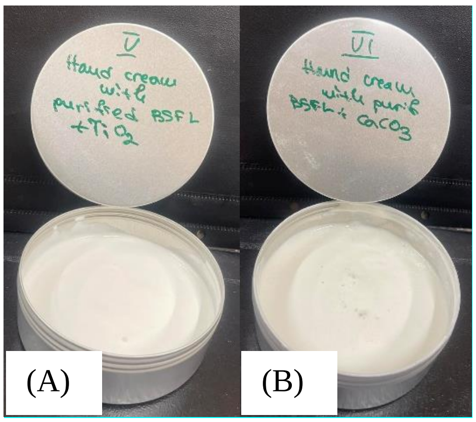
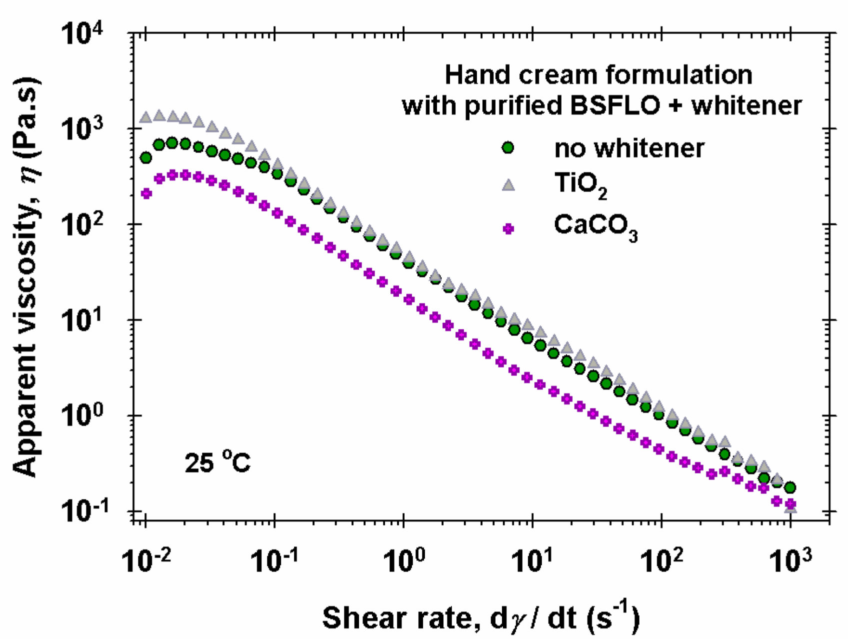
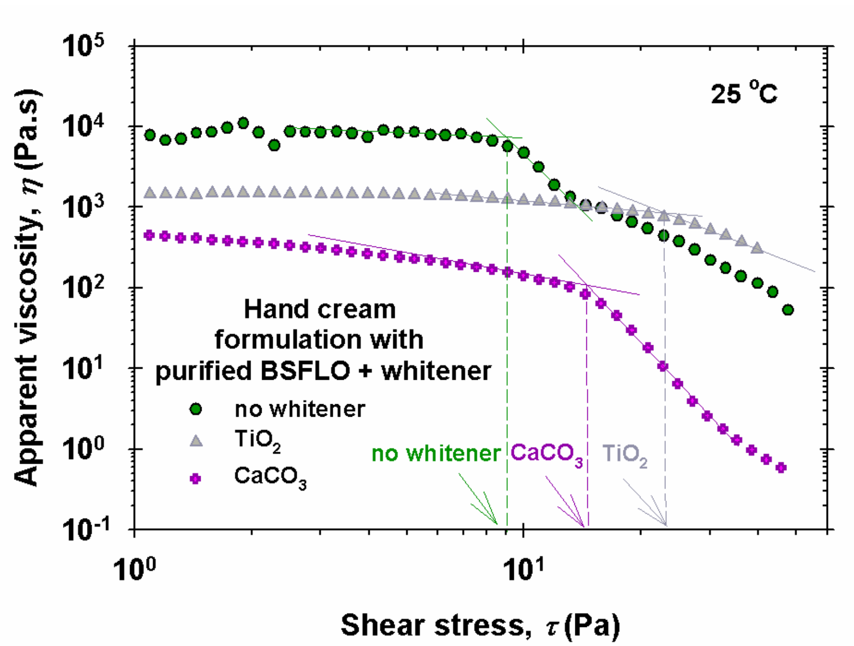


Figure 8. Hand cream formulations with purified BSFLO and added 2wt% whitening pigments: (A) TiO_2 and (B) $CaCO_3$.

Microscopic observations (Fig. 5) and rheological characterization (Figs. 6 and 7) showed good structure and consistemcy of the frmulations with BSFLO. However, the color of the emulsions was yellow (see Fig. 4), which could not be well accepted by the users expecting white cream as those with the coconut and palm kernel oil. Widely used strategy to obtain white formulations is to use a whitening pigment as titanium dioxide (TiO_2) or calcium carbonate ($CaCO_3$), for example [15]. We prepared formulations with the purified BSFLO by adding either 2 wt% titanium dioxide ot calcium carbonate. The hand creams produced using these two components are shown in Fig.8. As it can be seen, the addition of TiO_2 and $CaCO_3$ whitened significantly the color. The rheological properties of these two hand creams were also checked since the inorganic particles were expected to impact the viscosity. The measured dependences of the viscosity vs. shear rate are shown in Fig. 9A. The addition of TiO_2 led to an increase in the viscosity (up to almost 1300 Pa.s) at shear rates lower than 0.1 s^{-1}. On the other hand, the presence of $CaCO_3$ led to 2 times decrease in the viscosity in almost the whole interval of studied shear rates. In the presence of the whitener, the yield stress of the hand creams increased as it is shown in Fig. 9B. The values were as follows: with the addition of TiO_2 – 22.8 Pa and with $CaCO_3$ – 14.4 Pa. These values were comparable to the ones measured with the formulations containing coconut or palm kernel oil (25 and 27.4 Pa, respectively).



(A)



(B)

Figure 9. Rheological properties of hand cream formulation with purified BSFLO and added whiteners: (A) dependence of the viscosity as a function of the shear rate; (B) dependence of the viscosity as a function of the applied shear stress (yield stress measurement).

Discussion.

The cream formulations with the crude BSFLO were yellow and color and with the characteristic smell of the oil. The purification of the oil was a necessary step for obtaining cosmetic emulsions with good texture and sensorial properties.

The purified oil after the application of the five stage purification procedure was more transparent, lighter in color, and almost odorless oil than the original crude oil (see Fig. 1). The viscosity of the purified BSFLO was almost 10% lower than that of the crude oil, and furthermore coincided with that of the coconut oil (see Fig. 2).

The prepared hand cream emulsions with the purified BSFLO had the viscosity (Figs. 6 and 9A), yield stress (Figs. 7 and 9B), stability, color (Fig. 8) and odor as those with coconut or palm kernel oil. Light yellow color of the cream was successfully whitened with small amount of white pigment in the formulation. The inclusion of 2 wt% pigments did not lead to qualitative changes of the flow properties and the stability. The obtained model hand cream formulations showed characteristics suitable for market products and convinsingly demonstrated the potential for the successful inclusion of the BSFL oil in leave-on cosmetic products.

Conclusion.

Black fly soldier oil is a promising natural resource with tremendous potential for future development. Our study showed that model cosmetic emulsions (hand cream) with purified BSFLO had very similar properties (viscosity, relative size of oil droplets, stability to storage at different temperatures, and centrifugation at high speeds) compared to emulsions containing coconut or palm kernel oil. The applied five stage purification significantly improved the color and the odor of the oil, which resulted in almost odorless emulsions with very light yellow color. The light coloring was successfully removed by using white pigments. The formulated creams with the purified BSFLO and titanium dioxide had color, gloss, and yield stress as those of the creams with the coconut oil and palm kernel oil.

Future toxicological assessment would allow faster inclusion of the oil BSFLO as a cosmetic ingredient which could successfully replace coconut or palm kernel oil in many applications.

Acknowledgments

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Conflict of Interest Statement.

None

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