Physicochemical Characterization of Home-Made Soap from Waste-Used Frying Oils

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Keywords: Syntrophomonas sapovorans, long-chain fatty acids, waste utilization and management, reverse system logistics, microbial communities, soap degradation

Abstract:

The study aimed to describe the utilization of waste frying oils, originated mainly from households, in home-made soap production and to emphasize the advantages of soap biodegradation in comparison to biological treatment of oils. The physicochemical analyses of soaps were used to check the differences between the samples made of fresh and fried oils. Significant ($p < 0.05$) difference between the soaps made of fresh/fried olive oil pair was obtained, while the rapeseed sample pair did not differ significantly ($p < 0.05$). Malondialdehyde (MDA) exhibited notable differences with an increase from 1.94 ?g/g to 2.33 ?g/g for olive oil fresh/fried pair and from 3.43 ?g/g to 4.10 ?g/g for rapeseed?palm oil fresh/fried pair. The studies addressing the soap biodegradation process revealed that soaps are degrading up to four times faster than oils in waste processing plants. Literature data showed the syntrophic ways of soap degradation and degradation solely done by sulfate-reducing bacteria. Obtained results, same as literature data, indicated that soaps produced from fried plant oils represent acceptable products from the economic and environmental point of view. Soap production can be considered one of the possible ways toward reduction of waste oil disposal.

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Physicochemical Characterization of Home-Made Soap from Waste-Used Frying Oils

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Keywords: soap degradation; microbial communities; reverse system logistics; waste utilization and management; *Syntrophomonas sapovorans*; long-chain fatty acids

1. Introduction

Together with the World population growth, the amount of generated waste is increasing accordingly [1]. Frying oils used for processing different kinds of food at temperatures between 160 and 200 °C are no longer usable for human consumption and represent a large source of waste. The reactions which occur during frying in those oils are hydrolysis, thermal degradation, oxidation and polymerization, and they can result in highly toxic products [2]. It is estimated that the world's food industry, restaurants and households together produce about 200 million tons of this waste annually with an increasing trend [3,4]. The vegetable oils used in industry and restaurants are mainly collected and recycled, but there is still a high percentage of frying oils disposed of in the sewage of households. According to Greenea (2018), in European Union countries, 51% of produced waste frying oil comes from households, of which only a few percent have been collected and recycled [5]. This improper disposal creates big environmental problems, which, as a consequence, have clogging in the drainage systems, negative impact on wildlife, production of toxic substances and rancid odors [6,7].

The wastewater treatment requires the engagement of high levels of energy, which is additionally aggravated by sticking the oil to the apparatuses, corrosion occurring on the equipment and low

efficiency of the taken measures [8]. Nowadays, it is clear that reverse logistics systems are essential from an environmental and economic point of view and that every potential reuse of byproducts should be investigated [9]. Waste frying oil represents low-cost raw material for many applications like biodiesel, lubricants, resins and soaps production or use as a fermentation media component. In addition to this, its proper reuse solves alarming environmental problems that occurred by improper disposal of this waste [2,10]. Some assumptions are that 1 L of oil might pollute 500,000 L of natural water in which it was poured [2].

The production of soaps creates no byproducts, and the minimal amount of energy is required for the saponification reaction, and it is less harmful to nature [11,12]. They are also called natural soaps and are defined as alkali salts of fatty acids, mainly from vegetable origin [13]. The process of soap making could apply to the production of soaps from waste frying oils, giving it attributes of the technology with a green perspective [14]. Although natural soaps were considered environmentally friendly, there are still some adverse effects on the ecosystem, which can be observed (such as forming the insoluble films on water surfaces that decrease entry of the oxygen to the water) [15]. The soap as a constituent of the wastewater can be successfully treated by chemical (coagulation) and biologic means (using microorganisms) [16]. Some bacteria from the species *Syntrophomonas* can degrade the soap under anaerobic conditions and use it as a source of carbon [17]. Over the years, there has been a need to improve natural soaps to meet higher hygiene standards for the human population and to create synthetic soaps (detergents) made of substances like alkyl benzene sulfonate (ABS), linear alkylbenzene sulfonate (LAS) and benzalkonium chloride (BAC). The creation of those more effective cleaning agents significantly increased their negative effects on the environment due to their low biodegradability, high toxicity and the possibility to spread to other ecosystems in the water cycling process. The impact of the detergents mentioned above on sewage biologic treatment systems can be so strong and cause a 40% efficiency decrease [18]. Thus, it is necessary to work on the development of natural soaps as the base for cleaning products. Ingredients for making the natural soaps are often easily very accessible to small manufactures and households.

The topic, concerning the utilization of waste frying oils in soap production, has not been studied often in a scientific manner and to our knowledge, there is no work on the subject of the utilization of these oils in the production of home-made soaps. This method of waste oil utilization is not well known among entrepreneurs. Consequently, the main goal of the research is to emphasize not negligible amounts of the waste frying oils coming from households that could be used for soap production.

The entire biodegradation mechanism of natural soaps has not been described fully according to the literature sources. The problem of waste frying oil originated from households was poorly studied, and the production of soaps comes as one of the possible useful practices of its utilization.

The study aims to focus on home-made soaps and the possibility of utilizing large amounts of waste frying oils that are disposed of in the sewage by households. The study also reviews the possible further biologic degradation pathways of home-made soap as part of household waste. The study's hypothesis was to create soaps made from waste frying oils comparable in physicochemical properties to those produced from fresh oils. In this case, this can be offered as one of the solutions for household waste. The study aimed to emphasize the issue of soaps as waste material since literature data indicate that soaps biodegrade faster than waste frying oils.

2. Materials and Methods

2.1. Preparation of Soap Samples

The samples of soaps were prepared using cold saponification from two types of oils. The oils used for the experiment were olive oil (originated from Spain; purchased in the Czech Republic) and rapeseed–palm oil (80:20), originated from the Czech Republic and purchased in the Czech Republic. The first sample series of each oil was made from fresh oil and the second from fried oil. Frying of oils was carried out in laboratory conditions by consecutive heating the oil to 200 °C

and cooling it for 20 min at room temperature (three cycles of heating and cooling were repeated). The soaps were made according to the work of Adigun (2019), with slight modifications [19]. The recipe consisted of 280 g of oil, 36.04 g of NaOH for olive oil soap and 34.10 g of NaOH for rapeseed–palm oil soap and 106.40 g of distilled water. The NaOH was first dissolved in water and cooled to the room temperature and then mixed with oil using the blender (3–5 min). During the mixing process, the heat was generated, but it did not exceed 45 °C. The mixture was then poured into the silicone molds after 24 h pulled out and placed on filter paper to mature on the air. After 4 weeks of maturing, samples were subjected to the analysis.

2.2. Assessment of the Physicochemical Properties of the Soap

Analysis of the physicochemical properties of soap was done by using some standard procedures. Measured parameters were pH, moisture content (dry matter), total alkali, total fat content, hardness, adhesion and malondialdehyde (MDA).

For pH measurements, 2 g of soap powder was weighed and dissolved in 10 mL of distilled water [20]. The measurements were done using the instrument pH meter GRYF 259, Havlickuv Brod, Czech Republic.

Moisture content was measured in 5 g samples, accurately weighed in dried moisture dishes on an analytical balance model ALS 250-4A, Kern, with the sensitivity of 0.1 mg. Samples were dried in an oven at 105 °C for about 6 h until a constant sample mass was reached. The percentage of moisture was calculated using the following formula:

$$\% \text{ moisture} = (\text{m(sample)} - \text{m(dried sample)})/(\text{m(sample)}) \times 100 \quad (1)$$

The total alkali was determined by titrating excess acid in the aqueous phase using a standard NaOH solution [20,21]. To the weight of a soap sample of 10 g, 100 mL of neutralized ethanol and 5 mL of 1 N H2SO4(aq) solution was added. The mixture was heated until the total dissolution of the soap sample and then titrated with 1 N NaOH using the phenolphthalein indicator. The total alkali was obtained with the formula:

$$\% \text{ Total alkali} = (\text{V(Acid)} - \text{V(Base)})/(\text{m(sample)}) \times 3.1 \quad (2)$$

The total fat matter was measured by dissolving the sample in hot ethanol and measuring the insoluble matter in alcohol [20]. A sample of 10 g of soap sample was weighed, 150 mL of warm neutralized ethanol and heated. The dissolved solution was filtered, and the remaining residues on the filter dried in the oven at 110 °C for one hour and weighed again. The total fat matter was obtained with the formula:

$$\% \text{ Total fat matter} = 100 - (\text{moisture content} + \text{matter insoluble in alcohol})/1.085 \quad (3)$$

The texture of the samples was measured on the texturometer TA.XT plus, Stable Microsystems. The hardness and adhesion results were obtained using the 5 mm diameter cylinder stainless P/5 probe. The penetration depth of the probe was set to 5 mm.

The MDA evaluation was done using the thiobarbituric acid (TBA) method according to the work of Khalifa (2016) with some modifications [22]. The 1.5 g of sample was placed into a centrifugal tube, added 1 mL of EDTA and mixed, then 5 mL of 0.8% BHT was added and mixed again. Ten percent TCA solution was added to the mixture and homogenized for 30 s at 10,000 rpm. After homogenization, the tube was centrifuged for 5 min at 3500× *g* at 4 °C. The upper layer was taken from the tube, and the lower layer was filtered. The filtrate was added to the 10 mL volumetric flask, and 10% TCA was added to the mark. From this flask, 4 mL was taken, and 1 mL of TBA was added. Like the standard solutions of MDA, these were incubated for 90 min at 70 °C. After incubation, all was carried to the ice

bath for 2–3 min and then rested at the room temperature for 45 min. Absorption was measured using a spectrophotometer at 532 nm. The results were calculated using standard calibration curves.

2.3. Statistics

Mean values ± standard deviation is presented in the tables. All chemical analyses were done in triplicate. Texture analysis was done on five positions of the soap. Statistical significance at $p < 0.05$ was determined by the one-way ANOVA analysis of variance and parametric Tukey's post hoc test (in the case when Levene's test showed equal variances $p > 0.05$) and nonparametric Games–Howell post hoc test (in the case when Levene's test showed unequal variances $p < 0.05$) for finding differences within groups. The IBM SPSS Statistics computer program was used to make a statistical analysis.

3. Results and Discussion

The experimentally produced soaps were evaluated by chemical parameters, including pH, dry matter, total alkali, total fat and malondialdehyde (MDA) content. The obtained results are presented in Table 1.

Table 1. Chemical properties of obtained four samples of soap.

Sample	pH	Dry Matter (%)	Total Alkali (%)	Total Fat (%)	MDA (μg/g)
Fresh olive oil	9.61 ± 0.01 ^{c}	92.31 ± 1.33 ^{c}	0.06 ± 0.00	91.53 ± 0.93 ^{c}	1.94 ± 0.00 ^{a}
Fried olive oil	9.96 ± 0.03 ^{b}	97.55 ± 0.10 ^{b}	0.03 ± 0.00	96.24 ± 0.19 ^{b}	2.33 ± 0.01 ^{b}
Fresh rapeseed–palm oil	9.53 ± 0.02 ^{a}	89.06 ± 0.63 ^{a}	0.00 ± 0.00	88.57 ± 0.60 ^{a}	3.43 ± 0.01 ^{c}
Fried rapeseed–palm oil	9.61 ± 0.05 ^{c}	89.61 ± 1.40 ^{a}	0.00 ± 0.00	88.89 ± 1.27 ^{a}	4.10 ± 0.01 ^{d}

Different lowercase letters (^{a}, ^{b}, ^{c}, ^{d}) indicate statistically significant differences ($p < 0.05$) between rows. The results are presented as the mean values ± standard deviation; all analyses were done in triplicate.

The pH of the samples was very similar in all tested samples, and it was in a range from 9.53 to 9.96. When comparing the samples made from fresh and fried oil, there was a significant ($p < 0.05$) difference between the results. Compared to the other studies, some close results for pH were found in the testing of soaps made of waste cooking oils [23], where the results were in a range from 9.96 to 11.30. Human normal and healthy skin has a pH in the range of 5.4 to 5.9 [24], and any introduction of soap with high pH can affect the skin pH balance and its flora. On the other hand, most tested commercial soaps had a pH between 9 and 10 [24].

The moisture content of all tested soaps was very low. It ranged from 2.45% to 10.94%. There was a significant ($p < 0.05$) difference between samples from fresh and fried olive oil, though between samples made from fresh and fried rape–palm oil, a significant difference was not obtained. The other studies reported much higher moisture content that ranged from 24.90% to 43.24% [23]. The results found for some commercial soaps ranged from 30–35% of moisture content [23]. The present study's lower moisture content could be explained by a different recipe for soap preparation and not adding any substances or additives that help in water retention and its moisturizing effect. The high moisture content supports hydrolysis and alterations inside the soap itself. Some best soap producers declare a maximum of 14% of moisture in their products [25].

The results for the total alkali content show low values, which range from 0.00% to 0.06%. The lower this value is, the better is the quality of the soap [25]. Total fat content is one of the most important indicators of the soap quality [25], and the higher content of fat indicates better quality. A higher concentration of fatty acids has good effects on the skin in the sense of rehydration and overall enhanced cleansing properties [25]. The results of the total fat content of made soaps revealed very high values. The soap made from fried olive oil had a total fat content of 96.24% and showed a difference against all other tested samples. It was followed by soap made from fresh olive oil with 91.53%, then fried rapeseed oil with 88.89% and fresh rape–palm oil with 88.57% of total fat content. Same as with moisture content, the samples made of fresh and fried olive oil showed significant

($p < 0.05$) difference, and there was no significant difference between samples made of fresh and fried rape–palm oil.

MDA is known as a marker for lipid peroxidation [26]. No literature data indicates the use of MDA determination as an indicator of the soap quality parameter. The results from the analysis show the increase of MDA in fried samples when compared to the content of MDA in samples of soap. There was a significant ($p < 0.05$) difference between all tested samples regarding the obtained values of MDA. The value for MDA in fried olive oil was 2.33 µg/g; fresh olive oil was 1.94 µg/g. Results from fried and fresh rapeseed–palm oil were 4.10 µg/g and 3.43 µg/g, respectively. That only confirmed the assumption before the testing was done, that MDA levels in fried samples will be higher due to higher levels of peroxidation during the oil burning process.

The textural properties of all obtained soap samples are shown in Table 2. Soap texture measurements have been poorly described in the literature [27]. Consequently, it should be indicated that the comparison with the results of other authors is not possible, or it is not giving clear conclusions. Hardness results indicated significant differences between the samples. As expected, the sample with the least moisture showed the biggest hardness. Hence, for the fried olive oil soap hardness was 8538 g, following with fresh olive oil with 5268 g, then fried rapeseed oil with 3841 g and rape–palm oil soap with 3619 g. Statistically, there was no significant ($p > 0.05$) difference between samples made of fresh and fried olive oils and the significant ($p < 0.05$) difference was obtained between the samples made of fresh and fried rape–palm oils.

Table 2. Textural properties of obtained soap samples.

Sample	Hardness (g)	Adhesion (g)
Fresh olive oil	5268 ± 327 ^{a}	−728 ± 83 ^{a}
Fried olive oil	8538 ± 450 ^{a,c}	−1159 ± 310
Fresh rapeseed–palm oil	3619 ± 130 ^{b}	−441 ± 85 ^{b}
Fried rapeseed–palm oil	3841 ± 45 ^{c}	−525 ± 132

Different lowercase letters (^{a}, ^{b}, ^{c}) indicate statistically significant differences ($p < 0.05$) between rows. The results are presented as the mean values ± standard deviation; all analyses were done in 5 replicates.

Same as for hardness, the result for adhesion showed the highest value for samples with the driest content. Results are the following: fried olive oil (1159 g), fresh olive oil (728 g), fried rapeseed oil -535 g and fresh rape–palm oil (441 g). There was no statistically significant ($p < 0.05$) difference between the sample pairs made of fresh and fried oils.

Soap odor can be described as a common odor of soaps produced without addition of fragrances. The noticeable differences between the two soap samples series were not observed. Certainly, the addition of pleasant fragrances would make these products more acceptable for consumers.

Soap Degradation Processes

Two types of oils were used to produce soaps: olive and rapeseed–palm oil. The dominant fatty acids found in these two types of oils are presented in Table 3.

Table 3. The approximate composition of major fatty acids found in olive, rapeseed and palm oil (data from USDA 2020 [28], Montoya et al. 2014 [29], Matthaus et al. 2016 [30]).

Fatty Acid	Olive Oil	Rapeseed Oil	Palm Oil
Oleic acid (C18:1)	55–83%	56.5–65%	40%
Linoleic acid (C18:2)	3.5–21%	17–21%	10%
Palmitic acid (C16:0)	7.5–20%	4.2–5%	44%
Stearic acid (C18:0)	0.5–5%	1.5–2.5%	5%

During the soap making process, fatty acids present in the oil create alkaline salts that build the soap structure. The process of soap transformation is transforming barely dissolved oils to the soaps that create stable emulsions in water. This process can enhance microbial degradability, and it has been used as the first step in some polluted water cleaning systems [31]. The lipid hydrolysis inhibition was also reported, in the case when the lipases partially fail to activate in high product concentration [32]. According to Lefebvre (1998), the results for saponified grease indicated three to four times faster degradation compared to the not saponified grease, with the success yield over 98%, the raw grease was degraded with the success yield around 87%. Because of the good solubility and improved bioavailability, soaps themselves present better substrates for microbial degradation than the oils from which they are made [33].

Mechanism of biodegradation of long-chain fatty acids (with 14 C atoms or more [34]) is done by β-oxidation, which results in successive removal of two carbon atoms, resulting in a shorter chain fatty acid and acetyl-CoA. Degradation of fatty acids depends on their solubility, and since lower chained fatty acids are more soluble, they also degrade at higher rates. In the case of fatty acids mainly found in tested oils (used in our study), it means that palmitic acid will degrade faster than stearic acid. The degradation of oleic and linoleic fatty acids is more challenging in the sense of biodegradation in comparison to the palmitic and stearic acid [35]. On the other hand, unsaturated fatty acids are more soluble than saturated ones and, thus, more accessible to the microorganisms [36]. The process of biodegradation of fatty acids is complex, with the need for symbiotic action of more microorganisms with different functional roles [37]. The representative of isolated bacteria with those properties was *Syntrophomonas sapovorans*, also called soap devouring. *Syntrophomonas sapovorans* degrades soap (long-chain fatty acids) in the presence of hydrogen consuming bacteria in anaerobic conditions, reported by Roy, 1986 [38]. The name of the genus *Syntrophomonas* comes from the word syntrophy, which is explained as the way of functioning of two, in this case, microorganisms in mixed culture that give each other a growth factor by degrading the same substrate [39]. The capability of utilizing long-chain fatty acids is shared by *S. sapovorans*, *S. curvata*, *S. zehnderi*, *S. acidothropicus*, *S. palmitatica*, *Thermosyntropha lipolytica* and *Thermosyntropha tengcongensis* [17,40–42]. The predominant microorganisms found to degrade long-chain fatty acids in rich swine slaughterhouse waste were *Clostridium, Syntrophomonas* and *Methanospirillum*. The reported long-chain fatty acids in this animal origin waste were similar to the ones contained in oils used in our study [43]. The literature mentioned *Clostridium bryantii* as bacteria capable of degrading the fatty acids with eleven carbon atoms and less. Still, due to its physiology and other properties, it was renamed to *Syntrophomonas bryantii*. *Clostridium* sp. is also known to have a role in hydrolysis and acidogenesis [44,45]. *Methanospirillum* is responsible for converting hydrogen and carbon dioxide into methane, promoting further degradation of fatty acids, and without these methanogens, the degradation would not be possible [46].

The functioning of syntrophic bacteria is also possible in the presence of hydrogen-consuming sulfate-reducing bacteria [17]. This bacterial group can compete with molecular hydrogen with other bacteria by inhibiting the biodegradation process. This process can also be inhibited by hydrogen sulfide produced in sulfate reduction dissimilation by sulfate-reducing bacteria [47,48].

However, in sulfate-rich wastewaters, sulfate-reducing bacteria can out-compete syntrophic bacteria in long-chain fatty acids degradation processes. Many sulfate-reducing bacteria can partially or completely oxidize long-chain fatty acids without the need for syntrophic partners in those conditions. Among sulfate-reducing bacteria, 15 genera within the orders of *Desulfobacterales*, *Desulfuromonadales*, *Syntrophobacterales* and *Clostridiales* were isolated with this capability [49].

In Yoochatchaval's work (2016), the bacterial genus *Syntorophomonas* were used to degrade oleic and palmitic acid. Strains *Syntrophomonas sapovorans* and *Syntrophomonas zehnden* were identified as representative of these bacterial genera on substrate originating from palm oil mill effluent [50]. Fatty acid chain reduction leads to a higher number of microorganisms capable of degrading it. *Algorimarina butyrica, Desulfotomaculum thermocisternum, S. wolfei, S. bryantii, S. cellicola, S. erecta sporosyntropha, S. lipocalidus* are the strains which can degrade fatty acids from 4 do 12 C

atoms [46,51]. The final step is the direct use of acetate, formate and hydrogen by methanogens. Acetate can also be degraded by syntrophic bacteria [52]. The inhibition of this overall long-chain fatty acids degradation process can occur because, in higher concentrations, fatty acids have toxic effects on the devouring bacteria by adsorption on the cell surface and limiting the transport of metabolites. Methanogen microorganisms are also sensitive to a high concentration of long-chain fatty acids [53]. Though other bacterial families were found in some long-chain fatty acid digestion processes, it is still unclear if families other than Syntrophomonadaceae and Syntrophaceae can degrade these fatty acids [54].

It is worth mentioning that cold saponification of oils can, in practice, hardly be 100% completed, but since this topic has been poorly studied, there is very little data on it. In the work of Adigun (2019), the mentioned levels of saponified fatty acids in home-made soaps made by cold saponification ranged between 70% and 85% [19]. Hence, as the constituents of final soap products, we also have triacylglycerols, diacylglycerols, monoacylglycerols and free fatty acids, mainly consisting of unsaturated fatty acids [13]. Many lipase-secreting bacteria are capable of hydrolyzing the remaining acylglycerols in the soap. Among them, the ones that were isolated on frying oil substrate with the highest lipase activity are from *Pseudomonas*, *Bacillus* and *Candida* genera [55].

4. Conclusions

Our results emphasize issues concerning the disposal of used frying oil, mainly accumulated by culinary preparations in homes and restaurants. Soap production from used/fried different types of culinary oils represents an alternative, more ecological treatment of the waste that can be indubitably harmful to the environment. The study highlighted different chemical properties of soaps produced from fresh and fried oil. Significantly higher malondialdehyde contents in soaps made from fried oil are distinguishing these soaps. On the other hand, literature data indicate that waste treatment of soap is way easier than waste treatment of fried/used oil. Indeed, further study will mark more ecologically and economically sustainable soap production with favorable consumers' properties.

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