**Supplementary Information**

**From Kitchen to Cosmetics: Study on the physicochemical and antioxidant properties of Waste Cooking Oil-Derived Soap**

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***3.1 Chemicals***

The chemicals used for the soap-making are cooking oil samples (pure-5 cycle), sodium hydroxide (NaOH) pallets, Distilled water, phenolphthalein indicator, sulphuric acid, hydrochloric acid, calcium chloride, barium chloride, methyl red, sodium thiosulphate, potassium iodide, starch indicator, potassium dichromate, Iodine monochloride, Hanus solution, starch, Calcium nitrate, carbon tetrachloride, ethanol. All chemicals were analytical grade purchased from sigma Aldrich with above 97% purity.

***3.2 Equipment***

Sartorious analytical balance, Remi pH meter , hot air oven, remi heating mantle, ultrasonicator fume hood, vacuum pump, blender were also utilised for the synthetic process. UV-Visible spectrophotometer was used for evaluating the antioxidant potential of prepared soap (Shimadzu UV 2600).

***3.3 Collection of Samples***

The samples of different heating cycles of cooking oil are collected from households in the month of January, 2024. The same oil used with different heating cycles is first collected as a pure sample numbered as 1 then the same oil is used for 5 times for frying to different dishes and collected after each cycle and filtered(Fig. 1).

*4.1.1. Iodine Test*

In the test, 0.25 g of the oil sample is dissolved in carbon tetrachloride (CCl₄) and reacted with 5 mL of Hanus solution. The reaction mixture is incubated in the dark for 30 minutes, preventing light from interfering with the reaction. After the reaction, potassium iodide (KI) is added, which releases any unreacted iodine as molecular iodine (I₂). This iodine is then titrated with sodium thiosulfate (Na₂S₂O₃), using an ethanolic starch indicator to mark the endpoint of the titration when the blue color disappears. The iodine value is then calculated as per equation 1.

Here, B and S = Amount of titrant used for blank and sample, respectively

M = Molarity of Na2S2O3

W = weight of oil

*4.1.2. Saponification value*

It is calculated as the amount of alkali required to saponify per gram of oil (Equation 2). For this, 2g of sample were weighed and added to 25 ml of KOH*EtOH*. The reaction mixture was heated at ambient conditions to obtain a clear solution. The flask must be covered with the foil paper to minimize evaporation. The clear solution indicates the complete saponification of the reaction mixture. Further, the reaction mixture was titrated against 0.5M HCl using phenolphthalein indicator.

B and S = Amount of titrant used for blank and sample, respectively

M = Molarity of HCl and,

Molecular weight of potassium hydroxide = 56.1 g/mol

***5.1 pH Test***

The pH is the degree of measure of acidity or alkalinity of a solution. In this regard, 10% soap solution was prepared and heated to diaalove the soap completely. Further, the pH of the solution was measured using pH meter calibrated at 4 and and the pH of theses soaps is compared with commonly used laundry soap bars Fena bar, rin bar and tide bar.

***5.2 Moisture Content***

Moisture content (MC) is determined by the amount of water in soap sample in order to check the shelf life of soap as excess water causes the soft texture, hydrolysis and increases microbial growth. It is determined using the procedure prescribed in AOCS((Nurdiyanah et al., 2023);(Legesse, 2020)). In this regard, 5g of oil sample was heated in hot air oven at 103˚C ± 2˚C for 2h and weighed and further placed for every 1 h until the constant weight was observed. The moisture percent was evaluated using below equation:

Where, Cw, Cs, and CL = weight of the dish, dish + sample, and crucible + sample after drying, respectively.

***5.3 Free Caustic Alkali***

Free caustic alkali is determined to check the harshness/roughness of any soap. The presence of free caustic alkali causes irritation to skin and harm to clothes. To check the amount of free caustic alkali, about 5g of soap sample mixed in 30ml of ethanol. After that, few drops of phenolphathelein indicator added and 10ml of 20% BaCl2 were added into the solution. The mixture was stirred properly and the resulting solution was titrated against titrant 0.05M H2SO4 till the solution became pink to colorless (Equation 4)

**Top of Form**

Where VA = volume of acid added

W = weight of soap sample used.

***5.4 Total alkali content***

The total alkali content in soap refers to the total amount of sodium hydroxide (NaOH) or potassium hydroxide (KOH), bicarbonates, and carbonates present in the finished soap. It was determined by following the prescribed procedures in the literature ((Legesse, 2020);(Mwanza and Zombe, 2020);(Habib et al., 2016)). About 2.5g of soap sample was mixed with 25ml of neutralized ethanol with continuous stirring. 1.25ml of 1N H2SO4 solution was added to the solution while mixing and heating until the amount of soap added was completely dissolved. Followed by letting the flask cooled at room temperature. Some amount of sulphuric acid added is used in neutralization and the remaining amount of acid added was estimated by back-titrating with 1N NaOH until a pink colour appears using phenolphthalein as indicator. The total alkali content was calculated using the equation:

VA= volume of acid added in experiment

VB = volume of base at end point

W = weight of soap used in experiment.

***5.5 Total Fatty matter (TFM)***

Total fatty matter (TFM) is defined as the amount of fats or oils that have been converted into soap during the process of soap-making. Higher TFM value generally indicates the soap with better cleansing properties. TFM can be separated from a sample after splitting with mineral acid, usually HCl. The TFM value was determined by reacting soap with acid in the presence of hot ethanol. About 2.5g sample of soap was added to 37.5ml of hot neutralized ethanol and warmed as long as the soap completely dissolved. The resulting mixture was filtered out and the residue was dried in an oven at 110°C for an hour before reweighing. The TFM value was obtained using the following equation:

MC= Moisture content

MIA= Matter insoluble in alcohol

Matter insoluble in alcohol can be calculated by method described in AOCS with some modifications. About 1.25g sample of soap was mixed in 18.75ml of heated ethanol and stirred until fully dissolved. The resulting solution was subsequently filtered through preweighed filter papers. The leftover material was dried in the oven for 30 minutes before cooling and weighing again.

The MIC was calculated using formula:

WS = weight of the sample + Filter paper

WFP = weight of filter paper

W = weight of sample

***5.6 Chloride percentage***

The chloride content is the total amount of sodium chloride (NaCl) or potassium chloride (KCl) present in soap. Excess chloride content causes cracks in soap and irritation and dryness to the skin. The amount of chloride content is determined by using the procedure described in literature((Vivian et al., 2014);). Almost 10g of soap sample was dissolved in distilled water, and further diluted to 100ml with DI water. The mixture was subjected to heat and stirred till a clear solution was obtained and transferred to a 250ml volumetric flask followed by addition of 15% calcium carbonate (Ca(NO3)2, 20 mL). The solution was vigorously stirred until entirely dissolved and volumed was made upto 250ml. The solution was filtered using suction filtration and Whatmann filter paper no.1. 100 mL of filtrate was titrated against the 10N H2SO4 using methyl red as indicator until the solution got pink colored. The pink color solution was again titrated with 0.1M AgNO3 as titrant using K2Cr2O7 as indicator till the brick red color appears. The amount of chloride content was determined using:

***5.7. Antioxidant Activity***

To evaluate the antioxidant activity of soap and oil, 2,2-diphenyl-1-picrylhydrazyl(DPPH) analysis has been done to assess the free radical scavenging activity. In this work, 1g of soap and oil samples were dissolved in ethanol (10 mL). In this process, each of test tube has filled with 200 µl of soap (EtOH in blank) and oil solution and 3ml of DPPH solution (0.004%). The prepared samples have been placed in dark for 30 minutes to carry out the reactions and the absorbance was measured using UV-visible spectrophotometer 2600(Shimadzu). The percent DPPH radical scavenging effect was calculated using the following equation:

where

A0 is the absorbance of DPPH solution without soap (control solution) and

A1 is the absorbance of DPPH solution with soap (test solution).

***6.1. Physical Characteristics***

*6.1.1, Cleansing Capacity of Soap*

Soap's cleansing power depends on the chemical composition, formulation, concentration, and physical properties such as lathering ability and water solubility. Soap molecules have surfactant qualities, which enable them to come into contact with both water and oil-based substances, thereby removing grease and dirt from the surface. The as-prepared laundry soap bar is tested by applying strains on pieces of white clothes. The strains of gravy, grease, coffee, and lipstick were applied on white cloth and left for one day to dry. Fig. 2 shows the strains on clothes after 24 hours of applying it. Each of the strain was applied five times to check the cleansing action of all the five soap samples.

*6.1.2 Emulsification Test*

Emulsification is the process of mixing of two or more immiscible substances to form a homogeneous mixture which is in stable form, known as emulsion. The testing of emulsifying capacity was done to check the ability of soap to lift and suspend oils and dirt from the fabric. In this regard, in a test tube or small beaker, equal parts of water and oil were mixed. Add a few drops of the soap solution (typically 1-2 mL) into the water-oil mixture.The mixture was shaken vigorously for 5 minutes by applying a stopper and the extent of emulsification was noted. After mixing, formation of an emulsion was observed and a successful emulsification will appear as a homogeneous, milky solution without the separation of oil and water layers.. Soap molecules are having both hydrophilic and hydrophobic ends which form a bridge between water and oil allowing them to mix and form emulsions.

*6.1.3 Foam Ability Test*

To analyse foam ability, the samples were tested by introducing an equivalent amount of soap and DI water in a test tube, the solution was shaken vigorously for a few minutes and the amount of leather formed was observed and recorded(Profile and Profile, 2018) and the comparison test

*6.1.4 Texture and color*

The color and texture of soap vary according to the various preparation method utilize for soap-making. The hard or soft texture of soap depends on the stirring time of mixture of lye water with oil during the process.

*6.1.5 Acidity/Alkalinity of soap Test*

In a test tube, about 2g of soap sample was mixed with DI water. After the complete mixing of the solution, a few drops of phenolphthalein indicator were added and the color change was observed.

***6.2. Chemical Property***

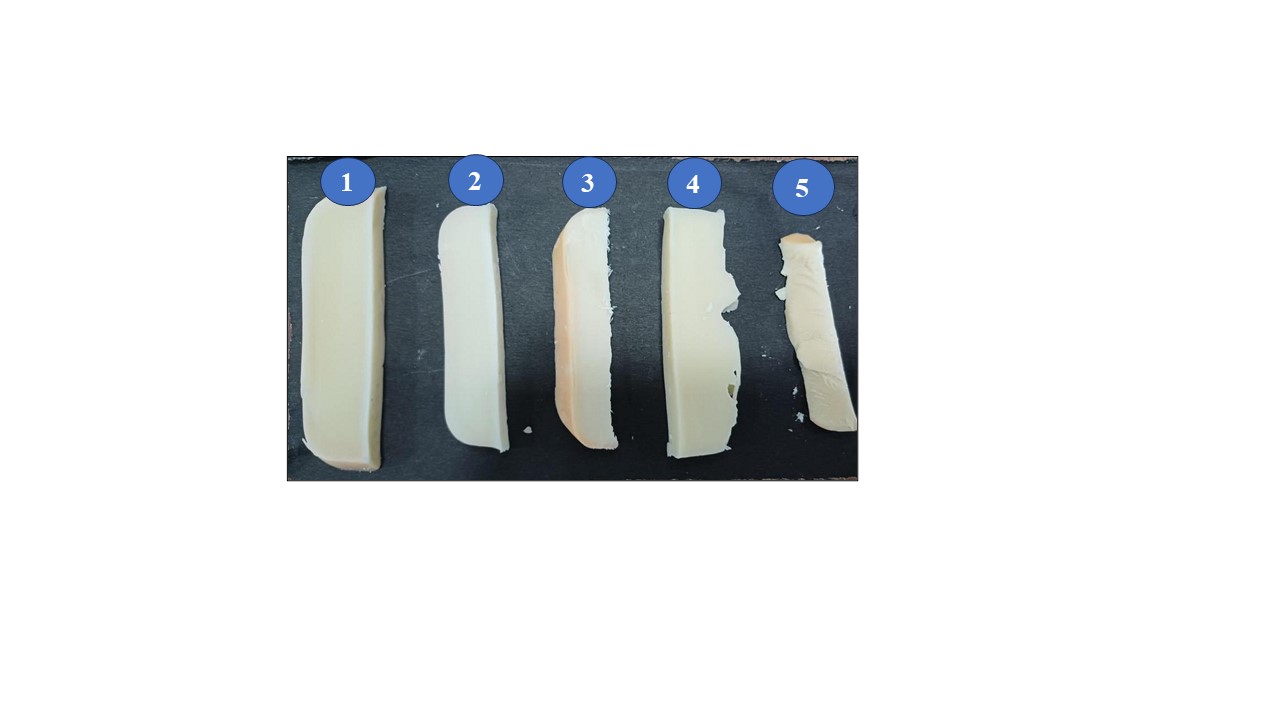
The chemical properties of soap have been tested by using NaCl, KCl, NH4Cl, CaCl2, MgCl2, and FeCl3 to check the effectiveness of soap in different water conditions. The procedure has been followed using the literature work of *Atiku et. al.* (Atiku et al., 2014). In the test tube, about 5ml of soap solution was made, and 2ml of 4% each of NaCl, KCl, CaCl2, and FeCl3 was added in different test tubes and each test tube was shaken vigorously to completely mix the solution and the precipitates formed were observed.



**Fig S1**: Mechanistic representation of formation of soap



**Fig S2**: General structure of Soap



**Fig. S3**: Texture and color of soap

**Table S1**: Iodine value and saponification values of oil samples

|  |  |  |
| --- | --- | --- |
| **Oil Sample** | **Iodine value(I2/100g)** | **Saponification value (mg KOH/g)** |
| Sample 1 | 1.0152 | 195 |
| Sample 2 | 1.47204 | 198 |
| Sample 3 | 1.67508 | 205 |
| Sample 4 | 1.67504 | 245 |
| Sample 5 | 1.7766 | 253 |

**Table S2**: Weigh loss during the curing time of soap samples.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Soap sample** | **After24 hours** | **10 days** | **12 days** | **15 days** | **17 days** | **20 days** | **1 month** | **35 days** |
| 1 | 68.48 | 64.56 | 64.33 | 64.017 | 63.49 | 63.09 | 62.91 | 62.90 |
| 2 | 69.91 | 65.91 | 65.72 | 65.50 | 65.27 | 64.98 | 64.67 | 64.65 |
| 3 | 51.34 | 47.48 | 47.18 | 46.84 | 46.59 | 46.12 | 45.73 | 45.72 |
| 4 | 72.40 | 69.40 | 69.21 | 68.87 | 68.82 | 68.12 | 68.09 | 68.07 |
| 5 | 53.65 | 51.56 | 51.40 | 51.16 | 51.04 | 50.46 | 50.28 | 50.27 |

**Table S3**: pH values of prepared soap bars

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sample bar** | **pH** | **Moisture content(%)** | **Free caustic alkali(mg/g)** | **Total alkali content (mg/g)** | **Total Fatty matter (%)** | **Chloride content (%)** |
| Sample 1 | 9.27 | 6.03 | 0.0558 | 0.29 | 86.180 | 0.152 |
| Sample 2 | 9.31 | 5.07 | 0.0744 | 0.34 | 86.213 | 0.111 |
| Sample 3 | 9.42 | 5.50 | 0.116 | 0.43 | 86.195 | 0.140 |
| Sample 4 | 10.21 | 4.18 | 0.1178 | 0.58 | 83.013 | 0.181 |
| Sample 5 | 10.34 | 4.46 | 0.2914 | 0.73 | 75.580 | 0.175 |

**Table S4**: Testing of physical properties of soap samples

|  |  |  |
| --- | --- | --- |
| **Sr. No.** | **Parameters** | **Results** |
| 1 | Emulsification | Emulsification was observed |
| 2 | Color | Pale white |
| 3 | Texture | Moderate |
| 4 | Foam test | Heavy foam |

**Table S5**: The results obtained from soap samples are shown in Table 6.

|  |  |  |
| --- | --- | --- |
| **Sr. No.** | **Parameter** | **Result** |
| 1 | NaCl | Foam with white precipitates |
| 2 | KCl | Foam with white precipitates |
| 3 | CaCl2 | White heavy precipitates |
| 4 | FeCl3 | Dark brown with precipitates |
| 5 | Phenolphthalein | Dark pink |
| 6 | HCl | Colourless |

Table S6. Comparison table of Physicochemical properties of soaps derived from different types of oil with current work

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sr. No. | Oil samples | Saponification value(mg KOH/g) | Iodine value(I2/100g) | Moisture content(%) | pH | Reference |
| 1. | Shea butter and  Palm kernel oil | 175.30-249.18 | 65.99±1.27  18.58±0.86 | 3.75-4.70 | - | (Zauro et al., 2016) |
| 2. | Neem seed oil | 146.73 | 65.93 | 13.64 | 10.46 | (Tijjani, 2022) |
| 3. | Cotton seed oil | 189 | 94.7 | 7.21 | 4.82 | (Adelola and Ndudi, 2012) |
| 4. | Waste cooking oil | 252.45-194.25 | - | 16.41-19.70 | 9.77-9.56 | (Abera et al., 2023) |
| 5. | Castor oil | 180.3 | 68 | 3.8 | 9.46 | (Mishra, 2013) |
| 6. | Waste cooking oil | 196.6-205 | 1.00-0.62 | 6.67 to 14.47 | 9.31 to 10.56 | (Legesse, 2020) |
| 7. | Balanites aegyptiaca seed oil | 168.6 | 78.7 | 0.27 | 8.92 | (Manji et al., 2013) |
| 8. | Used cooking oil | 195-253 | 1.0152-1.7766 | 6.03-4.46 | 9.72-10.34 | This work |