

Recycling waste cooking oil into soap: physicochemical characterization and multivariate assessment of experimental formulations

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Abstract

Waste cooking oil is an abundant food-related residue that can be valorized into useful products within circular economy strategies. This study aimed to prepare five soap formulations from waste edible oil-derived raw materials and to evaluate their physicochemical properties and antioxidant activity in order to identify the most balanced formulation. The waste oil was purified and used for soap production by alkaline saponification. The obtained formulations were assessed in terms of moisture content, foam height, pH, total fatty substances, total alkali content, and DPPH radical scavenging activity. The moisture content ranged from 8.78% to 20.53%, with the lowest value observed for F3 and the highest for F5. Foam height varied from 9.63 to 24.67 cm, and the highest foaming performance was recorded for F3. The pH values ranged from 7.03 to 10.21, indicating clear differences in alkalinity among the formulations. Total fatty substances ranged from 66.11% to 83.91%, with F4 showing the highest value, while total alkali content ranged from 0.19% to 1.10% and remained below the cited limit in all samples. Antioxidant activity was weak for all formulations, although F4 showed the lowest IC₅₀ value and therefore the strongest activity among the soap samples. The integrated evaluation indicated that F3 had the most balanced physicochemical profile, whereas F4 showed the most favorable antioxidant performance. These findings confirm that waste edible oil can serve as a promising raw material for soap production, although formulation optimization remains essential.

Keywords: *waste cooking oil; soap production; waste valorization; physicochemical characterization; circular economy.*

Introduction

Waste cooking oil (WCO) is one of the most abundant lipid-rich residues generated by households, restaurants, and food-service facilities worldwide. When disposed of improperly, it can cause sewer blockage, increase the burden on wastewater-treatment systems, and contribute to soil and water pollution. At

the same time, WCO is increasingly regarded not only as a problematic waste stream, but also as a valuable secondary feedstock that can be redirected into the production of bio-based materials and products. Recent reviews emphasize that the sustainable management of WCO should not be limited to disposal control alone, but should also include practical valoriza-

tion routes that support waste reduction, resource recovery, and circular-economy strategies (Caporusso et al., 2025; Sankhyan et al., 2025; Beghetto, 2025a; Beghetto, 2025b; Mannu et al., 2025; Mannu et al., 2019). The increasing interest in WCO valorization is closely linked to the chemical instability of oils during frying. Repeated heating promotes oxidation, hydrolysis, polymerization, and isomerization, which alter the fatty acid profile, increase degradation products, and reduce the suitability of oils for repeated food use. The extent of these changes depends on frying temperature, exposure time, oxygen availability, food moisture, and the original oil composition. Therefore, the quality of post-use oil may vary considerably, and this variability directly affects the behavior of WCO as a recycled raw material. Recent studies and reviews consistently show that any strategy for WCO reuse should take into account the degradation status of the oil, because the physicochemical history of the feedstock can influence the quality of the final product obtained from it (Abrante-Pascual et al., 2024; Bazina and He, 2025; Chen et al., 2025). A wide range of valorization pathways has been proposed for WCO, including biodiesel, lubricants, surfactants, polymeric materials, and other non-fuel products. In recent years, the discussion has gradually expanded from simple recycling feasibility toward broader environmental-engineering questions, such as circular-product design, waste hierarchy, and the environmental value of converting waste into technically acceptable materials. Studies on non-fuel applications have shown that WCO can be transformed into value-added products beyond energy conversion, which is especially relevant for sectors seeking simple, decentralized, and low-cost reuse routes. In this broader context, soap production is a particularly attractive option because it combines waste reduction with direct product generation and does not require highly complex conversion technologies (Foteinis et al., 2020; Cheng et al., 2024; Awogbemi et al., 2024; Oparanti et al., 2025; Dordević et al., 2025; Kumar et al., 2025). Among the available WCO-recycling routes, soap production is one of the simplest and most practical because it is based on the well-known saponification process and can be implemented with relatively simple equipment. Previous studies have shown that used frying oils can be incorporated into soap formulations with acceptable physicochemical properties, and that the final product quality depends strongly on raw-material composition, additives, and processing conditions. Antonić et al. (2022) demonstrated that soaps made from waste-used frying oils may exhibit measurable

differences in hardness, physicochemical behavior, and quality depending on the oil source and degradation degree. Abera et al. further showed that combining WCO with plant-derived material may improve product performance, while Zayed et al. extended this idea by incorporating orange peel and spent coffee grounds into homemade soaps. More recent work by Soni et al. also confirmed the relevance of physicochemical and antioxidant evaluation for WCO-derived soap formulations. Together, these studies indicate that soap production from WCO should be considered not merely as a disposal solution, but as a formulation-dependent valorization pathway requiring careful quality assessment (Antonić et al., 2020; Antonić et al., 2021; Abera et al., 2023; Zayed et al., 2024; Soni et al., 2024; Ahadito and Afriani, 2024; Octarya et al., 2025; Glevitzky et al., 2025; Jara-Vélez et al., 2025). The quality assessment of soap formulations is usually based on physicochemical indicators such as moisture content, pH, foaming behavior, total fatty matter or total fatty substances, and total alkali content, because these variables are directly related to product hardness, storage stability, cleansing efficiency, and formulation balance. Studies on both experimental soaps and commercial products consistently use these parameters as the basis for formulation comparison. At the same time, antioxidant activity has increasingly been considered as an additional characteristic, especially in formulations containing recycled oils or waste-derived additives. Although antioxidant performance is not the primary criterion for soap quality, it can still provide useful information about formulation behavior and broaden the comparative evaluation of recycled soaps. Recent studies on both WCO-derived and commercial soaps support the inclusion of antioxidant-related analysis as a supplementary indicator alongside standard physicochemical parameters (Antonić et al., 2020; Zayed et al., 2024; Soni et al., 2024; Nova et al., 2025). Despite the growing body of work on WCO valorization, several gaps remain in the literature. First, many published studies focus primarily on demonstrating that soap can be produced from WCO, whereas fewer compare several formulations using a unified set of quality indicators in order to identify the most balanced sample. Second, antioxidant activity is still included less consistently than the core physicochemical parameters. Third, although recent studies increasingly frame WCO reuse within circular-economy and environmentally oriented product development, more comparative formulation studies are still needed in order to connect waste valorization with measurable product quality.

Therefore, the aim of the present study was to prepare five soap formulations based on waste edible oil-derived raw materials and to evaluate their moisture content, foam height, pH, total fatty substances, total alkali content, and antioxidant activity in order to identify the most balanced formulation from the standpoint of product quality and sustainable waste utilization.

Materials and methods

Raw materials and reagents

Waste cooking oil (WCO) derived from used edible sunflower oil was selected as the main raw material for soap production. The oil residues were collected from urban households and public catering facilities in Shymkent, Kazakhstan. The WCO samples ranged in color from yellowish to dark brown-black, had a strongly burnt odor, and showed a viscous consistency. The density of the waste oil was 1075 kg/m³, the refractive index at 20°C was 1.893, the viscosity at 20°C was 0.1891 Pa·s, the acid value was 0.74 mg KOH/g, and the peroxide value was 5.72 mmol O/kg. Five soap formulations were prepared and coded as F1, F2, F3, F4, and F5. The composition of the formulations is presented in Table 1. Sodium hydroxide pellets, sodium chloride, sulfuric acid, methanol, diethyl ether, 2,2-diphenyl-1-picrylhydrazyl (DPPH), L-ascorbic acid, anhydrous sodium sulfate, and hydrogen peroxide were of analytical grade and used without further purification. As the animal fat source, beef fat was used to provide soap hardness and fatty characteristics. The fat had a yellowish color and a characteristic natural tallow odor. Its density was 938 kg/m³, solidification temperature was 30–40°C, melting temperature was 40–51°C, viscosity at 20°C was 0.0150 Pa·s, and iodine value ranged from 32 to 47%. Liquid glass was also used during soap preparation as a binder. Distilled water was used throughout the study.

Pretreatment of waste cooking oil

Waste cooking oil (WCO) derived from used edible sunflower oil was selected as the main raw material for soap production. The oil residues were collected from urban households and public catering facilities. The WCO samples ranged in color from yellowish to dark brown-black, had a strongly burnt odor and taste, and showed a viscous consistency. The density of the waste oil was 1075 kg/m³, the refractive index at 20°C was 1.893, the viscosity at 20°C was 0.1891 Pa·s, the acid value was 0.74 mg KOH/g, and the peroxide value was

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Characterization of pretreated oil

The pretreated waste cooking oil was characterized prior to soap preparation by determining its iodine value and saponification value. These indicators were selected because they provide important information about the chemical nature of the purified oil and its suitability for soap production. The iodine value was used to estimate the degree of unsaturation of the oil, whereas the saponification value was used to characterize the amount of alkali required for saponification of the lipid material. Both parameters were determined according to the analytical procedures described in the cited literature. All determinations were performed in triplicate before soap preparation and were used for physicochemical characterization of the purified waste cooking oil.

Preparation of soap formulations

Five soap formulations (F) were prepared and coded as F1, F2, F3, F4, and F5. The composition of the experimental formulations is presented in Table 1. All formulations were prepared by alkaline saponification using the same general procedure. Briefly, 50 g of animal fat was heated to 70 °C and mixed with 135 mL of pretreated waste cooking oil residue. Separately, 25 g of sodium hydroxide was dissolved in 32 mL of distilled water to prepare the aqueous alkaline solution. Because the dissolution of sodium hydroxide is strongly exothermic, the solution was stirred and allowed to cool before use. The temperature difference between the heated oil phase and the alkaline solution was maintained within ±10 °C. After reaching the required temperature range, the alkaline solution was gradually added to the heated oil mixture under continuous stirring

at 14 rpm. The mixture was stirred until a homogeneous soap mass was formed and the saponification reaction was completed. The reaction time was 45–60 min, depending on the ambient temperature and alkali concentration. To remove undesirable soluble impurities, the obtained soap paste was treated with 7–8% NaCl solution. After salting-out, a dense and concentrated soap mass was obtained. At the final stage, 10 g of liquid glass was added as a binder, and the mixture

was stirred at 14 rpm until homogeneous. The soap mass was then poured into molds, cooled, and dried at room temperature. The five formulations differed in their raw material composition and additive content as summarized in Table 1. In particular, F5 was prepared as a glycerin-containing soap formulation. After molding, all soap formulations were stored under the same conditions prior to physicochemical and antioxidant analyses.

Table 1. Composition of the experimental soap formulations.

Formulation	Waste cooking oil residue	Animal fat	NaOH	Water	NaCl solution for salting-out	Liquid glass	Formulation note
	mL	g	g	mL	%	g	
F1	135	50	25	32	7–8	10	Base formulation
F2	135	50	25	32	7–8	10	Modified formulation
F3	135	50	25	32	7–8	10	Modified formulation
F4	135	50	25	32	7–8	10	Modified formulation
F5	135	50	25	32	7–8	10	Glycerin soap formulation

Determination of moisture content

Moisture content was determined gravimetrically. Grated soap samples were dried in an oven at 104 °C for 1.5 h, cooled to room temperature in a desiccator, and weighed. Drying and weighing were repeated until the difference between two consecutive measurements was less than 0.01 g. Moisture content was calculated as:

$$\text{Moisture content (\%)} = \frac{W1 - W2}{W1} * 100 \quad [1]$$

where, W1 is the initial sample weight and W2 is the constant dry weight. All measurements were performed in triplicate.

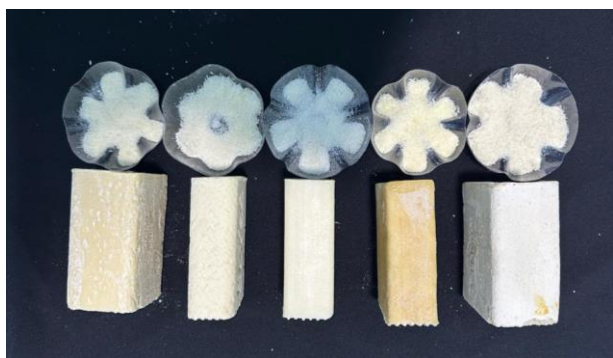


Figure 1. Soap samples taken to determine moisture content

Determination of foam height

Foam height was used as an indicator of foaming ability. For each sample, 10 g of soap was dissolved in 90 mL of distilled water in a 500 mL graduated cylinder. The cylinder was shaken vigorously 20 times, and the height of the foam was measured above the 100 mL mark. Measurements were performed in triplicate, and the average value was recorded.

Determination of pH

A 5% aqueous soap solution was prepared with hot distilled water and then allowed to cool to room temperature. The pH of the clear solution was measured using a calibrated pH meter [insert model/manufacturer]. Each sample was measured three times, and the average value was used for analysis.

Determination of total fatty substances

Total fatty substances (TFS) were determined by acid decomposition and solvent extraction. Briefly, 5 g of soap sample was dissolved in 100 mL of distilled water in a conical flask. After complete dissolution, 0.5 M H₂SO₄ was added until phase separation occurred, followed by an additional 10 mL excess. The liberated fatty matter was extracted with diethyl ether in a separatory funnel. A double extraction was carried out

using 50 mL portions of diethyl ether. The collected fat layer was dried and weighed. TFS was calculated as:

$$\text{TFS (\%)} = \frac{M_d}{M_i} * 100 \quad [2]$$

where, M_i is the initial mass of the soap sample and M_d is the mass of the recovered fatty matter.

Determination of total alkali content

Total alkali content was determined from the aqueous phase obtained during the TFS procedure according to the cited standard method. This subsection must be checked against the laboratory protocol before submission, because the current draft states that the aqueous layer was titrated with 0.5 M NaOH, which should be verified for chemical correctness. After verification, the final text should clearly specify the titrant, indicator, endpoint, and calculation formula used for total alkali determination. Measurements were carried out in triplicate.

Determination of antioxidant activity

Antioxidant activity of the soap samples was evaluated using the DPPH free radical scavenging assay. Soap extracts were prepared in methanol, and a concentration series of 25, 50, 75, 100, and 150 mg/mL was tested. Each sample solution (1 mL) was mixed with 1.5 mL of DPPH solution (0.04 mg/mL), thoroughly vortexed, and incubated for 30 min at room temperature in the dark. Absorbance was measured using a UV-Vis spectrophotometer [insert model/manufacturer]. L-ascorbic acid was used as the reference antioxidant. Radical scavenging activity was calculated as:

$$\text{Inhibition (\%)} = \frac{A_0 - A_s}{A_0} * 100 \quad [3]$$

where, A_0 is the absorbance of the control solution and A_s is the absorbance of the tested sample. The IC_{50} value was determined by linear regression from the plot of inhibition percentage versus concentration.

Statistical analysis

All measurements were performed in triplicate where replicate determinations were available, and the results are presented as mean \pm standard deviation (SD). Descriptive statistics were calculated for the main physicochemical and functional indicators of the soap formulations, including moisture content, foam height, pH, total fatty substances, total alkali content, and antioxidant activity expressed as IC_{50} . Pearson's correlation analysis was carried out using the mean values of the

measured formulation indicators in order to identify linear relationships among the studied variables. Correlation coefficients (r) were used to evaluate the strength and direction of the associations between the physicochemical and functional characteristics of the soap formulations. Principal component analysis (PCA) was applied as an exploratory multivariate tool to visualize the overall relationships among the soap formulations and the measured quality indicators. The PCA dataset included the quantitative variables reported in the study, namely iodine number, saponification degree, antioxidant activity of the soap solution, antioxidant activity of waste oil, moisture content, foam height, pH, total fatty substances, total alkali content, and IC_{50} . Prior to analysis, the variables were standardized using z-score transformation. Because replicate measurements were available only for selected variables, PCA was interpreted as an exploratory comparative approach for integrated assessment of formulation behavior rather than as a confirmatory multivariate model. Statistical analyses were performed using IBM SPSS Statistics 26.0 (IBM Corp., 2019) and RStudio (Posit team, 2025). Statistical significance was accepted at $p < 0.05$.

Results

The five soap formulations (F1–F5) were comparatively evaluated using a set of physicochemical and functional indicators, including moisture content, foam height, pH, total fatty substances, total alkali content, and antioxidant activity. The obtained results revealed clear differences among the formulations and made it possible to identify the samples with the most balanced quality profile.

Moisture content of the soap formulations

The moisture content of the tested soap formulations ranged from 8.78% to 20.53% (Figure 2). The lowest value was recorded for F3 (8.78%), whereas the highest moisture content was observed for F5 (20.53%). Formulations F1, F2, and F4 showed similar intermediate values of 10.14%, 10.93%, and 10.24%, respectively. According to the cited reference range of 10–15%, formulations F1, F2, and F4 were within the commonly accepted interval, while F3 showed a lower moisture value and F5 exceeded this range. The elevated moisture content of F5 may be associated with the glycerin-containing nature of this formulation, whereas the lower value of F3 suggests improved drying and storage stability (Figure 2). In general, the ob-

tained moisture values were comparable with those reported in the literature for soap products prepared from conventional and waste-derived raw materials. The observed differences among the formulations indicate that moisture content is one of the key parameters distinguishing the quality profile of the experimental soaps and should be considered together with foaming behavior, pH, total fatty substances, and total alkali content in the overall evaluation of formulation performance.

Foaming properties of the soap formulations

The foaming behavior of the prepared soap formulations differed markedly among the tested samples (Fig. 3). The average foam height ranged from 9.63 to 24.67 cm, indicating substantial variation in foaming performance between formulations. The highest foaming ability was observed for F3 (24.67 ± 0.47 cm), followed by F4 (20.97 ± 0.40 cm), whereas the lowest value was recorded for F1 (9.63 ± 0.41 cm). Formulations F2 and F5 showed intermediate and relatively close values of 14.47 ± 0.50 cm and 14.03 ± 0.39 cm, respectively. Thus, the overall order of foaming performance can be presented as $F3 > F4 > F2 \approx F5 > F1$. A comparison of the mean values shows that the foaming height of F3 was approximately 2.6-fold higher than that of F1, while F4 exceeded F1 by about 2.2 times. In contrast, the difference between F2 and F5 was relatively small, amounting to less than 0.5 cm. These results indicate that the experimental formulations can be clearly differentiated according to foaming performance and that the effect of formulation composition on foam development was not minor, but pronounced. Since foaming height is commonly considered one of the practical quality indicators of soap, the observed variation suggests that F3 and F4 had the most favorable foaming profile among the tested formulations. The repeat measurements also showed good internal consistency within each formulation. The dispersion among the three measurements was low for all samples, with standard deviations below 0.5 cm, which indicates satisfactory repeatability of the foaming test. This is important because it confirms that the observed differences between formulations reflect stable formulation dependent behavior rather than random measurement fluctuation. In particular, F3 combined the highest average foam height with a narrow spread of replicate values, which strengthens the conclusion that this formulation had the strongest and most reproducible foaming performance. A similarly stable pattern was observed for F4 and F5, while F2 showed slightly greater varia-

tion, although still within a narrow range. All tested formulations exceeded the minimum reference value of 1.5 cm per 100 mL of solution cited in the manuscript, which means that all soaps demonstrated acceptable foaming capacity under the applied test conditions. At the same time, the measured range of 9.63–24.67 cm was notably higher than the comparative literature interval of 2.30–8.50 cm cited in the current draft. This suggests that the prepared formulations, especially F3 and F4, exhibited comparatively strong foam formation. Therefore, foaming ability can be regarded as one of the key distinguishing functional parameters of the developed waste-oil-based soap formulations.

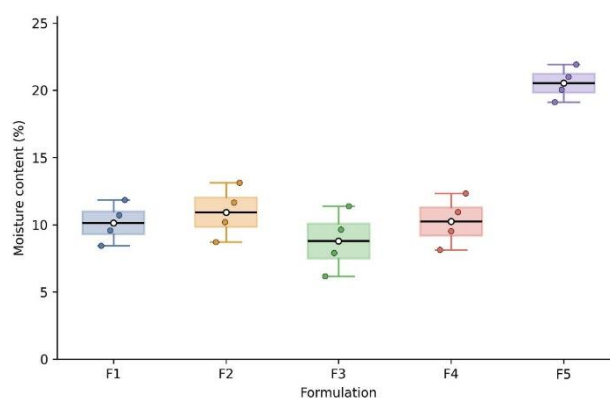


Figure 2. Moisture content of the soap formulations. Values are presented as mean \pm SD.

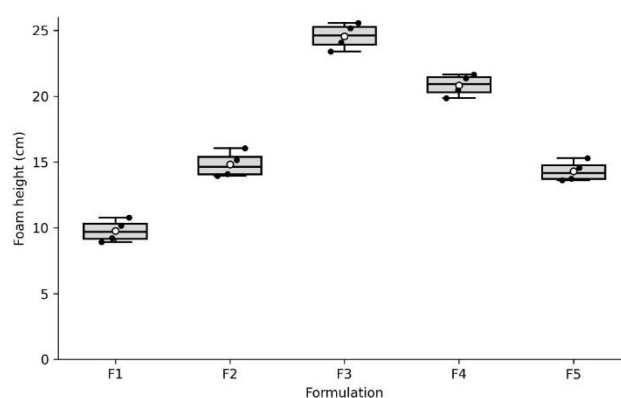


Figure 3. Foam height of the soap formulations. Values are presented as mean \pm SD.

pH of the soap formulations

The pH values of the tested soap formulations showed clear differences among the samples and ranged from 7.03 to 10.21 (Table 5, Figure 4). The lowest pH was recorded for F1 (7.03), followed by F3 (7.38) and F2 (7.88), whereas the highest value was observed for F4 (10.21). Formulation F5 also showed a relatively high pH value of 9.51, indicating a more alkaline character

than F1–F3. Thus, the formulations can be divided into two groups according to pH behavior: F1–F3 with near-neutral to mildly alkaline values and F4–F5 with more pronounced alkalinity. The repeat measurements demonstrated high internal consistency for all formulations. For F1, the three pH values were 7.03, 7.05, and 7.02; for F2, 7.90, 7.86, and 7.88; for F3, 7.41, 7.35, and 7.38; for F4, 10.21, 10.18, and 10.23; and for F5, 9.48, 9.52, and 9.53. The narrow spread of replicate values indicates good repeatability of the pH measurements and supports the reliability of the observed differences among formulations. In particular, the stability of the replicate values suggests that the differences between F1–F3 and F4–F5 reflect actual formulation-dependent variation rather than random analytical fluctuation. From a comparative perspective, the pH difference between the most acidic and the most alkaline formulation was substantial. F4 exceeded F1 by more than 3 pH units, while F5 also remained clearly above the values recorded for F1–F3. This pattern is important because pH is one of the core quality indicators of soap formulations and reflects the overall balance between the fatty phase and the alkaline component used during saponification. The relatively low pH values of F1–F3 indicate milder formulations, whereas the higher pH values of F4 and F5 suggest a stronger alkaline character. The obtained pH range of 7.03–10.21 is comparable with the literature values cited in the manuscript, including 7.29–11.53, 10.50–10.80, 9.6–10.4, and 8.6. At the same time, the present results show that the experimental soap formulations were not uniform in alkalinity and that pH can serve as an effective parameter for differentiating their quality profiles. Among the tested formulations, F1, F2, and F3 may be considered more favorable from the standpoint of moderate pH, while F4 and F5 were distinctly more alkaline. Therefore, pH should be considered together with total alkali content and total fatty substances in the overall comparative evaluation of the developed waste-oil-based soap formulations (Fig. 4).

Total fatty substances content

The total fatty substances (TFS) content of the tested soap formulations ranged from 66.11% to 83.91% (Table 6, Figure 5), indicating clear differences in the proportion of active fatty material among the formulations. The highest TFS value was recorded for F4 (83.91%), followed by F3 (81.75%), F2 (80.15%), and F1 (78.29%), whereas F5 showed the lowest value (66.11%). Thus, the formulations can be ranked as fol-

lows: $F4 > F3 > F2 > F1 > F5$. The obtained values show that four formulations, namely F1–F4, were relatively close to each other and all exceeded the 76% threshold cited in the manuscript for high-quality category I soaps. In contrast, F5 remained clearly separated from the other formulations, with a TFS content lower by more than 12 percentage points compared with F1 and by nearly 18 percentage points compared with F4. Nevertheless, the value of 66.11% still places F5 above the 63% level cited for category II standard soaps. These results indicate that F1–F4 had a more favorable fatty composition profile than F5, while F5 represented the least enriched formulation in terms of total fatty substances. From a comparative perspective, the relatively narrow range observed among F1–F4 suggests that these formulations had a generally similar and consistently high proportion of fatty matter. By contrast, the distinctly lower value of F5 points to a formulation-specific difference that may reflect variation in raw material composition, the degree of saponification, or the proportion of incompletely converted or non-fatty components. This interpretation is in line with the statement already present in the manuscript that low total fat content may be associated with incompletely reacted alkali. The TFS interval obtained in this study (66.11–83.91%) is also comparable with the literature ranges cited in the current draft, including 59.00–91.00%, 29.6–79.2%, 54–68%, and 60.20%. At the same time, the present data show that the experimental soaps, especially F2–F4, were positioned in the upper part of these comparative intervals. This supports the conclusion that most of the developed formulations had a favorable TFS profile, while F5 should be considered separately because of its markedly lower value. Overall, total fatty substances content was one of the key parameters distinguishing the quality profile of the tested waste-oil-based soap formulations (Fig. 5).

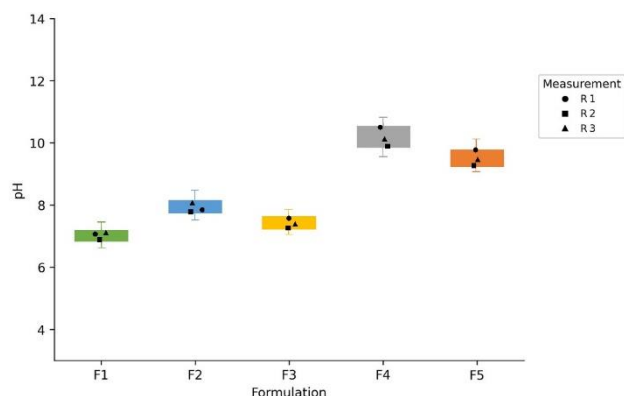


Figure 4. pH values of the soap formulations. Values are presented as mean \pm SD.

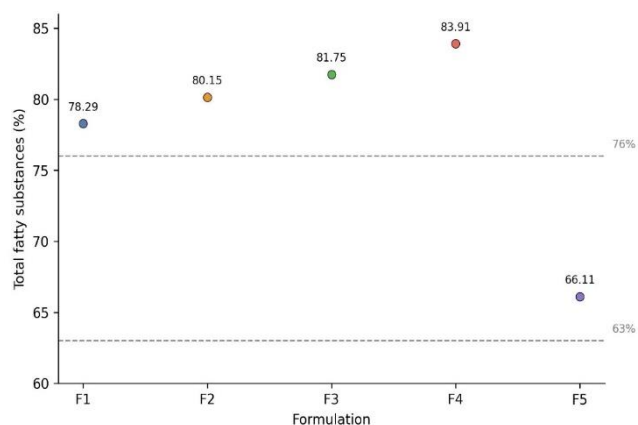


Figure 5. Total fatty substances content of the soap formulations.

Total alkali content

The total alkali content of the tested soap formulations ranged from 0.19% to 1.10% (Table 7, Fig. 6). The highest value was recorded for F5 (1.10%), whereas the lowest alkalinity was observed for F4 (0.19%). Intermediate values were found for F1 (0.77%), F2 (0.58%), and F3 (0.29%). Thus, the formulations can be ranked in descending order of total alkali content as follows: F5 > F1 > F2 > F3 > F4. The obtained data show a clear separation between F5 and the remaining formulations. The alkali content of F5 was almost 5.8 times higher than that of F4 and nearly 1.4 times higher than that of F1. At the same time, F3 and F4 formed a lower-alkali group, both remaining below 0.30%, whereas F1 and F2 occupied an intermediate position. This pattern indicates that the formulations differed substantially in residual alkalinity and that total alkali content is one of the strongest discriminating parameters among the tested soap samples. The repeat measurements also demonstrated high consistency within each formulation. The standard deviations were low for all samples, indicating good repeatability of the titration procedure and supporting the reliability of the observed inter-formulation differences. In particular, F4 and F5 showed very narrow variation among repeats, while F1–F3 also remained highly stable. Such repeatability strengthens the conclusion that the differences in total alkali content reflect true formulation-dependent variation rather than random analytical fluctuation. All measured values remained below the 2% limit cited in the manuscript, indicating that none of the formulations exceeded the referenced upper threshold for total alkali content. The obtained range of 0.19–1.10% is also comparable with previously reported values of 0.76% and 0.13–1.60%, and lower than the interval of 1.61–2.96% cited from other studies. Overall, the present re-

sults suggest that all formulations met the referenced requirement for total alkali content, although F5 was clearly distinguished by its comparatively higher residual alkalinity (Fig. 6).

Antioxidant activity

The antioxidant activity of the tested soap formulations was evaluated using the DPPH free radical scavenging assay and expressed as IC_{50} values (Table 8, Fig. 7). The obtained results showed that all soap formulations had relatively high IC_{50} values, ranging from 346.74 to 515.48 mg/mL, which indicates weak antioxidant activity according to the criterion cited in the manuscript for samples with IC_{50} values above 250 mg/mL. Among the tested formulations, the lowest IC_{50} value was recorded for F4 (346.74 mg/mL), indicating the strongest antioxidant activity within the soap group. In contrast, F5 showed the highest IC_{50} value (515.48 mg/mL) and therefore the weakest free radical scavenging ability. The remaining formulations occupied an intermediate position, with IC_{50} values of 431.64 mg/mL for F1, 423.16 mg/mL for F2, and 406.32 mg/mL for F3. Thus, the antioxidant activity of the soap formulations increased in the following order: F5 < F1 < F2 < F3 < F4. A comparison of the values shows that the antioxidant performance of the formulations was markedly lower than that of the reference antioxidant. L-ascorbic acid demonstrated an IC_{50} of 26.34 mg/mL, which was substantially lower than all values recorded for the soap samples. This difference confirms that, although the experimental soaps exhibited measurable radical scavenging activity, their antioxidant effect remained considerably weaker than that of the standard compound. Nevertheless, the inter-formulation differences among the soaps were sufficiently pronounced to distinguish the most active and least active samples. In particular, F4 showed an IC_{50} value lower by nearly 169 mg/mL than F5 and lower by about 85 mg/mL than F1, indicating a clearly more favorable antioxidant profile among the tested formulations (Fig. 7). The results presented in the manuscript also indicate a dose-dependent increase in radical inhibition for all tested soap samples, suggesting that higher soap concentrations led to greater DPPH scavenging. This pattern supports the validity of the calculated IC_{50} values and confirms that the antioxidant response was concentration-dependent. At the same time, the absolute IC_{50} values show that antioxidant activity was not a dominant functional property of the soap formulations and should therefore be interpreted as an additional comparative indicator rather than a

primary quality criterion. From this perspective, F4 can be regarded as the most promising formulation in terms of antioxidant activity, whereas F5 showed the least favorable result. The DPPH assay therefore enabled a clear differentiation of the tested soap formulations according to their antioxidant behavior. Although all soaps showed weaker activity than L-ascorbic acid, the variation in IC_{50} values was sufficient to distinguish formulations with more favorable and less favorable antioxidant profiles. In combination with the physicochemical indicators, these findings support the identification of F3 and F4 as the most promising formulations among the tested samples.

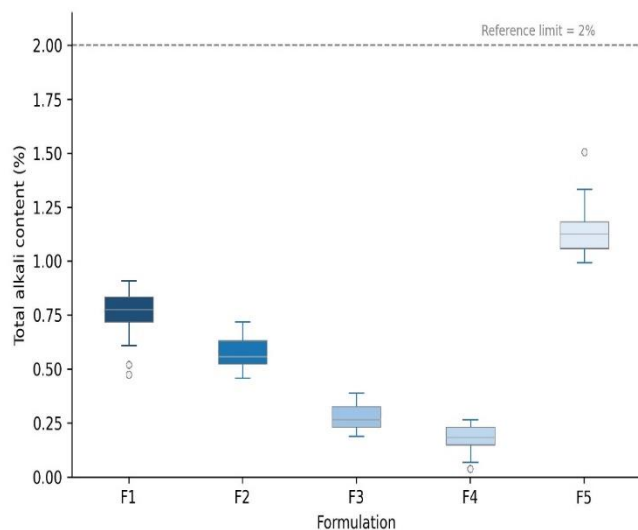


Figure 6. Boxplot of total alkali content in the soap formulations. The dashed line indicates the reference limit of 2%.

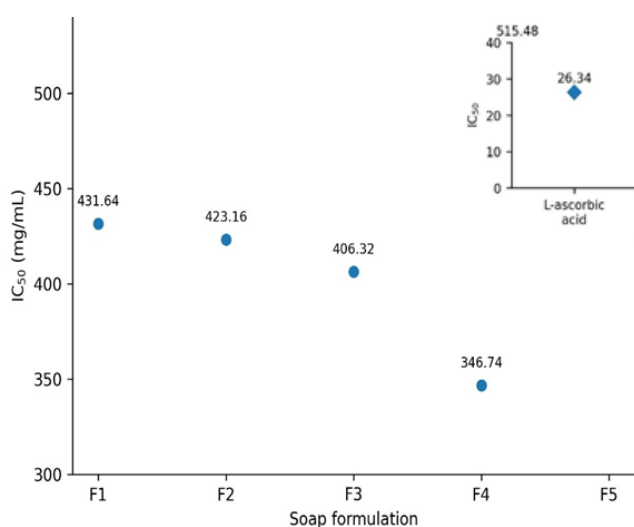
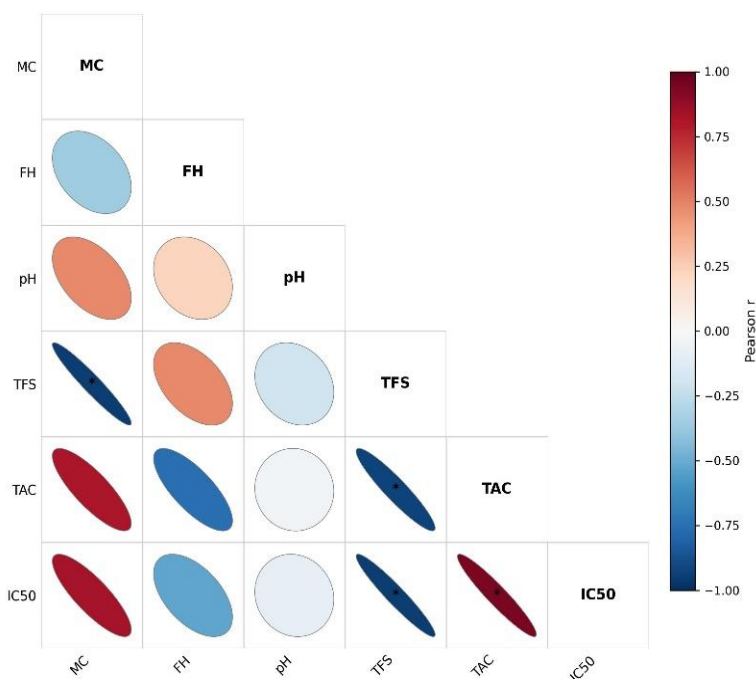


Figure 7. IC_{50} values of the soap formulations in the DPPH assay. The inset shows the IC_{50} value of L-ascorbic acid.

Pearson correlation and principal component analysis

Pearson's correlation analysis was performed using the mean values of the measured physicochemical and functional indicators in order to identify the main relationships among the tested soap formulations. The analysis showed several pronounced associations between the studied variables. The strongest negative correlation was observed between moisture content and total fatty substances ($r = -0.95$), indicating that formulations with higher moisture tended to have lower total fatty substance content. A similarly strong negative relationship was found between total fatty substances and IC_{50} ($r = -0.95$), whereas total alkali content showed a strong positive correlation with IC_{50} ($r = 0.94$). Since a lower IC_{50} corresponds to stronger antioxidant activity, these results indicate that formulations with higher total fatty substance content and lower residual alkalinity tended to demonstrate more favorable antioxidant performance. In addition, total fatty substances were strongly negatively correlated with total alkali content ($r = -0.93$), while moisture content showed positive relationships with both total alkali content ($r = 0.82$) and IC_{50} ($r = 0.83$). By contrast, pH showed only weak correlations with most of the remaining variables, suggesting that it behaved more independently within the studied dataset. The correlation pattern also provides a useful comparative interpretation of formulation quality. Foam height showed a moderate positive association with total fatty substances ($r = 0.48$) and a marked negative relationship with total alkali content ($r = -0.75$), suggesting that better foaming performance tended to occur in formulations with lower residual alkalinity and relatively higher fatty matter. Although some of the remaining pairwise relationships were weaker, the matrix as a whole indicates that the quality indicators did not vary independently. Instead, the tested formulations followed a structured pattern in which higher moisture and higher total alkali content were generally associated with less favorable quality characteristics, whereas higher total fatty substances and lower IC_{50} values reflected a more advantageous formulation profile. Because only five formulations were compared, these relationships should be interpreted as exploratory, but they still provide a coherent multivariate picture of the data structure (Fig. 8). Principal component analysis (PCA) was performed as an exploratory multivariate approach using the quantitative indicators measured for the soap formulations, including iodine number, sapo-

**Figure 8**

Pearson correlation heatmap of the studied soap-quality indicators.

nification degree, antioxidant activity of the soap solution, antioxidant activity of waste oil, moisture content, foam height, pH, total fatty substances, total alkali content, and IC₅₀. The first two principal components explained 89.4% of the total variance, with PC1 accounting for 54.1% and PC2 for 35.2%, indicating that the main structure of the dataset was well represented in the two-dimensional PCA space (Fig. 9). The PCA plot revealed a clear differentiation among the tested formulations. F5 was distinctly separated from the remaining soap samples, indicating that this formulation had the most contrasting overall profile in the dataset. This separation was mainly associated with higher moisture content, higher total alkali content, higher IC₅₀ values, and generally less favorable quality characteristics. In contrast, F1–F4 were distributed on the opposite side of the ordination space, reflecting comparatively more balanced and favorable formulation properties. The loading pattern showed that PC1 was positively associated with iodine number, saponification degree, antioxidant activity of the soap solution, antioxidant activity of waste oil, moisture content, pH, total alkali content, and IC₅₀, whereas total fatty substances contributed in the opposite direction. This suggests that PC1 primarily separated formulations characterized by higher residual alkalinity, higher moisture, and weaker antioxidant performance from those with higher total fatty substance content and a more favorable overall physicochemical profile. PC2 was influenced mainly by foam height, pH, and total

fatty substances in the positive direction, while total alkali content and IC₅₀ contributed negatively. This axis provided an additional differentiation of the soap formulations according to foaming behavior and quality balance. In particular, F4 occupied the most favorable position along this axis, which is consistent with its high total fatty substances content, low residual alkalinity, and comparatively strong antioxidant activity. F3 was also located in the more favorable region of the PCA plot, reflecting its strong foaming ability and balanced physicochemical characteristics. The relative arrangement of the formulations in the PCA space supported the trends observed in the univariate analysis. F3 and F4 were associated with the most favorable quality indicators, although for partly different reasons: F3 due to its balanced physicochemical profile and high foaming performance, and F4 due to its high total fatty substances content and stronger antioxidant activity. F1 and F2 occupied intermediate positions, indicating moderate and relatively stable quality profiles. In contrast, F5 remained clearly separated from the other formulations, confirming that it represented the least favorable sample in terms of the combined multivariate profile (Fig. 9). Taken together, the PCA provided an integrated view of the relationships among the measured formulation properties and confirmed that the soap samples differed not only in individual indicators, but also in their overall multivariate structure. The analysis supports the conclusion that F3 and F4 were the most promising formulations, whereas F5 was the most

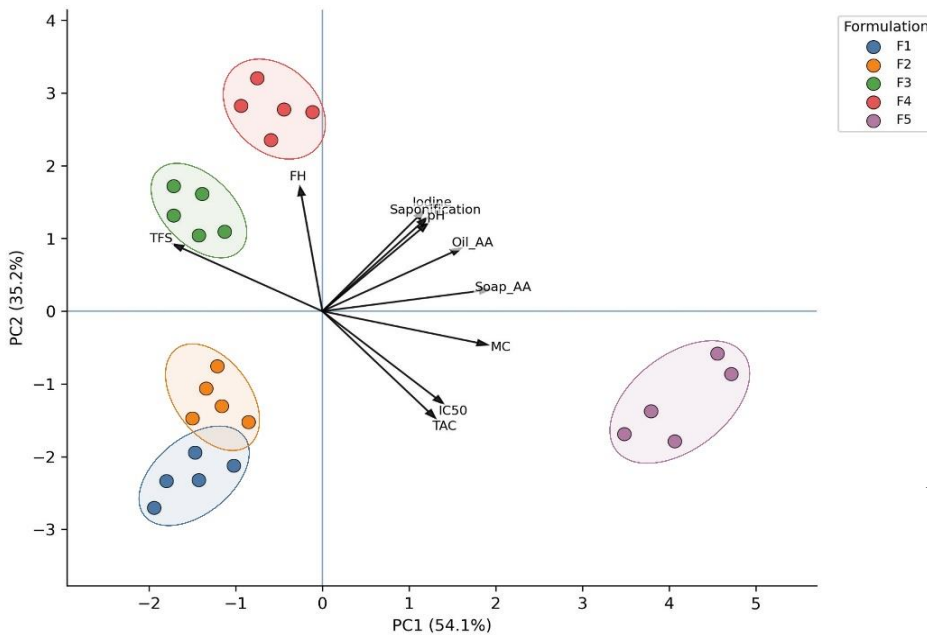


Figure 9
Principal component analysis (PCA) plot of the soap formulations based on the quantitative indicators measured in the study.

divergent and least favorable sample in the tested set. Integrated heatmap analysis of standardized quality indicators (Fig. 10) further supported the comparative interpretation of the soap formulations. The heatmap showed that F3 and F4 were characterized by the most favorable overall quality profiles, as reflected by relatively low moisture content, low total alkali content, and advantageous values of foam height, total fatty substan-

ces, and antioxidant-related parameters. In contrast, F5 displayed the most unfavorable combined profile, with high moisture content, high total alkali content, low total fatty substances, and the highest IC₅₀ value. Formulations F1 and F2 occupied intermediate positions, indicating moderate and relatively balanced quality characteristics.

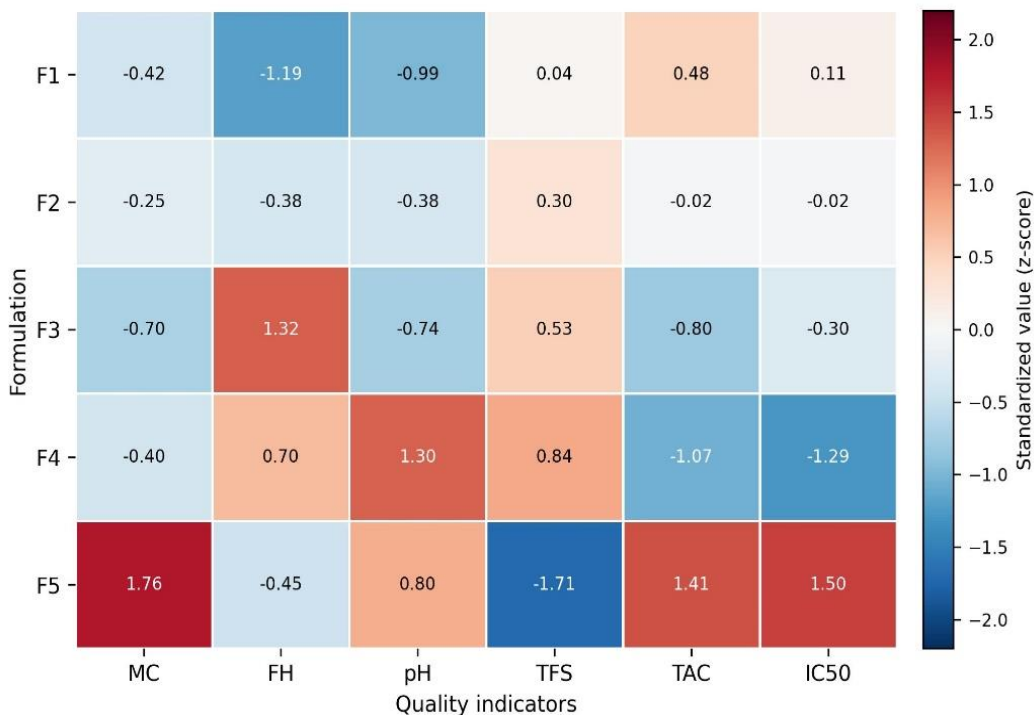


Figure 10
Integrated heatmap of standardized quality indicators of the soap formulations. Values are expressed as z-scores.

Discussion

The results of the present study confirm that waste edible oil-derived raw materials can be successfully valorized into soap formulations with acceptable physicochemical characteristics, but they also show that formulation-dependent differences strongly influence the final product profile. This interpretation is consistent with recent studies demonstrating that used frying oils can be converted into soaps of acceptable quality, although the final properties depend on raw-material composition, frying history, additives, and saponification conditions (Antonić et al., 2020; AntoniĆ et al., 2021; Abera et al., 2023). In a broader environmental-engineering context, this is also in line with recent reviews that frame waste cooking oil as a relevant secondary feedstock within circular-economy and waste-valorization strategies, while emphasizing that product quality remains a key condition for meaningful reuse (Caporusso et al., 2025; Kumar et al., 2025). One of the clearest patterns in the present dataset was the contrast between F3/F4 and F5. F3 combined the lowest moisture content with strong foaming performance, relatively low pH, favorable total fatty substances content, and low total alkali content, whereas F5 showed the highest moisture content, the highest residual alkalinity, the lowest total fatty substances content, and the weakest antioxidant activity. This pattern is important because moisture, total fatty substances, and residual alkali are core formulation-quality parameters in soap studies, and they often reflect the balance between the fatty phase, the alkaline phase, and the extent of soap curing or formulation stabilization (Nova et al., 2025). The elevated moisture content of F5 is also compatible with its glycerin-containing character in your manuscript, but the present data suggest that this did not translate into the most favorable overall formulation profile when considered together with total fatty substances and total alkali content. The foaming results provide an additional practical dimension to this interpretation. In the present study, F3 and F4 showed the strongest foam formation, while F1 produced the lowest foam height. Earlier work on soaps made from waste cooking oil similarly reported that formulation composition can substantially affect lather formation and cleansing-related behavior, and Abera et al. showed that blending WCO with plant-derived material improved foaming performance relative to WCO soap alone. Likewise, recent studies on WCO-derived soaps have emphasized that formulation design, rather than waste oil use itself, is often the decisive factor controlling functional quality (Abera et al., 2023; Zayed et al., 2024; Soni et al., 2024). From this perspective, the strong performance of F3 suggests that the most successful WCO-based formulations are those in which waste-derived raw materials are not merely reused, but are incorporated into a compositionally balanced system. The pH and total alkali results should be interpreted together rather than separately. In the present work, F1–F3 showed lower pH values than F4–F5, while F4 simultaneously had the lowest total alkali content

despite its relatively high pH. This indicates that pH alone does not fully describe formulation quality and that residual alkalinity must be considered alongside pH when evaluating soap mildness and formulation balance. Comparable studies on both WCO-derived soaps and commercial soaps also use pH and alkali-related indicators jointly, because the final formulation behavior depends on the combined effect of these variables rather than on a single metric (Antonić et al., 2020; AntoniĆ et al., 2021; Nova et al., 2025). Therefore, the present results support a more integrated interpretation in which F3 appears favorable because of its balanced pH and low total alkali content, while F4 remains attractive because its low residual alkalinity offsets, to some extent, its more alkaline pH profile. The antioxidant assay added a useful, though secondary, dimension to formulation comparison. All tested soaps showed weak antioxidant activity in absolute terms, since all IC₅₀ values were well above the reference threshold used in the manuscript, and all remained far weaker than L-ascorbic acid. However, the differences among formulations were still informative, with F4 showing the lowest IC₅₀ and F5 the highest. This agrees with recent studies reporting that WCO-based soaps may exhibit measurable antioxidant behavior, especially when formulation composition or waste-derived additives contribute to radical scavenging potential, although antioxidant performance is usually modest compared with standard antioxidants (Zayed et al., 2024; Soni et al., 2024; Nova et al., 2025). In this sense, antioxidant activity in the present study should be interpreted not as the main functional attribute of the soaps, but as an additional comparative indicator that helped distinguish F4 from the remaining formulations. The multivariate analyses strengthened the same general conclusion. Pearson correlation indicated that higher moisture and higher total alkali content were associated with less favorable formulation behavior, whereas higher total fatty substances were associated with lower IC₅₀ values and more advantageous overall profiles. PCA and the integrated heatmap showed that F3 and F4 occupied the most favorable multivariate positions, whereas F5 was clearly separated as the least favorable sample. Although the PCA in this study should be treated as exploratory because not all variables were available as full independent multivariate replicate sets, its interpretation is consistent with the broader trend in recent WCO valorization studies: formulation quality depends on the combined interaction of several variables rather than on a single performance indicator (Caporusso et al., 2025; Kumar et al., 2025). Thus, the convergence between univariate and multivariate results increases confidence in the identification of F3 and F4 as the most promising formulations. From an applied perspective, the present results support the environmental relevance of soap production as a practical valorization route for waste cooking oil. Reviews published in 2025 emphasize that WCO recycling should increasingly be eva-

luated through the lens of product-oriented circular systems, not only through energy conversion or disposal avoidance (Caporusso et al., 2025; Kumar et al., 2025). The present study contributes to that direction by showing that the environmental benefit of reuse depends not only on converting waste into a product, but also on achieving a technically acceptable and compositionally balanced formulation. In that sense, F3 and F4 appear to be the best candidates for further development, whereas F5 illustrates that not every waste-derived formulation automatically yields a favorable quality profile. At the same time, several limitations should be acknowledged. First, some formulation differences were captured mainly through final soap-quality indicators. Second, antioxidant activity was assessed only by the DPPH assay, and no microbiological, dermatological, or storage-stability validation was included. Third, the multivariate analysis remains exploratory because the study was based on a limited number of formulations and not all variables were represented by independent full replicate structures. These limitations are also consistent with the broader literature, where many WCO-soap studies remain formulation-oriented and would benefit from more systematic optimization, stability testing, and expanded functional validation (Zayed et al., 2024; Soni et al., 2024). Taken together, the discussion indicates that F3 showed the most balanced physicochemical profile, whereas F4 combined the highest total fatty substances content with the strongest antioxidant activity. In contrast, F5 consistently showed the least favorable combined profile due to its high moisture content, high residual alkalinity, low total fatty substances content, and weak antioxidant performance. These findings demonstrate that waste edible oil can serve as a promising raw material for soap production, but that the final quality depends critically on formulation optimization and integrated evaluation of product properties.

Conclusions

This study demonstrated that waste edible oil-derived raw materials can be successfully used for the preparation of soap formulations with acceptable physicochemical characteristics, confirming their potential as a promising feedstock for waste valorization. However, the obtained results also showed that the quality of the final soap strongly depended on formulation composition, and that the tested samples differed substantially in moisture content, foaming performance, pH, total fatty substances, total alkali content, and antioxidant activity. Among the tested formulations, F3 exhibited the most balanced physicochemical profile, combining low moisture content, the highest foam height, moderate pH, high total fatty substances content, and low residual alkalinity. F4 was also identified as one of

the most promising formulations because it showed the highest total fatty substances content together with the strongest antioxidant activity. In contrast, F5 consistently demonstrated the least favorable combined profile due to its high moisture content, high total alkali content, low total fatty substances content, and weak antioxidant performance. The multivariate analyses, including Pearson correlation, PCA, and the integrated heatmap, supported the same general interpretation and confirmed that F3 and F4 were the most favorable formulations, whereas F5 was the most divergent and least favorable sample in the tested set. These findings indicate that the environmental value of waste cooking oil recycling depends not only on converting waste into a new product, but also on achieving a technically acceptable and compositionally balanced formulation. Thus, waste edible oil can be regarded as a promising raw material for soap production within circular-economy and waste-management strategies. Future studies should focus on clearer formulation optimization, long-term storage stability, microbiological validation, and broader functional evaluation in order to support the further development of waste-oil-based soap products.

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Abbreviations: The following abbreviations are used in this manuscript:

WCO Waste cooking oil
TFS Total fatty substances
PCA Principal component analysis

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