

Article

Valorization of Waste Cooking Oils into Antimicrobial Soaps with Honey, Propolis, and Essential Oils

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Abstract

The valorization of waste cooking oils (WCOs) provides a strategy to reduce environmental impact while converting residues from the food industry into valuable products. This study developed and characterized antimicrobial soaps from purified WCOs (sunflower, palm, and pumpkin oils) enriched with natural bioactive ingredients. WCOs were purified by filtration, treatment with 10% NaCl, and bleaching with 3% H₂O₂, followed by cold saponification with NaOH. Twelve soap formulations were prepared, including six enriched with bee products (propolis, poly-floral honey, linden, acacia, honeydew, and sunflower) and six enriched with essential oils (EOs) (clove, rosemary, mace, nutmeg, white pepper, and juniper). The WCOs, natural bioactive ingredients, and soaps were characterized using physico-chemical methods (FTIR, GC-FID, phenols, flavonoids, etc.), while their antibacterial activity was determined against two microbial strains: *Staphylococcus aureus* and *Escherichia coli*. The antimicrobial activity of soaps is related to their alkaline pH, while the addition of honey, propolis, or EOs contributes to additional antimicrobial effects. Among honey- and propolis-enriched soaps, those with propolis produced the largest inhibition zones (up to 8.67 mm for *S. aureus* and 7.0 mm for *E. coli*). EO-based soaps exhibited higher activity, with rosemary EO-based soap showing the largest zones (up to 9.5 mm for *S. aureus* and 7.5 mm for *E. coli*). These data support the potential of enriched soaps containing honey, propolis, and EOs for antimicrobial applications, highlighting their value as a sustainable alternative in the valorization of WCOs.

Keywords: waste cooking oils; sustainable recycling; honey; propolis; essential oils; soap formulation; antimicrobial activity



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1. Introduction

Biowaste and other bio-derived residues, such as waste cooking oils (WCOs), are attractive raw materials with useful chemical properties [1]. Recent research has focused on reducing the carbon footprint of technological processes or reusing waste as part of the economic circularity paradigm [2].

As the population has grown, the demand for vegetable oils has increased. This rise in demand has mainly been driven by the food, biodiesel, and pharmaceutical industries [3]. The market is dominated by palm, soybean, and sunflower oil [4], and vegetable oils are widely used in the food industry, including in deep-frying processes. WCOs contain degradation products formed through thermal oxidation, isomerization, and oligomerization side reactions, all facilitated by high temperatures [5]. Improper disposal of WCOs poses significant environmental risks. WCOs discharged into sewage, soil, or water bodies can cause clogging of drainage systems, water contamination, and adverse effects on aquatic ecosystems due to oxygen depletion and chemical toxicity [6]. Moreover, thermal degradation and the accumulation of WCOs in the environment may contribute to the formation of persistent organic pollutants and increase the chemical oxygen demand of wastewater [7]. Valorization of WCOs through saponification, biodiesel production, or other circular economy approaches mitigates these environmental impacts while converting waste into value-added products [8,9]. It is estimated that around 2 million tons of WCOs are generated annually in the EU, posing a significant environmental concern [10].

WCOs are typically defined as vegetable oils subjected to multiple frying cycles, leading to chemical degradation. International guidelines indicate that oils are considered to be WCOs when the total polar compounds exceed 24%, or after several frying cycles, depending on the oil type, frying temperature, and type of food that is fried. WCOs are defined as inedible oils recovered from food processing activities and consist of mono-, di-, and triglycerides, as well as various free fatty acids (FFAs, 5–20% *w/w*) [11]. Due to high-temperature processing, WCOs also contain oxidation derivatives (aldehydes, alcohols, epoxy products, etc.), free radicals, and other degradation products, the concentration of which depends on the frying conditions and duration [12]. Practical safety thresholds for total polar compounds (TPCs) in frying oils are established in many countries to ensure food quality. Although there are no precise EU-wide regulatory limits for TPCs in frying oils, national guidelines recommend that fresh vegetable oils contain 0–18% TPCs, with values between 19 and 24% considered a critical range. Oils with values above 24% are generally classified as waste oils or unsuitable for food use. These thresholds are widely referenced in the scientific literature as practical criteria for evaluating the degree of oil degradation before reuse or valorization, reflecting common food safety and quality practices in different regions [13,14].

WCOs are generated from domestic, commercial, and industrial frying. They can be valorized in biodiesel, bio-lubricants, animal feed, asphalt rejuvenation, and soap manufacturing, within the framework of circular economy strategies [4,6]. However, their practical use is challenged by high variability in chemical composition, the accumulation of oxidation and polymerization products, and potential environmental and health risks if they are improperly managed. These limitations require a careful assessment of oil degradation and appropriate purification or processing prior to valorization [15].

From a technological and managerial perspective, the valorization of WCOs involves major challenges related to the variability of the raw material (type of oil, frying conditions and temperature, level of contamination), the complexity of collection networks—with heterogeneous sources such as food service establishments, the food industry, and household sectors—and the selection of optimal technologies for large-scale industrial conversion [16]. Moreover, life cycle assessment studies indicate that achieving a genuine environmental benefit from WCO recycling depends on the efficiency of collection, the proper purification of the waste stream, and the appropriate integration of secondary products into existing value chains [10,17]. In the absence of efficient collection and valorization mechanisms, these wastes can become major sources of soil and water pollution [18,19]. Numerous studies have demonstrated that WCO can be transformed into valuable products through eco-friendly saponification processes; however, most reported approaches rely on con-

ventional soap-making protocols and provide limited insight into the influence of WCO purification on product quality and functionality [20].

Bioactive ingredients have gained increasing attention in cosmetic and personal care products due to their numerous beneficial properties. Honey exhibits broad-spectrum antimicrobial effects due to its high sugar content, low pH, and H_2O_2 and phenolic compounds [21]. Honey is known for its antioxidant and moisturizing effects, which contribute to hydration of the skin and protection against microbial contamination [21]. Propolis, a resinous substance produced by bees, contains flavonoids, phenolic acids, and esters that contribute to its strong antibacterial, antifungal, and anti-inflammatory activities, making it a valuable natural additive to enhance the hygienic and therapeutic properties of soaps [22,23]. Essential oils (EOs), derived from plants and rich in terpenes and phenolic compounds, provide antimicrobial, antioxidant, and aromatic properties, while also improving the sensory experience of the final product [24,25].

Despite known benefits, the incorporation of multiple bioactive ingredients into soap produced from WCOs has scarcely been investigated, particularly regarding antimicrobial performance and compatibility with recycled lipid matrices. When incorporated into soap formulations derived from purified WCOs, these bioactive ingredients are expected to enhance not only cleansing efficiency but also antimicrobial protection, an aspect that has received limited attention in previous WCO-to-soap studies [26]. Although the antimicrobial properties of honey, propolis, and EOs are well established, their application has been mainly investigated in formulations based on virgin or refined lipid matrices. In parallel, studies on WCO valorization in soaps are largely based on conventional saponification approaches, with limited consideration of how WCO purification and bioactive enrichment influence the functional performance of the final product.

In this context, the present work addresses this gap by valorizing WCOs collected from food processing facilities through a defined purification process, followed by saponification and enrichment with selected EOs, honey, and propolis. The novelty of this study lies in the combined approach of WCO purification and bioactive functionalization, supported by physicochemical characterization and antimicrobial testing of the final soap formulations. By moving beyond conventional WCO-to-soap protocols, this work demonstrates the potential of recycled lipid resources to yield value-added, functional cosmetic products, contributing to sustainable waste management and circular economy principles. This study was designed to test the hypothesis that the antimicrobial performance of soaps produced from purified WCOs is influenced by the chemical composition of the bioactive ingredients incorporated, particularly their profiles of phenolic and volatile compounds.

2. Materials and Methods

2.1. Sample Collection, Processing, and Storage

2.1.1. Waste Cooking Oil

The sampling of vegetable oils used was carried out from various units in Alba Iulia, Romania. Table 1 presents the types of oils tested and the specific frying parameters that correspond to each of them.

2.1.2. Bee Products

The bee products used in this study were obtained from *Apis mellifera carpatica* bees, collected between July and August 2025 from apiaries located in Alba County, Romania. Honey and propolis were sourced from authorized local beekeepers, according to Directive 2001/110/EC [27] and relevant national legislation governing apicultural products. Their use as cosmetic ingredients is allowed under Regulation (EC) No 1223/2009 [28], provided that safety and quality requirements are met.

Table 1. Types of collected WCOs and their corresponding frying conditions.

Type of Used Oil	Status (Refined/Unrefined)	Field of Use	Type of Fried Food	Frying Time/Preparation, min	Temperature, °C	Number of Usage Cycles	Oil-to-Food Ratio	Type of Frying
Sunflower	Refined	Fast food	French fries	5–10	175–190	10–15	5:1	Deep frying
Palm	Refined	Bakery/ Pastry	Donuts, Fried buns	2–5	170–180	8–12	3:1	Deep frying
Pumpkin	Unrefined	Restaurant	Vegetables/ Mushrooms	2–5	120–140	3–5	4:1	Light sauté/ shallow frying

Note: Collected oils were confirmed as WCOs with total polar compounds (TPCs) > 24%.

Honey. Honey samples were collected from beekeepers according to the geographical location and the type of flowers from which the bees collected the nectar. Five types of honey were obtained, as declared by the beekeepers: H1—polyfloral honey, H2—linden honey, H3—acacia honey, H4—honeydew honey, and H5—sunflower honey. Additionally, the botanical origin of honey was determined by melissopalynological analysis, through microscopic identification and quantification of pollen grains following standardized methods [29]. For each sample, 500 g of honey were collected in sterile brown glass containers with airtight lids, gently homogenized, and transported in insulated containers. The samples were stored at room temperature, protected from light and heat, until analysis the following day.

Propolis. Propolis samples were collected from the same beekeepers, who provided the honey directly from the hives using a sterilized stainless-steel tool. Due to the small amount obtained from each apiary (35–75 g), a composite sample (P_{tot}) was prepared by combining and homogenizing all collected propolis. The samples were stored in sterile, airtight glass containers at 4 °C in the dark until analysis.

For the aqueous extract, 50 g of purified, finely ground propolis was mixed with 250 mL of distilled water (1:5 *w/v*) and maintained under reflux for 1 h. After cooling, the suspension was successively centrifuged at 12,000 rpm and 10,000 rpm using a Centra CL2 centrifuge (Thermo Fisher Scientific Inc., Waltham, MA, USA), and the supernatant was concentrated by evaporation at approximately 20% of the initial volume, yielding a theoretical extract concentration of 1 g/mL. The extract was stored in sealed bottles in a dark, dry place until use [30]. A working concentration of 0.1 g/mL was used to prepare soaps enriched with propolis.

2.1.3. Essential Oils

The EOs were purchased from Esentivia Company, Drâmbăr, Alba County, Romania, and were supplied in airtight brown glass containers. The oils used were: EO1—clove (*Syzygium aromaticum*) oil, EO2—rosemary (*Rosmarinus officinalis*) oil, EO3—mace (aril of *Myristica fragrans*) oil, EO4—nutmeg (seeds of *Myristica fragrans*) oil, EO5—white pepper (*Piper nigrum*) oil, and EO6—juniper (*Juniperus communis*) oil.

2.2. Physico-Chemical Analyses of the Functional Ingredients

For honey, the following parameters were determined: water content, pH, water activity, free acidity, color (Pfund scale), ash content, electrical conductivity, hydroxymethylfurfural (HMF) content, sugar profile (reducing and invert sugars), pollen spectrum of botanical origin, and total phenolic content [29,31–39].

For propolis, the analyses included the total phenolic content, the total flavonoid content, and the titratable acidity [31,38,40,41].

EOs were characterized in terms of density, refractive index, optical rotation, total phenolic content, total flavonoid content, and chemical composition by GC-FID analysis [42–46].

The detailed analytical procedures for honey, propolis, and EOs are presented in Supplementary Materials S2: Analytical procedures for honey, propolis, and essential oils (EOs).

2.3. Physico-Chemical Treatments Applied to Waste Vegetable Oils

The waste oils were filtered through a 400-micron mesh. Solid samples (palm oil) were melted with gentle stirring in a water bath at approximately 10 °C above the melting point of the oil. For purification, 200 g of filtered oil was heated to 90 ± 5 °C and washed with a hot brine solution (20 mL) at 10% NaCl, *w/v* (SALROM, Ocna-Dej, Romania). The ratio of waste oil to brine solution was 10:1 (*v/v*). The hot mixture (waste oil and brine) was homogenized using a stirrer at 60 rpm for 30 min. The mixture was transferred to a separating funnel and left for 8 h to allow phase separation into the oil phase and the lower aqueous phase (brine solution + impurities). Then, 150 g of purified oil was heated to 70 ± 5 °C and homogenized with a 3% H₂O₂ solution (*v/v*) (Vitalia Pharma, Bucharest, Romania) at 80 rpm for 30 min, maintaining the temperature at 70 ± 5 °C. The mixture was transferred to a separating funnel for 1 h of decantation. The upper phase constituted the purified and bleached oil [47,48].

2.4. FT-IR Analysis

The FT-IR spectra of 2 mL from WCO and purified oil were recorded using a Bruker Vertex 70 v spectrometer (Bruker Optik GmbH, Rosenheim, Germany) attached to a A225/Q Platinum ATR unit with a single reflection diamond crystal (Bruker Optik GmbH, Rosenheim, Germany). Measurements were carried out in the 4000–400 cm^{−1} range, with a resolution of 4 cm^{−1} and 128 scans/sample.

2.5. Recipe for Cold-Process Soap Preparation

For soap preparation, the recipe included an oil phase, composed of vegetable oils, and an alkaline phase, prepared by dissolving NaOH in distilled water [49,50]. Distilled water was used at approximately 25–30% of the total oil mass. The required amount of NaOH (m_{NaOH}) was calculated based on the saponification values (SVs) specific to each vegetable oil (Equation (1)):

$$m_{NaOH} = \sum_i (m_{oil_i} \cdot SV_i) \quad (1)$$

where m_i = mass of each oil used (g) and SV_i = saponification value corresponding to each type of oil (g NaOH per g of oil).

To adjust the amount of NaOH (Equation (2)), the desired superfat (SF) percentage was applied, representing the oil that remained unconverted into soap, contributing to a milder and more moisturizing product:

$$m_{NaOH, adjusted} = m_{NaOH} \cdot \left(1 - \frac{SF}{100}\right) \quad (2)$$

where m_{NaOH} is the amount calculated for complete saponification and SF is the desired superfat percentage (usually 5–8%).

The entire amount of NaOH was gradually added to distilled water with continuous stirring. The caustic soda solution (lye) at 60 °C was then homogenized with the mixture of oils, also heated to the same temperature. Homogenization continued until a viscous mixture was obtained. The mixture was poured into a mold and left to harden [51]. The resulting solution was then combined with the oil mixture to initiate the saponification process. The alkaline solution needed to be at a temperature similar to that of vegetable oils (approximately 60 °C) to avoid thermal shock. After reaching the “trace” stage, the composition was enriched with the bioactive ingredients as follows: EOs (2% of the total

oil mass), honey (3% of the total oil mass), and propolis extract (1% of the total oil mass). These were added and homogenized into the soap mass after cooling to approximately 40 °C in order to preserve their functional properties.

2.6. Physico-Chemical Analysis of Plain Soap

pH. A sample of 10 g of finely grated soap was mixed thoroughly with 100 mL of distilled water, producing minimal foam. This was made up to prepare a 10% soap solution. The mixture was left undisturbed for 24 h to allow maximum dissolution of the soap. The pH was then measured using the pH meter HI2211 (Hanna Instruments, Bucharest, Romania).

Water Content. A total of 5 g of accurately weighed soap sample was dried in an oven at 101 °C until a constant weight was achieved [52].

Free caustic alkali. Five grams of soap, accurately weighed, was dissolved in 30 mL of ethanol, to which 10 mL of 20% BaCl₂ solution (Merck KGaA, Darmstadt, Germany) and phenolphthalein (Sigma-Aldrich, St. Louis, MO, USA) as an indicator were added. The solution was then titrated with 0.05 M aqueous H₂SO₄ (Merck KGaA) [53,54]. Calculation: Free caustic alkali = $0.31 \cdot V_{\text{acid}} / W$, where V_{acid} is the volume of 0.05 M aqueous H₂SO₄ used for titration and W is the exact weight of the soap sample.

Determination of total dissolved solids (TDSs): A 10 mL sample of the soap solution was filtered through Whatman No. 42 paper, and the filtrate was poured into a clean beaker. The solvent was completely evaporated on a hot plate, leaving only the dissolved solids, which were then weighed. TDSs were calculated as the difference between the weight of the beaker with the dried residue (W_2) and the empty beaker (W_1): $\text{TDS} = W_2 - W_1$ [51,55].

Total Solids (TSs): A 10 mL sample of the soap solution was transferred into a pre-weighed, dry beaker (W_1). The sample was evaporated to dryness on a hotplate at 105 °C until a constant weight was achieved. After cooling in a desiccator, the beaker with the dried residue (W_2) was weighed. $\text{TS} = W_2 - W_1$ [51,55].

Foam Height. A soap solution was prepared by stirring 2 g of finely grated soap in 100 mL of distilled water for 5 min, until complete dissolution and foam formation. The resulting solution was poured into a large, graduated cylinder with a diameter of 4 cm. After standing for 1 min, the height of the foam column was measured using a ruler and expressed in cm [56,57].

2.7. Antimicrobial Analysis of Soaps with Functional Ingredients

Each soap sample was grated using a sterile grater. A one-gram portion of the soap was then dissolved in 9 mL of sterile distilled water to prepare the stock solution. The soaps dissolved completely in the water, without generating foam or bubbles during the process. The resulting stock solutions were transferred into tightly sealed containers and stored in a refrigerator until further analysis. For the evaluation of antibacterial activity, three concentrations of soap solutions were prepared in sterile distilled water: 500 mg/mL, 250 mg/mL, and 125 mg/mL.

The in vitro antimicrobial activity of the various soap samples was assessed using the agar well diffusion technique against two bacterial strains: *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923), both obtained from MicroBioLogics Inc. (St. Cloud, MN, USA). Bacterial cultures were grown in 10 mL of nutrient broth (Merck KGaA) and incubated at 37 °C for 18 h. The turbidity of each culture was standardized to a 0.5 McFarland standard (Thermo Fisher Scientific Inc., Waltham, MA, USA) using a McFarland densitometer (Mettler Toledo, Columbus, OH, USA), which corresponds to a bacterial suspension with approximately 1.5×10^8 colony-forming units/mL (CFU/mL).

The antimicrobial activity of the soap samples was assessed on Mueller–Hinton agar plates (Merck KGaA) using the agar well diffusion method. The agar medium was poured into 9 cm Petri dishes to a depth of approximately 4 mm. For inoculation, 0.1 mL of bacterial suspension was evenly spread over the agar surface using a sterile glass spreader. Following inoculation, the plates were allowed to rest at 37 °C for 15 min to enable absorption of the bacterial inoculum into the agar.

Wells of 6.0 mm in diameter were created using a sterile stainless-steel tube by gently pressing into the solidified medium. Each well was filled with 100 µL of the prepared soap solutions. Commercially available Safeguard antibacterial soap was used as a positive control, while sterile distilled water served as the negative control.

After sample loading, the plates were incubated at 37 °C for 24 h. The zones of inhibition were measured (in millimeters) using a DIN 862 ABS digital caliper (Fuzhou Conic Industrial Co., Ltd., Fuzhou, China). All assays were conducted in triplicate, and the mean values were calculated and are reported as mean $\pm$ standard deviation [58].

2.8. Statistical Analysis

The antimicrobial activity of the soap formulations was assessed based on the mean diameter of *E. coli* and *S. aureus* inhibition zones (mm). For each concentration level (125, 250, and 500 µg/mL), a one-way analysis of variance (ANOVA) was performed using soap formulation as the independent factor. The assumptions of normality and homogeneity of variances were evaluated using residual diagnostics and Levene's test, respectively. When Levene's test was nonsignificant ($p > 0.05$), homogeneity of variances was assumed and a standard one-way ANOVA was applied, followed by Tukey's Honestly Significant Difference (HSD) post-hoc test. In cases where Levene's test revealed significant heterogeneity of variances ($p < 0.05$), Welch's ANOVA was used, followed by the Games–Howell post-hoc test for multiple comparisons. When a statistically significant overall effect was detected ($p < 0.05$), multiple pairwise comparisons were carried out using the appropriate post-hoc test (Tukey's HSD or Games–Howell, depending on variance homogeneity) to identify which soap types differed significantly in their mean inhibition zones. The level of significance was set at $\alpha = 0.05$ for all analyses. Statistical computations were performed in OriginPro 2025 (OriginLab Corporation, Northampton, MA, USA). Results are presented as mean values accompanied by post-hoc grouping letters (Tukey or Games–Howell, as appropriate) to indicate statistically homogeneous subsets of formulations.

Physico-chemical parameters were determined in triplicate and are presented as the mean $\pm$ standard deviation. These analyses were primarily descriptive and intended for characterization and quality assessment of raw materials and products; therefore, no inferential statistical comparisons were applied unless explicitly stated.

3. Results

3.1. Analysis of Bioactive Components in Soaps

Tables 2 and 3 present the results of the physicochemical analysis of bee products collected from Alba County, Romania.

Honey samples from Alba County showed moisture content between 15.2% and 18.1%, values within the accepted quality standards. The pH ranged from 3.66 to 4.20 and free acidity from 11.9 to 28.8 meq/kg, with manna honey displaying the highest acidity. The water activity of the investigated honey samples (0.568–0.581) confirms their microbiological stability, as these values are below the threshold required for microbial proliferation. Propolis exhibited a slightly higher value (0.652), yet its rich content of bioactive compounds contributes to maintaining its stability. Electrical conductivity distinguished floral honeys (0.15–0.59 mS/cm) from honeydew honey (1.02 mS/cm). All samples presented very low

HMF levels (<2 mg/kg), confirming freshness. Easily hydrolysable sugars ranged from 2.30% to 3.59% and inverted sugar from 73.26% to 79.87%, with the highest values in acacia and sunflower honeys. Melissopalynological analysis validated the monofloral origin of linden, acacia, and sunflower honeys, which contained 30–40% specific pollen. The content of bioactive compounds varied among samples: phenolics ranged from 64.9 to 90.1 mg GAE/100 g, and flavonoids from 2.7 to 5.5 mg QE/100 g, with acacia honey showing the highest values. Propolis was significantly richer, with ~160 mg GAE/g phenolics and 75 mg QE/g flavonoids, confirming its superior antioxidant potential compared to honey.

Table 2. Results of the physical and botanical parameters of the bee products.

Sample	Bee Product	Moisture Content, %	a_w	Phund Value, mm	Color Category *	Proportion of Specific Pollen Grains, %
H1	Poly-floral honey	15.72 ± 0.21	0.571 ± 0.016	52	Light amber	-
H2	Linden honey	17.29 ± 0.35	0.574 ± 0.031	40	Extra light amber	30.0 ± 1.4
H3	Acacia honey	18.13 ± 0.40	0.568 ± 0.020	9	Extra white	29.2 ± 1.1
H4	Manna honey	16.2 ± 0.19	0.581 ± 0.013	94	Amber	-
H5	Sunflower honey	16.4 ± 0.27	0.576 ± 0.028	39	Extra light amber	40.1 ± 2.2
P _{tot}	Propolis	5.11 ± 0.21	0.652 ± 0.19	-	-	-

Abbreviations: a_w —water activity. Note: * United States Standards for Grades of Extracted Honey [59]. Data are presented as mean ± standard deviation ($n = 3$).

Table 3. Chemical parameters of bee products.

Sample	pH	Acidity, meq/kg	Ash, %	EC, mS/cm	HMF, mg/kg	EHS, %	Inverted Sugar, %	Phenols H: mg GAE/100 g; P: mg GAE/g	Flavonoids H: mg QE/100 g; P: mg QE/g
H1	3.66 ± 0.18	21.7 ± 0.9	0.40 ± 0.05	0.591 ± 0.020	1.21 ± 0.07	2.30 ± 0.17	73.26 ± 1.58	83.52 ± 0.26	4.68 ± 0.15
H2	4.20 ± 0.30	11.9 ± 0.2	0.32 ± 0.03	0.542 ± 0.013	1.10 ± 0.10	2.73 ± 0.48	74.36 ± 1.23	71.03 ± 0.52	2.79 ± 0.11
H3	3.71 ± 0.26	12.3 ± 0.5	0.21 ± 0.02	0.159 ± 0.027	0.68 ± 0.12	2.90 ± 0.30	79.87 ± 1.93	90.11 ± 1.17	5.54 ± 0.16
H4	3.94 ± 0.32	28.8 ± 0.8	0.35 ± 0.07	1.025 ± 0.036	1.09 ± 0.09	2.71 ± 0.50	79.50 ± 1.3	64.90 ± 0.28	3.66 ± 0.19
H5	3.85 ± 0.29	22.5 ± 0.4	0.37 ± 0.04	0.501 ± 0.025	1.00 ± 0.13	3.59 ± 0.29	79.26 ± 1.83	66.33 ± 0.84	3.32 ± 0.20
P _{tot}	-	84.6 ± 0.9	2.55 ± 0.08	0.411 ± 0.020 *	-	-	-	159.83 ± 2.05	74.68 ± 0.59

Abbreviations: H—honey; P—propolis; EC—electrical conductivity; HMF—hydroxymethylfurfural; EHSs—easily hydrolysable sugars; GAE—gallic acid equivalents; QE—quercetin equivalents. Note: * to the extract; Data are presented as mean ± standard deviation ($n = 3$).

Table 4 presents the results of the physico-chemical analysis conducted on commercially available EOs.

Table 4. The results of the physical and chemical analysis for EOs.

Sample	Essential Oil	Density at 20 °C, g/cm ³	Refractive Index at 20 °C	Optical Rotation at 20 °C	Phenols, mg GAE/g	Flavonoids, mg QE/g
EO1	Clove	1.0490 ± 0.0005	1.5315 ± 0.0013	(+)0.2	0.800 ± 0.10	0.074 ± 0.010
EO2	Rosemary	0.9070 ± 0.0003	1.4701 ± 0.0017	(+)8.7	nd	0.096 ± 0.025
EO3	Mace	0.8952 ± 0.0002	1.4790 ± 0.0015	(+)18.2	nd	0.080 ± 0.017
EO4	Nutmeg	0.8850 ± 0.0001	1.4925 ± 0.0012	(+)22.5	nd	0.065 ± 0.011
EO5	White pepper	0.8875 ± 0.0002	1.4772 ± 0.0019	(+)4.1	nd	nd
EO6	Juniper	0.8602 ± 0.0001	1.4739 ± 0.0016	(+)6.0	nd	nd

Note: The total phenolic content and flavonoids were determined using different colorimetric assays with different sensitivities and selectivities. Total phenols were quantified by the Folin–Ciocalteu (limit of quantification 0.05 mg GAE/g), while flavonoids were determined using the AlCl₃ method (limit of quantification: 0.01 mg QE/g). Values reported as “nd” indicate concentrations below the respective quantification limits of each method. Data are presented as mean ± standard deviation ($n = 3$).

The physico-chemical and bioactive profiling of commercial EOs revealed species-specific differences. Density at 20 °C ranged from 0.8602 g/cm³ (juniper) to 1.0490 g/cm³

(clove), with higher values associated with oils rich in phenolic compounds. Refractive indices varied between 1.4701 and 1.5315, the highest observed in clove oil, reflecting the greater content of aromatic compounds with higher molecular weight. Optical rotation was positive for all samples (+0.2° in clove to +22.5° in nutmeg seeds), indicating differences in the composition of optically active isomers.

Phenolic and flavonoid contents were generally low, with clove oil showing the highest phenolic content (0.800 mg GAE/g). Flavonoids were detected at low concentrations (0.065–0.096 mg QE/g), while some oils, such as white pepper and juniper, contained undetectable levels.

Volatile phenols differed considerably among species: clove was rich in thymol, carvacrol, and eugenol; rosemary contained fewer volatile phenols and mainly partially non-volatile compounds such as carnosol and rosmarinic acid; while mace, nutmeg, white pepper, and juniper exhibited very low levels, dominated by terpenes. The remaining non-volatile phenols remained in the aqueous residue or degraded during distillation, so they were not present in the EOs but only in hydroalcoholic or aqueous extracts.

These results highlight the significant phytochemical variation among EOs, directly reflecting their botanical origin, chemical composition, and the volatility of phenolic compounds. Clove oil was characterized by high levels of bioactive volatile phenols, whereas other species contained mostly non-volatile phenols or terpenes, which limited their representation in the EO fraction.

It should be noted that the Folin–Ciocalteu method is nonspecific and may have reacted with other reducing compounds in the extracts, potentially leading to an overestimation of total phenolic content.

The GC-FID chromatograms of the investigated EOs (Supplementary Materials Figure S1a–f and Table 5) provide a detailed qualitative and quantitative profile of their volatile constituents, enabling the identification and comparison of the main compounds across different botanical species.

Table 5. Major compounds and their percentages in essential oils: EO1—clove; EO2—rosemary; EO3—mace (aril of *Myristica fragrans*); EO4—nutmeg (seeds of *Myristica fragrans*); EO5—white pepper (*Piper nigrum*); EO6—juniper (*Juniperus communis*).

EO1—Clove		EO2—Rosemary		EO3—Mace (Aril)		EO4—Nutmeg (Seeds)		EO5—White Pepper		EO6—Juniper	
Compound	%	Compound	%	Compound	%	Compound	%	Compound	%	Compound	%
Eugenol	69.15	1,8-Cineole	42.31	Myristicin	43.08	Myristicin	27.31	β -Caryophyllene	49.74	α -Pinene	46.96
β -Caryophyllene	23.61	α -Pinene	14.12	Safrole	5.16	Terpinen-4-ol	17.76	α -Cubebene	5.48	β -Myrcene	18.44
Humulene	3.08	Camphor	11.05	β -Pinene	5.11	Methyl eugenol	8.38	Limonene + β -Phellandrene	3.25	Sabinene	5.51
Eugenyl acetate	1.63	β -Pinene	7.83	Terpinen-4-ol	4.43	γ -Terpinene	4.56	α -Humulene	2.90	Limonene	4.45
-	-	Camphene	5.01	Methyl eugenol	3.93	β -Pinene	4.30	δ -3-Carene	1.99	Germacrene D	2.58
-	-	Limonene	2.76	Limonene + β -Phellandrene	3.66	α -Pinene	2.80	α -Copaene	1.98	β -Caryophyllene	1.99
-	-	β -Caryophyllene	2.67	α -Pinene	3.47	Limonene + β -Phellandrene	1.64	Terpinen-4-ol	1.56	α -Humulene	1.34
-	-	Borneol	0.95	γ -Terpinene	3.29	α -Terpineol	1.51	β -Pinene	1.37	δ -Cadinene	1.33
-	-	Linalool	0.83	Sabinene	2.81	Terpinolen	1.18	Linalool	0.91	Germacrene B	1.00
-	-	α -Terpineol	0.72	Elemicin	2.54	Linalool	0.50	α -Terpineol	0.29	β -Elemene	0.92
-	-	-	-	Eugenol	0.95	β -Caryophyllene	0.41	trans-Nerolidol	0.69	α -Cubebene	0.60
-	-	-	-	α -Terpineol	0.72	Safrole	3.15	Caryophyllene oxide	1.27	α -Copaene	0.44
-	-	-	-	β -Caryophyllene	0.68	Elemicin	3.72	β -Farnesene	1.25	-	-
-	-	-	-	-	-	Sabinene	1.34	β -Elemene	1.04	-	-
-	-	-	-	-	-	trans-Sabinene hydrate	0.32	-	-	-	-
-	-	-	-	-	-	Para-cymene	0.23	-	-	-	-
-	-	-	-	-	-	α -Thujene	0.72	-	-	-	-
-	-	-	-	-	-	α -Felandrene	0.37	-	-	-	-

GC-FID analysis of clove (*Syzygium aromaticum*) EO revealed a profile dominated by eugenol, which is the main bioactive constituent responsible for its potent antimicrobial, analgesic, and antioxidant activities. The oil also contained significant amounts of β -caryophyllene (23.61%) and smaller proportions of humulene and eugenyl acetate.

The chemical profile of rosemary EO is dominated by 1,8-cineole (42.31%), followed by α -pinene (14.12%) and camphor (11.05%). Other notable constituents included β -pinene, camphene, limonene, and β -caryophyllene, while minor compounds such as borneol, linalool, and α -terpineol were also detected in lower proportions.

GC-FID analysis of aril from mace EO revealed a profile dominated by myristicin (43.08%), accompanied by safrole, β -pinene, terpinen-4-ol, and methyl eugenol. Other relevant constituents included limonene with β -phellandrene, α -pinene, γ -terpinene, sabinene, and elemicin, while several compounds such as eugenol, α -terpineol, and β -caryophyllene were detected in minor proportions.

Nutmeg EO revealed a profile dominated by myristicin (27.31%), which is the main phenylpropanoid responsible for the characteristic aroma and bioactivity of the oil. Other major constituents included terpinen-4-ol (17.76%) and methyl eugenol (8.38%), both contributing to antimicrobial and aromatic properties. Monoterpenes such as γ -terpinene (4.56%), β -pinene (4.30%), and α -pinene (2.80%), along with limonene and β -phellandrene (1.64%), form the hydrocarbon fraction that shapes the volatile profile of the oil. Minor oxygenated terpenes, including α -terpineol, terpinolen, and linalool, together with sesquiterpenes such as β -caryophyllene, safrole, and elemicin, contribute to both aroma complexity and bioactive potential. Other minor monoterpenes identified were sabinene, trans-sabinene hydrate, para-cymene, α -thujene, and α -felandrene.

GC-FID analysis of the white pepper revealed a chemical profile dominated by β -caryophyllene (49.74%), which is the main sesquiterpene contributing to the oil's anti-inflammatory and antimicrobial activities. Other major constituents included α -cubebene (5.48%), limonene + β -phellandrene (3.25%), and α -humulene (2.90%), which collectively contribute to the characteristic aroma and synergistic bioactivity. Monoterpenes such as δ -3-carene (1.99%), α -copaene (1.98%), terpinen-4-ol (1.56%), and β -pinene (1.37%) form the hydrocarbon fraction, enhancing volatility and aroma complexity. Minor oxygenated terpenes, including linalool, α -terpineol, trans-nerolidol, as well as sesquiterpene derivatives like caryophyllene oxide, β -farnesene, and β -elemen, further contribute to the therapeutic potential of the oil.

GC-FID analysis of the juniper EO revealed a profile dominated by α -pinene (46.96%), which defines the main monoterpene fraction and contributes strongly to the characteristic aroma and potential anti-inflammatory properties. Other major constituents included β -myrcene (18.44%), sabinene (5.51%), and limonene (4.45%), forming the hydrocarbon monoterpene fraction that enhances volatility and aroma complexity. Sesquiterpenes such as β -caryophyllene (1.99%), germacrene D (2.58%), α -humulene (1.34%), δ -cadinene (1.33%), and germacrene B (1.00%) were also detected, contributing to the bioactive potential of the oil, particularly its antimicrobial and anti-inflammatory properties. Minor sesquiterpenes, including β -elemene, α -cubebene, and α -copaene, further enrich the chemical complexity.

3.2. FT-IR Analysis of Used and Purified Oils

Figure 1 shows the FT-IR spectra of sunflower oil, used (green trace) and purified (sky blue trace); palm oil, used (red trace) and purified (purple trace); and pumpkin oil, used (blue trace) and purified (mustard yellow trace).

The FTIR spectra of used and purified oils (Figure 1) show characteristic absorption bands typical of triglyceride structures, with minor differences related to oil degradation and purification. In the spectrum of WCO, a broader absorption band around 3400 cm^{-1}

was observed, which can be attributed to O–H stretching vibrations associated with moisture and oxidation products. For all samples, the band at approximately 3006 cm^{-1} corresponded to =C–H stretching and indicated the presence of unsaturated fatty acids, while the bands at 2922 and 2852 cm^{-1} were attributed to asymmetric and symmetric CH_2 stretching vibrations of aliphatic chains. The intense band at 1745 cm^{-1} reflected the C=O stretching of triglyceride esters. After purification, slight changes in band intensity were observed, suggesting a possible removal of polar degradation compounds. Bands at 1465 and 1377 cm^{-1} corresponded to CH_2 and CH_3 bending vibrations. The region 1235 – 1160 cm^{-1} and the band at 1098 cm^{-1} were assigned to C–O–C stretching vibrations of ester linkages. The band near 720 cm^{-1} was assigned to CH_2 rocking, typical of long-chain fatty acids.

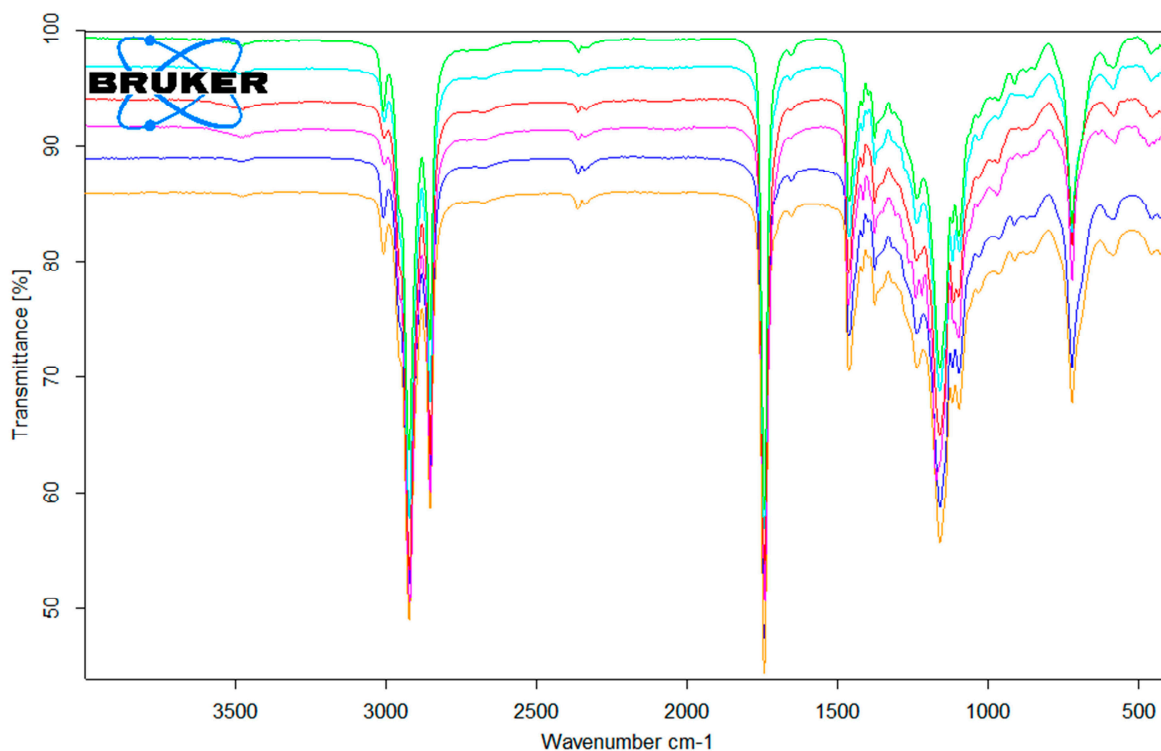


Figure 1. FTIR spectra of used and purified oils: used oils are represented by green (sunflower), red (palm), and blue (pumpkin) traces, while purified oils are shown in sky blue (sunflower), purple (palm), and mustard yellow (pumpkin) traces.

The similarity of the FTIR spectra before and after purification indicates that the treatment did not modify the main chemical structure of the oils, while the removal of impurities and polar compounds occurred without generating new IR-active functional groups.

Compared to purified oils, the used oils exhibited a reduced intensity of the unsaturation band at $\sim 3006\text{ cm}^{-1}$ and an increase in the carbonyl band at $\sim 1745\text{ cm}^{-1}$, indicating the oxidation of unsaturated fatty acids and formation of secondary oxidation products such as aldehydes/ketones. These spectral changes were more pronounced in pumpkin oil, likely due to its high content of polyunsaturated fatty acids and increased susceptibility to thermal degradation. The preservation of the main ester and methylene bands after purification confirms that the purification process effectively removed oxidation products while maintaining the fundamental lipid structure of the oils.

3.3. Technological Process for Soap Production

In general, WCO, previously subjected to filtration and decantation to remove solid impurities and water, is reacted with a NaOH solution, thereby producing soap and

glycerol as the main products. The process may include additives such as fragrances, colorants, or stabilizers, depending on the desired final application. This valorization method contributes to reducing the environmental impact of food waste and aligns with trends in the circular economy.

Figure 2 presents the technological process for the production of soaps from WCO, along with the equipment corresponding to each stage, detailing the sequence of physical and chemical processes through which residual oils are transformed into finished soap products.

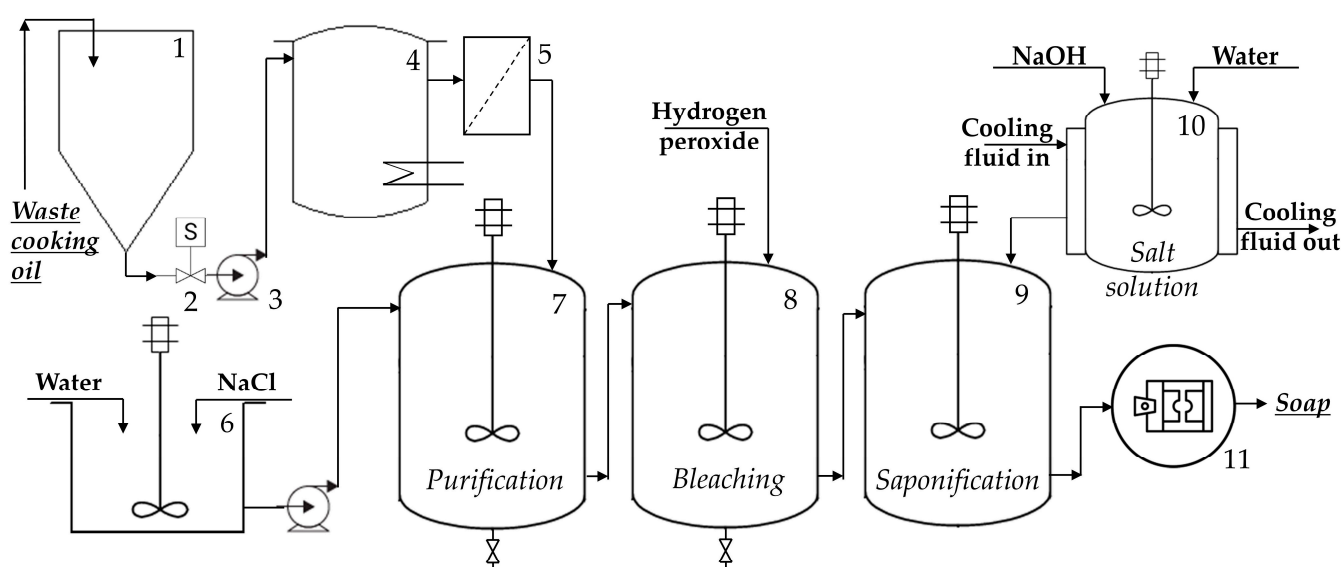


Figure 2. Process flow diagram for soap production from WCO, including the main stages and corresponding equipment. Description: 1—Waste oil storage tank; 2—Solenoid valve; 3—Electric pump; 4—Preheating tank; 5—Filtration; 6—Brine preparation tank (with agitator); 7—Mixing reactor for oil purification by decantation; 8—Mixing reactor for oil bleaching by decantation; 9—saponification reactor; 10—Caustic soda dissolving tank; 11—Soap molding machine.

Figure 2 highlights both the necessary technological operations—such as filtration, purification, bleaching, saponification, and molding—and the corresponding industrial equipment for each stage:

1. Collection and storage of WCOs: WCOs are collected from restaurants, households, or food industries and stored in stainless steel or food-grade plastic tanks, avoiding contamination or mixing with other wastes.
2. Mechanical filtration: Solid impurities, food residues, and water are removed using nylon mesh filters, filter presses, fine metal sieves, or gravitational decantation, with the WCOs maintained at 40–55 °C to ensure a suitable viscosity while minimizing oxidation and polymerization.
3. Physico-chemical purification with brine (NaCl): Filtered oil is mixed with a 10% (*w/v*) NaCl solution at a 1:1 volume ratio (e.g., 100 mL of WCO with 100 mL of NaCl solution) and stirred for 30–60 min at 30–40 °C to remove polar compounds, proteins, and other soluble impurities.
4. Decantation and phase separation: The mixture is left to settle at room temperature (~ 25 °C) for 2 h, allowing the oil layer (top) to separate from the aqueous layer containing heavy impurities (bottom). Centrifugation (300–500 rpm, 5–10 min) can speed up the process. The purified oil is collected from the top, resulting in minimal sample loss (<5%).

5. Bleaching with H_2O_2 : The purified oil is treated with 3% H_2O_2 (1–2% *v/v* relative to the oil) at pH 7–8 and 40–60 °C for 30 min under gentle stirring to remove colorants and improve appearance.
6. Post-bleaching separation: The mixture is decanted (at room temperature for 2 h) or centrifuged (300–500 rpm, 5–10 min) to separate the bleached oil from the aqueous phase; residual peroxide is checked with potassium iodide–starch paper. The bleached oil is collected, resulting in minimal sample loss (<3%). The oil is then stored in dark, airtight containers at room temperature until further processing.
7. Saponification with caustic soda (NaOH): The bleached oil is mixed with an excess of NaOH at a 1:0.134 molar ratio (oil:NaOH) at ~60 °C and stirred for 30–60 min until complete saponification of triglycerides in soap.
8. Glycerin separation: Glycerin by-product is removed by decantation (1–2 h) or centrifugation (300–500 rpm, 5–10 min); a small portion may remain (~ 5% superfat) to improve moisturizing and texture.
9. Washing and neutralization: The soap is washed with warm demineralized water or 5–10% saline at 30–40 °C for 10–15 min, allowed to settle, and drained. pH is adjusted for safety and then the soap is cooled and solidified.

3.4. Chemistry of the Soap Production Process

The saponification process leads to the formation of soap, facilitating the removal of FFA, which can have a negative impact on the quality of the soap. The complete conversion of FFA from WCO during saponification increases the stability of the final product and improves its foaming properties. In addition, the reuse of WCO through saponification provides an important environmental benefit, as it transforms waste into a valuable product that supports the principles of sustainability.

Soap molecules are typically sodium or potassium salts of long-chain fatty acids, consisting of a polar carboxylate group at one end and a nonpolar hydrocarbon chain at the other. The hydrophobic tail dissolves readily in nonpolar substances, consistent with the principle of “like dissolves like.” The general molecular structure of soap is presented in Figure 3.

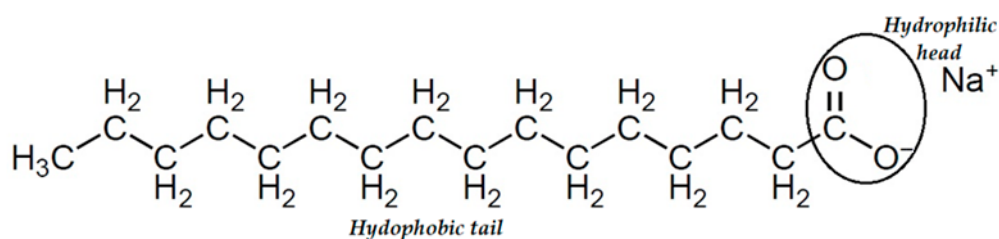


Figure 3. Amphiphilic structure of soap showing polar head and nonpolar tail.

The saponification process is a base-catalyzed hydrolysis of triglycerides present in oils, where NaOH cleaves the ester bonds of the glycerides [60]. This reaction is essentially irreversible under conditions of high temperature and excess alkali, which differentiates it from simple hydrolysis. The reaction yields glycerol as a coproduct and sodium salts of long-chain fatty acids, which precipitate as crude soap. Depending on the method, glycerol may be separated in industrial production or retained in artisanal soaps, where it contributes moisturizing properties [61].

The molecular scheme of triglyceride saponification in the presence of NaOH is shown in Figure 4.

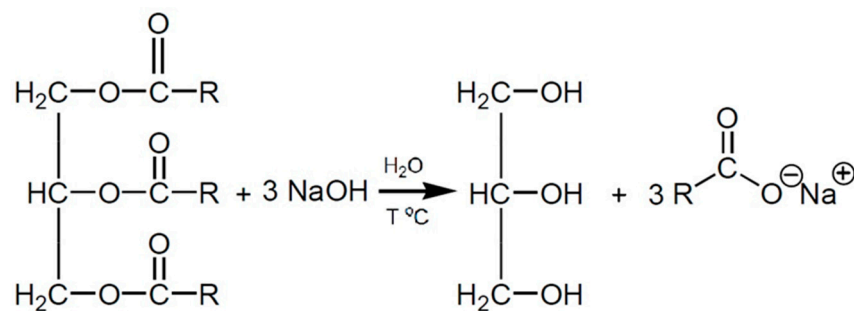


Figure 4. Reaction scheme of triglyceride saponification with NaOH. R—long hydrocarbon chains of carbon atoms, called fatty acid chains.

The saponification of FFA is represented by the reaction



Saponification not only produces soap with improved functional properties but also valorizes WCO in a sustainable way.

3.5. Soap Formulation

All soap formulations were prepared using the same base composition and saponification parameters, resulting in comparable alkalinity. The pH of the plain soap base after curing was 9.87 ± 0.04 , and the incorporation of bioactive ingredients at low concentrations (1–3% *w/w*) did not significantly modify the final pH. Therefore, pH was considered constant across all tested samples.

Table 6 presents the composition of the formulation for functional soaps.

Table 6. Composition of the soap formulations obtained.

No.	Ingredient	Quantity, g	SV, g NaOH/g Oil
1	Purified sunflower oil	60	0.134
2	Purified palm oil	170	0.141
3	Purified pumpkin oil	170	0.136
4	Sodium hydroxide	52.37	-
5	Distilled water	125.5	-
6	Essential oil	8	-
	Honey	12	-
	Propolis extract	4	-

Note: SV—saponification value.

The proportions of oils used in the soap formulation were 15% sunflower oil, 42.5% palm oil, and 42.5% pumpkin seed oil, calculated relative to the total oil mass (400 g). At the final stage of soap preparation, different soap formulations were obtained by incorporating a single bioactive ingredient per batch, based on the total oil content. Specifically, honey was added at 3% (12 g), EOs at 2% (8 g), or propolis extract at 1% (4 g), depending on the formulation.

The amount of NaOH and distilled water was calculated (Equations (4)–(7)) for a superfat (SF) of 5% as follows:

$$\begin{aligned}
 m_{\text{NaOH}} = & (\text{amount of sunflower oil} \times 0.134 + \text{amount of palm oil} \times 0.141 \\
 & + \text{amount of pumpkin oil} \times 0.136), 0\% \text{ SF}
 \end{aligned} \quad (4)$$

$$m_{NaOH} = \text{amount of NaOH, g SF 0\%} - \frac{5}{100} \times \text{amount of NaOH, g 0\% SF} \\ = \text{amount of NaOH, g 5\% SF} \quad (5)$$

$$m_{NaOH} = (60 \times 0.134 + 170 \times 0.141 + 170 \times 0.136) = 55.13 \text{ g NaOH, 0\% SF} \quad (6)$$

$$m_{NaOH} = 55.13 - \frac{5}{100} \times 55.13 = 52.37 \text{ g NaOH, 5\% SF} \quad (7)$$

A plain soap, prepared from the same oil base without bioactive ingredients, was also prepared and used as a control for all physico-chemical analyses to assess the baseline properties of the soap matrix. For the antimicrobial activity assays, a commercial soap-Safeguard (Procter & Gamble (Pvt.) Ltd., Karachi, Pakistan) was used as a reference, since plain soap typically exhibits only modest antibacterial effects and does not provide a relevant biological comparison.

3.6. Analysis of Plain Soaps

Table 7 presents the main parameters used for the characterization of plain soap (soap without bioactive ingredients), compared with the analyzed commercial soap.

Table 7. Physico-chemical parameters for the quality assessment of plain soap.

Parameter	Experimental Soap Without Bioactive Ingredients	Commercial Soap—Safeguard
pH	9.87 ± 0.04	10.30 ± 0.07
Moisture content, %	15.30 ± 0.08 *	8.20 ± 0.09
Total Dissolved Solids, TDSs	0.46 ± 0.02	0.61 ± 0.05
Total Solids, TSs	1.25 ± 0.09	1.15 ± 0.06
Free caustic alkalinity, %	0.01 ± 0.00	0.00 ± 0.00
Foam height, cm	10.50 ± 1.00	12.40 ± 2.00

Note: * Measured 4 weeks after soap preparation. Values are reported as mean ± standard deviation ($n = 3$).

Table 7 presents the main parameters used for the characterization of plain soap (soap without bioactive ingredients) compared with the analyzed commercial Safeguard soap. The pH and free caustic alkalinity values indicate good skin compatibility and safety for both products. The experimental soap shows a higher moisture content, which may affect hardness and shelf life. Total dissolved solids and total solids are similar for both soaps, suggesting comparable concentration and solid content. The foam height is slightly higher in commercial soap, reflecting a better foaming capacity. Overall, the experimental soap demonstrates satisfactory physicochemical properties, close to those of the commercial product, despite the absence of bioactive additives.

3.7. Antimicrobial Properties of Soaps

The antimicrobial activity of the formulated soaps was evaluated using the diameter of the inhibition zone (mm) against *E. coli* and *S. aureus*.

Tables 8 and 9 summarize the results for soaps enriched with various types of honey and propolis, while Tables 10 and 11 present the data for soaps containing different EOs. Each sample was tested at three concentrations (500, 250, and 125 mg/mL), and the inhibition zones were measured to assess the efficacy of the bioactive ingredients in limiting bacterial growth. For comparison, a commercial soap (Safeguard) was included as a reference. The results highlight the influence of the type and concentration of bioactive ingredients on the antibacterial properties of the soaps.

Table 8. Diameter of the zone of inhibition (mm) against *E. coli* for soap samples containing honey or propolis.

Concentration	Sample Inhibition Diameter Area (mm)						
	Soap with Polyfloral Honey—BPS1	Soap with Linden Honey—BPS2	Soap with Acacia Honey—BPS3	Soap with Manna Honey—BPS4	Soap with Sunflower Honey—BPS5	Soap with Propolis—BPS6	Safeguard—Commercial Soap—SGS
500 mg/mL	6.00 ± 0.50 ^{cde}	6.50 ± 0.50 ^{bc}	3.83 ± 0.29 ^f	4.83 ± 0.29 ^{ef}	5.17 ± 0.29 ^{de}	7.00 ± 0.87 ^{bc}	9.83 ± 0.29 ^a
250 mg/mL	4.83 ± 0.29 ^{de}	6.50 ± 0.00 ^b	3.50 ± 0.50 ^f	4.33 ± 0.29 ^{ef}	4.17 ± 0.29 ^{ef}	5.83 ± 0.29 ^{bcd}	8.17 ± 0.29 ^a
125 mg/mL	4.00 ± 0.00 ^{ef}	6.50 ± 0.00 ^a	4.17 ± 0.29 ^{def}	3.50 ± 0.50 ^f	3.67 ± 0.29 ^{ef}	5.00 ± 0.50 ^{bcd}	7.17 ± 0.29 ^a

Note: BPS—Bee Product Soap. Means followed by the same letter are not significantly different by the Tukey test at 5% level. Values are reported as mean ± standard deviation ($n = 3$).

Table 9. Diameter of the zone of inhibition (mm) against *S. aureus* for soap samples containing honey or propolis.

Concentration	Sample Inhibition Diameter Area (mm)						
	Soap with Polyfloral Honey—BPS1	Soap with Linden Honey—BPS2	Soap with Acacia Honey—BPS3	Soap with Manna Honey—BPS4	Soap with Sunflower Honey—BPS5	Soap with Propolis—BPS6	Safeguard—Commercial Soap—SGS
500 mg/mL	7.83 ± 0.29 ^{cde}	8.33 ± 0.29 ^{cd}	6.00 ± 0.50 ^g	7.17 ± 0.29 ^{ef}	6.67 ± 0.29 ^{fg}	8.67 ± 0.29 ^{bc}	19.00 ± 0.00 ^a
250 mg/mL	7.00 ± 0.50 ^{cde}	6.83 ± 0.58 ^{cde}	5.17 ± 0.29 ^g	6.00 ± 0.00 ^{efg}	5.67 ± 0.29 ^{fg}	7.83 ± 0.29 ^{bc}	17.17 ± 0.29 ^a
125 mg/mL	6.50 ± 0.50 ^{bc}	6.00 ± 0.00 ^{bcd}	4.17 ± 0.29 ^{ef}	5.17 ± 0.29 ^{def}	5.67 ± 0.76 ^{bcd}	6.67 ± 0.58 ^b	14.00 ± 0.00 ^a

Note: BPS—Bee Product Soap. Means followed by the same letter are not significantly different by the Tukey test at 5% level; Values are reported as mean ± standard deviation ($n = 3$).

Table 10. Diameter of the zone of inhibition (mm) against *E. coli* for soap samples containing EOs.

Concentration	Sample Inhibition Diameter Area (mm)						
	Soap with Clove EO—EOS1	Soap with Rosemary EO—EOS2	Soap with Aril of Mace EO—EOS3	Soap with Nutmeg EO—EOS4	Soap with White Pepper EO—EOS5	Soap with Juniper EO—EOS6	Safeguard—Commercial Soap—SGS
500 mg/mL	7.00 ± 0.50 ^{bc}	7.50 ± 0.00 ^b	7.17 ± 0.29 ^{bc}	6.00 ± 0.50 ^{cde}	6.17 ± 0.29 ^{cd}	6.83 ± 0.29 ^{bc}	9.83 ± 0.29 ^a
250 mg/mL	5.67 ± 0.76 ^{bcd}	6.17 ± 0.29 ^{bc}	6.00 ± 0.00 ^{bc}	4.83 ± 0.29 ^{de}	5.17 ± 0.29 ^{cde}	6.17 ± 0.29 ^{bc}	8.17 ± 0.29 ^a
125 mg/mL	5.17 ± 0.29 ^{bc}	5.33 ± 0.29 ^b	5.00 ± 0.00 ^{bcd}	4.33 ± 0.29 ^{cdef}	4.17 ± 0.29 ^{def}	4.50 ± 0.50 ^{bcd}	7.17 ± 0.29 ^a

Note: EOS—Essential Oil Soap. Means followed by the same letter are not significantly different by the Tukey's test at 5% level. Values are reported as mean ± standard deviation ($n = 3$).

Table 11. Diameter of the zone of inhibition (mm) against *S. aureus* for soap samples containing EOs.

Concentration	Sample Inhibition Diameter Area (mm)						
	Soap with Clove EO—EOS1	Soap with Rosemary EO—EOS2	Soap with Aril of Mace EO—EOS3	Soap with Nutmeg EO—EOS4	Soap with White Pepper EO—EOS5	Soap with Juniper EO—EOS6	Safeguard—Commercial Soap—SGS
500 mg/mL	8.50 ± 0.50 ^{bc}	9.50 ± 0.00 ^b	7.33 ± 0.29 ^{def}	6.50 ± 0.50 ^{fg}	6.50 ± 0.00 ^{fg}	7.83 ± 0.76 ^{cde}	19.00 ± 0.00 ^a
250 mg/mL	7.17 ± 0.29 ^{bcd}	8.17 ± 0.29 ^b	6.67 ± 0.29 ^{def}	5.00 ± 0.00 ^g	5.50 ± 0.50 ^g	7.17 ± 0.29 ^{bcd}	17.17 ± 0.29 ^a
125 mg/mL	6.50 ± 0.50 ^{bc}	6.17 ± 0.29 ^{bcd}	5.00 ± 0.50 ^{def}	4.00 ± 0.00 ^f	4.17 ± 0.29 ^{ef}	5.33 ± 0.29 ^{cde}	14.00 ± 0.00 ^a

Note: EOS—Essential Oil Soap. Means followed by the same letter are not significantly different by the Tukey test at 5% level. Values are reported as mean ± standard deviation ($n = 3$).

The results indicate that all soaps containing honey and propolis demonstrated measurable antimicrobial activity against *S. aureus* and *E. coli*, although the effectiveness varied according to both the soap concentration and the type of ingredient incorporated in the formulation. Overall, the inhibition zones decreased as the concentration was reduced, reflecting a clear dose–response relationship. The inhibition zones were larger in the case of the *S. aureus* strain than in the case of the *E. coli* strain. Among the formulations, the soap prepared with propolis produced the largest inhibition zones, suggesting a stronger antibacterial potential. When compared with the experimental soaps, the commercial Safeguard sample consistently produced larger inhibition zones across all concentrations,

confirming its superior antimicrobial performance, likely due to the inclusion of specific antibacterial agents in its formulation.

The results revealed that all tested soaps containing EOs displayed moderate antimicrobial activity against both bacterial strains, with inhibition zones generally larger than those recorded for soaps containing honey and propolis. A concentration-dependent trend was observed, as the antimicrobial activity decreased with lower bacterial concentrations. Among the formulations, the rosemary EO-based soap consistently produced larger inhibition zones, suggesting greater antibacterial effectiveness. In contrast, the soaps formulated with nutmeg EO and white pepper EO showed comparatively weaker activity. Like the results observed for soaps containing honey and propolis, the commercial Safe-guard soap exhibited the largest inhibition zones across all concentrations, confirming its superior performance, likely attributed to the presence of dedicated antibacterial agents in its industrial formulation.

The antimicrobial performance of the formulated soaps was mainly governed by several key parameters. The most influential factor was the type and chemical composition of the incorporated bioactive ingredient, with phenolic-rich components such as propolis and clove EO showing stronger antibacterial effects. The concentration of the soap solution also played a critical role, as a clear dose–response relationship was observed for all formulations. In addition, bacterial susceptibility significantly influenced the results, with Gram-positive *S. aureus* being more sensitive than Gram-negative *E. coli*.

In contrast, formulation-related parameters such as the soap base composition and pH were kept constant across all samples and therefore did not contribute to the observed differences in antimicrobial activity.

The differences in antimicrobial activity between the soap formulations can be mechanistically linked to the phenolic and flavonoid content of the incorporated bioactive ingredients. Phenolic compounds are known to disrupt bacterial cell membranes, alter permeability, and induce protein denaturation, leading to cell lysis. In the present study, soaps containing propolis, which exhibited the highest total phenolic and flavonoid content, consistently produced larger inhibition zones against both bacterial strains compared to honey-based formulations. Similarly, clove EO, characterized by a high phenolic content, demonstrated enhanced antibacterial performance relative to EOs with lower phenolic levels.

GC-FID analysis further supports these observations by revealing clear differences in the dominant chemical constituents of the EOs. Clove oil was dominated by eugenol, a phenylpropanoid with well-documented antimicrobial activity attributed to its ability to disrupt cytoplasmic membranes and interfere with enzyme systems. Rosemary oil, rich in 1,8-cineole and camphor, exhibited moderate antibacterial activity consistent with the membrane-disrupting effects of oxygenated monoterpenes. In contrast, EOs dominated by hydrocarbon monoterpenes or sesquiterpenes, such as juniper and white pepper oils, displayed weaker antimicrobial effects, reflecting the lower reactivity of these compounds toward bacterial membranes.

The higher susceptibility of *S. aureus* compared to *E. coli* can be explained by differences in the structure of the cell. The outer membrane of Gram-negative bacteria acts as an additional permeability barrier, limiting the diffusion of hydrophobic phenolic and terpenoid compounds, whereas Gram-positive bacteria are more readily affected by membrane-active agents. This structural difference accounts for the consistently larger inhibition zones observed against *S. aureus* across all formulations.

Although the overall inhibition zones are relatively modest, the differences among formulations correlate with the phenolic and flavonoid content of the bioactive ingredients,

indicating that the observed antimicrobial activity is mainly attributable to the added compounds rather than the alkaline soap matrix.

A one-way ANOVA was conducted separately for each soap concentration (125, 250, and 500 µg/mL) to evaluate the effect of formulation type on *E. coli* growth inhibition. The results indicated highly significant differences among the 14 formulations at all concentrations: 125 µg/mL, $F_{\text{value}} = 33.43$, $p < 0.0001$, $R^2 = 0.940$; 250 µg/mL, $F_{\text{value}} = 35.97$, $p < 0.0001$, $R^2 = 0.943$; 500 µg/mL, $F_{\text{value}} = 35.39$, $p < 0.0001$, $R^2 = 0.942$.

Levene's tests for homogeneity of variances were nonsignificant (all $p > 0.05$), which confirms that the assumption of equal variances was satisfied. Tukey's HSD post-hoc analysis revealed that at 125 µg/mL, SGS (7.17 mm) and BPS2 (6.50 mm) formed the top efficacy group and did not differ significantly from each other ($p > 0.05$), whereas all other soaps showed significantly smaller inhibition zones ($p < 0.05$). At 250 µg/mL, SGS (8.17 mm) remained the most active formulation, followed closely by BPS2 (6.50 mm; $p > 0.05$ vs. SGS), while BPS3–BPS5 exhibited the weakest activity ($p < 0.001$ vs. SGS). At the highest concentration, 500 µg/mL, SGS (9.83 mm) continued to display the greatest inhibition, but EOS2 (7.5 mm), EOS3 (7.17 mm), EOS1 (7.00 mm), and BPS6 (7.00 mm) achieved statistically comparable effects ($p > 0.05$ vs. SGS).

Antibacterial efficacy increased significantly with concentration, indicating a clear dose-dependent response. SGS consistently showed the strongest inhibitory activity, while several essential-oil-based soaps (particularly EOS2 and EOS3) approached similar performance at higher doses. Conversely, BPS3 remained significantly less effective at all concentrations ($p < 0.0001$).

Regarding the effect of soap formulation on *S. aureus* growth inhibition, one-way ANOVA showed significant differences among the 13 formulations at all concentrations: 125 µg/mL: $F_{\text{value}} = 121.38$, $p < 0.0001$, $R^2 = 0.982$; 250 µg/mL: $F_{\text{value}} = 252.54$, $p < 0.0001$, $R^2 = 0.991$; 500 µg/mL: $F_{\text{value}} = 235.61$, $p < 0.0001$, $R^2 = 0.991$.

Levene's test indicated unequal variances for the 125 µg/mL and 500 µg/mL data sets ($p < 0.05$), but not for the 250 µg/mL set ($p > 0.05$), so results at 125 and 500 µg/mL should be interpreted with caution. Tukey's HSD post-hoc comparisons showed that none of the tested soap formulations matched SGS in mean inhibition-zone diameter at any concentration—SGS formed a unique top group (SGS: 14 mm at 125 µg/mL; 17.17 mm at 250 µg/mL; 19.00 mm at 500 µg/mL), and all other formulations were significantly lower (Tukey grouping letters: SGS alone in group A at each concentration). The next-best formulations across concentrations were BPS6/EOS1/EOS2 (means ≈ 6.7 – 9.5 mm depending on concentration), but these remained significantly lower than SGS.

Antibacterial activity increased with concentration for most formulations (dose-dependent response), yet SGS maintained a clear superiority against *S. aureus* across the tested range.

Because Levene's test indicated significant heterogeneity of variances for the *S. aureus* data at 125 and 500 µg/mL, Welch's ANOVA followed by the Games–Howell post-hoc test was applied to provide variance-robust statistical comparisons. Welch ANOVA revealed significant differences among the groups for all three datasets (125: $F_{(13,14.28)} = 19.60$, $p < 0.001$; 250: $F_{(13,12.88)} = 24.49$, $p < 0.001$; 500: $F_{(13,11.72)} = 43.61$, $p < 0.001$). Games–Howell post-hoc tests indicated that the SGS category consistently showed significantly higher values compared to most BPS and EOS categories, with the magnitude of differences increasing with the dataset level.

4. Discussion

Although the valorization of WCOs into soaps and the incorporation of natural bioactive ingredients have been individually reported, this study provides an integrated and

comparative approach that has not been previously addressed. The novelty of the present work lies in the combined valorization of purified WCOs and chemically characterized bee products and EOs within a single, controlled soap matrix, followed by a mechanistic correlation between phenolic composition, GC-FID chemical profiles, and antimicrobial performance. By systematically comparing different classes of bioactive compounds under identical formulation, this study advances the understanding of how chemical composition governs the functional properties of sustainable soap formulations.

The extensive physico-chemical characterization was therefore not intended as an independent analytical study of raw materials, but as a supporting tool to interpret differences in antibacterial performance among soap formulations.

4.1. Preliminary Sustainability and Valorization Assessment

The use of WCOs as raw materials for soap production represents a valorization pathway that combines waste management with the generation of value-added functional products. WCOs are widely generated household and food-service residues, and their improper disposal is associated with environmental concerns, including water contamination, sewer blockages, and increased treatment costs. Converting this lipid-rich waste stream into soap contributes to waste reduction and mitigates the environmental burden associated with uncontrolled disposal.

From a resource-efficiency perspective, the valorization of WCOs aligns with circular economy principles by extending the life cycle of edible oils beyond their primary use. Instead of relying exclusively on virgin vegetable oils, which require agricultural land, water, and energy inputs, the proposed approach reintroduces a secondary raw material into the production cycle. This strategy supports more sustainable material use and reduces pressure on primary natural resources commonly employed in the oleochemical and soap industries [4].

The saponification process itself is well established, technically simple, and compatible with small- and medium-scale production, suggesting favorable prospects for scalability. From a preliminary economic standpoint, WCOs represent a low-cost and readily available feedstock compared to refined oils, potentially lowering the cost of raw materials in soap formulation. This aspect is particularly relevant for decentralized or community-based production models, where waste-derived inputs can enhance local value creation [62].

In addition to the valorization of WCOs, the incorporation of honey, propolis, and EOs further contributes to the biobased character of the formulations. These natural additives are known for their antimicrobial properties and may reduce the need for synthetic antibacterial agents, which are increasingly scrutinized due to environmental persistence and potential health concerns. The observed antimicrobial activity of the experimental soaps indicates that functional performance can be achieved while maintaining a predominantly natural composition.

It should be noted that the present sustainability evaluation is qualitative and preliminary in nature. A comprehensive assessment, including quantitative life cycle analysis and techno-economic evaluation, would be required to fully substantiate the environmental and economic advantages of WCO-based antimicrobial soaps on an industrial scale. Nevertheless, the results support the concept that transforming WCOs into antimicrobial soaps constitutes a viable valorization strategy with promising sustainability implications.

WCOs represent one of the most abundant and complex categories of organic waste generated globally, as inevitable by-products of the modern food chain. Primarily originating from frying and cooking processes, these oils gradually lose their functional properties and, if not properly managed, become a major source of pollution. At the same time, WCOs have valuable potential as a renewable raw material for the production of biofuels,

polymeric materials, or “green” chemical agents [4]. WCOs, primarily produced after frying processes that exceed optimal usage time, represent a major portion of lipid residues from the food industry. Compared to virgin oils, WCOs have a more complex composition, containing degraded triglycerides, FFA, oxidation and volatile compounds, metal traces, and food particles. This complexity requires thorough pretreatment and careful selection of recycling and valorization methods [10,63].

Globally, WCOs are estimated to range between 41 and 67 million tons per year. Their abundance and composition highlight their potential as a valuable raw material for valorization processes within the framework of a circular economy [62]. Policies and management schemes dedicated to WCOs are becoming increasingly relevant in the context of the transition toward a circular economy. A study conducted in the U.K. by Gong et al. [16] analyzed various WCO collection and recycling models, including the use of blockchain for traceability. The study showed that WCO recycling poses not only technological but also infrastructural, regulatory, and trust-related challenges across the value chain. Munir et al. [63] in their research demonstrate that standardizing flows and optimizing collection routes for WCOs can significantly reduce losses and operational costs, generating sustainable added value at the regional level and enhancing the efficiency of WCO management systems.

Furthermore, beyond energy applications, Musharavati et al. [64] report the conversion of WCOs into polyol intermediates for polyurethane synthesis, confirming the feasibility of obtaining WCO-derived compounds. These results highlight the great potential of this “waste-to-materials” pathway for the development of sustainable polymeric materials such as foams and elastomers.

The valorization of WCOs through their transformation into soap represents a concrete example of applying the principles of sustainability and the circular economy. Instead of being improperly discarded and polluting the soil or water, these oils are reintegrated into the economic cycle as a useful and environmentally friendly product: soap [65]. This process reduces the negative environmental impact and minimizes waste and the consumption of new resources, thus contributing to a model of responsible production and consumption [66]. Furthermore, by preventing the contamination of water and soil, the initiative supports the goals related to clean water and sanitation [67]. At the same time, reusing existing resources instead of producing new raw materials reduces greenhouse gas emissions, thereby having a positive impact on climate action [68]. Thus, the transformation of WCOs into soap perfectly illustrates the transition to a circular economy, where waste becomes a resource and innovation supports sustainable development [69]. In the current context of the transition to sustainable production and consumption models, the valorization of WCOs has gained strategic importance in both environmental protection and the advancement of the circular economy. Recent studies [1,18,70] underscore the potential of these residual streams to be reintegrated into local production cycles through the development of biodegradable soaps, thus generating added value while minimizing environmental impact.

From an environmental protection and sustainability perspective, the conversion of WCOs into biodegradable soaps simultaneously contributes to reducing the pollution risks associated with their discharge into sewage systems [71]. A recent study [72] has shown that it is technologically feasible to produce antimicrobial soaps from WCOs enriched with EOs, which exhibit good functional properties and biodegradability. From a sustainability perspective, a recent Life Cycle Assessment by Mwamba et al. [73] demonstrated that integrating WCOs with plant-based residues such as citrus peels in soap production significantly reduces environmental impacts compared to the use of virgin oils. The study

also confirmed the environmental and economic advantages of this circular approach, highlighting its feasibility for small and medium-sized enterprises.

On an industrial scale, the use of NaCl for WCO purification could generate substantial saline wastewater. Implementing brine recovery, recrystallization, and appropriate wastewater treatment would be essential to ensure that the process remains environmentally sustainable [74].

From an economic and value-chain-resilience perspective, community initiatives that collect WCOs and convert them into soaps demonstrate a favorable cost–benefit ratio. These projects reduce waste management expenses while generating local micro-income. Municipal programs offering training and demonstration workshops have fostered household and small-scale adoption, enhancing community engagement and reducing uncontrolled discharges [51].

These bio-inspired approaches contribute to the simultaneous valorization of multiple waste streams—WCOs and plant-based products and residues—and lead to high-value-added products tailored for local markets and community-level ecological education initiatives [65,75].

4.2. Antibacterial Activity of Bio-Enriched WCO-Based Soap Formulations

In addition to sustainability considerations, current research directions focus on combining WCOs with natural products or extracts to obtain solid soaps with antibacterial properties. In this study, EOs were selected to represent different botanical origins and dominant chemical classes relevant for antimicrobial activity in soap formulations. These oils were selected to enable a systematic comparison between different chemical classes (phenolics, oxygenated monoterpenes, and hydrocarbon terpenes) and their contribution to antibacterial performance in an identical soap matrix.

Expanding beyond their use in soap manufacturing, WCOs are increasingly being incorporated into biopolymer formulations, offering an effective means to reduce petrochemical plastic use and carbon footprint while reinforcing circular economy principles [76–78].

The adherence to ISO 22716:2007 Good Manufacturing Practices demonstrates [79] that the developed formulation meets essential cosmetic safety, labeling, and quality criteria. The compliance of the soap formulation with Regulation (EC) No 1223/2009 on cosmetic products [28] indicates that the product not only meets European safety and quality standards but also strengthens its potential for practical application and market acceptance.

Many studies [80–82] have investigated the antimicrobial activity of various honeys and propolis, highlighting their potential as natural agents against foodborne and environmental pathogens. Bee products possess antibacterial properties, their effectiveness being commonly measured by the zone of inhibition in laboratory settings [83]. When incorporated into soap, honey can retain some of its antimicrobial properties, although its efficacy depends on factors such as concentration, honey type, and the presence of other ingredients. The antimicrobial activity of honey is multifactorial: it contains the enzyme glucose oxidase, which produces H_2O_2 with natural antiseptic effects; it has a low pH (3.2–4.5) that inhibits bacterial growth; and its high sugar content creates a hypertonic environment that dehydrates bacterial cells [84]. In addition, compounds like methylglyoxal in Manuka honey provide a strong non-peroxide antibacterial effect, while flavonoids and phenolic acids contribute antioxidant and antimicrobial properties [79]. However, in soap formulations, these effects differ from those in medical-grade applications: dilution during washing activates H_2O_2 but also lowers the concentration of active compounds. Moreover, since soap's contact time with the skin is brief, its main antibacterial effect is mechanical, removing microbes through rinsing rather than destroying them chemically [84].

The antimicrobial activity observed in the tested formulations is the result of two complementary mechanisms. The base soap matrix contributes primarily through its alkaline pH and surfactant action, which can disrupt microbial cell membranes and reduce bacterial viability. In contrast, the added bioactive ingredients provide an additional, ingredient-specific antimicrobial contribution, reflected by the increased inhibition zones observed in enriched soaps compared to plain soap formulation.

While the base soap itself is expected to exhibit a baseline antibacterial effect due to its alkalinity, the comparative results among enriched formulations indicate that bioactive additives modulate and enhance this effect in a concentration-dependent manner, with EOs showing the strongest contribution, followed by propolis and honey. A quantitative separation of the antimicrobial effects of pH and bioactive additives was not performed in the present study and represents a limitation that should be addressed in future research through pH-controlled assays [85].

The antibacterial activity was evaluated against Gram-positive- (*S. aureus*) and Gram-negative-strain (*E. coli*), selected to assess potential differences related to bacterial cell wall structure [86].

Regarding the antibacterial efficacy, both honey- or propolis- enriched soaps exhibited moderate, concentration-dependent inhibitory activity. A greater efficacy in bacterial inhibition was observed in soaps enriched with EOs, followed by those with propolis and then those with honey.

The antimicrobial results indicate that the soaps enriched with EOs were particularly effective against *S. aureus*. This increased activity can be attributed to the presence of highly bioactive, hydrophobic compounds such as eugenol in clove oil and 1,8-cineole in rosemary oil, which are known to disrupt bacterial cell membranes and interfere with metabolic functions [87]. The higher susceptibility of *S. aureus* compared to *E. coli* is consistent with its Gram-positive cell wall structure, which facilitates the penetration of hydrophobic antimicrobial agents.

Although the inhibition zones obtained for the experimental soaps were smaller than those of the commercial Safeguard soap, the results demonstrate that the incorporation of natural bioactive ingredients can meaningfully enhance the antimicrobial performance of soaps based on waste-derived oils, highlighting their potential as environmentally friendly and safer alternatives to synthetic antibacterial products. It is known that honey has antibacterial properties, which differ according to its properties and botanical and geographical origins [81,88]. Compared to honey, propolis from Romania has been shown to have a more potent antimicrobial effect [89,90]. This also explains why soaps with the addition of aqueous propolis extract had stronger antibacterial activity than those with the addition of honey. However, soaps with EOs had an even stronger antibacterial effect due to the components present in these oils, such as terpenoid components or phenolic components with antimicrobial properties [91,92].

The effect of soaps on the *S. aureus* strain was greater than that on the *E. coli* strain. This is consistent with other studies that showed similar results, but using commercial medicated soaps [93]. The antibacterial activity of our soaps was, however, lower than that of the commercial Safeguard soap, the explanation being its content of active antibacterial agents such as piroctone olamine as well as citric acid and sodium citrate for pH regulation.

The experimental soaps developed in this study, including formulations with bee products and EOs, show promising physico-chemical and preliminary antimicrobial properties, suggesting potential applications as natural and eco-friendly personal care products. These formulations could be further explored for cosmetic, hygienic, or therapeutic uses, particularly in markets seeking natural alternatives to conventional soaps. However, this study has some limitations. Only preliminary antimicrobial tests were performed on a

limited number of bacterial strains, and certain physico-chemical properties, such as hardness, saponification value, surfactant properties, and long-term storage stability, were not fully assessed. Future research will address these aspects and expand testing to additional microbial strains to better evaluate efficacy and safety. Furthermore, scaling up the production process and assessing consumer acceptability are needed before practical applications are realized. From a safety perspective, all formulated soaps exhibited pH values within the typical range reported for traditional alkaline soaps (pH 9–10), which are generally considered acceptable for rinse-off cosmetic products. The free caustic alkalinity values were negligible, indicating complete saponification and a low risk of skin irritation.

The bioactive ingredients used (honey, propolis, and EOs) are widely applied in cosmetic formulations and are generally recognized as safe when used at appropriate concentrations. However, potential skin sensitivity or allergenic responses, particularly associated with EOs and propolis, cannot be fully excluded and require further dermatological evaluation [94].

From a regulatory perspective, the proposed soap formulations are intended as cosmetic products and would need to comply with relevant cosmetic regulations (e.g., EU Regulation (EC) No. 1223/2009), including ingredient concentration limits, safety assessment, labeling requirements, and stability testing prior to commercialization. These aspects were beyond the scope of the present study and represent important directions for future work [95].

The one-way ANOVA statistical method also revealed that the next-best formulations across concentrations were BPS6, EOS1, and EOS2 samples for both studied strains, but none of them were close to the activity registered by the commercial soap. For future work, the use of EOs, especially clove and mace together with propolis, will be considered due to the concerted effect of their active properties regarding the inhibition of certain bacterial strains in soap formulations.

5. Conclusions

This study demonstrates a sustainable method for the recycling of WCOs into value-added soaps with satisfactory physico-chemical properties. Incorporation of natural bioactive ingredients, such as honey, propolis, and EOs, enhanced the antimicrobial activity against *S. aureus* and *E. coli*. The soaps containing EOs demonstrated the highest antibacterial effectiveness, followed by the one with propolis and then those with honey. The enhanced antimicrobial activity is attributed to the combined effects of the alkaline nature of the soaps and the bioactive compounds present in the natural additives. Among EOs, rosemary and clove showed the strongest antibacterial potential, which is correlated with their higher phenolic and volatile bioactive contents, particularly eugenol and 1,8-cineole, which disrupt bacterial membranes. *S. aureus*, being Gram-positive, is more susceptible than Gram-negative *E. coli*.

The enriched soaps demonstrate both cosmetic and antimicrobial potential while promoting the sustainable reuse of WCOs. This approach supports the principles of the circular economy by transforming waste from the food industry into value-added, eco-friendly soap products. Future studies could focus on optimizing bioactive concentrations, expanding antimicrobial testing to additional microbial strains, and evaluating long-term stability and consumer acceptability of formulations.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/chemengineering10020031/s1>, Figure S1a: GC-FID chromatogram of essential oil: EO1—clove; Figure S1b: GC-FID chromatogram of essential oil: EO2—rosemary; Figure S1c: GC-FID chromatogram of essential oil: EO3—mace (aril of *Myristica fragrans*); Figure S1d: GC-FID chromatogram of essential oil: EO4—nutmeg (seeds of *Myristica fragrans*).

tica fragrans); Figure S1e: GC-FID chromatogram of essential oil: EO5—white pepper (*Piper nigrum*); Figure S1f: GC-FID chromatogram of essential oil: EO6—juniper (*Juniperus communis*); Supplementary Materials S2: Analytical procedures for honey, propolis, and essential oils (EOs).

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Abbreviations

The following abbreviations are used in this manuscript:

WCO	waste cooking oil
EO	essential oil
FFA	free fatty acid

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