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Scale-Up of a Medicinal and Aromatic Plant-Based Shampoo Formulation: Physicochemical Quality, Microbiological Safety, and Stability Analyses

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Abstract
This study investigated the scale-up process of a plant-based shampoo formulation developed for dry and damaged hair, evaluating its quality, safety, and stability parameters across laboratory, pilot, and industrial production scales. The formulation comprised nonionic and amphoteric surfactants (decyl glucoside, coco glucoside, cocamidopropyl betaine), moisturizing and antistatic agents (D-panthenol, sodium lactate), plant-derived keratin, and essential oils of medicinal lavender and rosemary. The formulation was initially developed at a 100 mL laboratory scale. Following systematic optimization of process parameters—including mixing speed, mixing time, phase addition sequence, and mechanical stirring conditions—the formulation was successfully scaled to a 3,000 mL pilot scale and subsequently to a 40,000 mL industrial production scale. Physicochemical parameters including pH, viscosity, and density were monitored at each stage using calibrated instrumentation: a digital pH meter, a Brookfield-type rotational viscometer, and volumetric density calculations. Microbiological safety and antimicrobial efficacy assessments were conducted on the industrial-scale batch in accordance with relevant ISO standards. Microbiological analyses included total aerobic mesophilic microorganism enumeration and screening for *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli*, and *Candida albicans*. Preservative system efficacy was evaluated via challenge testing per TS EN ISO 11930, with logarithmic reductions in microbial counts recorded. Accelerated stability studies encompassing varied temperature, centrifugation, and storage conditions confirmed consistent pH, viscosity, physical appearance, and microbiological integrity throughout. The results demonstrated that the formulation retained its physicochemical characteristics across all production scales while meeting microbiological safety requirements. These findings highlight the industrial scalability of naturally derived hair care formulations and emphasize the critical role of rigorous quality control protocols in cosmetic manufacturing.

Keywords: Scale-up, Shampoo formulation, Medicinal and aromatic plants, Preservative efficacy, Herbal cosmetics, Process validation, Stability studies

1. INTRODUCTION

The demand for natural and plant-based products in the global cosmetic market has increased significantly in recent years. According to market research data, the organic shampoo market was valued at approximately USD 4.5 billion in 2023 and is expected to expand at an annual growth rate of 6.5% until 2030 (Grand View Research, 2024). The primary drivers of this growth include increasing consumer awareness of synthetic surfactants and preservatives (sulfates, parabens), demand for eco-friendly formulations, and the growing importance of ingredient transparency in personal care products (Mordor Intelligence, 2025; Shim et al., 2024). This trend necessitates scientifically based product development studies in which natural raw materials are incorporated into formulations not merely as attractive marketing elements but with proven functional efficacy.

Shampoo formulations are multi-component, multi-phase systems consisting of surfactant systems, moisturizing agents, thickeners, preservatives, and functional active ingredients. In these systems, surfactant selection plays a decisive role in the performance and dermatological compatibility of the formulation. Glucoside-derived nonionic surfactants, decyl glucoside and coco glucoside, are obtained from renewable plant sources such as corn and coconut; they are among the preferred primary surfactants in natural cosmetic formulations due to their compliance with ECOCERT certification, biodegradability, and low irritation

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potential (Gubitosa et al., 2019). Amphoteric cocamidopropyl betaine accompanies this surfactant system as a foam-enhancing, thickening, and irritation-reducing co-surfactant; its pH-independent zwitterionic structure contributes to the stability of the formulation and its mild conditioning effect on hair (Burnett et al., 2012).

In addition to the surfactant system, the selection of active ingredients is also critically important for the efficacy of formulations developed for dry and damaged hair. D-panthenol (Provitamin B5) is one of the most widely used active moisturizing ingredients in hair care products; it penetrates the hair fiber to provide long-term moisture retention, increases resistance to mechanical stress, and improves the elasticity of the hair strand (Shin et al., 2021). Sodium lactate contributes to water-binding capacity with its humectant property and supports the pH balance of the formulation. Hydrolyzed vegetable keratin, due to its biocompatible amino acid sequence with the hair's own structural protein, adsorbs onto damaged areas of the hair cuticle to form a repair film; it is noteworthy as an ingredient approved as safe in cosmetics by the Cosmetic Ingredient Review (CIR) Expert Panel (Burnett et al., 2021).

The use of essential oils in plant-based formulations assumes biologically active functions beyond merely contributing to aromatic quality. Medicinal lavender (*Lavandula angustifolia* Mill.) essential oil exhibits antimicrobial, anti-inflammatory, and scalp-soothing effects through its bioactive components, primarily linalool and linalyl acetate, and has also been reported to potentially increase the number of hair follicles (Abelan et al., 2022). Rosemary (*Salvia rosmarinus* Spenn.) essential oil has been extensively studied in the literature as an active ingredient supporting hair growth, improving scalp circulation, and exhibiting antimicrobial efficacy through antioxidant compounds such as rosmarinic acid, carnosic acid, and ursolic acid; some studies have reported potential hair growth-supporting effects, with preliminary comparisons to minoxidil noted in the literature (Panahi et al., 2015; Patel et al., 2025), though direct efficacy evaluation was beyond the scope of the present study.

The successful transfer of formulations designed with a balanced combination of these active and functional ingredients from laboratory scale to industrial production depends on the scientific and technical management of the scale-up process. Scale-up encompasses many interrelated variables such as mixing dynamics, heat transfer, vessel geometry, and component homogeneity; if these parameters cannot be reproducibly established in large-scale equipment, product quality and stability may be compromised (Durgun & Algın Yapar, 2021). For cosmetic products to be placed on the market in compliance with legal requirements, microbiological compliance and antimicrobial efficacy tests are among the mandatory prerequisites; the challenge test conducted according to TS EN ISO 11930 constitutes the internationally recognized reference method for evaluating the adequacy of the preservative system.

However, studies that simultaneously document systematic scale-up validation and comprehensive quality characterization at industrial scale for shampoo formulations containing natural active ingredients remain quite limited in the literature. The aim of this study is to document the scale-up process of a plant-based shampoo formulation containing a nonionic/amphoteric surfactant system based on decyl glucoside, coco glucoside, and cocamidopropyl betaine, D-panthenol, sodium lactate, vegetable keratin, and medicinal lavender and rosemary essential oils from 100 mL laboratory scale to 40,000 mL industrial production scale; to monitor physicochemical properties at each production stage; and to comprehensively evaluate microbiological safety, antimicrobial efficacy, and stability analyses at industrial scale.

2. MATERIAL AND METHOD

2.1. Formulation development

A plant-based shampoo formulation for dry and damaged hair was developed through a multi-phase production process. The components of the formulation are presented in Table 1. All components were weighed using a Weightlab Instruments WL-303L precision balance (max 300 g, d = 0.001 g).

During the production process, Phase B (xanthan gum + glycerin mixture) was stirred on a magnetic stirrer at 350 rpm for at least 5 minutes. Phase C (surfactant mixture) was then slowly added to Phase B without foaming. Phase A components were weighed in a separate beaker, stirred for 5 minutes, and then slowly added to the mixture, stirred gently for 15–20 minutes at intervals of a few minutes to ensure homogeneous gum distribution. The surface of the resulting mixture was covered with stretch film and allowed to rest for 30

minutes. Before adding Phase D, the pH of the main mixture was checked and adjusted to the target range of 4.5–6.0 using 10% (w/v) citric acid solution. In the final step, the preservative was added, and the pH was checked again.

Table 1. Components of the plant-based shampoo formulation

Phase	Function	Ingredient / INCI Name	Concentration (%)
A	Solvent	Distilled Water / Aqua	20-30
A	Antistatic / Humectant	Provitamin B5 / D-Panthenol	2-5
A	Moisturizer	Sodium Lactate	1-3
A	Active	Herbal Keratin / Hydrolyzed Vegetable Keratin	1-5
B	Humectant	Glycerin / Glycerin	3-7
B	Viscosity Agent	Xanthan Gum / Xanthan Gum	0.1-05
C	Surfactant, nonionic	Decyl Glucoside / Decyl Glucoside	20-30
C	Surfactant, nonionic	Coco Glucoside / Coco Glucoside	10-20
C	Surfactant, amphoteric	Cocamidopropyl Betaine / Cocamidopropyl Betaine	10-20
C	Fragrance, Active	Medicinal Lavender Oil / *Lavandula angustifolia* Flower Oil	0.05-0.2
C	Fragrance, Active	Rosemary Oil / *Salvia rosmarinus* Leaf Oil	0.2-0.6
D	Preservative	Lexgard Natural / Glyceryl Caprylate and Glyceryl Undecylenate	1-2
D	pH Adjuster	Citric Acid Monohydrate (10% w/v) / Citric Acid Monohydrate	q.s.

*q.s.: quantum satis (to achieve target pH range of 4.5–6.0). Concentration ranges are provided as general guidelines; exact concentrations are withheld due to proprietary know-how considerations.

2.2. Scale-up

The formulation was developed progressively at three different production scales in order to evaluate manufacturability and homogeneity. The same production procedure was applied at each scale with appropriate equipment for the batch volume (Figure 1). Scale-up mixing parameters were adjusted based on the constant tip speed criterion to ensure consistent shear conditions across scales. At pilot scale (3,000 mL), mixing was conducted using an Emsan planetary stand mixer with an impeller diameter of approximately 0.15 m at ~76 rpm, yielding a tip speed of approximately 0.60 m/s. At industrial scale (40,000 mL), a vacuum-equipped stainless steel planetary mixer with an impeller diameter of approximately 0.38 m was operated at 30 rpm, maintaining a consistent tip speed of ~0.60 m/s. The mixing Reynolds number ($Re = \rho N D^2/\mu$), calculated using measured density ($\rho = 1013$ kg/m³) and viscosity ($\mu = 3.2$ Pa·s) values from Table 4, was estimated at $Re \approx 9$ (pilot) and $Re \approx 23$ (industrial), confirming laminar flow conditions at both scales, consistent with the rheological behavior of xanthan gum-based viscous cosmetic systems (Durgun & Algın Yapar, 2021).

At laboratory scale (100 mL), mixing was performed using a combination of a mechanical stirrer and a magnetic stirrer. At pilot scale (3,000 mL), a mixing blender was used; the increased batch volume was mixed while maintaining homogeneity. At industrial scale (40,000 mL), production was carried out with an integrated equipment system consisting of a vacuum-equipped stainless steel main mixer, a preparation mixer, and a homogenizer (Figure 1). For the production procedure, Phase B was first prepared; glycerin and xanthan gum were transferred to the main mixer in sequence and stirred at 30 rpm for 30 minutes. The mixture was then rested for 60 minutes to allow adequate wetting and swelling of the xanthan gum. Subsequently, Phase C (surfactant mixture) was added to the preparation mixer in sequence, slowly mixed at 20 rpm for 5 minutes, and transferred onto Phase B in the main mixer via the vacuum system. The mixture obtained in the main mixer was stirred for an additional 10 minutes at 30 rpm, after which Phase A components were weighed in sequence and slowly added to the mixing system through the product loading chamber at 30 rpm. Stirring was continued at 30 rpm for 20 minutes to ensure homogeneous distribution of the gum. Before adding Phase D, the pH of the main mixture was measured, and it was adjusted to the target range of 4.5–6.0 using citric acid

solution. The preservative was then added to the system, mixed, and the final pH value was recorded. In the final stage, the product was transferred from the main mixer to the filling machine (Figure 2) with a homogenizer running at 110 rpm, filled, and labeled.

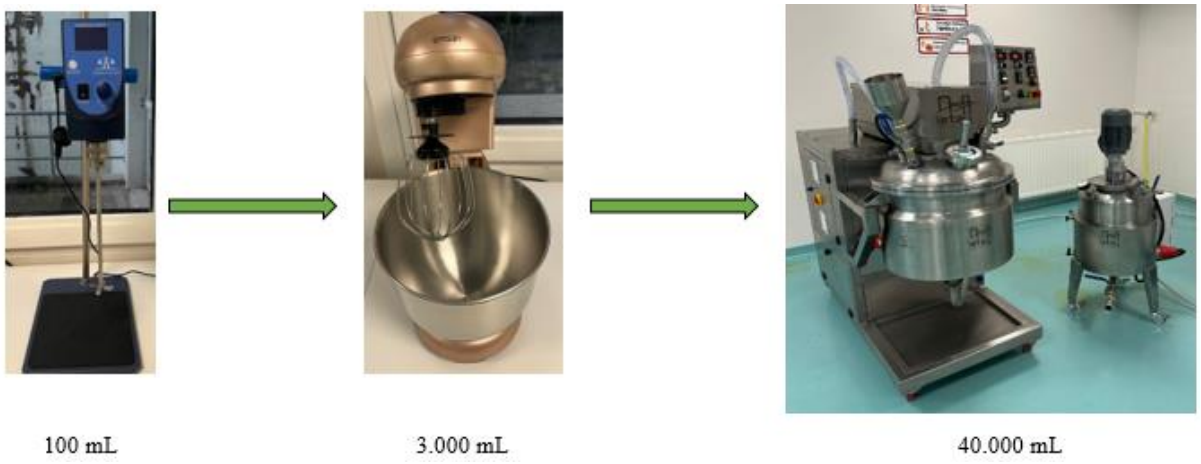

Figure 1. Mixing equipment used during the scale-up phase

Figure 2. Filling equipment used during the scale-up phase

2.3. Physicochemical characterization

Organoleptic properties (color, odor, and general appearance) of samples produced at each scale were evaluated visually and sensorially by three trained observers under standardized laboratory lighting conditions using a consensus-based assessment approach. pH measurements were performed at room temperature (25 ± 1 °C) using a Thermo Scientific Orion Star A215 pH/conductivity meter (Thermo Fisher Scientific, USA) calibrated with standard buffer solutions before each measurement session; measurements were performed in triplicate, and mean values were recorded. The coefficient of variation (CV) across production scales was calculated to assess inter-scale variability of physicochemical parameters. Viscosity measurements were carried out using a Brookfield-type rotational viscometer equipped with Spindle 4 at 50 rpm under constant temperature conditions (25 ± 1 °C). Density was calculated based on the mass measurement of a defined sample volume. For evaluation of phase separation, samples were centrifuged at 3,000 rpm for 30 minutes at 25 °C using a Nuve NF 1200R refrigerated centrifuge (Nuve, Turkey), and visual inspection was performed after the process to observe phase separation, sedimentation, or changes in homogeneity (Pekcan, 2018).

2.4. Microbiological analysis

Microbiological analysis was carried out in accordance with the guidelines established by the Turkish Medicines and Medical Devices Agency (TITCK) for cosmetic products (TITCK, 2005). Analyses were

performed only on the industrial-scale batch (40,000 mL) as it represents the final production scale for commercial application. The total count of aerobic mesophilic microorganisms was determined according to the TSE EN ISO 21149:2017 standard. Detection of specified microorganisms (*Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739), *Candida albicans* (ATCC 10231), and mold/yeast) was performed using relevant TSE EN ISO reference standards (Orth et al., 2006). The culture media and microbiological analysis methods used for the tested microorganisms are given in Table 2. All microbial reference strains were initially grown in Brain Heart Infusion Broth for 18–24 hours. Bacterial suspensions were turbidimetrically standardized to 0.5 McFarland (approximately equivalent to 1×10^8 CFU/mL) and then diluted to a working concentration of 5×10^5 CFU/mL in sterile physiological saline before use.

Table 2. Microbiological analysis methods

Microorganism	Method	Medium
Staphylococcus aureus ATCC 6538	Enrichment & Inoculation of Culture Media	Baird Parker Agar Medium
Pseudomonas aeruginosa ATCC 9027	Enrichment & Inoculation of Culture Media	Cetrimide Agar Medium
Escherichia coli ATCC 8739	Enrichment & Inoculation of Culture Media	MacConkey Agar Medium + Levine Eosin - Methylene Blue Agar Medium
Candida albicans ATCC 10231	Enrichment & Inoculation of Culture Media	Sabouraud 4% Dextrose Agar + Supplement
Moulds - Yeast	Pour Plate or Spread Plate Technique	Sabouraud 4% Dextrose Agar + Supplement
Total Aerobic Mesophilic Microorganisms	Pour Plate or Spread Plate Technique	Tryptic Soy Agar with Polysorbate 80 and Lecithin

2.5. Antimicrobial analysis (Challenge test)

The efficacy of the preservative system was evaluated by antimicrobial analysis in accordance with the TS EN ISO 11930:2019 standard. The antimicrobial analysis was performed on the industrial-scale batch (40,000 mL) in accordance with the guidelines established by TITCK for cosmetic products (TITCK, 2005). Five standard microorganisms were used as test strains: *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739), *Candida albicans* (ATCC 10231), and *Aspergillus brasiliensis* (NCPF 2275). Each formulation sample was inoculated with a standardized microbial suspension and stored under controlled conditions. Viable microbial counts were determined on days 7, 14, and 28 after inoculation, and logarithmic reductions relative to the initial inoculum were calculated for each strain. All tests were performed in triplicate, and the culture media and incubation conditions for each organism are presented in Table 3.

2.6. Stability Analysis

The purpose of stability testing is to provide evidence on how the quality of a product changes over time under the influence of various environmental factors such as temperature, humidity, and light, and to determine both the retest period and the shelf life and recommended storage conditions for the product (TITCK, 2017). The physicochemical stability of the formulation was evaluated under accelerated and long-term stress conditions to predict shelf life performance. Stability analyses were conducted on the industrial-scale batch (40,000 mL). These analyses were performed using a programmable stability cabinet (JSDS-300C, South Korea) capable of providing precise temperature control throughout all storage conditions. Samples were subjected to (i) 24-hour storage at −24 °C (freeze-thaw test); (ii) one-week cold storage at −5 °C; (iii) four-month storage at 25 °C in darkness at room temperature, with weekly evaluations in the first month and monthly evaluations in subsequent months; (iv) three-month storage at 45 °C at elevated temperature, with evaluations on day 3, weekly during the first month, and monthly during the 2nd and 3rd months (ICH, 1995; ICH, 2003). After

aging, color, odor, and general appearance were recorded by trained observers; pH was measured using a Thermo Scientific Orion Star A215 pH meter as described above; viscosity was determined using a Brookfield-type rotational viscometer; and density was calculated based on the mass measurement of a defined sample volume.

Table 3. Antimicrobial analysis methods

Microorganism	Method	Culture Media	Incubation Condition
Staphylococcus aureus ATCC 6538	Pour Plate	Tryptic Soy Agar	30 - 35 ℃
Pseudomonas aeruginosa ATCC 9027	Pour Plate	Tryptic Soy Agar	30 - 35 ℃
Escherichia coli ATCC 8739	Pour Plate	Tryptic Soy Agar	30 - 35 ℃
Candida albicans ATCC 10231	Pour Plate	Sabouraud 4% Dextrose Agar	30 - 35 ℃
Aspergillus brasiliensis NCPF 2275	Pour Plate	Potato Dextrose Agar	30 - 35 ℃

3. RESULTS

3.1. Physicochemical results

The physicochemical properties of the formulation were evaluated at all production scales, and the results obtained are summarized in Table 4. In organoleptic evaluation, a homogeneous, yellow, foaming gel appearance and characteristic lavender-rosemary odor were observed at all scales. No phase separation, sedimentation, or loss of homogeneity was detected at any scale after centrifugation testing.

Table 4. Physicochemical characterization results of the shampoo formulation at different production scales

Parameter	100 mL	3000 mL	40000 mL
pH (25 °C)	5.24 ± 0.18	5.36 ± 0.22	5.28 ± 0.11
Viscosity (mPa·s)	3200 ± 150	3150 ± 120	3180 ± 130
Density (g/mL)	1.012 ± 0.002	1.014 ± 0.002	1.013 ± 0.002
Appearance	Homogeneous, yellow, foaming gel	Homogeneous, yellow, foaming gel	Homogeneous, yellow, foaming gel
Odor	Characteristic lavender-rosemary	Characteristic lavender-rosemary	Characteristic lavender-rosemary
Centrifugation	No phase separation	No phase separation	No phase separation

3.2. Microbiological analysis results

The results of the microbiological quality analysis performed on the 40,000 mL industrial-scale batch are presented in Table 5. Total aerobic mesophilic microorganism count and total yeast/mold count were determined as <10 CFU/g in triplicate; both parameters remained well below the acceptable limit of $<10^2$ CFU/g (TITCK, 2005). The absence criterion was met for all specified pathogenic microorganisms, including *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*, and *Candida albicans*.

Table 5. Microbiological analysis results of the shampoo formulation (40,000 mL)

Microorganism	Unit	Microbiological Analysis Result	Tolerance
Total Aerobic Mesophilic Microorganisms	CFU*/g	< 10	< 100
Staphylococcus aureus	/g or /ml	Absent	Absent
Pseudomonas aeruginosa	/g or /ml	Absent	Absent
Escherichia coli	/g or /ml	Absent	Absent
Candida albicans	/g or /ml	Absent	Absent
Total Mold and Yeast	CFU/g	< 10	< 100

**CFU: colony forming unit*

3.3. Antimicrobial analysis (Challenge test) results

The results of the challenge test performed according to the TS EN ISO 11930:2019 standard are given in Table 6. All five microorganisms tested showed progressive and significant reductions in viable counts after inoculation. For bacterial strains *S. aureus, P. aeruginosa*, and *E. coli,* the initial inocula were determined as 3.4×10^8, 2.7×10^8, and 2.2×10^8 CFU/g, respectively; on day 7, all bacterial strains were found to have fallen below the detection limit (<10 CFU/g). For fungal strains *C. albicans* and *A. brasiliensis*, the initial inocula were measured as 1.5×10^6 and 2.3×10^6 CFU/g, respectively; they fell below the detection limit by day 7 and remained at that level until day 28. The fall of all microorganisms below the detection limit on day 7 and maintenance of this level until day 28 demonstrates that the Category A criteria as defined in the TITCK Guide on Microbiological Control of Cosmetic Products are met and that the preservative system used in the formulation is effective (TITCK, 2005).

Table 6. Antimicrobial analysis results of the shampoo formulation (40,000 mL)

Microorganism	Day 0		Day 7	Day 14	Day 28
	N (CFU/g)	N_0 (CFU/g)	N_7 (CFU/g)	N_{14} (CFU/g)	N_{28} (CFU/g)
Staphylococcus aureus ATCC 6538	3.4×10^8	9.2×10^5	< 10	< 10	< 10
Pseudomonas aeruginosa ATCC 9027	2.7×10^8	1.1×10^4	< 10	< 10	< 10
Escherichia coli ATCC 8739	2.2×10^8	3.8×10^5	< 10	< 10	< 10
Candida albicans ATCC 10231	1.5×10^6	2.6×10^3	< 10	< 10	< 10
Aspergillus brasiliensis NCPF 2275	2.3×10^6	4.5×10^3	< 10	< 10	< 10

3.4. Stability analysis results

The stability analysis results obtained during the three-month monitoring period for the 40,000 mL industrial-scale sample are summarized in Table 7. Under all storage conditions (25°C and 40°C), the color of the formulation remained light yellow, the odor retained its characteristic properties, and no change in appearance or phase separation was observed. The pH value ranged between 5.10 and 5.34 throughout the monitoring period and remained within the target range of 4.5–6.0. Density values remained between 1.010 and 1.015

g/mL, and viscosity values ranged between 3122 and 3159 mPa·s across all time points, representing negligible variation consistent with the initial physicochemical characterization results (Table 4). No microbiological growth was detected at any time point.

Table 7. Physicochemical and microbiological stability results of the product (40,000 mL)

Parameter	Week 1	Month 1	Month 2	Month 3
	19.07.2024	12.08.2024	12.09.2024	12.10.2024
Color	Yellow	Yellow	Yellow	Yellow
Odor	Rosemary-Lavender	Rosemary-Lavender	Rosemary-Lavender	Rosemary-Lavender
Appearance	No Change	No Change	No Change	No Change
pH	5.22 ± 0.16	5.10 ± 0.12	5.34 ± 0.26	5.18 ± 0.17
Density (g/mL)	1.010 ± 0.001	1.015 ± 0.002	1.012 ± 0.002	1.010 ± 0.001
Viscosity (mPa·s)	3122 ± 125	3151 ± 130	3146 ± 120	3159 ± 140
Packaging	No Change	No Change	No Change	No Change
Phase Separation	Not observed	Not observed	Not observed	Not observed
Microbiological Growth	Not observed	Not observed	Not observed	Not observed

4. DISCUSSION

In this study, the findings suggested that the plant-based shampoo formulation could be transferred to industrial scale under the tested production conditions, indicating that the scale-up process can be managed with a systematic approach. The findings obtained are consistent with similar studies in the literature, while limited studies appear to report comprehensive validation of formulations containing natural active ingredients at industrial scale.

The physicochemical results obtained during the scale-up process revealed that the formulation exhibited high consistency between different production scales. pH values (5.24–5.36) remained in the acidic range compatible with hair and scalp physiology; no statistically significant difference was observed between scales in viscosity (3,150–3,200 mPa·s) and density (1.012–1.014 g/mL) values. The findings suggest that acceptable formulation consistency was maintained across scales (Durgun & Algın Yapar, 2021). The coefficient of variation across production scales remained below 1% for all physicochemical parameters (pH: CV = 0.6%; viscosity: CV = 0.8%; density: CV = 0.1%), indicating negligible inter-scale variability and confirming the reproducibility of the formulation across scales. Studies on physicochemical evaluation of shampoo formulations in the literature have emphasized that maintaining pH in the 5.0–6.0 range is of critical importance for surfactant efficacy and hair cuticle integrity (Dias et al., 2014). Similarly, viscosity values of 2,400–3,400 mPa·s reported in plant-based shampoo formulations containing glucoside-based surfactant systems (Salunkhe et al., 2022; Nile & Kulkarni, 2024) are consistent with the results obtained in our study, confirming that the xanthan gum/glycerin-based rheological system works effectively at industrial scale. The absence of phase separation at any scale in the centrifugation test supports the physical stability of the formulation and the emulsification performance of the surfactant system (Thompson et al., 2023).

The microbiological analysis results performed at industrial scale demonstrated that the formulation fully met microbiological compliance criteria during the production process. The maintenance of total aerobic mesophilic microorganisms and total yeast/mold counts at <10 CFU/g level indicates a safety margin far exceeding legal requirements in Turkey (TITCK, 2005). It is known that formulations with high water activity in cosmetic products increase the risk of microbiological contamination; therefore, hygiene control during the production process and equipment cleaning emerge as critical factors (Orth et al., 2006). The microbiological cleanliness achieved in our study is consistent with the expected advantage provided by the use of vacuum-equipped stainless steel equipment and the closed production system in this regard. Although the microbiological results demonstrated robust cleanliness at industrial scale, biofilm formation on stainless steel

surfaces remains a recognized challenge in cosmetic manufacturing, as sessile microbial communities exhibit significantly higher resistance to preservatives compared to planktonic cells (Orth et al., 2006). The use of a vacuum-equipped closed mixing system combined with strict hygiene protocols is considered to have minimized this risk in the present study. Nevertheless, long-term surface swab testing in accordance with ISO 22716 GMP guidelines is recommended for routine production monitoring.

The Lexgard Natural (Glyceryl Caprylate and Glyceryl Undecylenate)-based preservative system met Category A criteria, the highest level of protection under the TS EN ISO 11930:2019 standard, with all five microorganisms falling below the detection limit (<10 CFU/g) on day 7 and remaining at this level until day 28. Glyceryl caprylate is known to act by disrupting cell membrane permeability, with activity enhanced under acidic pH conditions (Hyldgaard et al., 2012; Tang & Du, 2024); the acidic pH range of the present study (5.24–5.36) may potentially contribute to this effect. In the context of the increasing departure from parabens and formaldehyde-releasing preservatives in the cosmetic industry (Chen & Chang, 2024), the Category A efficacy demonstrated by this naturally derived, ECOCERT-compatible preservative system carries important reference value for clean label formulation development.

The accelerated stability analysis revealed that the formulation maintained its physicochemical and microbiological stability under all stress conditions (−24 °C freeze-thaw, −5 °C cold storage, 25 °C room temperature, and 45 °C elevated temperature) throughout the three-month monitoring period. The maintenance of pH in the 5.10–5.34 range and the absence of phase separation demonstrate the resistance of the formulation to oxidative and thermal stress. In accelerated stability testing of cosmetic products, stability at 45 °C for 90 days is correlated with approximately 2 years of shelf life under room conditions (ICH, 2003). In this context, the findings of our study suggest potential shelf stability under the tested conditions, though long-term real-time stability studies are required for definitive shelf life determination. The ability of the xanthan gum-based rheological system to preserve viscosity against temperature changes demonstrates that natural polymer-based thickeners constitute a reliable stabilization alternative at industrial scale (Gubitosa et al., 2019). It should be noted that viscosity was assessed using single-point Brookfield measurements throughout the stability monitoring period, with values remaining within a narrow range (3122–3159 mPa·s), confirming rheological stability. While flow curve analysis would provide more comprehensive characterization of the non-Newtonian behavior of xanthan gum-based systems, single-point viscometry remains the standard approach in cosmetic quality control practice (TITCK, 2017). The presence of medicinal lavender and rosemary essential oils in the formulation assumed antimicrobial and antioxidant support functions beyond aromatic quality contribution; this functional contribution may have laid the groundwork for the positive results in both the challenge test and stability analyses (Abelan et al., 2022; Panahi et al., 2015).

From a regional development perspective, this study represents a tangible R&D output with potential for industrial application. The systematic approach applied, integrating formulation development, process validation, and quality control, provides a repeatable methodological framework for similar natural cosmetic formulation studies.

5. CONCLUSIONS

In this study, the scale-up process of a plant-based shampoo formulation containing a nonionic/amphoteric surfactant system based on decyl glucoside, coco glucoside, and cocamidopropyl betaine, D-panthenol, sodium lactate, vegetable keratin, medicinal lavender, and rosemary essential oils from 100 mL laboratory scale to 40,000 mL industrial production scale was completed; physicochemical properties were monitored at each production stage and quality, safety, and stability analyses were performed at industrial scale in accordance with applicable regulatory guidelines.

Physicochemical characterization results were found to be consistently maintained at all production scales. No phase separation was observed at any scale in the centrifugation test; organoleptic properties (homogeneous yellow gel appearance and characteristic lavender-rosemary odor) remained unchanged. These findings suggest that the three-stage scale-up strategy (laboratory → pilot → industrial) applied was effective in maintaining formulation integrity.

In microbiological analyses performed at industrial scale, total aerobic mesophilic microorganisms and total yeast/mold counts remained at <10 CFU/g level; absence criteria were met for *Staphylococcus aureus*,

Pseudomonas aeruginosa, *Escherichia coli*, and *Candida albicans*. As a result of the challenge test conducted according to TS EN ISO 11930:2019 standard, the Lexgard Natural (Glyceryl Caprylate and Glyceryl Undecylenate)-based preservative system brought all five tested microorganisms below the detection limit on day 7 and maintained this level until day 28, fully meeting TITCK Category A criteria. This result suggests that naturally-derived preservative systems, which are increasingly important as alternatives to parabens and formaldehyde-releasing preservatives, can show sufficient efficacy at industrial scale.

The maintenance of the product's stability throughout the three-month monitoring period in accelerated stability analyses may support future shelf-life assessment under commercial distribution and storage conditions. In terms of practical recommendations, the process parameters and quality control data during the transition to industrial scale in this study may serve as a methodological reference for manufacturers developing similar plant-based cosmetic formulations. It is recommended to complete verification of real-time long-term stability of the formulation, in-use stability tests, and process validation studies within the scope of ISO 22716 Good Manufacturing Practices (GMP). It would also contribute scientifically to include in vitro and in vivo evaluations regarding hair fiber penetration, moisturizing effect, and conditioning performance in future study plans to support the efficacy of the formulation.

From a regional development perspective, this study represents a tangible R&D output with potential for industrial application. The systematic approach applied, integrating formulation development, process validation, and quality control, provides a repeatable methodological framework for similar natural cosmetic formulation studies.

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AUTHOR CONTRIBUTIONS

SDK: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review and editing, Project administration, Funding acquisition. BK: Investigation.

DECLARATIONS

Ethics Approval and Consent to Participate

This study did not involve any human participants or animals. Therefore, ethics committee approval was not required.

Conflict of Interest

The authors declare that there is no conflict of interest.

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