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FORMULATION OF AN EXFOLIATING SHOWER GEL USING BIOACTIVE INGREDIENTS EXTRACTED FROM AGRICULTURAL BY-PRODUCTS (*PERSEA AMERICANA* KERNEL AND *ANANAS COMOSUS* WASTE)

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ABSTRACT

In this work, an exfoliating shower gel was formulated and fortified with bioactive ingredients from *Persea americana* seeds and bromelain extracted from *Ananas comosus* wastes. *Persea americana* seed powder was obtained by grinding dry seeds. The powder was characterized using physicochemical parameters to evaluate its exfoliating potentials. A protease enzyme, bromelain was extracted from *Ananas comosus* wastes, purified and characterized by determining its sensitivity to temperature and pH. A mixing plan was developed using the Centurion software, including three main factors: Sodium Laureth Sulfate, Texapon and Natrosol, to optimize the formulation. The responses studied were pH, viscosity, and foaming power. The analysis of the response surfaces led to the selection of optimal formulation parameters like viscosity (12500 cP), pH (6.27) and foaming power (153 mL). The gel's exfoliating efficiency was evaluated in vitro via a hydrolysis

test of the Bovin Serum Albumine. Due to bromelain content, the gel has favorable proteolytic properties needed to digest and erode dead cells form the skin. Microbiological analyzes confirmed the hygienic compliance of the product. The evolution of pH, viscosity and enzymatic activity with temperature were followed through stability studies. Results show that the gel has good exfoliating power, skin tolerance and satisfactory stability.

KEYWORDS: Bioactive ingredients, bromelain, *Persea americana*, exfoliating shower gel, Natrosol.

1.0 INTRODUCTION

The global cosmetics industry is undergoing a profound transformation, characterized by a growing collective awareness of the environmental and health impact of beauty products as the range of chemical substances employed in their production keeps expanding (Wirtu, 2024). The use of a broad spectrum of chemical substances in cosmetics can open up the leeway to various forms of chemical intoxication, allergic reactions and other adverse effects of protracted chemical exposure (Gutierrez, 2023). Hence for cosmetic purposes, there is an expanding demand for more natural, biodegradable, and sustainably sourced formulations which are compatible with environmental and health requirements. Plant-based ingredients in cosmetic products are increasingly preferred due to their perceived benefits for the skin, as well as their reduced ecological pollutant load and for their contribution to the circular economy (Hirata et al., 2022).

Among agricultural by-products, avocado seeds and pineapple waste represent a group of often overlooked biomass resources, neglected and hardly valorized (Adaramola et al., 2016). However, these biomass waste are laden with bioactive constituents which can be exploited for cosmetic and other health benefits. Avocado kernels are known for their polyphenol, flavonoid, and other antioxidant content, giving them anti-inflammatory, antimicrobial, and photoprotective properties (Setyawan et al., 2021; Jimenez et al., 2020; Bahru et al., 2019; Lara-Flores et al., 2018; Wang et al., 2010). Their texture, once ground into a powder, also makes them an excellent physical exfoliant (Bhise et al., 2022). Exfoliating particles are used to remove dead cells from the body thus rendering the skin clean, healthy and nourished. Cosmetic substances prepared from natural ingredients are usually preferred because they are safer for use, have reduced side effects and usually possess antioxidant, anti-aging and humectant properties which are desirable quality parameters (Mahajan et al., 2020). The choice of this type of product is motivated by the widespread need of exfoliating shower gels

and by the potential of *Persea americana* kernel powder to replace micro plastics or other synthetic exfoliants often incorporated into shower gel products (Bom et al., 2020).

Bromelain is a chemical substance that is made up of important proteolytic enzymes. It is present in some parts of the pineapple plant from where it can be extracted and used in a variety of industrial applications (Chanyakan et al., 2026; Gharge et al., 2024). Bromelain occurs virtually in every part of the pineapple plant with some variation depending on tissue type. The bromelain in pineapple fruits is responsible for the proteolytic activity in the edible portion of the fruit (Rico et al., 2020;). Bromelain is widely studied for its ability to degrade proteins hence projecting its gentle enzymatic exfoliating, anti-inflammatory, and healing properties appropriate for use in cosmetic formulations (Báez et al., 2007). Bromelain is a powerful group of protein-digesting enzymes naturally extracted from the pineapple plant. It is highly valued for its skincare and medicinal ability to gently dissolve dead skin cells, reduce severe inflammation, and accelerate tissue repairs (Varilla et al., 2021). Some researchers and scientists have valorized these waste biomass components separately to meet certain health and technological needs (Abubakar et al., 2017) but until now, the synergistic effects of these two products in one formulation tailed to meet targeted health needs have not been verified to the best of our knowledge. Combining these two natural active ingredients in a single cosmetic formulation, such as an exfoliating shower gel, offers a promising prospect for skincare that is effective, natural, and environmentally friendly. This work is part of an approach to valorizing local natural bio resources through innovative formulations to develop an exfoliating shower gel combining bioactive ingredients from *Persea americana* seed extracts and bromelain extracts from *Ananas comosus* waste to reap the synergistic health benefits for public health enhancement.

2.0 MATERIALS AND METHODS

2.1 Materials

The materials that were utilized in this research work targeting a shower gel formulation included agricultural by-products such as *Persea americana* (avocado) kernels and *Ananas comosus* waste (peels and stems). The biological materials (agricultural by-products) were obtained from farmland and market areas in the Center Region of Cameroon, using recommended sampling methods. Other materials used in this study included materials for the base formulation such as vegetable oil, glycerin, EDTA, distilled water, natrosol,

Texapon, sodium lauryl sulfate, cocoamido propylbetain, methylparaben and materials for mixing, characterization and physico-chemical analysis.

2.2 METHODS

A variety of methods were used to bring our exfoliating shower gel to the lime light. These ranged from sample pre-treatment methods, extraction of bioactive substances from agro-waste products, formulation of base gel, incorporation of bioactive substances into base formulation, analysis of raw materials and final products. The first part of this section has to do with the production and characterization of *Persea americana* seed powder.

2.2.1. Production of *Persea americana* Seed Powder

In this procedure, avocado seeds gathered from farm lands and market places were brought to the lab. The seeds were manually sorted, weighed to get rid of debris. The sorted seeds weighed, then washed, crushed and dried in the sun for ten days. The crushed dry seeds were ground to powder and sieved to separate coarse particles. The fine powder was packed in polythene bags and stored at ambient temperature for subsequent analysis and related uses.

2.2.2. Determination of Particle Size

Exfoliating powder is characterized by particle size and the sensation on the skin. Particles that are too large will be abrasive and irritating while particles that are too fine will not have a perceptible exfoliating effects. After obtaining the dry *Persea americana* kernel powder, 100 g were weighed and sieved using a stack of sieves with different mesh sizes (from largest to smallest). The amount retained by each sieve was then weighed, and the mass percentage of each fraction was determined. The particle size distribution curve was plotted according to the following formula:

$$Particle\ size\ percentage\ (\%P) = \left(\frac{M_i}{M_t}\right) \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 01$$

with %P, the particle size percentage of each fraction; mi, the mass on a given sieve; Mt, the total sieved mass.

2.2.3 Determination of Dispersibility

Dispersibility describes the ability of a powder to diffuse and spread evenly in a liquid without quickly settling. It is a qualitative observation. To determine dispersibility, 2 g of *Persea americana* kernel powder were weighed and then placed in a beaker containing 100 mL of neutral gel. The mixture was then vigorously stirred for 5 minutes to ensure initial

homogeneous dispersion. The immediate appearance of the mixture (homogeneity, presence of agglomerates) was noted. The mixture was left to stand for 2 hours without stirring. An observation was made and then another after 48 hours.

2.2.4 pH and Moisture Content Determinations of *Persea americana* Powder

2 g of *Persea americana* seed powder were weighed and then placed in a beaker containing 20 mL of water. The suspension was then stirred for 5 min at room temperature and left to stand for 10 min. Finally, the pH of the supernatant was measured using a calibrated pH meter. The moisture content represents the amount of residual water present in the powder, expressed as a percentage of its total mass. The moisture content was determined using a modified method of Lestyo et al (2026). Gravimetric methods were similarly used to determine the moisture content of *Persea americana* seed powder (Alang et al., 2026; Magdalene, 2020; Camuffo, 2018). An empty beaker was weighed (M_1). Then, 5 g of powder were added (M_2), and the beaker was placed in an oven at 105°C until a constant mass was reached. After cooling, the beaker containing the dried *Persea americana* seed powder was weighed again (M_3). The moisture content was determined using the following formula:

$$\%\ \text{Humidity} = \frac{\textit{Weight of moisture}}{\textit{Weight of moist sample}} \times 100 = \left(\frac{M_1 - M_3}{M_2 - M_1}\right) \times 100 \quad \text{.....} \quad 02$$

2.2.5 Evaluation of Antioxidant Properties

The antioxidant properties were determined to evaluate the ability of *Persea americana* kernel powder to neutralize free radicals responsible for cellular aging. These antioxidant properties were determined using a modified method of Baliyan et al. (2022). The free radical scavenging activity (RSA) of the *Persea americana* seed powder was then carried out as follows; 2 g of *Persea americana* kernel powder were macerated in 10 mL of 98% ethanol for 24 h. The resulting mixture was filtered, and the extract was collected. Then, a 2%w/v solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) was prepared using 98% ethanol as solvent. 5 mL of the macerated *Persea americana* extract were mixed with 5 mL of the DPPH solution in a test tube and the mixture was incubated for 30 min at room temperature in the dark. A control set-up was prepared by mixing 5 mL of ethanol and 5 mL of DPPH in another test tube without the *Persea americana* extract. The absorbance of each tube was read at 517 nm. The inhibition level was determined using the following formula (Valko et al., 2007):

$$\% \text{ Antioxidant activity} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100 \quad \text{..... 03}$$

With A_0 is the absorbance of the control tube and A_1, the absorbance of the reactive mixture extract.

2.2.6 Extraction of *Ananas comosus* waste powder and bromelain

Ananas comosus peels and stems were collected from the field and manually sorted to separate waste. The sorted peels and stems underwent size reduction and then were washed and dried in an oven at 70^{o}C for 5 days. The dried raw materials were then ground to powder and sieved to get fine powder that corresponds to the formulation specification.

To extract and purify bromelain from the obtained powder, 200 g of *Ananas comosus* waste powder were weighed and placed in a beaker. Then 200 mL of 0.5 M phosphate buffer (pH = 6.8) serving as extraction solvent was added. The resulting mixture was stirred manually for 30 min in an ice bath and allowed for 24 hours for extraction to occur. The mixture was then filtered through filter paper to separate the bromelain-rich solution from the solid residue. The filtrate was transferred into a beaker containing 100 mL of ammonium sulfate and the mixture was stirred for 5 minutes in an ice bath. The new mixture was filtered through filter paper to separate the precipitate from the filtrate. The resulting precipitate was kept refrigerated until use and then yield calculation followed. The bromelain yield refers to the ratio between the weight of the extracted compound and the weight of the pineapple waste powder used. The bromelain yield was calculated using the following formula:

$$\% \, Yield = \left(\frac{M_1}{M_0}\right) \times 100 \quad \text{..... 04}$$

With M_1 , mass of the precipitate in grams, and M_0, mass of pineapple waste powder in grams.

2.2.7 Bromelain Solubility Tests in Different Solvents

The solubility of bromelain refers to the maximum amount of bromelain that can dissolve and saturate a specific amount of solvent to form a homogeneous stable solution at a given temperature and pressure (Letcher, 2007). To determine the relative solubility of bromelain in different solvents, 100 mg of pineapple waste powder were weighed and transferred into 4 test tubes each. 5 mL of a specific solvent (water, 98% ethanol, 30% ethanol, ether) was added to each tube. The tubes were shaken for 2 minutes and then allowed to stand. The

bromelain extraction procedure earlier reported above was repeated for the mixtures in each test tube.

2.2.8 Proteolytic Activity of Bromelain (GDU Determination)

Bromelain is a proteolytic enzyme that is extractible from fresh *Ananas comosus* and *Ananas comosus* biomass. It can hydrolyze the protein chains in gelatin transforming it into a liquid solution. As the gelatin's solid structure is contingent on a tangled web of intact protein molecules, bromelain's action liquefies the gelatin and prevents it from solidifying. Bromelain operates as a biological catalyst (enzyme) that targets and hydrolyzes the peptide bonds linking amino acids in collagen, the prime source of gelatin (Anbesaw, 2021). To determine the proteolytic activity of bromelain, 5 g of gelatin were weighed and placed in 100 mL of phosphate buffer. The mixture was gently heated (without boiling) to dissolve the gelatin. The solution was then cooled to 37°C. A 0.5%w/v solution of bromelain was prepared using a 50 mL of phosphate buffer. In a test tube containing 5 mL of gelatin solution, 5 mL of bromelain solution were added and homogenized. The tube was placed in a water bath and the absorbance was measured at 280 nm using a spectrophotometer. A stopwatch was used to measure the liquefaction time. The appearance of the tube was observed every 5 minutes by tilting it at a 45-degree angle for 20 minutes. The endpoint corresponding to the time at which the gelatin was completely liquefied, was recorded for each sample. A blank was prepared without bromelain to ensure that the gelatin did not liquefy spontaneously. The proteolytic activity of bromelain was expressed in gelatin digesting units per milligram (GDU/mg) as follows (Munir and Ramli, 2023).

The GDU/mg was calculated using the following formula:

$$Gelatin\ digesting\ units\ (GDU) = \frac{A_s - A_b}{W_B\ (mg)} \times C.F \quad \text{.....05}$$

Where; $A_s\ is$ the absorbance of the sample, $A_b\ is$ the absorbance of the blank and C.F is the conversion factor.

2.2.9. Shower Gel Formulation Matrix

A mixing plan was developed so as to determine the optimal formulation conditions for the body shower gel and then select the proportions of ingredients at which the body shower gel would have desirable characteristics that meet specification requirements. The desired characteristics for a shower gel include; a good foaming power, a pH value between 4.5 and 6.5, and a viscosity between 10,000 and 15,000 cP (Ngan et al., 2020). Other ingredients in

the formulation included Texapon or Sodium laureth ethyl-sulfate (SLES) for surfactant and foaming roles, sodium lauryl sulfate (SLS) a surfactant and foaming agent, and Natrosol, which improve the rheological properties and stability of the gel. All these constituted the variable factors for the mixing plan. The output or responses monitored were the pH, foaming power, and viscosity. The composition of the shower gel developed from the mixing plan was arrived at using the experimental matrix below (Table 1). This experimental matrix was found by compiling the various factors in the STATGRAPHICS software, using the fundamental stress law for mixtures. The output characteristics (responses) were then recorded.

Table 1: Experimental Matrix.

Trials (T)	Texapon (%w/v)	SLS (%w/v)	Natrosol (%w/v)	Responses or Output Signals		
				pH	Foaming power	Viscosity
T_1	0.100	0.045	0.015			
T_2	0.100	0.040	0.020			
T_3	0.095	0.050	0.015			
T_4	0.090	0.050	0.020			
T_5	0.098	0.046	0.016			
T_6	0.098	0.043	0.019			
T_7	0.096	0.048	0.016			
T_8	0.093	0.048	0.019			
T_9	0.100	0.043	0.017			
T_{10}	0.096	0.049	0.015			
T_{11}	0.095	0.045	0.020			
T_{12}	0.093	0.050	0.017			
T_{13}	0.096	0.046	0.018			

2.2.10 Formulation of Shower Gel Base

The formulation of body wash products with prime constituents from nature is currently very promising (Oyi et al., 2010). Using the formulation matrix above, 100 g of the base formulation of the shower gel was prepared in two main steps. The first step had to do with the preparation of the aqueous and oily phases and then the second step involved the mixing and homogenization of the two phases. In order to prepare the aqueous phase, 75 g of water heated to about 40^{o}C was measured into a plastic basin and 0.5 g of EDTA were added. The mixture was homogenized until total dissolution of the EDTA. Then, 1.5 g of Natrosol were added to this mixture, stirring vigorously to prevent lump formation. The new mixture was allowed to hydrate for approximately 15-20 minutes. In a separate basin, 10 g of Texapon was mixed with 4 g of sodium lauryl sulfate and homogenized before being gradually added

to the previous mixture. Next, 4 g of cocoamido propylbetaine and 0.5 g of methylparaben were carefully added to the resulting mixture, and the final solution was left at rest for 30 minutes, thus making up the aqueous phase. For the oily phase, 2 g of sunflower oil and 5 g of glycerin were weighed and placed in a plastic bowl. The mixture was stirred for 5 minutes to achieve uniformity for the oily phase. The oil phase was added to the aqueous phase with vigorous stirring until a viscous, thick mixture was obtained. The production of this formulation base may be summarized by the following unit operations in Figure 1 below.

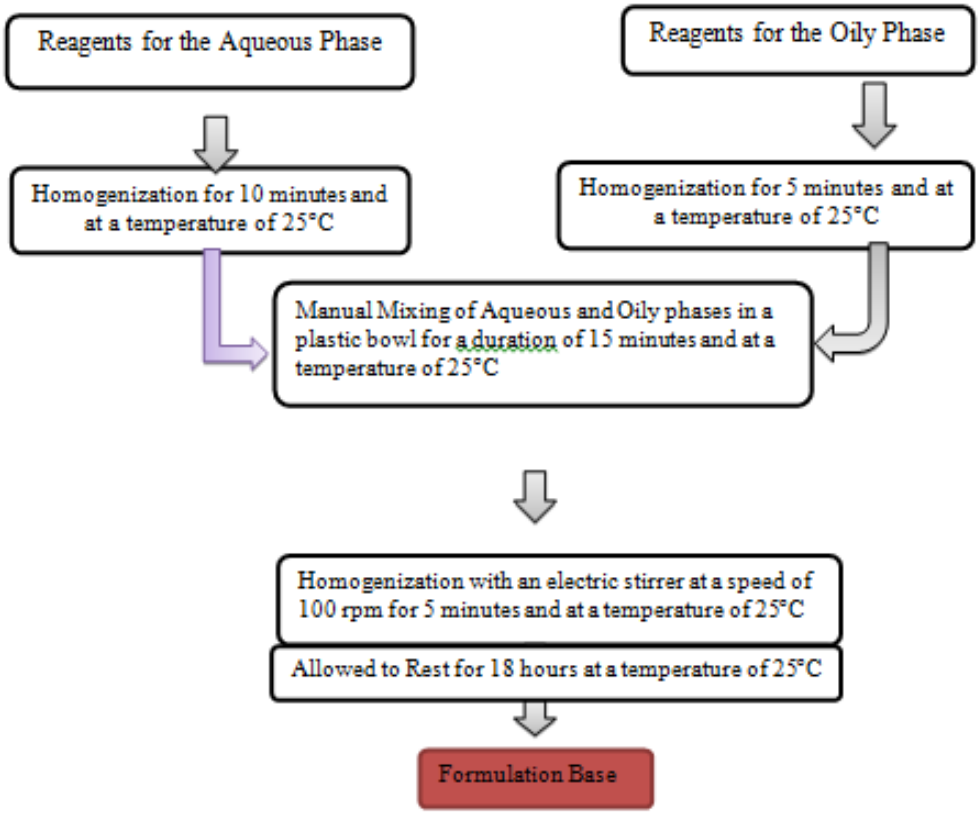


Figure 1: Process Flow Diagram for the Formulation Base of the Shower Gel.

2.2.11. Incorporation of bioactive ingredients into the base formulation

There are no strict orthodox regulatory standards specifying a precise concentration of *Persea americana* seed powder in shower gel. However, standard cosmetic formulation practices generally use a percentage concentration (%w/w), ranging from 0.5% to 3% of the total formulation weight, which provides a gentle exfoliating effect without irritating the skin (Ana et al., 2022). Nevertheless, this percentage depends on several factors, such as particle size, dispersion within the gel, skin type, gel texture, and personal preferences. To achieve this objectives, 2%w/w of *Persea americana* avocado seed powder was measured out and sprinkled into the determined quantity of shower gel and mixed with an electric stirrer at 100

rpm for about five minutes until complete homogenization and desired color was obtained. The exact amount of powder to be incorporated into 100 g of gel base was calculated using the formula below:

$$Quantity\ of\ powder\ (g) = \left(\frac{\%\times Total\ weight\ of\ formulation}{100}\right) \quad \text{.....} \quad 08$$

The incorporation of the avocado seed powder into the formulation was closely followed by the incorporation of bromelain. The appropriate dose of bromelain was diagnosed so as to ensure effective exfoliating properties without irritating the skin. Although there are no strict regulatory standards for its use in cosmetics, formulation practices recommend a typical concentration of 0.1–1% w/w of the formulation's total weight (Iara et al., 2014). Then 0.5%w/w of bromelain was incorporated into the bases containing avocado seed powder, followed by vigorous stirring to homogenize the final formulation.

2.2.12 Evaluation of the physicochemical properties of the formulated gel

The formulated gel underwent physicochemical characterization in order to evaluate its degree of compliance to the laid down specifications. The pH of the formulated shower gel was measured using a properly calibrated Consort pH meter (C3010). The viscosity of the liquid gel was determined using the NDJ-9S viscometer.

The foaming power of a soap is the ability of the soap to form lather while in use (Haruna et al., 2022; Kunatsa and Katerere 2021). The foaming power of the gel base was determined using a modified method of Issa et al. (2020*)*. This method involved mixing 10 g of shower gel with 100 mL of distilled water in a beaker, stirring until completely dissolved. This solution was then poured into a 500 mL graduated cylinder containing 200 mL of distilled water. The cylinder was shaken manually to create foam. After a few minutes of rest, the height of the dense part of the foam is measured. The foaming power was determined using the following formula (Ngan et al., 2020).

$$Foaming\ power\ (\%) = \left(\frac{H_2 - H_1}{H_2}\right) \times 100 \quad \text{.....} \quad 06$$

Where H_1 is the initial foam height, and H_2 is the foam height after agitation.

2.2.13 Stain Removal Ability

The stain removal power of the formulated shower gel was determined using the method of Manallah and Terras (2022). In this method, a soap solution was prepared by dissolving 10 g of gel in 50 mL of lukewarm water. Then a few drops of this solution were added to a fabric stained with a common substance like coffee. The fabric was rubbed for close to 30 seconds to allow the gel to penetrate its fibers. The fabric was then rinsed with clean water for about 10 seconds and left to dry in the air. Finally, the degree of cleaning was visually compared. The more faded the stain, the greater the stain removal power. The following values were assigned to quantify idiosyncratic responses; no stain removal (0), partial stain removal (1) and complete stain removal (2).

2.2.14. Density and Viscosity Evaluation of Shower Gel Base Formulation

The Viscosity was determined using the Brookfield HB-DVIII Ultra viscometer. The appropriate viscometer spindle was immersed in a sufficient quantity of gel sample. Once the instrument was switched on, the viscosity was measured with a No. 4 rotor at 620 rpm at 24.9°C. The density of a liquid soap refers to the ratio of a product's mass to its volume. In this study, the density was measured and expressed in g/mL using ASTM D4052 standard analytical methods.

2.2.15. Microbiological Analysis of the Formulated Gel

Microbiological analysis allowed us to study the effectiveness of antimicrobial preservation, or challenge test. This was accomplished through an artificial contamination test and the control of microbial contamination (microbiological cleanliness) by counting microorganisms (Fan and Song, 2008). The ISO 16212 and ISO 21149 methods were used for the enumeration of fungi (yeasts and molds) and mesophilic anaerobic flora, respectively. Culture media adapted to the microorganisms being sought were used, in particular the plate contour agar (PCA) for the detection of mesophilic anaerobic flora and Sabouraud Dextrose Agar (SDA)+ CYCLOHEXIMIDE for the detection of fungi. The different culture media were prepared as recommended by the manufacturer (Kusumo et al., 2022). For the enumeration, 5 g of the sample were placed in a beaker, and 45 mL of physiological saline was added. The mixture was stirred using a pipette. Decimal dilutions (10^{-1}, 10^{-2}, 10^{-3}) were performed on the sample, starting from the original sample. The mixing was carried out in a sterile environment, near a Bunsen burner flame. The different samples were inoculated in Petri dishes using a 1 mL pipette. These were once again carried out near a flame, starting

with the least concentrated solution and moving towards the most concentrated. The Petri dishes were incubated at 30°C for 48 hours for the enumeration of total mesophilic aerobic flora, and at 35°C for 6 days for the enumeration of fungi.

2.2.16 Stability Study of the Formulated Gel

A gel is said to be stable if there is no macroscopically or microscopically visible changes during its period of use at a given temperature. For the stability study, samples were taken and placed in test tubes. Each sample was subjected to different temperature conditions: 45°C in an oven, 5°C in a refrigerator, and room temperature (25°C). For the optimal product, parameters such as pH and viscosity were recorded on days 0, 7, 14, 21, and 28 at these different temperatures.

2.2.17. Evaluation of the Formulated Gel's Effectiveness (In vitro test with BSA)

Evaluating the efficacy of our gel for skin exfoliation is a complex process requiring rigorous methods to obtain reliable results. The chosen method was the spectrophotometric method at 280 nm on a Bovine Serum Albumin (BSA) substrate. This method consists of measuring the enzymatic activity of bromelain by monitoring the hydrolysis of a specific substrate such as BSA. During this hydrolysis, bromelain cleaves the peptide bonds. The aim here is to evaluate bromelain's ability to degrade proteins, simulating the removal of dead skin cells. To accomplish this task, 1 g of BSA was dissolved in 100 mL of 0.1 M phosphate buffer. In a test tube, 3 mL of the gel to be tested was mixed with 3 mL of BSA solution. The mixture was incubated at 37 °C for 30 min. Next, 5 mL of 10%w/v trichloroacetic acid (TCA) solution was added to the mixture to precipitate the unhydrolyzed proteins. Finally, the absorbance of the supernatant was measured at 280 nm. The tests were repeated every 7 days for 28 days. The ability of bromelain to degrade proteins was evaluated using the following formula (Rathnavelu et al., 2016).

$$\text{Relative activity } (\%) = \left(\frac{Abs_{jx}}{Abs_{j0}}\right) \times 100 \quad \text{.....07}$$

With Abs_{jx} refers to the absorbance on day x (x = 7, 14, 21, 28); Abs_{j0} is the absorbance on day 0.

2.2.18. Sensory or in Vivo Tests

These tests were tailored to gather objective opinions on the organoleptic qualities of the product in order to validate its acceptability. Ten volunteer participants aged 20 to 35 years

were chosen for this category. Those chosen were people who regularly use shower gels and have no record of skin disorders emanating from such uses and had no allergies to the ingredients and were not simultaneously using other exfoliating products during the study. The placebo (similar shower gel base without exfoliating active ingredient) served as the control. The gels were applied for 14 days as follows: placebo (Days 1-7), wash-out (Days 8-10), exfoliating shower gel (Days 11-17).

The volunteers used the shower gel or placebo once a day on their entire body during their usual shower. They were instructed to massage the skin for 2 minutes before rinsing thoroughly. The volunteers then completed a self-assessment questionnaire at the end of the first week and at the end of the study period. An adapted Vickers rating scale was used for each criterion (0, No effect/very weak/not at all satisfied; 5, Moderate effect/moderately satisfied; 10, Very strong effect/very strong/completely satisfied).

2.2.19. Effect of Temperature on the Exfoliating Power of the Gel

Evaluating the effect of temperature on the exfoliating activity of the gel containing bromelain helped to determine the optimal conditions for enzymatic efficacy and product's stability. In order to investigate temperature effects on the exfoliating power of our gel, 2 g of phosphate buffer were introduced into three test tubes each containing 10 mL of the gel. Next, the tubes were shaken and placed in a water bath at different temperatures (30, 45, 60°C) and then at 5°C for 30 minutes. Then 1.0 mL of gelatin solution was added to each tube, and the tubes were incubated at the same temperature as the pretreatment for 30 minutes. The tubes were then placed in an ice bath to quench the reaction. Finally, the absorbance was measured at 280 nm.

2.2.20. Sensory Analysis of the Formulated Gel

The various formulated gels underwent sensory analysis to identify the most acceptable gel for users. A panel of 15 people aged 20-35 was recruited for this purpose. This panel consisted solely of women, as they are generally the largest consumers of cosmetic products. The parameters used for this sensory analysis were texture, fluidity, foaming power, spreadability, and stain-removing power. The subjects evaluated the products sequentially, knowing they could retest the reference products as many times as necessary. The 15 panelists evaluated the seven formulated samples for each parameter.

3.0 RESULTS AND DISCUSSION

3.1 Characteristics of *Persea americana* Kernel Powder

The physicochemical properties of avocado kernel powder are reported in Table 2 below;

Table 2: Characteristics of *Persea americana* Kernel Powder.

Parameter	Value
Particle size (µm)	125 – 250
pH	5.8
Humidity (%)	8
Antioxidant activity (%)	77.5

The study was conducted on particles ranging in size from 125 to 250 µm. The pH of the avocado kernel powder is 5.8. This pH is close to the physiological pH of skin (6.0) and clearly indicates that avocado kernel powder can be used to manufacture a shower gel that compatible with the normal human skin without adverse effects (Otto Braun-Falco, 1987). The moisture content of the avocado kernel powder is 8%. This moisture level, below 10%, indicates that its incorporation into the gel will not promote the growth of microorganisms such as mold. The antioxidant activity of avocado seed powder was 77.5%. This also indicates that the avocado kernel powder has a strong antioxidant potential that can be used to protect the skin against free radicals' adverse effects such as irritations.

The dispersibility results were collected by recording a series of visual observations over a period of 48 hours. After mixing the avocado powder with the neutral gel, a homogeneous mixture was formed without agglomerates and created a stable dispersion suitable for incorporation into cosmetic formulations. After two hours, the suspension was still stable with no sedimentation and good dispersion stability. Some slight settling was observed after 48 hours with a still cloudy supernatant.

3.2. Characteristics of Bromelain

The extraction yield of bromelain was 3.2%. This is a reasonable yield for a method using phosphate buffer as the extraction solvent. Table 3 summarizes the physicochemical parameters of bromelain.

Table 3: Characteristics of Bromelain.

Parameter	Observed Value
Yield	3.2%
Appearance	Beige Amorphous powder
Solubility	Soluble in water

	Partially soluble in dilute ethanol Insoluble in ether
pH	6.4

The proteolytic activity of the bromelain biocatalyst was monitored for 20 minutes and observations after every 5 minutes, and the results are presented in Table 4.

Table 4: Influence of bromelain on the gelatin solution.

Time (minutes)	Observation
0	Homogeneous appearance Translucent and viscous substance (gel does not flow) No phase separation
5	Softening of the gel Less rigid, less homogeneous gel, Observable deformation
10	Loss of consistency Heterogeneous mixture (liquid +gel fragments) Soft gel
15	Liquid gel Smooth and rapid flowClear solution
20	Complete liquefaction

This table shows that the gelatin completely liquefies at 20 minutes. Furthermore, the GDU/mg obtained was 0.00238. This indicates that the extracted bromelain has slightly low proteolytic activity. This could be due to the extraction conditions and impurities that might have inhibited the enzyme. The results of the effect of temperature on bromelain showed that the bromelain extracted from pineapple wastes demonstrated good stability at temperatures ranging from 5 to 25°C and began to lose stability above 45°C.

3.3 Formulation Base Results

The mixing design method was used to obtain the formulation base that served as the matrix for the active ingredients in the gel. The mixing design results are given in Table 5 below.

Table 5: Mixing Design Results.

	FACTORS			RESPONSES		
TRIAL	A (Texapon) %	B (SLS) %	C (Natrosol) %	pH	Viscosity (cP)	Foaming power (mL)
E_1	0.100	0.045	0.015	5.49	11555	196
E_2	0.100	0.040	0.020	6.28	16620	188
E_3	0.095	0.050	0.015	6.32	11800	174
E_4	0.090	0.050	0.020	5.79	16445	128
E_5	0.098	0.046	0.016	6.02	13658	180
E_6	0.098	0.043	0.019	6.07	15560	178
E_7	0.096	0.048	0.016	6.43	13445	170
E_8	0.093	0.048	0.019	6.11	15780	142
E_9	0.100	0.043	0.017	5.65	14640	192

E_{10}	0.096	0.049	0.015	6.07	12500	176
E_{11}	0.095	0.045	0.020	5.82	16600	167
E_{12}	0.093	0050	0.017	6.34	14258	140
E_{13}	0.096	0.046	0.018	6.27	14763	170

The analysis of experimental results allowed us to identify formulations that meet the expected specifications.

Model Validation

The model validation criteria are listed in Table 6.

Table 6: Models Obtained Based on Validation Guidelines.

	R^2	**Adjusted R^2**
pH	0.93	0.86
Viscosity	0.97	0.94
Foaming power	0.97	0.95
Acceptability	≥ 0.9	≥ 0.8 %

Based on the results above, the model is validated because $R^2 \geq 0.9$ for all parameters. This indicates that the fitted model explains more than 80% of the variability in pH, viscosity, and foaming power of the formulated gel.

3.4 Mathematical Modeling Equations

The mathematical models for the formulation are given by the following equations;

$$pH = 2.65A + 5.23B - 6.58C + 8.52AB + 30.03AC + 20.31BC - 30.74ABC$$

…….... 08

$$Viscosity = 10498.36A + 10773.81B + 6837.18C + 6205.74AB + 33060.67AC + 31390.20BC - 25410.72ABC$$

……09

$$Foaming\ power = 216.90A + 127.13B + 354.10C + 55.79AB - 328.67AC - 348.05BC + 72.68ABC$$

……10

Where A, B, and C representing Texapon, SLS, and Natrosol, respectively.

The resulting pH-related mathematical model indicates that Texapon and Sodium lauryl sulfate (SLS) tend to increase pH, while Natrosol has the opposite effect. The binary interaction of each component contributes positively to the pH increase. This is not the case for the ternary interaction of the components, which has a significant negative effect on the pH.

The resulting viscosity-related mathematical model shows that Texapon (7906.53), SLS (7926.33) and Natrosol (6293.48) have positive effects on viscosity. Thus, increasing the concentration of any one of these components tends to increase viscosity. This is also observed with the binary interaction of the factors. The ternary interaction, with a high negative coefficient (-53078.0), indicates that the three components have an overall antagonistic effect.

The resulting mathematical model related to the foaming power shows that each component individually contributes positively to increasing foaming power. Conversely, the binary interaction terms have negative coefficients, indicating that these combinations lead to a reduction in the foaming power.

3.5 Analysis of Variance (ANOVA)

ANOVA, or analysis of variance, is a statistical method used to test general differences between the arithmetic means of two or more groups of data, allowing for the evaluation of multiple factors and their interactions in factorial design experiments. It compares variance between group means against variance within the groups to produce an F-statistic (ratio of two variances). ANOVA simultaneously compares means across several groups to determine if observed differences are due to chance or reflect genuine distinctions (Sawyer, 2009): The ANOVA results are shown in Table 7:

Table 7: ANOVA of the Effect of Factors.

Source	Sum of Squares	DDL	Quadratic mean	F-Ratio	Probability
pH	0.9360	6	0.1560	14.17	0.0026
Viscosity	3.626×10^7	6	6.043×10^6	34.01	0.0002
Foaming power	5023.97	6	837.33	39.62	0.0001

The table above assesses the significance of the special cubic model used to study the effects of combining the components on the output like pH. The results indicate that the model is highly significant, with a probability (P-value) of 0.0011, below the critical threshold of 0.05. This means that there is a statistically significant relationship between the product's pH and the proportions of the different formulation components at the 95% confidence level. There is also a highly significant statistical relationship between viscosity and the interacting components. This means that the proportions of the ingredients of the shower gel formulation have a real and measurable impact on the product's viscosity (P-value 0.0001). The same is true for foaming power (P-value = 0.0001).

3.6 Influence of Factors on Different Responses

3.6.1 The pH

Figure 2 shows the effects of the different factors (SLS, Texapon, Natrosol) on the product's pH.

A ternary diagram here, refers to a triangular graph employed to show the proportions of three variables whose values always add up to a fixed total sum such as 100%. Each vertex of the triangle signifies 100% of one specific component and 0% of the other two. The side opposite a vertex represents 0% of that component. Grid lines running across the space inside the triangle enabling us to read the exact mix of all three parts for any single point.

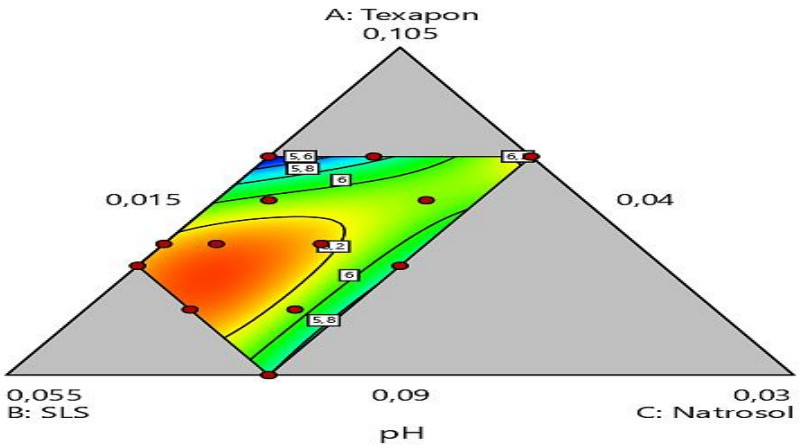


Figure 2: pH Response Surface.

This Figure 2 represents a ternary diagram illustrating the contours of the estimated pH response surface as a function of the proportions of three components. Analysis of this figure shows that the pH increases primarily with an increase in the concentration of natrosol, particularly when combined with a low SLS content. Conversely, combinations rich in SLS or Texapon tend to slightly decrease the pH. Therefore, the optimal region for obtaining a slightly acidic pH for cosmetic applications is located in the center of the diagram, where the proportions of natrosol are moderate and those of SLS are relatively low.

3.6.2 Foaming Power

This influence of studied parameters on the foaming ability of the gel can be explained through Figure 3.

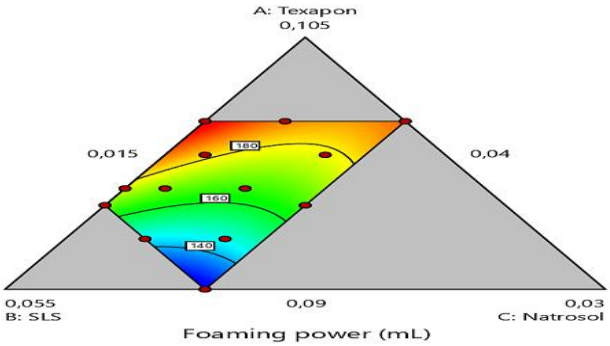


Figure 3: Foaming Power Response Surface.

Analysis of this ternary diagram shows that optimal foaming power is achieved when the formulation is rich in Texapon with a moderate concentration of SLS and a low concentration of natrosol. Too high a concentration of natrosol reduces foam by increasing viscosity, while an excess of SLS can impair foam stability. Thus, the balance between surfactants and thickening agent is essential to optimize the product's foaming properties.

3.6.3 Viscosity

The effect of the different factors on viscosity is shown in Figure 4.

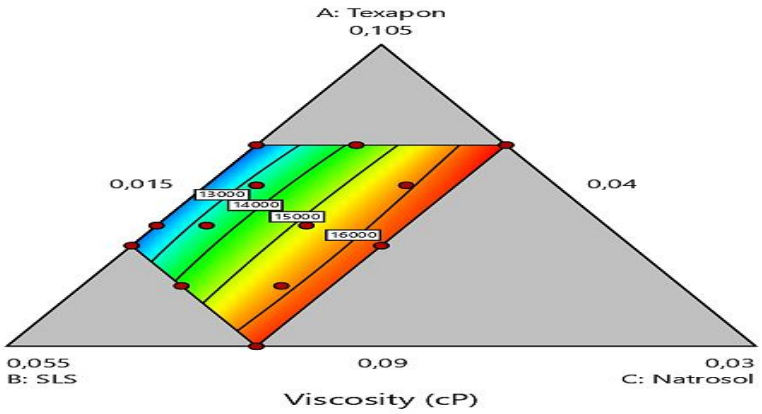


Figure 4: Viscosity Response Surface.

This diagram shows the viscosity contours as a function of the proportions of three components: Texapon, SLS, and Natrosol. A clear increase in viscosity is observed in areas rich in Natrosol, which is expected since this gelling agent is known to thicken formulations. Conversely, areas rich in Texapon or SLS exhibit lower viscosities. Thus, the response surface analysis shows that the determining factor in the viscosity of the shower gel is the Natrosol content. On the other hand, increasing the Texapon or SLS content has a lesser, or even limiting, effect on viscosity. Therefore, the ideal formulation in terms of viscosity relies on optimizing the Natrosol content while maintaining consistent concentrations.

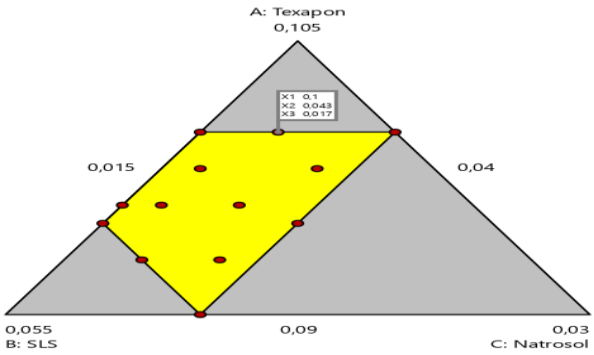


Figure 5: Overlay curve.

3.6.4 Overall Effect

The results of the multi-response optimization allowed us to obtain the formulation base into which the active ingredients were incorporated. The physicochemical parameters (pH, viscosity, and foaming power) of the base gel are reported in the table 8 below. These parameters fall within the ranges specified in the technical specifications.

Table 8: Optimal Level of Factors.

Factor	Low	High	Optimum
Texapon	0.090	0.100	0.100
SLS	0.040	0.050	0.043
Natrosol	0.015	0.020	0.017

3.7. Different Grades of Base Formulation

In formulating the base gel, several formulas were used to produce slightly different grades of shower gels before the bioactive ingredients were incorporated into each. The formulae for the selected grades of base formulations were guided by Table 5. Table 9 presents the basic formulation formulas selected in accordance with the specifications.

Table 9: Basic formulation formulas selected according to the specifications.

	FACTORS			RESPONSES		
TRIAL	A (Texapon)	B (SLS)	C (Natrosol)	(pH)	(Viscosity)	(Foaming power)
	%	%	%		cP	mL
E_1	0.100	0.045	0.015	5.49	11555	196
E_3	0.095	0.050	0.015	6.32	11800	174
E_5	0.098	0.046	0.016	6.02	13658	180
E_7	0.096	0.048	0.016	6.43	13445	170
E_{10}	0.096	0.049	0.015	6.07	12500	176
E_{12}	0.093	0.050	0.017	6.34	14258	140
E_{13}	0.096	0.046	0.018	6.27	14763	170

3.8. Sensory analysis of gels made from the base formulations

Sensory analysis was performed on products made using these formulas to determine consumer preference. Figure 6 summarizes the results of the panel selection.

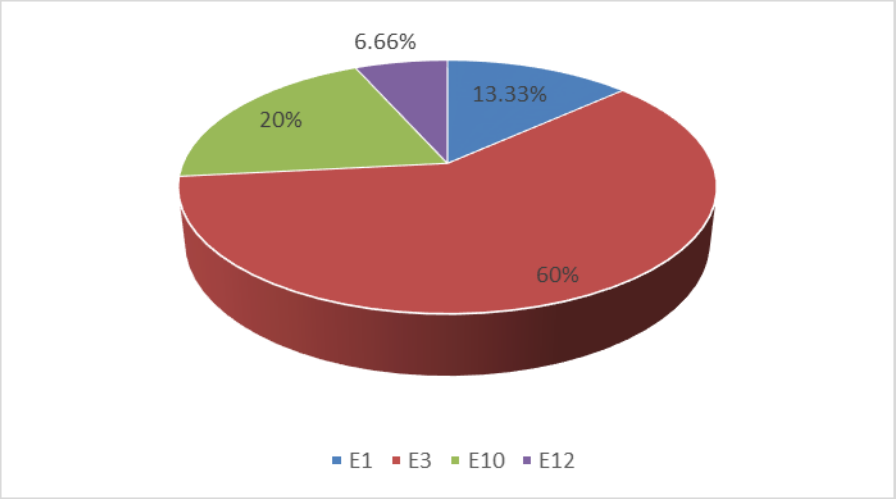


Figure 6: Results of the panel selection.

The results show that the volunteers found the gel obtained from the formulation base E_3 to be the most acceptable. The organoleptic characteristics of this gel closely match those of our specifications.

3.9 Microbiological control of the formulations base

After obtaining the formulation base, we performed a microbiological analysis. The results show that the formulation base contains microorganisms that can cause skin irritation, which does not meet the requirements of the standard indicated in the specifications. These microbes could emanate from raw materials and reagents due to the product packaging or the ineffectiveness of the preservative used (0.5% methylparaben). Table 10 presents the microbiological composition of the formulation base.

Table 10: Microbiological load of gel before and after correction of preservative concentration.

	Base Formulation without preservative		
Culture Medium	10^{-1}	10^{-2}	10^{-3}
SDA + CYCLOHEXIMIDE	+	+	+
PCA	*	*	*
	Base Formulation with preservative		
Culture Medium	10^{-1}	10^{-2}	10^{-3}
SDA + CYCLOHEXIMIDE			
PCA			

+ indicates the actual presence of fungi

* indicates the actual presence of total flora

- absence of colonies (fungi, total flora)

After observing the colonies, the concentration of the preservative (methylparaben) in the base formulation was increased to 0.75% and 1.0%. Then a new analysis was performed using the new proportions. The new results show that no microbial flora was detected. The results are simply expressed as the presence or absence of microbial colonies. From the results obtained, it can be observed that the microbial load dropped to zero for doses of 0.75% and 1% methylparaben. It may be concluded that the preservative proportions initially used were ineffective. Thus, the percentage at which the microbial load is zero is 0.75%.

3.10. Evaluation of gel stability after incorporation of active ingredients

The stability of the gel was studied over a period of one month, as a function of time and under the influence of different temperatures (5°C, 25°C, and 45°C). The effects of temperature on the physical properties of the gel were noted. Table 12 presents the physical appearance of the gel over time under the effect of temperature.

Table 11: Variation of physical properties of the gel with temperature over time.

TEMPERATURE	DAY				
	0	7	14	21	28
5°C	One phase	One phase	One phase	One phase	One phase
25°C	One phase	One phase	One phase	One phase	One phase
45°C	One phase	One phase	One phase	Two phases	Two phases

The results show that between 5°C and 25°C, the physical appearance of the gel remains unchanged (one phase). However, from 45°C onwards, the physical appearance of the gel starting changing after three weeks at higher temperature. The physical appearance remained unchanged from day 1 to day 21, then started to change after the 21st day of the study, notably through phase separation. These results show that the increase in temperature contributes to destabilizing the gel. This could be due to the fact that the surfactants used have lost their stabilizing properties between the different phases of the system. To remedy the situation, hydrocolloids such as xanthan gum, guar gum amongst others which reduce water activity could be used. Indeed, these texturizing agents form a three-dimensional network that limits the mobility of water molecules in the medium. These agents allow for a

more or less effective interaction between water molecules and the reactants (Mansuang et al., 2011).

3.11 Evaluation of pH of gel over time under different temperature conditions

Figure 7 shows the evolution of the pH of the formulated gel as a function of time at different temperatures.

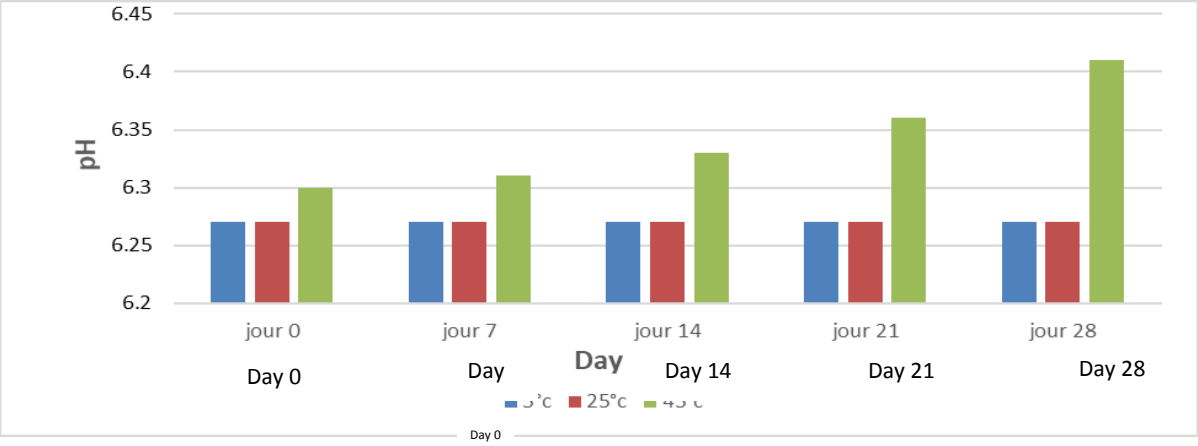


Figure 7: pH Evolution Over Time and at Different Temperatures.

This figure shows that the pH remains stable between 5°C and 25°C, but increases over time at 45°C. In other words, the formulated gel is not pH stable at high temperatures. This could be due to the fact that high heat can trigger chemical reactions between the gel's components, leading to the formation of new products that can alter the pH. The increase in pH indicates that the gel is becoming more alkaline, which can occur due to the release of OH^- ions.

3.12 Effect of Temperature on Gel Viscosity

Viscosity is an important physical property of liquid soaps that can be used as a product quality parameter. Viscosity is important both for stability and use of cosmetic products (Sanela et al., 2024). The viscosity of shampoos and liquid soaps is between 400 and 4000 cP. Figure 8 shows the evolution of gel viscosity over time and different temperatures.

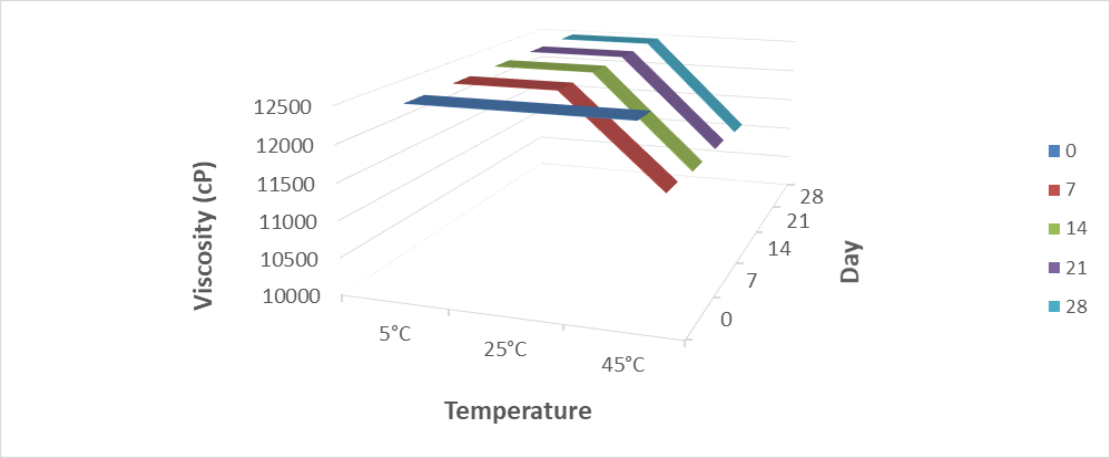


Figure 8: Evolution of gel viscosity over time and at different temperatures.

At 5°C and 25°C: the viscosity remains generally stable over time. The gel withstands these temperatures well. At 45°C, a decrease in viscosity is observed from the first week. This indicates that an increase in temperature contributes to reducing the gel's viscosity. This phenomenon may be due to the loss of the stabilizing properties of the surfactants used at 45°C, or to accelerated chemical interactions (oxidation, hydrolysis), or to a destabilization of the viscoelastic network formed by the gelling agents. To remedy this problem, thickeners more resistant to heat, such as xanthan gum, or more stable acrylate polymers, such as carbopol ETD 2020, could be used to strengthen the gel network. These texturizing agents form a three-dimensional network that limits the mobility of water molecules in the medium, thus allowing a more or less good interaction between water molecules and the reagents likely to interact (Mansuang et al., 2011).

3.13 **Physicochemical Properties of the Formulated Gel**

The table 13 below presents the physicochemical properties of the formulated gel, compared to those of the SISLEY reference gel. It shows that the pH of the gel is slightly higher than that of the reference gel. However, the density and foaming power are almost the same. The formulated gel has a lower density than the reference shampoo, while the foaming power of the reference gel is slightly higher than that of the formulated gel.

Table 12: Comparison of the characteristics of formulated gel with those of the reference gel.

Properties	Shower Gel	Reference Gel
pH	6.27	5.80
Density	0.93 g/cm³	1.02g/cm³
Foaming power	112 mL	118 mL
Viscosity	12500 mPa.s	11800 mPa.s

3.14 Sensory test results

The table 14 below presents the average scores obtained for the exfoliating shower gel and the placebo on a scale of 10.

Table 13: Sensory evaluation results.

Evaluation Criteria	Exfoliating gel /10	Placebo /10
Exfoliation sensation	8.5	2,1
Skin softness after rinsing	7.8	4.5
Ease of rinsing	7.6	8.3
Sensation of irritation	1.2	0.8
Scent and texture	8.6	8.8
Overall satisfaction	8.5	6.0

The values are average scores given by the volunteer panel members. These results show that the formulated gel has an exfoliating action compared to the placebo, which scored lowest in evaluation such as 2.1 on 10. The same is true for the skin's harshness. Furthermore, the scores obtained for the fragrance and texture criteria are virtually identical for the formulated gel and the placebo, indicating that the incorporation of avocado kernel powder did not negatively alter the product's fundamental sensory properties. Finally, the scores obtained for the irritation sensation criteria are low, indicating that the exfoliating shower gel is well tolerated and that the concentration and particle size of the avocado kernel powder are appropriate for daily use without causing significant discomfort. The overall satisfaction rating is further proof that the volunteers found the shower gel acceptable.

CONCLUSION

The main objective of this work was to formulate a natural exfoliating shower gel based on avocado kernel powder and bromelain extract as bioactive ingredients. The prime interest was targeting the use of agro-food by-products to valorize local biomass, sanitize the environment while simultaneously meeting current cosmetic requirements for more skin-friendly products. The various formulation stages allowed for optimization of the gel's composition, incorporating mild foaming agents, a natural gelling agent (natrosol), active ingredients such as vegetable oil, and a suitable preservative. The avocado kernel powder, after characterization (particle size, moisture content, pH, dispensability, antioxidant activity), demonstrated interesting mechanical exfoliating properties. Bromelain was extracted from pineapple waste (peels, leaves and stems), purified and tested for its enzymatic activity. The results confirming its effectiveness as a gentle enzymatic exfoliant. The physicochemical tests performed (pH, viscosity, foaming power, stability) revealed that

the resulting formula complied with cosmetic standards, exhibited good skin tolerance, and had a pleasant sensory profile. Its exfoliating efficacy was also confirmed in vitro through tests on gelatin and protein substrates. This work is therefore part of a sustainable and innovative approach to valorization of local agricultural waste and biomass, safeguard cosmetic efficacy and promoting environmental sanity.

AUTHORS' CONTRIBUTION

The following authors contributed to the realization of this manuscript as follows; **Alang Michael Bong** initiated the research, developed the manuscript and worked on the references. **Adjia Robert** proof read the manuscript, cross-checked the scientific principle and edited the work. **Olga Nana** verified and corrected botanical names of biological raw materials and checked conformity to scientific principles. **Donfack Demanou Paola** carried laboratory analyses ensuring experimental accuracy and precision. **Kom Regonne Raissa** edited the manuscript and ensured overall conformity.

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