Asian Journal of Medicine and Health Sciences Vol 7 Issue 2, November 2024

ORIGINAL ARTICLE

Formulation of Bergamot Essential Oil-Loaded Emulsion for Hyperhidrosis and Bromodosis: Stability and Antibacterial Assay

Siti Hajar Musa*, Nurul Izzah Ismail, Faizan Naeem Razali.

Faculty of Pharmacy and Health Sciences, Royal College of Medicine Perak, Universiti Kuala Lumpur, 30450 Ipoh, Perak, Malaysia

Corresponding Author
Siti Hajar Musa
Pharmaceutical Technology
Royal College of Medicine Perak, Universiti Kuala Lumpur, Ipoh, Malaysia.
Email: sitihajar.musa@unikl.edu.my

Submitted: 25/08/2024. Revised edition: 18/10/2024. Accepted: 20/10/2024. Published online: 01/11/2024.

Abstract

The current marketed product of antiperspirant could temporarily block the production of excessive sweat in hyperhidrosis patients. The sweat leads to the growth of bacteria, especially in the foot area. Therefore, the chemical compound in bergamot essential oil which is linalool, able to provide antibacterial properties to the emulsion. In this study, the formulation of antiperspirant lotion containing bergamot essential oil as an antibacterial agent has been formulated and optimized at different content of oil, surfactant and aluminium chloride. The formulated emulsion has then been evaluated based on its stability and antibacterial activity. The formulation consisting of 25% aluminium chloride, 3% bergamot essential oil, 6% Tween 80, and 0.5% Phenonip (F1) was selected as the most optimum formulation. It demonstrated good stability in the overtime stability analysis, with no phase separation, no precipitation, no colour changes, and only an insignificant decrease in pH at various storage temperatures (4, 25 and 34°C). In contrast, the other formulated emulsions (F2 to F8), were found to be unstable at high-temperature storage (34°C). The stability of the emulsions was further confirmed by the positive results obtained from the accelerated and freeze-thaw stability analyses. Disk diffusion analysis indicated that the formulated emulsion with a high content of bergamot essential oil highly inhibits the growth of Gram-positive bacteria. However, the use of bergamot essential alone failed to inhibit the growth of Gram-negative bacteria due to the presence of lipopolysaccharide in the bacteria cell wall, which blocks the permeation of antibacterial agents. The newly optimized antiperspirant emulsion could overcome the problem of excessive production of sweat as well as inhibit the growth of bacteria that cause foot odour.

Keywords: *antiperspirant, bergamot essential oil, emulsion, formulation, topical.*

Introduction

The skin is the largest human organ, composed of three layers, the epidermis, dermis, and hypodermis (or subcutaneous layer) which serves different functions and has distinct structures [1]. The skin's surface is a waterproof, semi-permeable membrane that acts as a skin barrier from foreign substances and pathogens. Moreover, it protects the internal organ from trauma such as chemical, thermal, and ultraviolet radiation. Skin is a natural sensor detecting the surroundings through a host of nerve endings, stabilizing body temperature, increasing metabolic function, and synthesizing vitamin D [2]. The dermis has multiple layers of connective cells located between the epidermis and the subcutaneous layer. The layer is composed of elastic tissue, collagen, vasculature, nerves, hair follicles, and sweat glands. It aids the thermoregulation of the body by secreting sweat and assists in external sensation due to the presence of the nerves ending [3].

A chronic sweating disorder called hyperhidrosis is a clinical condition where the patient secretes an excessive volume of sweat [4]. It is a dysfunction of the sympathetic and parasympathetic nervous system that seriously affects the patients' social life. Most of them experience shaking hands and unwanted sweat patches. General hyperhidrosis affects the whole body and is caused by infections, endocrine disturbances, neurological disorders, medications, intoxication, or withdrawal of alcohol or other substances. Whereas focal hyperhidrosis is the excessive production of sweat in specific areas, such as the feet, hands, armpits, and face. Primary focal hyperhidrosis can develop in healthy persons since it was suggested to have a genetic predisposition. Between 30% and 65% of patients have a positive family history. Secondary focal hyperhidrosis, on the other hand, is caused by defects in the central or peripheral nervous system [5]. The presence of carbonic anhydrase II (CAII) in the pyramidal-shaped secretory coil clear cells of the glands act as hyperhidrosis glands. It is considered a specific marker for the sweating disorder, as this enzyme is absent in apocrine glands [4]. Topical therapy is considered the first-line treatment for focal hyperhidrosis. It contains aluminium chloride as a therapeutic agent, which block the sweat output [6]. The salt precipitates with mucopolysaccharides, resulting in damage to the luminal epithelial cells. Hence, the precipitation forms a plug that blocks the eccrine duct from secreting sweat onto the skin surface. Long-term blockage may lead to the functional degeneration of the eccrine glands with loss of secretory function [7].

Bromodosis or commonly known as foot odour is a disturbing and embarrassing condition caused by odour-forming bacteria [8]. The smell is produced by the bacteria from the breakdown of carbohydrates, peptides and fatty acids into smaller molecules. The sweat secreted by the eccrine gland does not produce odour, but the moisture environment favours the growth of the bacteria that produce odorous metabolites. In addition, the presence of exo-enzymes (lipase, protease) has a connection to the foot odour problem. Feet with strong odours have significantly more population of bacteria produced than feet with weaker odours [9]. Essential oils are an alternative and affordable antibacterial treatment for bromodosis [8]. Bergamot essential oil is widely used as antibacterial agent in pharmaceutical products [10]. The oil is extracted from the Citrus bergamia Risso's peel that contains highly therapeutic action compounds such as linalyl acetate, linalool and geraniol which effectively inhibit the growth of bacteria [11].

Currently, the main option for primary focal hyperhidrosis is a topical antiperspirant containing aluminium chloride [12]. The mechanism of action of the aluminium salt is to block the eccrine sweat gland and precipitate with mucopolysaccharides. The precipitation raises the pH value from neutral to basic which results in the damaging of epithelial cells along the duct then forming a plug that blocks the sweat output [13].

However, the current treatment only blocks the activation of the eccrine gland without addressing the issue of bacteria growth is caused by excessive sweat produced. The secretion of sweat enhances the growth of microbial flora, especially in closed areas such as the feet and armpits. Therefore, the formulation of an antiperspirant cream combining bergamot essential oil is believed to address both hyperhidrosis and bacterial overgrowth, thereby boosting patients' confidence in their daily social life.

Materials and methods

Formulation of antiperspirant lotion containing antibacterial agent

Emulsions of different compositions (F1 to F8) were prepared by using aluminium chloride (20 to 30%), bergamot essential oil (3 to 9%) and Tween 80 (3 to 9%). Oil and aqueous phases were prepared separately. The aqueous phase contains stock solution of aluminium chloride and water, while the oil phase contains bergamot essential oil and Tween 80. Phenonip was utilized as preservative. The stock solution of aluminium chloride and distilled water was prepared by the ratio of 1:1 (w/w). The aqueous phase and the oil phase were prepared separately in a water bath at 50^{o}Cand stirred on a hot plate. The oil phase was gradually added into the aqueous phase while strirring with an overhead stirrer (HS-100D, Daihan Laboratory) and continuously stirred for 3 hrs at 500 rpm speed. The sample was then further mixed with a high-shear homogenizer (Ultra-Turrax T25, IKA) at 11,000 rpm for 15 mins. The composition for each formulation (F1 to F8) is shown in Table 1**.**

pH stability test

All emulsions were assessed using a digital pH meter (pH 2700, EUTECH). The pH of the emulsion was adjusted to a range of 4.0 to 5.5 was using 0.2M sodium hydroxide. The analysis of pH was carried out weekly for a month at different storage temperatures (4, 25 and 34°C). The measurements for each sample were done in triplicate [14].

Accelerated stability test

The centrifugation process was conducted using a centrifuge (Model 4000, KUBOTA). Each emulsion (F1 to F8) was placed in a test tube and centrifuged at 2,300 rpm speed for 15 mins. The stability of the emulsion was evaluated based on its phase appearance [14].

Overtime stability test

All the formulated emulsion (F1 to F8) were placed and kept at different storage temperatures (4, 25 and 34°C) for 4 weeks. Physical observations and pH measurements were carried out weekly, with each measurement taken in triplicate [14].

Freeze-thaw stability test

The formulated emulsion (F1) was incubated at different temperature cycles; (i) 4 and 25°C (ii) 25 and 35°C and (iii) 4 and 35°C for 24 hrs each. The study was carried out for 6 cycles. Physical observation and pH evaluation were recorded after the cycles finished [14].

Disk diffusion assay

The disk diffusion analysis was carried out using *Staphylococcus epidermidis* (Gram positive bacteria) and *Escherichia coli* (Gram negative bacteria). Each bacteria colony was cultured from a bacteria stock for three days. The colonies were transferred into Mueller Hinton broth solution and incubated for 2 hrs at 37°C. The bacteria broth undergoes a turbidity test using spectrophotometer at a wavelength of 600 nm within 0.08 to 0.13 mg/L. The inoculation of plate was carried out by streaking the agar with the swab containing the inoculum. The surface was allowed to dry for 5 mins. Three disks with a diameter of 6 mm filled with 20 µL of test compound were place on each plate; (i) pure bergamot oil (positive control) (ii) antiperspirant emulsion without bergamot oil (negative control) and (iii) formulated emulsions (samples). The plates were then incubated for 24 hrs at 37°C. The

inhibition zone was observed and measured using a ruler graduated to 0.5 mm [15].

Statistical analysis

All analyses were carried out in triplicate and all data are shown as mean ± standard deviation (n=3).

Results and discussion

Accelerated stability analysis

All formulated emulsions (F1 to F8) were found to be stable due to the absence of physical changes, phase separation and precipitation (Figure 1). Emulsion stability was determined based on the ratio of the total liquid phase volume that separated after a specified time of the centrifugation process to the volume of the sample [16]. The higher centrifugation speed and longer centrifugation time were able to accelerate the separation process. According to Kusumastuti et al. [17], the emulsification efficiency for centrifugation process increased to 97% at 3500 rpm speed for 15 mins.

The stability was achieved due to a proper method had been used during the emulsification process. The mixing process of the aqueous and oil phases was conducted using low and high-speed energy emulsification techniques to form a stable emulsion. Particle size, distribution of particle size, density between the dispersed and continuous phases, and chemical integrity of the dispersed phase are the factors that influence emulsion stability [18]. A fine emulsion can be produced from large particles that are broken down into smaller droplets by the application of mechanical energy [18]. In this study, high shear homogenizer was used to apply a high mechanical impact towards the mixture of both phases during the homogenization process. Therefore, a stable emulsion was proven to be achieved by this combination of emulsification methods.

pH stability analysis

The emulsions withstanding both different conditions with a slight insignificant decrease (p<0.05) of pH. The results proved the ability of the emulsions to retain a stable system by maintaining its pH values (Figure 2). In addition, any topical application should not compromise the acidic pH of the natural human skin (pH 4.0 to 5.5) [19]. The acid surface of the skin is important for controlling the growth of skin microflora, maintaining a good skin condition, and forming an optimal structure of lipid barrier and stratum corneum homeostasis [20]. Hence, the slight change of the emulsion pH within the ideal range for skin is acceptable.

Emulsion physical analysis

Table 2 shows the physical and chemical stability of the emulsions were examined through a long-term stability study for four weeks. The study has been carried out to determine the recommended storage condition and the shelf-life of the emulsions to ensure its safety and effectiveness. Stability testing aims to provide information on the changes of the emulsion's quality over a longer period under the influence of certain environmental factors such as temperature, humidity, and light. The test period is related to the stability of the product which should be long enough to prove no degradation has occurred [21].

Effect of bergamot essential oil content

F1, F2 and F3 were varied in terms of the bergamot essential oil content (3, 6 and 9% respectively) with constant amount of tween 80 (6%) and aluminium chloride (25%). F1 was stable at all storage temperatures with no sign of phase separation. However, at 34℃ storage temperature, layer separation was observed in F2 and F3. In this study, increased bergamot essential oil content directly decreased the amount of water per formulation. The water content and the temperature could affect the stability of the emulsion [22]. A study discovered by Raya et al. [23] proved that the decrease in water content decreases the rate of emulsification. Therefore, this explains the instability of F2 and F3 compared to the stable F1 that has the highest water content compared to F2 and F3. The

stability of the emulsion is depending on the densities between oil phase and aqueous phase, as well as the unfavourable contact between molecules in both phases [8].

According to Goodarzi and Zendehboudi [22], the phase ratio can alter the viscosity of the emulsion and thus, the coalescence rate rapidly occurs due to the increase of flow resistance between the particles in the emulsions. Concerning the storage temperature, high temperature increases the demulsification process by increasing the water particle collisions. The viscosity was observed to be decreased by the increasing temperature (34°C). Hence, the stability of the F2 and F3 were reduced due to the high ratio of oil/water phase and high storage temperature. Moreover, the observation of physical appearance for F1, F2 and F3 shows that there was no growth of mould was observed in this stability analysis. This proved the emulsions were formulated with a sufficient amount of preservative and successfully inhibits the growth of mould.

At high temperature (34°C), F2 to F8 turned into a light-yellow colour from the initial bright yellow colour, except for F1 that remained its initial phase colour. Exposure to high temperatures has a crucial impact on the essential oil integrity [24]. Chang et al. [25] mentioned that linalool, a thermal sensitive compound presents in bergamot essential oil, undergoes decomposition of chemical structure through the dehydroxylation process. Alteration of chemical stability can cause instability of the emulsion causing destabilization processes such as physical changes, colour changes, and oil and aqueous phase separation [26]. Hence, the physical instability of the emulsions is also being affected by the degradation of the chemical structure of bergamot essential oil causes by high temperature storage.

Effect of Tween 80 content

F4, F2 and F5 were formulated with various content of tween 80 (3, 6, and 9%, respectively) with a constant amount of bergamot essential oil (6%) and aluminium chloride (25%). These three emulsions were found to be stable at 4 and 25°C storage temperatures, but not stable at higher temperatures. Theoretically, as the content of the surfactant increased, the stability of the emulsion increased too. Henriquez stated that the high concentration of surfactant in the aqueous phase allows the saturation of surfactant molecules on the interface and then accumulates on the particle in the emulsion [27].

At some point, the surfactant molecules start forming micelles where the hydrophobic side is removed from the aqueous environment. However, the high temperature allows the process of reverse micelles where it increases the disproportionation process of the surfactant molecules which increases the particle screening and decreases the maximum particle mobility [28]. The emulsion instability forms a floating droplet on the surface, cohesion between droplets, and finally leads to creaming and separation [29]. Even though the emulsion was formulated with a high concentration of surfactant, the high temperature can alter the process of forming a micelle which results in instability of the formulations.

Effect of aluminium chloride content

F6, F7, and F8 were formulated with various contents of aluminium chloride (20, 25, 30%, respectively) with constants amount of bergamot essential oil (10%) and tween 80 (6%). Best formulation was possessed by F7 and F8 where the emulsions were stable at both 4 and 25°C. In contrast with F6, the emulsion was not stable even at 25°C. This finding could be explained by the effect of aluminium chloride presented in each emulsion.

According to Clark [30], a solution of aluminium chloride (1 mol/dm^3) has a pH reading of 2 to 3. Emulsion droplets were reported to be more stable under more acidic and saline conditions. Due to the increases of the emulsifier ability to

absorb on the oil/water interface and produce smaller emulsion droplets during the preparation process of the emulsions [31]. In this study, F6 contain the least amount of aluminium chloride than F7 and F8 which directly caused its less acidic system. Therefore, it was proven that higher physical stability can be achieved by formulating an acidic emulsion system.

Freeze-thaw stability analysis

From the stability evaluation studies, F1 was selected to be the optimum composition with the greatest stability among the formulated emulsions. F1 was stored in two-cycle of freeze and thaw analysis under different investigation conditions: (i) 4 and 25°C (ii) 25 and 34°C (iii) 4 and 34°C. The emulsion was examined for a week under extreme temperature changes and were proven to own a good stability system against deterioration due to the absence of physical changes (**Figure 3**). In addition, the stability of emulsion has also been supported by insignificant small changes (p<0.05) of pH within the acceptance range of skin use (4.0 to 5.5).

The freeze-thaw stability test is used to analyse the stability of emulsion under various conditions. The cyclic temperature stress was designed to provide a knowledge of the product to mimic likely with the conditions in marketplace storage [21]. Magnusson et al. mentioned that the freeze-thaw stability is mainly collated with the composition and crystallization behaviour of oils and the freezing conditions of the emulsion [32]. Hence, the emulsion was confirmed to have an acceptable stability system when encounter extreme changes in storage conditions.

Disk diffusion analysis

Table 3 shows the disk diffusion analysis of *Staphylococcus epidermidis* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria) using the disk diffusion method. According to Reller et al. (2009), the main reasons for the testing are to determine the resistance of possible drugs towards common pathogens and to ensure susceptibility to drugs of choice for certain infections [33]. Linalool is a component that contains in bergamot essential oil that seizes anti-bacterial properties [34]. The acidity of the bergamot essential oil can provide antibacterial properties due to the high content of ascorbic acid and citric acid. The pH of the bergamot juice is found to be lower than pH 3.05 but similar to or higher than pH 2.43 [35]. Bacteria have high sensitivity towards low pH since the increase of bergamot essential oil's hydrophobicity will raise the rate of dissolution of the membrane lipid's target bacterium [36].

From the result, the Gram-positive bacteria were more susceptible compared to Gram-negative bacteria. Both positive (bergamot essential oil) and negative control (emulsion without bergamot essential oil) exhibited smaller inhibition zones than obtained by the emulsion sample. The inhibition zone for the (positive control) was smaller than the emulsions (F1 to F8). The zone of inhibition of emulsions (F1 to F8) for Gram-positive bacteria indicates that the combination of the aluminium chloride as the active ingredient in antiperspirants emulsion with the bergamot essential oil was able to boost its ability to inhibit the *Staphylococcus epidermidis* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria). The largest inhibition zone caused by the formulated emulsions has proven the efficacy of incorporating bergamot essential oil into the formulation.

The resistance mechanism creates by the bacteria protects them from antibacterial activity of bergamot essential oil. The low resistance of the bacteria to the bergamot essential oil is due to the lack of an outer membrane found in Gram-negative bacteria, but the thick layer of the peptidoglycan layer that surrounds the outer membrane provides the survival mechanism for the bacteria to live in harsh environment [37]. Peptidoglycan layer of the bacteria is the best target for inhibition activity for antibacterial

agents by altering the peptidoglycan synthesis [38].

Lazarotta et al. [11] stated that the Minimal Inhibitory Concentration (MIC) of the bergamot essential oil for Gram-negative bacteria is 125 μg/mL. Some negative bacteria especially *Escherichia coli* have developed resistance to most available antibacterial agents [39]. The lipopolysaccharide on the bacteria wall is able to block the permeation of hydrophobic properties of bergamot essential oil and avoids the accumulation of the oil in the target cell membranes [39]. The complexation of the cell wall increases the survival rate of the bacteria toward the antibacterial activity of the bergamot essential oil.

Conclusions

The result achieved in the present study shows that F1 is the optimum formulation for antiperspirant lotion containing bergamot essential oil as an antibacterial agent. It possessed good stability at all storage temperatures (4, 25 and 34°C), no phase separation, no precipitation, no colour change, and minimal pH changes. The freeze-thaw stability showed great stability throughout the six cycles of storage in various temperature conditions, demonstrating an ideal property for shipping and transportation of the emulsion. Disk diffusion analysis proved that the addition of bergamot essential oil into the antiperspirant formulation was able to boost the ability to inhibit the growth of *Staphylococcus epidermidis* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria). Unlike the pure bergamot essential oil, it has failed to inhibit the growth of *Escherichia coli* due to the presence of lipopolysaccharide that blocks the permeation of the antibacterial agents. Overall, the results obtained in this study pointed out the successful development of an antiperspirant lotion containing bergamot essential oil, which might be used as an emulsion that blocks the production of sweat and inhibits the growth of bacteria.

Conflicts of interest

The authors have declared that no competing interests exist.

Acknowledgement

The authors are grateful to Universiti Kuala Lumpur, Royal College of Medicine Perak, Malaysia for funding this study.

Authors' Contribution

SHM: Conceived the research, conducted the analysis, and drafted the manuscript.
NII: Study the theory and performed the analysis.
FNR: Verified the analytical methods.
All authors discussed the results and contributed to the final manuscript.

Table 1. Composition of antiperspirant lotion with bergamot essential oil.

Ingredients	Formulation composition (%)							
	F1	F2	F3	F4	F5	F6	F7	F8
Aluminium chloride	25	25	25	25	25	20	25	30
Bergamot essential oil	3	6	9	6	6	10	10	10
Tween 80	6	6	6	3	9	6	6	6
Phenonip	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Table 2. Physical observations of F1 to F8 after 4 weeks of storage.

After 4th weeks of observation	Storage temperature	F1	F2	F3	F4	F5	F6	F7	F8
Phase separation	4°C	✗	✗	✗	✗	✗	✗	✗	✗
	25°C	✗	✗	✗	✗	✗	✓	✗	✗
	34°C	✗	✓	✓	✓	✓	✓	✓	✓
Colour changes	4°C	✗	✗	✗	✗	✗	✗	✗	✗
	25°C	✗	✗	✗	✗	✗	✗	✗	✗
	34°C	✗	✓	✓	✓	✓	✓	✓	✓
Presence mould	4°C	✗	✗	✗	✗	✗	✗	✗	✗
	25°C	✗	✗	✗	✗	✗	✗	✗	✗
	34°C	✗	✗	✗	✗	✗	✗	✗	✗

✓: Phase separation/ colour changes/ Presence mould is visible.

✗: Phase separation/ colour changes/ Presence mould is not visible.

Table 3. Disk diffusion data of emulsions (F1 to F8) on *Staphylococcus epidermidis* and *Escherichia coli.*

Tested bacteria	**Tested emulsion**	**Inhibition zone (mm)**		
		Pure bergamot essential oil	**Control emulsion**	**Formulated emulsion**
Staphylococcus epidermidis	F1	11±0.16	26±0.12	29±0.17
	F2	10±0.12	25±0.17	30±0.16
	F3	10±0.12	26±0.12	32±0.17
	F4	11±0.13	27±0.16	30±0.12
	F5	10±0.16	26±0.16	28±0.17
	F6	9±0.17	25±0.12	29±0.17
	F7	9±0.12	26±0.15	27±0.17
	F8	9±0.17	26±0.10	26±0.17
Escherichia coli	F1	-	21±0.16	23±0.21
	F2	-	31±0.22	32±0.16
	F3	-	21±0.21	23±0.16
	F4	-	21±0.19	21±0.12
	F5	-	20±0.12	21±0.16
	F6	-	27±0.17	28±0.12
	F7	-	20±0.21	21±0.22
	F8	-	26±0.17	28±0.17

- : No inhibition zone

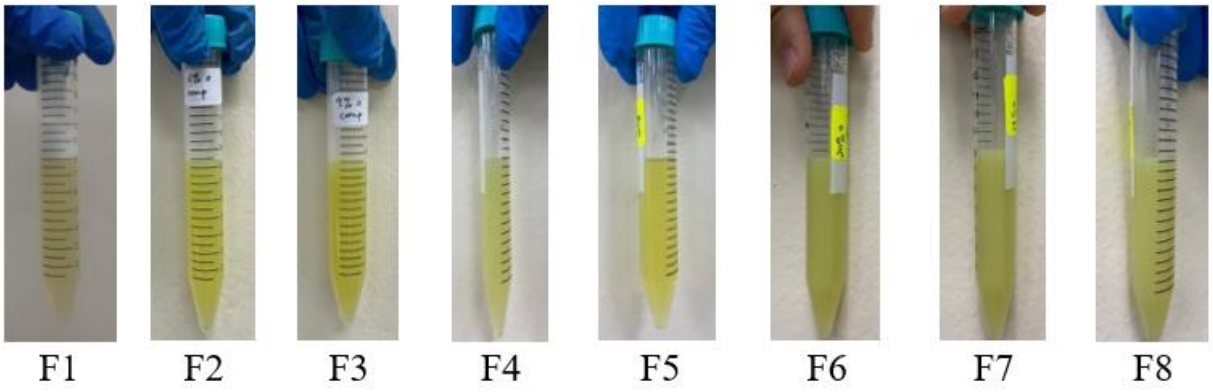

Figure 1. The physical evaluation of emulsions (F1 to F8) consisting of different composition of bergamot essential oil, aluminium chloride and tween 80 after the centrifugation process.

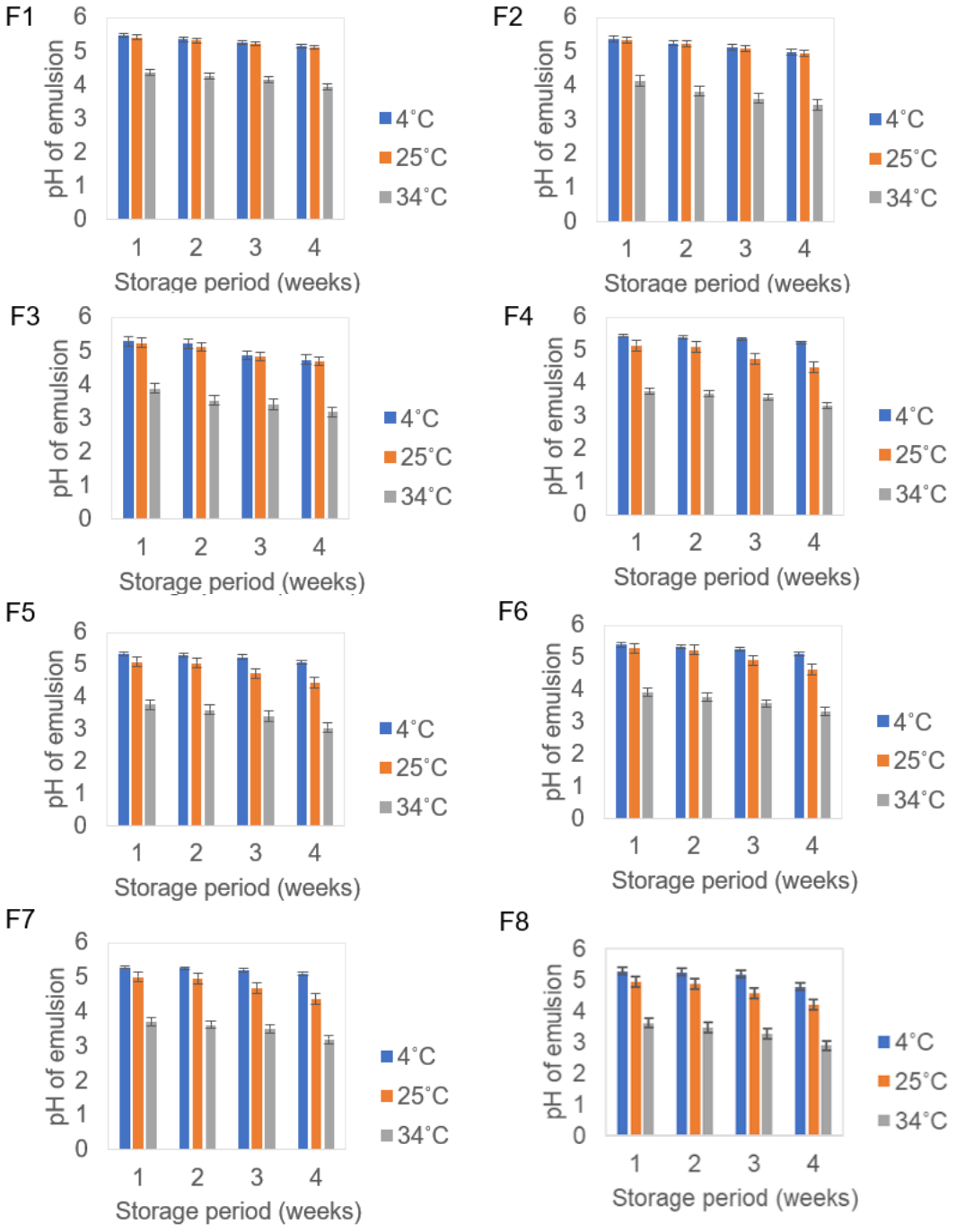

Figure 2. Weekly pH evaluation of emulsions (F1 to F8) for 1mth of storage at different temperatures: 4, 25 and 34°C.

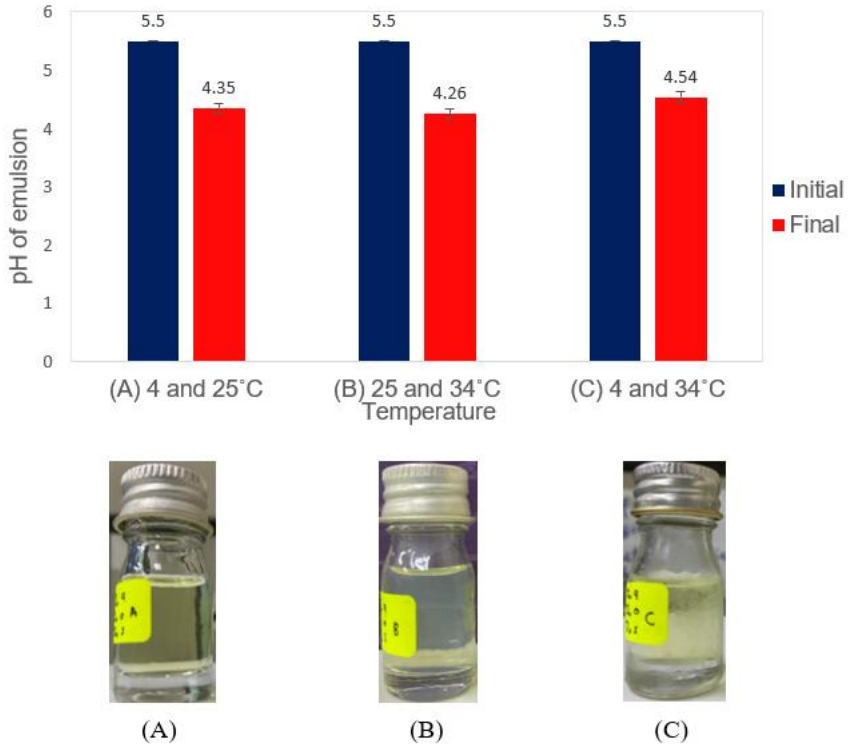

Figure 3. The pH value and physical observations for formulated emulsion after 6 cycles in freeze-thaw analysis.

References

[1]. Yousef, H., Ahaj. J., M. and Sharma, S. Anatomy, Skin (Integument), Epidermis. A service of the National Library of Medicine, *National Institutes of Health*. 2019; 101 (5): 392-399.

[2]. Abdo JM, Sopko NA, Milner SM. The applied anatomy of human skin: A model for regeneration. Wound Medicine. 2020; 28:1-10.

[3]. Mohamed SA, Hargest R. Surgical anatomy of the skin. Surgery (Oxford). 2022; 40(1):1-7.

[4]. Bovell DL, MacDonald A, Meyer BA, Corbett AD, MacLaren WM, Holmes SL, Harker M. The secretory clear cell of the eccrine sweat gland as the probable source of excess sweat production in hyperhidrosis. *Exp Dermatol*. 2011; 20:1011-1037.

[5]. Schlereth T, Dieterich M, Birklein F. Hyperhidrosis—Causes and Treatment of Enhanced Sweating. *Dtsch Arztebl Int.* 2009; 106(3):32-37.

[6]. Walling HW, Swick BL. Treatment options for hyperhidrosis. Therapy in Practice. 2011; 12(5):285-295.

[7]. Benohanian. Antiperspirants and Deodorants. *Clin Dermatol*. 2001; 19:398-405.

[8]. Van Vuuren S, Ramburrun S., Kamatou G, Viljoen, A. Indigenous South African essential oils as potential antimicrobials to treat foot odour (bromodosis). *South African Journal of Botany*, 2019; 126, 354-361.

[9]. Ara K, Hama M, Akiba S, Koike K, Okisaka K, Hagura T, Kamiya T, Tomita F. Foot odor due to microbial metabolism and its control. *Can J. Microbial*. 2006; 52:357-364.

[10]. Mandalari G, Bennett RN, Bisignano G, Trombetta D, Saija A, Faulds CB, Gasson MJ, Narbad A. Antimicrobial activity of flavonoids extracted from bergamot (Citrus bergamia Risso peel, a byproduct of the essential oil industry. *J. Appl. Microbiol*. 2007; 103(6):2056-2064.

[11]. Lazarotto M, Valério A, Boligon A, Tres MV, Scapinello J, Magro JD, Oliveira JV. Chemical composition and antibacterial activity of bergamot peel oil from supercritical CO_2 and compressed propane extraction. *Open Food Sci J*. 2018; 10:16-23.

[12]. Gregoriou S, Sidiropoulou P, Kontochristopoulos G, Rigopoulos, D. Management strategies of palmar hyperhidrosis: challenges and solutions. *Clin Cosmet Investig Dermatol.* 2019; 12:733-744.

[13]. Bretagne A, Cotot F, Arnaud-Roux M, Sztucki M, Cabane B,.Galey J–B. The mechanism of eccrine sweat pore plugging by aluminium salts using microfluidics combined with small angle X-ray scattering. *J Soft Matter.* 2017; 13(20):3812-3821.

[14]. Musa SH, Basri M, Fard Masoumi HR, Shamsudin N, Salim, N. Enhancement of physicochemical properties of nanocolloidal carrier loaded with cyclosporine for topical treatment of psoriasis: in vitro diffusion and in vivo hydrating action. *Int J Nanomedicine*. 2017; 2427-2441.

[15]. Baliga S, Mamtora DK, Gupta V, Shanmugam P, Biswas S, Mukherjee DN, Shenoy S. Assessment of antibacterial activity of levonadifloxacin against contemporary gram-positive clinical isolates collected from various Indian hospitals using disk-diffusion assay. *Indian J. Med. Microbiol*. 2020; 38(3-4), 307-312.

[16]. Küchler S, Schneider C, Lerche D, Sobisch TJLI. Process optimisation for making stable emul-sions using accelerated dispersion analysis by multisample analytical centrifugation. *LabPlus Int*. 2006; *20*(4):14.

[17]. Kusumastuti A, Anis S, Ahmad AL, Ooi BS, Buddin MS. Emulsion liquid membrane for heavy metals removal: emulsion breaking study. *J Teknol.* 2020; *82*(5).

[18]. Maphosa Y, Jideani VA. Factors affecting the stability of emulsions stabilised by biopolymers. *IntechOpen*. 2018; 5:65-81.

[19]. Ali SM, Yosipovitch G. Skin pH: From basic science to basic skin care. *Acta Derm Venereol*. 2013; 93:261-267.

[20]. Lambers H, Piessens S, Bloem H, Pronk, Finkel P. Natural skin surface PH is below 5, which is beneficial for its resident flora. *Int J Cosmet Sci.* 2006; 28:359-370.

[21]. Bajaj S, Singla D, Sakhuja N. Stability testing of pharmaceutical products. *J Appl Pharm Sci.* 2012; 2(03):129-138.

[22]. Goodarzi F, Zendehboudi S. A comprehensive review on emulsions and emulsion stability in chemical and energy industries. *CJCE*. 2019; 97:281-309.

[23]. Raya SA, Saaid IM, Ahmed AA, Umar, A. A critical review of development and demulsification mechanisms of crude oil emulsion in the petroleum industry. *J Pet Explor.* 2020; 10:1711-1728.

[24]. Turek C, Stintzing FC. Stability of essential oils: A review. *CRFSFS*. 2013; 12(1):40-53.

[25]. Chang HT, Lin CY, Hsu LS, Chang ST. Thermal degradation of linalool-chemotype cinnamomum osmophloeum leaf essential oil and its stabilization by microencapsulation with β-cyclodextrin. *Molecules*. 2021; 26(406):1-8.

[26]. Campelo HP, Junquiera LA, Resende JVD, Zacarias RD, Fernandes RVDB, Botrel DA, Borges SV. Stability of lime essential oil emulsion prepared using biopolymers and ultrasound treatment. *Int J Food Prop.* 2017; 1(20):564-576.

[27]. Henriquez CJM. W/O emulsions: formulations, characterization and destabilization. *Tag der mündlichen Prüfung*. 2009; 1:1-121.

[28]. Michor EL, Berg JC. temperature effects on micelle formation and particle charging with span surfactants in apolar media. Langmuir. 2015; 31:9602-9607.

[29]. Ishii F, Nii T. Lipid emulsions and lipid vesicles prepared from various phospholipids as drug carrier. *Colloid Interface Sci in Pharmaceutical Research and Development*. 2014; 22(2):469-501.

[30]. Clark J. Chlorides of Period 3 Elements. LibreTexts, 2021; 1:1-4.

[31]. Yu L, Li S, Stubbs LP, Lau HC. Effects if salinity and pH on the stability of clay-stabilized oil-in-water pickering emulsions. *SPE J.* 2020; 1:1-20.

[32]. Magnusson E, Rosen C, Nilsson L. Freeze-thaw stability of mayonnaise type oil-in-water emulsions. *Food Hydrocoll*. 2011; 25:707-715.

[33]. Reller LB, Weinstein M, Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: A review of general principles and contemporary practices. *Clin Infect Dis*. 2009; 49:1749-1755.

[34]. Fisher K, Phillips CA. The effect of lemon, orange and bergamot essential oils and their components on the survival of Campylobacter Jejuni, *Escherichia coli* O157, Listeria monocytogenes, Bacillus cereus and *Staphylococcus aureus* in vitro and in food systems. *J Appl Microbiol.* 2006; 101:1232-1240.

[35]. Giuffre AM. Bergamot (citrus bergamia, risso): the effects of cultivar and harvest date on functional properties of juice and cloudy juice. *Antioxid*, 2019; 8(221):1-21.

[36]. Cruz ARN, Velasco CEO, Alvarez FJC, Hernandez MAL, Lopez OV, Malo AL, Sosa RA. Effect of pH and mexican oregano (lippia berlandieri schauer) essential oil added to carboxymethyl cellulose and starch edible films on listeria monocytogens and *staphylococcus aureus*. *J Food Qual*. 2018; 1 (2018):6.

[37]. Jubeh B, Breijyeh Z, Karaman R. Resistance of gram-positive bacteria to current antibacterial agents and overcoming approaches. *Molecules*. 2020; 25:1-22.

[38]. Theuretzbacher U. Global antibacterial resistance: the never-ending story. *JGAR*. 2013; 1(13):63-69.

[39]. Gyawali R, Hayek SA, Ibrahim, SA. Plant extracts as antimicrobials in food products: mechanisms of action, extraction methods, and applications. *Handbook of Natural Antimicrobials for Food Safety and Quality*, 2015; 3:49-68.