

Full Length Research Paper

Cosmetic emulsion from virgin olive oil: Formulation and bio-physical evaluation

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The present study aimed to formulate and subsequently evaluate a topical skin-care cream (o/w emulsion) from virgin olive oil versus its vehicle (base). A formulation containing 3% virgin olive oil was developed by entrapping it in the oily phase of oil-in-water (o/w) emulsion. The base form did not contain natural oil. Lemon oil was incorporated to improve the odor. Both the base and formulation were stored at 8°C (in refrigerator) and at 25, 40 and 55°C (in incubator) for a period of four weeks to investigate their stability. The evaluation parameters consisted of color, smell, phase separation, centrifugation, liquefaction, and pH. The microbiological stability of the formulations was also evaluated. The findings indicate that the formulations with olive oil were efficient against the proliferation of various spoilage microorganisms, including aerobic plate counts as well as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and yeast and mould counts. Organoleptic stability of the creams was achieved during four weeks. The results presented in this study showed good stability throughout the experimental period. The newly formulated cream of virgin olive oil proved to exhibit a number of promising properties and attributes that might open new opportunities for the construction of more efficient, safe, and cost-effective skin-care, cosmetic, and pharmaceutical products.

Key words: Virgin olive oil, o/w emulsion, stability, pH.

INTRODUCTION

An emulsion is a complex mixture of two immiscible phases, with one phase dispersed in another. The macroscopic separation of the phases is prevented by the addition of a suitable surfactant (Imhof and Pine, 1997). In an emulsion, the therapeutic properties and spreading ability of the constituents are increased (Herbert, 1988). In fact, the development of liquid-liquid emulsion is a common practice in the food and pharmaceutical Industries. A system that consists of oil droplets dispersed

in an aqueous phase is called oil-in-water or O/W emulsion; a system that consists of water droplets dispersed in an oil phase is called water-in-oil or W/O emulsion (Timothy et al., 2008). While O/W emulsions are commonly used as water-washable drug bases and for general cosmetic purposes, W/O emulsions are widely used as emollients and for dry skin treatment (Magdy, 2004). Additional value can be conferred to these formulations by including active ingredients with specific cosmetic effects. Particularly advantageous cosmetic emulsion preparations are obtained when antioxidants are used as active ingredients (Bleckmann et al., 2006).

Due to their beneficial and therapeutic properties, natural plant extracts and their derived products have often been incorporated in the form of emulsions in recent pharmaceutical and cosmetics formulations and

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preparations (Khan et al., 2010). The latter are often reported to consist of a number of ingredients most of which come from natural origins, such as olive oil, almond, argan, fruit extracts, and essential oils. Of particular interest, olive oil is increasingly gaining momentum in both cosmetic and pharmaceutical preparations. In fact, the great therapeutic and health-promoting virtues of olive oil are well-known since ancient times. Olive oil is highly compatible with the acidity of skin, rich in precious substances, such as squalene, phytosterol, and tocopherol, as well as in other valuable vitamins (A and E) and fatty acids, such as oleic, linoleic and linolenic acids, which all have soothing, protective effects on the skin (Ruiz et al., 1999). Moreover, the antioxidants present in olive oil are able to scavenge free radicals and afford adequate protection against peroxidation (Ruiz et al., 1999; Owen et al., 2000).

The attractive biological properties of olive oil are also attributed to the presence of minor components, such as squalene and phytosterols, antioxidant compounds, such as tocopherols and particularly phenols (Owen et al., 2000). The most important classes of phenolic compounds in olive fruit include phenolic acids, phenolic alcohols, flavonoids, and secoiridoids (Soler-Rivas et al., 2000). The phenolic alcohols of olives are hydroxytyrosol and tyrosol (Romero et al., 2002). The tocopherols present in virgin olive oil are important for their nutritional value and antioxidant properties, in that they protect the fat components from autoxidation. They constitute the lipophilic antioxidant group and are noted for their effective inhibition of lipid oxidation in all vegetable oils. In fact, α -tocopherol, the most important antioxidant, accounts for about 95% of the total tocopherols in virgin olive oil (Paz Aguilera et al., 2005).

Furthermore, aldehydes and alcohols of six straight-chain carbons (C_6) and their corresponding esters are considered, from both qualitative or quantitative perspectives, to constitute the most important compounds in the aroma of virgin olive oil (Morales et al., 1999).

The oxidative stability, sensory quality, and health properties of virgin olive oil stem from a prominent and well-balanced chemical composition (Bendini et al., 2007). The latter is beneficial not only to human skin but also to the preservation of the shelf life of oils within emulsions and cream bases. In this context, cosmetics are occasionally contaminated with spoilage microorganisms and pathogens that may cause spoilage to the product and present serious health risks for the user (Cowen and Steiger, 1976; Becks and Lorenzoni, 1995; Behravan et al., 2005). Under inappropriate conditions, even the presence of non-pathogenic spoilage microbes in a cosmetic product may lead to troublesome diseases. A microbe contaminating a cosmetic product may, for instance, be invasive if one applies the cosmetic to cover a blemish or break in the skin (Food and Drug Administration, 2001). Recent advances in microbiology seem to indicate that the use of a variety of

chemical preservatives can prevent contamination by pathogens or spoilage microorganisms and help extend the shelf life of the product. Accordingly, the present study was undertaken to investigate the potential gain effects of using virgin olive oil with regards to the continuous search for enhanced formulation of cosmetic and pharmaceutical emulsions and, if any, to submit it to a battery of well-established tests for consistency and potential industrial application.

MATERIALS AND METHODS

Three kilograms of olive fruit samples (*Olea europaea* L.) were employed in the present study. The fruits were harvested from three olive trees, one kilogram each, at normal harvest period and at the same maturity stage of ripeness. After harvesting, the olive fruit samples were immediately transported to the laboratory where oil was extracted within 24 h using a small oleodoseur extraction unit. The olive oil samples obtained were then submitted to a set of experimental procedures and used to carry out the analytical formulations under investigation. Simulsol[®]4000 (Polyoxyl 40 hydrogenated castor oil) was a kindly obtained from seppic (France). paraffin oil (Merck KGaA, Darmstadt, Germany) was also employed in the current work.

Preparation of emulsions

In this study O/W emulsions were prepared by the addition of an aqueous phase to the oily phase through continuous agitation (Henriette, 1995).

Preparation of base

The oil phase consisted of paraffin oil and Simulsol[®]4000 heated up to 75°C. At the same time, an aqueous phase, that is, distilled water was heated to the same temperature. After heating, the aqueous phase was added to the oil phase drop by drop using a mechanical stirrer with constant stirring at 2000 rpm for 15 min until complete aqueous phase. Two to three drops of lemon oil were added during the stirring period when the temperature reached 60°C to give fragrance to the emulsion. After the addition of distilled water was complete, the speed of the stirrer was decreased to 1000 rpm for homogenization; agitation was maintained until the emulsion was cooled to room temperature.

Preparation of formulation

The oil phase comprised paraffin oil, Simulsol[®]4000, and virgin olive oil (3%) heated up to 75°C. The aqueous phase was added to the oil phase drop by drop with constant stirring using a mechanical stirrer set at 2000 rpm for 15 min until complete aqueous phase was added. During the stirring period and when the temperature reached 60°C, 2 to 3 drops of lemon oil were added to give fragrance to the formulation. After the complete addition of the aqueous phase, the speed of the stirrer was reduced to 1000 rpm for 5 min to achieve homogenization, and then further reduced to 500 rpm for 5 min to reach complete homogenization. Agitation was maintained until the emulsion was cooled to room temperature.

Properties of emulsions

Physical analysis

The obtained emulsion was submitted to a set of organoleptic

(color, thickness, look, feel) and physical (creaming and phase separation) analyses.

Types of emulsions

The type of emulsion was analyzed by diluting the emulsion with oil and water separately.

Stability tests

Stability tests were performed at different conditions for emulsions to investigate the effect of these conditions on the storage of emulsions. These tests were performed on samples kept at $8 \pm 0.1^\circ\text{C}$ (in refrigerator), $25 \pm 0.10^\circ\text{C}$ (in incubator), $40 \pm 0.1^\circ\text{C}$ (in incubator), and $40 \pm 0.1^\circ\text{C}$ (in incubator) with 75% relative humidity (RH). The physical, that is, color, and the organoleptic, that is, liquefaction and phase separation, characteristics of emulsions were observed at various intervals for 28 days.

Centrifugation tests

Centrifugal tests were performed for emulsions immediately after preparation. These tests were repeated for emulsions after 24 h, 7, 14, 21, and 28 days of preparation. They were performed at 5000 rpm and 25°C for 10 min by placing 10 g of the sample in centrifugal tubes.

pH determination

The pH value of freshly prepared emulsions and emulsions kept at different conditions were determined using a digital pH-Meter. The pH tests were repeated for multiple emulsions after 24 h, 3, 7, 14, 21, and 28 days of preparation.

Microbiological analysis

In order to assess the degree of contamination, 1 g of material was dispersed in a 4-ml sterile ringer solution containing 0.25% tween 80. Appropriate dilutions were made in the same dispersing vehicle, and 0.1 ml was plated out on the appropriate solid medium using the surface viable method. Emergent colonies were counted after the necessary incubation. All operations were carried out in duplicates (ISO NF- 21148, 2000).

Aerobic plate count

Aerobic plate counts (APC) were determined by inoculating 0.1 ml of the homogenate sample onto triplicate sterile plates of pre-poured and dried standard methods agar using the surface spread technique. The plates were then incubated for 48 h at 35°C (ISO NF- 21149, 2006). The standard methods agar is a standardized medium for the enumeration of microorganisms from materials of sanitary importance. Duplicates of each dilution (1 ml) of neutralized and non-neutralized samples were pour-plated using standard methods agar (Oxoid, Basingstoke, Hampshire, England) and incubated at $30 \pm 1^\circ\text{C}$ for 48 ± 3 h. Plates containing 25 to 250 colonies were selected and counted, and the average number of CFU/ml was calculated.

Pseudomonas aeruginosa count

P. aeruginosa were enumerated on *Pseudomonas* agar base (CM

559, Oxoid) supplemented with cetrimide, fucidin, and cephaloridine (CFC), providing a selective isolation medium for *P. aeruginosa*. Colonies were counted after 2 days of incubation at 25°C (ISO NF- 22717, 2006).

Staphylococcus aureus

Surviving population of *S. aureus* was determined by standard plating methods (ISO NF- 22718, 2008). At each sampling time, colonies of *Staphylococcus* were selected, Gram-stained, and observed for catalase and oxidase reactions to confirm the presence of *S. aureus*. Microbiological data were transformed into logarithms of the number of colony-forming units (CFU/g).

Yeast and mould counts

The method involved enumeration of colonies on sabouraud dextrose chloramphenicol agar medium. Enumeration was carried out as a pour plate, surface spread, or membrane filtration method (ISO NF- 16212, 2008). Microbiological tests were repeated for fresh and formulations at 25°C after 3, 5, 7, 10, 15, 20, 25, and 30 days of preparation.

RESULTS AND DISCUSSION

Stability of formulated emulsions

“Oil in water” (O/W) or “water in oil” (W/O) emulsions, which represent the majority of cosmetic and pharmaceutical creams, evolve with time. They are thermodynamically unstable, usually splitting into two distinct phases. This instability could be manifested at different time rates and through a variety of physicochemical destabilizing processes, for example, creaming (or sedimentation), flocculation, coalescence, or phase inversion (Masmoudi et al., 2005). In this study, formulations were placed in different storage conditions that is, at 8°C in refrigerator, at 25, 40, and 55°C in stability chambers. The samples were observed for change in color, liquefaction, and phase separation, as presented in Table 1.

Several mechanisms may deteriorate emulsions, including the swell of internal drops due to osmotic pressure, leading to passage of water from external phase to internal phase, the rupture of the oil layer or coalescence of the oil globules, or the coalescence of the internal water droplets (Florence and Whitehill, 1982). The findings revealed that the freshly prepared emulsion was white in color. In fact, no change in color was observed for emulsions at the different storage conditions, that is, 8, 25, 40, and 55°C , up to 28 days of observation. Little changes in color were observed for the emulsions using olive oil at 40 and 55°C (Table 1). The change in color appeared from the 21st day and persisted up to the 28th day of the period of analyses. The change in color at the end of the observation period was presumably due to the oily phase separation which was promoted at higher temperature.

Table 1. Physical characteristics of base (B) and formulation (F) kept at 8, 25, 40, and 55°C.

Parameter	°C	Fresh		12 h		24 h		36 h		2 day		3 day		7 day		14 day		21 day		28 day		
		B	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F	
Liquefaction	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
	55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Color	8	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW	W	Y	Y
	25	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW	W	Y	Y
	40	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW	W	Y	W	Y	Y
	55	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW	W	Y	W	Y	Y
Phase separation	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Centrifugation	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+
	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+
	40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
	55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+

- =No change; + =slight change; W= white; YW= yellowish white; Y= yellow.

The viscosity of emulsion is often reported to play a vital role in its flow properties (Nasirideen et al., 1998). As the emulsion is prepared, the time and temperature processes start to affect its separation, leading to a decrease in viscosity which, in turn, results in increased liquefaction (Hebert et al., 1988). In fact, liquefaction is a sign of instability, and might attribute to the passage of water from the internal phase to external phase as described in several reports in the literature (Kawashima et al., 1992). As far as the findings of the present study are concerned, no liquefaction was observed for the emulsions at any of the storage conditions under investigation, that is, at 8, 25, 40, and 55°C throughout the 28 days of observation. The absence of liquefaction provided strong evidence for the stability of the emulsions under investigation.

Creaming leads to phase separation and is often attributed to density differences between the two phases under the influence of gravity (Derick, 2000). The separated phase can cream or sediment either. Creaming is the upward movement of dispersed droplets while sedimentation is the downward movement of particles relative to the continuous phase (Hebert et al., 1988). Emulsions are thermodynamically unstable, and droplets (water) unite with each other to produce big droplets which, in turn, increase the rate of coalescence. The latter may lead to the destruction of emulsions, which happen when the adhesion energy between two droplets is greater than the turbulent energy, thus causing

dispersion (Abdurahman and Rosli, 2006). The findings of the present work revealed that all the formulation samples were stable at all storage conditions, that is, 8, 25, 40, and 55°C throughout the 28 days of the observation period.

Centrifugation test

The centrifugation test is based on the principle of using centrifugal force to separate two or more substances of varied densities, such as two different liquids or a liquid and a solid, and is a useful tool for assessing and predicting the shelf life of emulsions (Khan et al., 2010). In the case of base, no phase separation was observed after centrifugation in any of the samples kept at different storage conditions up to 14 days. A slight phase separation was, however, recorded on centrifugation from the 21st day and up to the 28th day of observation in the samples kept at 40 and 55°C. No other phase was promoted at higher temperature. separations were observed till the end of the experimental period.

In the case of Formulation, no phase separation was observed after centrifugation in any of the samples kept at different storage conditions up to 14 days of observation. A phase separation on centrifugation was at different storage conditions up to 14 days of observation. A phase separation on centrifugation was recorded in the samples kept at different storage conditions after the 21st

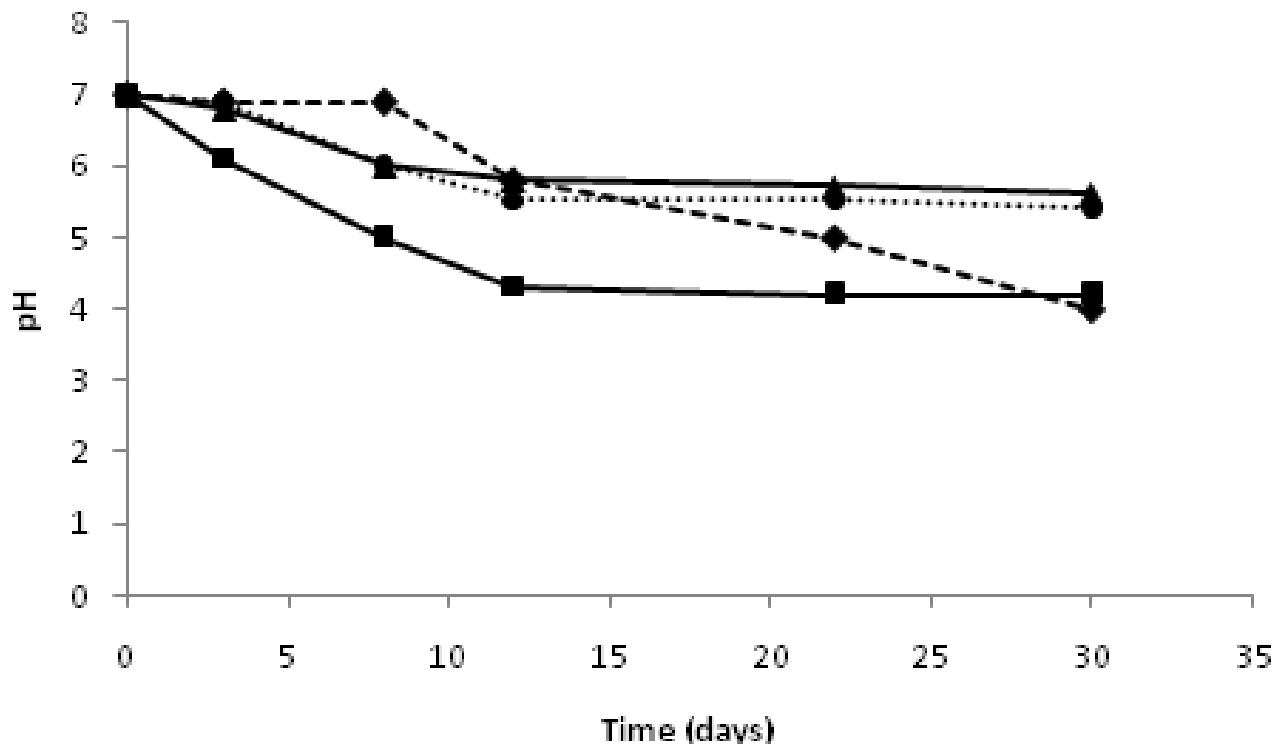


Figure 1. pH values of samples kept at 8°C (◆), 25°C(▲), 40°C(●), and 55°C(■).

day of observation, and no further increases in phase separation were observed in the samples kept at 8, 25, 40 and 55°C till the end of the experimental period. This indicated that the emulsions are stable at all storage conditions for 28 days. This was presumably due to the proper homogenization speed during emulsion formulation which might have prevented the breakage of the formulations during testing (Abdurahman and Rosli, 2006).

pH test

The most important parts of chemical stability are performances on accelerated testing and kinetics of pH profiles (Issa et al., 2000). As far as the effectiveness of the cream is concerned, the pH is often regarded as a significant parameter. The pH of human skin normally range from 4.5 to 6.0, and 5.5 is considered as an average pH of human skin. Therefore, in order for a formulation to possibly gain admission for industrial application, it should have a pH that is close to this range (Matousek et al., 2003). The multiple emulsions prepared in this work had a pH value of 7.15, which is close to the neutral pH. Moreover, the pH of the various emulsion samples kept at different storage conditions, that is, 8, 25, 40, and 55°C were noted to undergo a continuous decrease up to the 28th day of observation (Figure 1). The pH of the sample kept at 8°C was, for instance,

noted to continuously decrease from the 1st day and up to the last day (28 day), on which the pH was recorded to attain 5.2. The pH of the sample kept at 25°C was also noted to decrease continuously, reaching 4.0 on the 28th day of observation. Likewise, the pH values of the samples kept at 40 and 55°C showed continuous decreases, reaching 4.2 and 4.0 on the 28th day of observation, respectively. This decrease in pH was presumably due to the presence of fatty acids, such as palmitic acid, margaric acid, stearic acid, arachidic acid (saturated fatty acid), palmitoleic acid, oleic acid, linoleic acid, linolenic acid, and eicosenoic acid (unsaturated fatty acid) in virgin olive oil (Dhifi et al., 2005; Issaoui et al., 2009).

Microbiological evaluation

Aerobic plate count

The increase in storage time resulted in significant proliferations in aerobic plate counts regardless of the type of treatment being applied (Figure 2). The log mean count recorded for the aerobic plate count of the control base sample on day 0 was 3.68 log₁₀ CFU/ g. On day 30 of storage, the log mean count of aerobic plate count reached 6.27, which did not approximate the maximum limit of 6,9 log₁₀ CFU/ g for aerobic plate count recommended by ISO NF- 21149 (2006) in processed

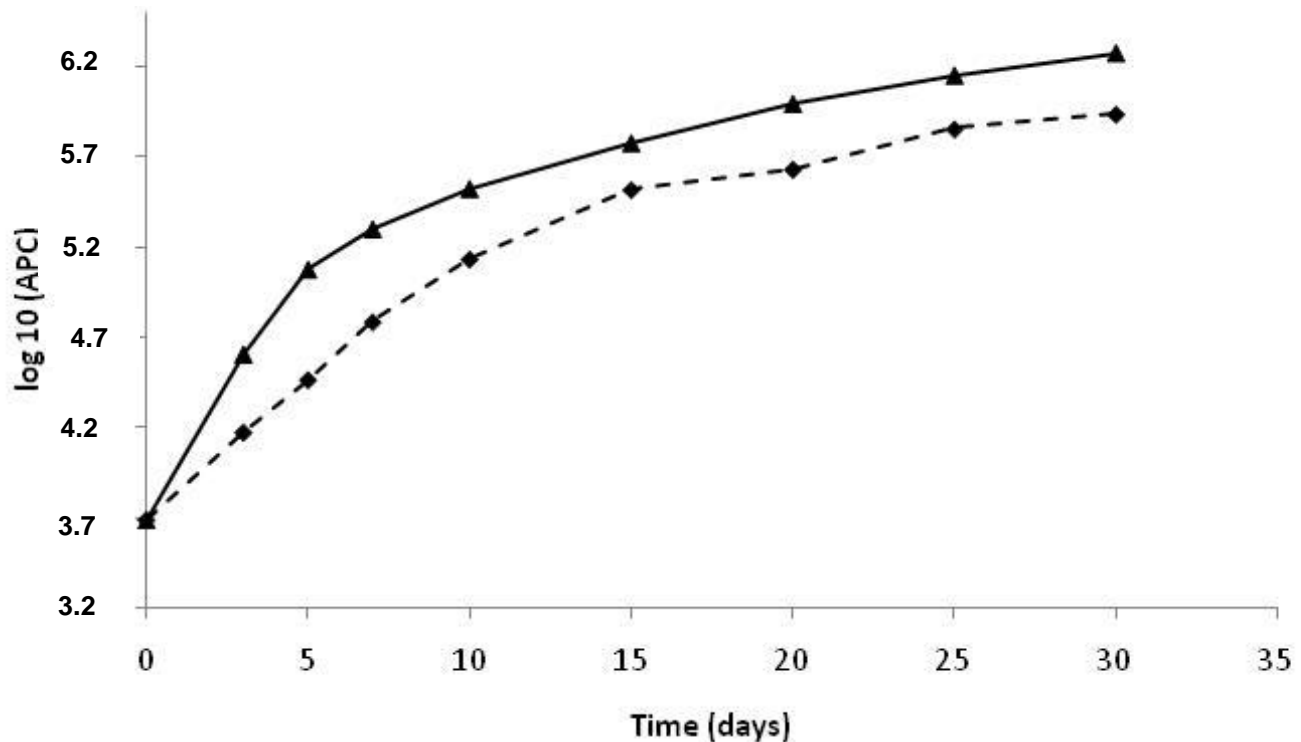


Figure 2. Aerobic plate count (APC) of base (▲) and formulated sample (◆) during storage period at 25°C.

cosmetics.

The aerobic plate count values recorded for the formulated samples using olive oil were, on the other hand, noted to show delayed growth when compared to the controls (base). On day 30 of storage, for example, the formulated samples were observed to show a delayed growth of aerobic plate count of about 0.33 log₁₀ CFU/ g (Table 1).

***Pseudomonas aeruginosa* and *staphylococcus aureus* counts**

The results from the *Pseudomonas* and *S. aureus* detection tests were negative, thus confirming that all the treated samples met the conventional standards specified with regards to fitness for human consumption (ISO NF- 22717, 2006; ISO NF- 22718, 2008).

Yeast and mould counts

Yeast and moulds have often been tested in cosmetic products to assess microbiological safety, sanitation conditions, and product quality during processing and storage (ISO NF- 16212, 2008). Despite the presence of yeast and mould in the samples, the levels of these microorganisms were noted to remain under the standard limit. In fact, the initial yeast and mould counts recorded for all treatments were below the detection limit (6, 21 log

10 CFU/ g) (ISO NF- 16212, 2008).

Moreover, the yeast and mould counts values recorded for the formulated samples using olive oil were noted to show delayed growth when compared to the base. By the end of the storage period, the formulated samples were observed to show a delayed growth of yeast and mould counts of about 0.22 log₁₀ CFU/ g (Figure 3). More interestingly, further microbiological tests of elaborated cream showed that the sample was stable during storage at 25°C.

Conclusion

The findings presented in the current study indicate that the application of an O/W emulsion with 3% of virgin olive oil yield good physical characteristics and microbiological stability, thus providing a safe and stable emulsion delivery system. The formulation and subsequent evaluation of the cosmetic emulsion from virgin olive oil presented here showed no phase separation in primary emulsion at different storage conditions for a period of 28 days. No phase separation in emulsion was also observed at different storage conditions throughout the 28 days of the experiment. Liquefaction of emulsion started in the samples at increased temperatures after the 21st day of storage. The phase separation on centrifugation was noted, in both primary and multiple emulsions, to start at higher temperature conditions after

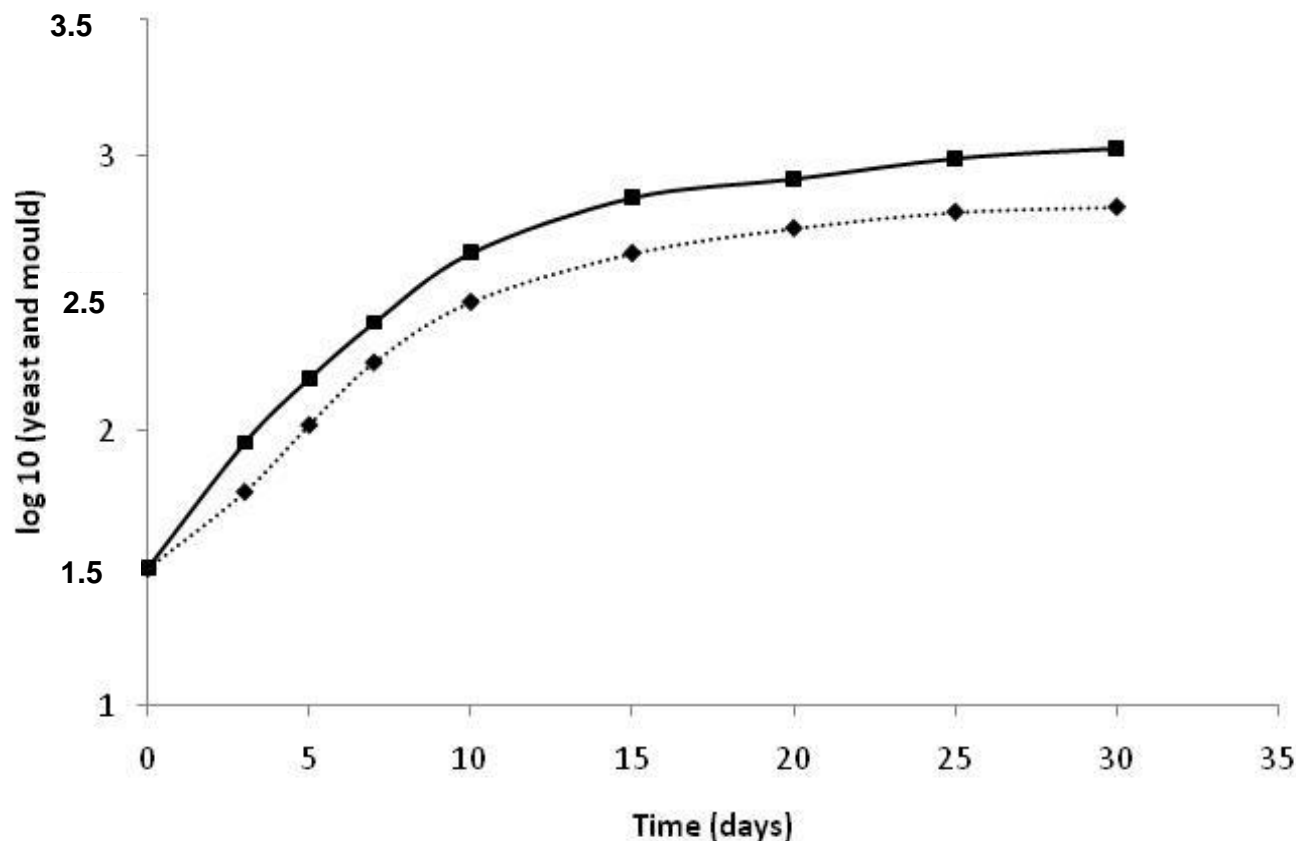


Figure 3. Yeast and mould count of base (▲) and formulated sample (◆) during storage period at 25°C.

the 21st day of storage. The pH values of emulsion at all the storage conditions were noted to decrease with the passage of time. The stability of the emulsions formulated in this study can, in fact, be improved by using better emulsifying agents. Microbiological assays (aerobic plate count, *P. aeruginosa*, *S. aureus*, and yeast and mould counts) on elaborated cream revealed that the sample was stable during storage at 25°C.

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REFERENCES

- Abdurahman HN, Rosli MY (2006). Stability investigation of Water-in-Crude oil emulsion. *J. Appl. Sci.* 6: 2895-2900.
- Becks V, Lorenzoni N (1995). *Pseudomonas aeruginosa* outbreak in a neonatal intensive care unit: a possible link to contaminated hand lotion. *Am. J. Infect. Control.* 23: 396-398.
- Behravan J, Bazzaz F, Malaekheh P (2005). Survey of bacteriological contamination of cosmetic creams in Iran. *Int. J. Dermatol.* 44: 482-485.
- Bendini A, Cerretani L, Carrasco-Pancorbo A, Gómez-Caravaca AM, Segura-Carretero A, Fernández-Gutiérrez A, Lercker G (2007). Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. *Molecules*, 12:1679-1719.
- Bleckmann A, Kropke R, Schneider GU (2006). Preparations of the W/O emulsion type with increased water content, and comprising cationic polymers, United States Patent, 7138128.
- Cowen RA, Steiger B (1976). Antimicrobial activity: a critical review of test methods of preservative efficacy. *J. Soc. Cosmet. Chem.* 27: 467.
- Derick R (2003). Fat crystals and emulsion stability, a review. *Food Res. Int.* 33: 3-14.
- Dhifi W, Angerosa F, Serraiocco A, Oumar I, Hamrouni I, Marzouk B (2005). Virgin olive oil aroma: characterization of some Tunisian cultivars. *Food Chem.* 93: 697-701.
- FDA (Food and Drug Administration) (2001). Bacteriological analytical manual. (8th ed), Washington, D.C.
- Florence AT, Whitehill D (1982). The formulation and stability of multiple emulsions. *J. Pharm. Pharmacol.* 34: 687.
- Henriette K (1995). Paraffinum Liquidum, B.P. Liquid paraffin. The British Pharmaceutical Codex. pp. 1911-1912.
- Herbert AL, Martin MR, Gilbert SB (1988). Pharmaceutical emulsions, pharmaceutical dosage forms: disperse system. Vol.1, 199-240, 285-288., Marcel Dekkar, New York and Basel.
- Imhof A, Pine DJ (1997). Stability of nonaqueous emulsions, *Journal Colloid Interf. Sci.* 192: 368-374.
- ISO NF- 16212 (2008). Cosmetics – Microbiology – Enumeration of

- yeast and mold.
- ISO NF- 21148 (2000). Cosmetics- Microbiology. Instructions for microbiological tests.
- ISO NF- 21149 (2006). Cosmetics – Microbiology – Enumeration and detection of aerobic mesophilic bacteria.
- ISO NF- 22717 (2006). Cosmetics- Microbiology. Detection of *Pseudomonas aeruginosa*.
- ISO NF- 22718 (2008). Cosmetics- Microbiology. Detection of *Staphylococcus aureus*.
- Issa TS, Philippe B, Raymond H, Michel H, Jacques D (2000). Improved kinetic parameter estimation in pH-profile data. *Int. J. Pharm.* 198: 39-49.
- Issaoui M, Ben Hassine K, Flamini G, Brahmi F, Chehab H, Ouni Y, Beligh M, Mokhtar Z, Hammami M (2009). Discrimination of some Tunisian olive oil varieties according to their oxidative stability, volatiles compounds and chemometric. *J. Food Lipids*, 16: 164-186.
- Kawashima Y, Hino T, Takeuchi H, Niwa T (1992). Stabilization of water/oil/water multiple emulsion with hypertonic inner aqueous phase. *Chem. Parma. Bull.* 40: 1240-1246.
- Khan BA, Akhtar N, Mahmood T, Qayum M, Zaman SU (2010). Formulation and pharmaceutical evaluation of a W/O emulsion of *Hippophae ramnoides* fruit extract. *J. Pharm. Res.* 3: 1342-1344.
- Magdy IM (2004). Optimization of Chlophensin emulgel formulation. *AAPS J.* 6:1-7.
- Masmoudi H, LE DY, Piccerelle P, Kister J (2005). The evaluation of cosmetic and pharmaceutical emulsions, aging process using classical techniques and a new method: FTIR. *Int. J. Pharmaceut.* 289: 117-131.
- Matousek JL, Campbell KL, Kakoma I, Solter PF, Schaeffer DJ (2003). Evaluation of the effect of pH on in vitro growth of *Malassezia pachydermatis*. *Can. J. Vet. Res.* 67: 56-59.
- Morales MT, Angerosa F, Aparicio R (1999). Effect of the extraction conditions of virgin olive oil on the lipoxygenase cascade: Chemical and sensory implications. *Grasas y Aceites.* 50:114-121.
- Nasirideen S, Kas HS, Oner F, Alpar R, Hincal AA (1998). Naproxen incorporated lipid emulsion. Formulation and stability studies. *J. Clin. Pharm. Ther.* 23: 57-65.
- Owen RW, Mier W, Giacosa A, Hull WE, Spiegelhalter B, Bartsch B (2000). Identification of lignans as major components in the phenolic fraction of olive oil. *Clin. Chem.* 46:976-988.
- Paz Aguilera M, Beltran G, Ortega GD, Fernandez A, Jimenez A, Uceda M (2005). Characterisation of virgin olive oil of Italian olive cultivars: 'Frantoio' and 'Leccino', grown in Andalusia. *Food Chem.* 89: 387-391.
- Romero C, Brenes M, Garcia P, Garrido A (2002). Hydroxytyrosol 4- β -glucoside, an important phenolic compound in olive fruits and derived products. *J. Agr. Food Chem.* 50:3835-3839.
- Ruiz MA, Navarro J, Gallardo V (1999). Dermatological applications of olive oil. *J. App. Cosmetol.* 17: 19-22.
- Soler-Rivas C, Espin JC, Wichers HJ (2000). Oleuropein and related compounds. *J. Sci. Food Agr.* 80:1013-1023.
- Timothy NH, Robert JP, George VF, Graeme JJ (2008). The role of particles in stabilizing foams and emulsions. *Adv. Colloid Interface.* 137: 57–81.