International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Development and Evaluation of Novel Cream of Microparticles Loaded Fish Oil and Natural Antioxidant

Mulchand Shende[1], Deepak Pawar[2]

[1,2]Pharmaceutics Department, Government College of Pharmacy, Kathora Naka, Amravati, Maharashtra, India, 444604.

Abstract

Herbal cosmetics are formulations derived from plant-based ingredients designed to enhance and maintain the skin's natural appearance. The objectives of this study are formulation and evaluation of herbal moisturizing cream containing microparticles loaded fish oil and natural ginger antioxidants. The encapsulation efficiency (EE) of fish oil ranged from 78.60 to 84.50%, whereas that of ginger extract ranged from 52.6 to 62.21%. The results showed that the encapsulates formulated with gum acacia and fish oil (E3) exhibited a lower TBA value (1.58 mg malonaldehyde kg^{-1}) than the E1 (9.85 mg malonaldehyde kg^{-1}) on the seventh day, indicating better oxidative stability. The formulated cream demonstrated good physical and chemical stability, with no changes in appearance, consistency, spreadability, homogeneity, pH, or viscosity over the study period. The cream exhibited a viscosity range of 47,899–79,970 cps, indicating good spreadability and easy application. Overall, the research concluded that the herbal moisturizer containing microencapsulated fish oil and natural ginger antioxidant was stable, effective, and safe for skin application. These findings suggest that the formulation has potential as a natural, antioxidant-rich topical product for skin hydration and protection.

Keywords: Microencapsulation, fish oil, natural antioxidant, moisturizing cream.

1. Introduction

Globally, Ayurveda, a traditional Indian system of medicine, is gaining increasing global recognition and popularity. In recent years, there has been a notable increase in animal-based research aimed at validating the pharmacological actions of various substances mentioned in Ayurvedic texts. The scientific community has shown growing interest in examining these claims and establishing evidence-based foundations for the ayurvedic principles. The use of dietary supplements, including omega-3 fatty acids, vitamins, minerals, amino acids, and natural substances like beta-carotene, quercetin, phytonutrients, and probiotics, commonly known as nutraceuticals, has notably increased [1]. Natural remedies are often perceived as safer and associated with fewer adverse effects compared to synthetic alternatives. Consequently, the value of herbal ingredients in *cosmeceuticals* has grown considerably within the personal care industry, leading to an increased demand for herbal cosmetics. Herbal formulations that are non-toxic, safe, effective, and enhance patient compliance are generally preferred over synthetic counterparts. Among nutraceuticals, omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are found in high concentrations in fatty fish, are

considered the most functionally significant. These polyunsaturated fatty acids are primarily derived from dietary sources, particularly from marine fish. Strong evidence from randomized controlled trials (RCTs) suggests that omega-3 fatty acids significantly reduce cardiovascular disease mortality [2]. However, despite their well-documented benefits, there are theoretical concerns that omega-3 fatty acids may increase lipid peroxidation by elevating the unsaturation index when incorporated into lipoproteins and cell membranes. Conversely, omega-3 fatty acids may exert antioxidant effects by enhancing the levels of anti-inflammatory molecules and suppressing lipid peroxidation [3-4].

Ginger (*Zingiber officinale*), a perennial herb with green leaves and yellowish-green flowers marked with purple, is widely utilized as both a culinary spice and a medicinal agent. Its rhizomes contain potent antioxidant compounds, such as phenolic constituents (6-gingerol and 6-shogaol), alanine, and vitamin C [5]. Owing to its strong antioxidant properties, ginger can neutralize or reduce free radicals generated by various biological processes, including those induced by trypanosomes [6]. The therapeutic efficacy of ginger is attributed to its carminative, absorbent, and aromatic properties [7]. Antioxidant compounds form the first line of defense against free radicals, which play a key role in the development of diseases such as cancer, asthma, metabolic disorders, and neurodegenerative diseases. Given the health benefits of omega-3 fatty acids and their susceptibility to degradation under environmental and processing conditions, microencapsulation has gained attention as a method for enhancing their stability [8]. Furthermore, there is growing interest in sourcing natural antioxidants from agro-industrial by-products, which represent a valuable and sustainable resource for developing health-promoting food products.

Microencapsulation is currently defined as a collection of technologies designed to protect sensitive compounds from environmental factors and control their release over time. This technique is an effective strategy for safeguarding unsaturated fatty acids from oxidation and other degradation reactions caused by light, temperature, oxygen, and humidity. The incorporation of natural antioxidants during encapsulation can further enhance the stability and shelf life of these compounds, mask unpleasant odors and flavors, and facilitate effective fish oil delivery.

Fish oil is widely recognized as a beneficial supplement for improving various skin conditions, including photoaging, skin cancer, allergies, dermatitis, wound healing, and melanogenesis. Increasing attention has been directed toward its role in skin protection and homeostasis, primarily because of the presence of omega-3 polyunsaturated fatty acids (PUFAs), namely docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Other PUFAs, such as α-linolenic acid (ALA) and linoleic acid (LA), have positive effects on skin health. The main mechanisms through which PUFAs mitigate cutaneous inflammation involve competition with arachidonic acid, thereby inhibiting the production of pro-inflammatory eicosanoids. Additionally, PUFAs in fish oil modulate cytokine synthesis and activity, promoting wound healing and tissue regeneration [8].

Omega-3 fatty acids are also known for their intense hydration properties. They improve the skin barrier function, reduce transepidermal water loss, and help maintain optimal hydration levels. Furthermore, the anti-inflammatory properties of fish oil may alleviate redness and irritation associated with inflammatory skin disorders, such as eczema and sunburn. The water content of the stratum corneum and lipid composition on the skin's surface must remain balanced to preserve both the appearance and functionality of the skin. As the outermost protective barrier of the body, the skin is constantly exposed to environmental stressors. This delicate balance can be disrupted by intrinsic and extrinsic factors. Frequent exposure to harsh cleansers, detergents, alcohol-based products, and hot water can strip essential lipids from the skin surface. The resulting disruption of the skin barrier often leads to increased

water loss, manifesting as dryness, roughness, scaling, cracking, redness, and tightness, sometimes accompanied by itching and stinging. The application of moisturizers helps restore and maintain skin health and integrity, imparting a youthful and hydrated appearance [9].

Compared to other semisolid formulations, moisturizing creams provide prolonged contact with the application site, enhancing their efficacy. These creams impart a smooth and elegant texture to the skin without excessive grease. The oil phase contributes emollient properties, restoring softness and suppleness to dry skin while facilitating the removal of waste materials from pores and providing a cooling effect. Such formulations are generally non-irritating, easily washable, and liquefy at body temperature, allowing efficient penetration through the epidermis. The aqueous phase of the cream further supports skin hydration and protection, helping to maintain its natural barrier function and overall health. Over the past six decades, extensive research has explored the role of fish oil in the prevention and treatment of various diseases. A compromised skin barrier often results in dryness, roughness, irritation, and discomfort because it loses its ability to retain moisture and block external irritants. Barrier-restoring creams and moisturizers help rehydrate the skin, improving its smoothness, elasticity, and overall skin appearance. Regular use of moisturizers enhances the water content of the stratum corneum, normalizes skin pH, supports lipid bilayer restoration, and strengthens corneocyte cohesion, thereby maintaining effective moisture retention. Additionally, moisturizers may provide anti-inflammatory, antipruritic, antimicrobial, photoprotective, and wound healing benefits. In this context, growing attention has been directed toward the microencapsulation of fish oil fatty acids with natural antioxidants, such as aqueous ginger extract, to enhance their stability and effectiveness. This study focused on developing and evaluating microencapsulated fish oil and natural antioxidant compounds for moisturizing cream formulations.

2. Materials and Methods

2.1 Materials

Fish oil (Seacod, Universal Medicare, Mumbai, India) and fresh ginger bulbs were purchased from a local market in Amravati, India. Gelatin powder (Type A, yellowish) was obtained from Hi-Media Laboratories, Mumbai, India. Acacia gum powder, stearic acid, cetyl alcohol, peppermint oil, propyl paraben, beeswax, glycerin, triethanolamine, and methyl paraben were obtained from Merck. All other chemicals and reagents used were of analytical grade.

2.2 Microencapsulation of fish oil and aqueous ginger extracts

Microencapsulation was performed following standard methods a three-step procedure emulsification, gelation and microencapsulation for preparing oil-containing microcapsules [10]. The composition of the emulsion used in this study is presented in Table 1. First, 9.5 g of 5% (w/w) gelatin solution and 18.5 g of 2% (w/w) acacia gum solution were prepared at 45°C. Then, 4.9 g of fish oil (25°C) was added to the gelatin solution and emulsified for 5 min at 2500 rpm using a laboratory mixer. Acacia gum solution and aqueous ginger extract were then added, and the pH of the mixture was adjusted to 4 using 50% (v/v) acetic acid. The mixture was stirred at 600 rpm for 15 min and slowly cooled (5°C every 10 min) until the temperature reached 4–7°C. Stirring was stopped, and the emulsion was allowed to stabilize at room temperature for 1 h.

The microcapsules were then prepared using a pilot-scale spray dryer (Hemraj Enterprises, Mumbai, India) under the following conditions: inlet temperature 160°C, outlet temperature 80°C, nozzle

diameter 3 mm, and air pressure 2.5 bar. The spray-dried fish oil microcapsules were collected and used for further analyses.

Table 1: Composition of emulsion for the preparation of microencapsulates

Compositions	E1	E2	E3
Fish oil (ml)	4.9	4.9	4.9
Acacia gum (g)	18.5	20.5	25.5
Bovine gelatin (g)	9.5	7.5	7.5
Aqueous ginger extract (g)	-	3	5
Distilled water (ml)	q. s. to 100 ml	q. s. to 100 ml	q. s. to 100 ml

2.3 Moisture content and encapsulation efficiency

The moisture content of the fish oil encapsulates was determined using the standard AOAC (2005) method [11]. The encapsulation efficiency (EE) was calculated based on the relationship between the total oil and surface (free) oil. The total oil content and free oil were determined using Soxhlet extraction and the method described by Tan et al. (2005), respectively [12]. For this, 0.5 g of the fish oil and natural antioxidant encapsulate was mixed with 20 ml of *n*-hexane and stirred for 15 min at room temperature. The mixture was then filtered and the solvent was evaporated. The extracted oil was then dried to a constant weight. The amount of ginger extract in the supernatant (free extract) and the extracted encapsulated portion was quantified using UV-Visible analysis. The entrapment efficiency was calculated based on the amount of encapsulated and total ginger extract. The encapsulation efficiency (EE) was calculated using Equation (1).

Encapsulation efficiency (EE) = [(AT-AU)/AT] ×100 (1)

Where, AT is the total amount of dug added and AU is the amount of unentrapped drug.

2.4 Flow properties and morphology of microcapsules

The bulk density (ρb) and tapped density (ρt) were determined, and the powder flowability was calculated from carr's index and the hausner ratio.

A 0.1 g microcapsule suspension was diluted in 5 ml of sodium dodecyl sulfate solution (0.1% w/v), stirred in an ultrasonic water bath at 35°C for 20 min, and then investigated using an optical microscope.

The morphology of the fish oil and natural antioxidant encapsulates was analyzed using scanning electron microscopy (Philips XL 30 SEM, Netherlands). Fish oil and natural antioxidant encapsulates were fixed onto double-sided adhesive carbon tabs mounted on SEM-tubs, coated with gold, and then examined using SEM (XL 40 Philips, Netherlands).

2.5 Superoxide radical scavenging *in-vitro* antioxidant capacity

Stock solutions of microparticle-loaded *Zingiber officinale* extract and ascorbic acid were prepared at a concentration of 1 mg/ml in purified water. From these, various dilutions (50, 100, 150, 200, and 300 µg) were made using phosphate buffer. To each reaction mixture, 0.05 ml of riboflavin (2 µM), 0.3 ml of the extract solution or ascorbic acid (at different concentrations), 0.2 ml of EDTA (6 µM containing 3 µg NaCN), and 0.1 ml of nitro blue tetrazolium (NBT, 50 µM) were added. The final volume was adjusted to 3 ml using phosphate buffer (58 mM, pH 7.8). The tubes were uniformly illuminated for 15 min, and the absorbance was measured at 560 nm [13-14]. The percentage inhibition of superoxide production by the extracts was determined by comparing the absorbance of the test samples with that of the control and calculated using Equation (2).

% inhibition = (Average control O. D–Test sample O. D)/ (Average control O. D) ×100 (2)

The optical density obtained with each concentration of the extracted microparticles and ascorbic acid was plotted on a graph, with concentrations on the X-axis and percentage (%) inhibition on the Y-axis. The graph was extrapolated to determine the concentration required for 50% inhibition.

2.6 Oxidative stability of microencapsulated fish oil and antioxidant

Lipid oxidation in fish oil and natural ginger extract was evaluated by measuring the Thiobarbituric Acid Reactive Substances (TBARS) using the method described by McDonald and Hultin [15]. For this, 2 ml of thiobarbituric acid reagent was added to a test tube containing 1 ml of the sample (5 mg of fish oil and antioxidant encapsulated in 1 ml of acetate buffer). A blank was prepared using 1 ml of distilled water instead of the sample. The tubes were sealed, mixed thoroughly by vortexing twice, and incubated in a boiling water bath (100°C) for 15 min. After incubation, the tubes were cooled in a cold-water bath for 10 min and centrifuged at 1,000 rpm for 15 min. The absorbance of the supernatant was measured at 532 nm, and the TBARS values were expressed as mg of malondialdehyde per kg of sample.

2.7 Formulation of topical moisturizing cream

All the equipment was thoroughly cleaned before use. Each ingredient was accurately weighed for preparation. The moisturizing cream formulations were prepared by combining two separate phases, as shown in Table 2.

Table 2: Compositions of topical moisturizing creams

S. No.	Ingredients	F_1	F_2	F_3
1	Microencapsulated fish oil (g)	1.25	1.25	1.25
2	Stearic acid (g)	3	3.5	4
3	Cetyl alcohol (g)	2	2	2
4	Bees wax (g)	1	1.75	1.25
5	Peppermint oil (ml)	3.25	3	3
6	Propyl paraben (g)	0.25	0.25	0.25
7	Glycerin (ml)	3	3	3
8	Triethanolamine (ml)	0.5	0.5	0.5
9	Methyl Paraben (g)	0.25	0.25	0.25
10	Purified Water (ml)	35.5	34.5	34.5

Phase 1: The solid ingredients (stearic acid, beeswax, and cetyl alcohol) were melted using indirect heat at 70–80°C. Mineral oil, propyl paraben, and microencapsulated fish oil were added to the mixture and stirred well.

Phase 2: In a separate beaker, the aqueous ingredients, glycerin, triethanolamine, methyl paraben, and water were heated to the same temperature as the oil phase.

While still hot, Phase 1 was gradually added to Phase 2 with continuous stirring. The mixture was stirred for 5 min, removed from heat, and stirred until a uniform moisturizing cream was formed. The prepared cream was then transferred to clean containers for storage.

2.8 Evaluation of moisturizing creams

2.8.1 Physical properties: The cream was evaluated for spreadability, extrudability, pH, color, odor, and overall appearance. These tests were repeated thrice, and the average values were reported.

2.8.2 Homogeneity: The homogeneity of the cream was assessed by observing its visual appearance.

2.8.3 Washability: A small amount of cream was applied to the hand and rinsed with running water.

2.8.4 Irritancy: A 1 sq. cm area on the left hand was marked. The cream was applied, and any irritation, redness (erythema), or swelling (edema) was checked at regular intervals for up to 24 h.

2.8.5 pH of cream: To measure the pH, 0.5 g of cream was dissolved in 50 ml of distilled water. The pH was measured using a digital pH meter.

2.8.6 Dye test: Scarlet red dye was mixed with the cream and examined under a microscope. In an oil-in-water (o/w) cream, the dispersed globules appear red, whereas in a water-in-oil (w/o) cream, they appear colorless.

2.8.7 Viscosity: The viscosity of the cream was measured using a Brookfield viscometer at 20 rpm using spindle no. LV-4.

2.8.8 Spreadability test: The cream sample was applied between the two glass slides and was compressed between the two-glass slides to uniform thickness by placing 100 gm of weight on upper slide for 5 minutes then weight was added to the weighing pan. The weight was detached, and any excess formulation was removed carefully. The lower slide was secured to the apparatus board, and the upper slide was attached using a non-flexible string bearing a 20 g load. The time required for the upper slide to slip was recorded [15].$S = m \times l / t$ (3)

Where, the time in which the upper glass slide moved s=weight tight to upper slide l =length moved on the glass slide t =time take

2.8.9 Extrudability: The amount of cream extruded from the tube was measured when pressure was applied to the tube. The better the extrudability, the larger the amount of cream that was extruded. The weight required to extrude 0.5 cm of cream in 5 s was measured [16].

2.8.10 Microbial growth test: The cream was inoculated into agar media using the streak plate method. After incubation at 37°C for 24 h, microbial growth was checked and compared to that of the control.

2.8.11 Saponification value: Two grams of the cream was refluxed with 25 ml of 0.5 N alcoholic KOH for 30 min. After adding phenolphthalein, the solution was titrated with 0.5 N HCl. The saponification value was calculated as follows [17]: Saponification value = (b-a) × 28.05/W (4)

Where, a = volume of titrate, b = volume of titrate, w = weight of substances in gram

2.8.12 Acid value: Ten grams of cream was dissolved in a 50 ml mixture of alcohol and ether. After refluxing, 1 ml of phenolphthalein was added and titrated with 0.1 N NaOH until a faint pink color appeared. The acid value was calculated using the following formula:Acid value = n × 5.61/w (5) Where, w = weight of the substances, n = the number of ml in NaOH required

2.8.13 *In-vitro* occlusitivity

Beakers with a width of 3.2 cm and a height of 4.6 cm were used. Each beaker was filled with 10 g of distilled water. The beakers were sealed with Whatman filter paper (0.45 µm pore size), and 200 mg of the sample was evenly spread on the paper. The beakers were kept at 37.2°C and 60-70% relative humidity (RH) for 48 h. After the incubation period, water flow was measured to assess the occlusivity of the sample formulations, including a prototype formulation and a negative control, where the filter paper was left uncovered. The occlusion factor (f) was calculated as follows: $f = (a - b)/a \times 100$ (6)

Where, a = water flux through uncovered filter (percent water loss), b = water flux through filter when covered by test preparation (percent water loss).

2.8.14 *Determination* of peroxide value

A modified method of Wheeler described in ISO 3960:2007 was used where lipid peroxide is reacted with potassium iodide (KI) to liberate iodine that is then titrated with a standard solution of sodium thiosulfate ($Na_2S_2O_3$). The quantity of liberated iodide indicates the peroxide content. The peroxide value (PV) is expressed as the amount of peroxide oxygen present per kilogram of fat (mmol O_2/kg). The aluminum tubes were stored at room temperature, protected from oxygen exposure, for a period of six months. All the developed formulations were also subjected to accelerated stability at testing at 40±2°C, 75% RH ± 5% RH for three months. For three months accelerated study, Peroxide value (PV) was assessed at one, two and three months[18].

2.8.15 Sensory evaluation

Sensory parameters of creams were evaluated at different storage conditions, that is 40±2°C, 75% RH± 5% RH for three months and under ambient condition for six months. The colors, odors and textures of the creams were evaluated and compared [18]. A standardized quantity of 1 g of cream was applied over the dorsal surface of the left hand and sensory properties descriptively graded using terms as (1) Excellent: smooth and homogeneous texture, light brown color, distinctive fishy odor, (2) Satisfactory: smooth texture, non-consistency, light brown color, distinctive fishy odor, (3) Unsatisfactory: rough texture, visible solid particles, non- consistency, dark brown color, rancid fishy odor.

3. Results and Discussion

In the ongoing trend toward creating natural and safe antibacterial agents with few side effects, the fatty acid extract from fish liver oil, while not highly potent, has demonstrated effectiveness as an antibacterial agent against Gram-positive bacteria. Therefore, fish oil presents significant promise as an active pharmaceutical component for new effective drug formulations, particularly for preventing and treating skin disorders. Microencapsulation of fish oil helps protect it from oxidation, light, heat, and moisture, preserving its beneficial properties. When combined in a formulation, the microencapsulated fish oil and ginger extracts can work synergistically to improve the skin's health. The fish oil provides essential fatty acids that support skin barrier function and hydration, while ginger's antioxidant and anti-inflammatory properties help protect the skin from environmental damage and promote healing. Microencapsulation improves the stability and effectiveness of both ingredients, ensuring they remain active and effective over time.

3.1 Characterization of microencapsulated fish oil and aqueous ginger extract

Moisture content of the fish oil and aqueous ginger extracts encapsulates was ranged between 2.5 and 3.6%. This is a little higher than the moisture content (2.89–3.02%) reported for tuna oil powders by Klay Pradit and Huang [19]. It may be due to wall material composition and encapsulation method used for the study. The flowability of powder is an important property in handling and processing operations such as storage, transportation, formulation, mixing, compression or packaging. They are generally assessed by Carr's index and Hausner ratio. The higher Carr‟s index and Hausner ratio means that the powder is more cohesive and less able to flow freely. The obtained carr's index (>25) and hausner ratio (>1.3) suggest limited flowability, a common characteristic of spray-dried microcapsules with higher oil

content. In the present study, higher Carr index (32.27-35.40) and Hausner ratio (1.5-1.7) was observed for fish oil encapsulates which indicated poor flowability of the spray dried powder (Table 3).

Table 3: Physiochemical properties of micro encapsules

Code	Moisture (%)	Encapsulation efficiency of fish oil/ginger extract	Carr's index	Hauser ratio
E1	2.5 ±0.05	78.6 ±0.05/52.6±0.1	32.27 ±0.35	1.6 ±0.2
E2	3.6 ±0.02	82.35 ±0.1/60.25± 2.05	35.40 ±0.1	1.7 ±0.1
E3	3.4 ±0.02	84.5 ±0.1/62.21± 1.55	33.5 ±0.25	1.5 ±0.15

Sharma *et al*. (2012) reported that the oil content of the powder negatively influences the bulk density of the powder which reduces the flowability [20]. Determination of free and encapsulated oil will provide the information of Encapsulation Efficiency (EE). In general, the encapsulation efficiency may be affected by many factors as emulsion composition, pH, type and content of emulsifiers, droplet size, type of wall material, fish oil concentration, and spray drying parameters as inlet and outlet air temperature, air flow rate, humidity and type of atomization [21]. Encapsulation Efficiency is used to assess the quality of the dried microcapsules because it has an effect on oxidation sensitivity and flow powder properties [22]. In the present study, the encapsulation efficiency (EE) of fish oil ranged from 78.60% to 84.50%, while that of ginger extract varied between 52.6% and 62.21% (Table 3). The high EE of fish oil suggests effective entrapment within the microparticles, likely due to the emulsifying properties of the wall materials used. The observed variations in EE can be attributed to differences in the core and wall material compositions. Gelatin is a water-soluble material with mechanical barrier forming ability in emulsification. Further, higher *EE* (84.5 ±0.1/62.21±1.55%) was observed for sample contained gum acacia as wall material. It has been reported that the use of acacia gum as wall material was able to provide good encapsulating properties, especially when used with carbohydrates [23].

Optical microscopy images revealed that the oil droplets within the powders were smaller than the overall particle size. The presence of larger oil droplets and smaller particles may contribute to a higher amount of non-encapsulated oil [24]. The amount of superficial oil can be affected by the water migration rate during the drying process, the crust formation and microparticles morphology, in other words, the driven force of heat and mass transfer will impact the microparticles characteristics [25]. SEM analysis of fish oil encapsulates prepared by spray drying showed spherical shape and wrinkle appearance. Spray-dried encapsulates showed a range of droplet sizes, meaning the process produced particles of different sizes. Kolanowski et al. (2006) also found that fish oil microcapsules made by spray drying were spherical and varied in size [26].

Moisture content of the fish oil encapsulates was ranged between 2.87 and 3.6%. The storage time influenced the moisture gain, which continuously increased, mainly between zero and 90 days. Between 0 and 90 days, the E2 and E3 sample did not show any variation in this parameter at 24±1°C and the both microcapsules (E2 and E3) showed no variation at either storage temperature (Table 4). Temperature also influenced the moisture gain, but less than storage time. In general, the E1 sample exhibited a higher moisture gain, which can be attributed to its hydrophilic wall material and small particle size; additionally, this sample showed a physical change (caking) during the first month of storage. High moisture content can cause physical, textural, chemical, and microbiological changes in the microparticles. Water activity is an important parameter for powdered product quality, and the lipid

oxidation rate may increase at a aw below 0.178 and decrease at aw above 0.669 due to the dilution of oxidation catalysts [27]. Samples stored at 24±1°C showed higher aw than those stored at 6±2°C (Table 4). According to Labuza et al. (2006), the aw of dehydrated foods can change if the package used is moisture permeable and when impermeable packages are subject to an upward temperature shift [28]. Different temperatures can affect the aw since the water binding, dissociation of water, and solubility of solutes in water, among other factors, will be changed. Similar moisture content and aw results are reported in the literature. Lavanya et al. (2020) obtained spray-dried microparticles loaded with fish oil with moisture content from 2.19 to 3.20% (db) and aw values from 0.35 to 0.48, which are considered good to have a safe product during the shelf life and for the high-quality standards of dried powders in the food industry [29].

Table 4: Moisture content and water activity of microparticles loaded with fish oil at zero, and 90 days of storage at 6 ± 2°C and 24 ± 1°C (mean ± SD, where n=3)

Days	Formulation	Storage condition (6 ± 2°C)		Storage condition (24 ± 1°C)	
		Moisture content (wt %)	Water activity (aw)	Moisture content (wt %)	Water activity (aw)
0	E1	2.87 ± 0.057	0.178 ± 0.006	2.87 ± 0.057	0.178 ± 0.06
	E2	2.72 ± 0.03	0.200 ± 0.004	1.72 ± 0.030	0.29 ± 0.04
	E3	3.6± 0.03	0.185 ± 0.01	3.6± 0.03	0.185 ± 0.01
90	E1	14.637 ± 0.064	0.669 ± 0.002	16.138 ± 0.174	0.698 ± 0.01
	E2	7.303 ± 0.015	0.694 ± 0.003	7.596 ± 0.041	0.720 ± 0.02
	E3	5.303 ± 0.05	0.614 ± 0.003	5.303 ± 0.05	0.614 ± 0.03

3.2

3.3 Superoxide radical scavenging *in-vitro* antioxidant capacity

The formation of free radicals can initiate numerous reactions, leading to extensive tissue damage. Lipids, proteins, and DNA are particularly vulnerable to oxidative attack by these radicals. Antioxidants help counteract oxidative stress by neutralizing and scavenging free radicals. Superoxide is produced from molecular oxygen due to oxidative enzymes of mitochondria [30]. The antioxidants scavenge the free radicals and inhibit the lipid peroxidation. The formulations at different concentrations were tested for antioxidant activity by *in-vitro* models namely; superoxide inhibition in comparison with standard (ascorbic acid) antioxidant [31]. Superoxide radical is considered a major biological source of reactive oxygen species. Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress [32]. The scavenging activity towards superoxide radicals is measured in terms of inhibition of superoxide radicals. *Zingiber officinale* and ascorbic acid at quantities of 50-300 µg produced dose dependent inhibition of superoxide radicals. The quantity of the microparticles *was* needed for 50% scavenging of superoxides was found to be 112-114±0.25µg. The quantity needed for the same effect by the known antioxidant, ascorbic acid was 114 µg. This indicates that the microcapsules E2 and E3 containing aqueous extract of *Zingiber officinale* possess antioxidant activity. The EC_{50} values of scavenging superoxide radicals for the selected microcapsules is given in Table 5. Though the antioxidant potential of fractions was found to be low ($P<0.05$) than those of ascorbic acid, the study revealed that *Zingiber officinale* have prominent antioxidant activity; the presence of phenolic compounds (containing phenolic

hydroxyls) is mainly found in these fractions and could be attributable to the observed high antiradical properties of these fractions.

Table 5: The IC_{50} values of scavenging and peroxidation effect of microparticles loaded with fish oil and aqueous extracts (µg/ml)

Microparticles	Superoxide radical scavenging (IC_{50} µg)
E1	-
E2	112±0.12
E3	114±0.25
Ascorbic acid	114±0.42

3.4 Oxidative stability of microencapsulated fish oil and antioxidant

Lipid oxidation of microencapsulated oils is affected by microcapsule characteristics. Oxidative stability was evaluated by measuring Thiobarbituric Acid Reactive Substances (TBARS). TBARS are secondary oxidation products formed from the breakdown of oxidized PUFAs [33]. Lipid oxidation was quantified using the TBARS method, a widely employed and sensitive assay for tracking secondary peroxidation products. Measuring secondary oxidation products is important in the determination of lipid oxidation in food products for human consumption because they are generally odor active, whereas primary oxidation products are colorless and flavorless. In the present study, initial *TBARS* values of encapsulates varied from 0.30 to 0.99 mg malonaldehyde kg^{-1} and it showed an increased trend during storage. Results indicated that encapsulates prepared by gum acacia with fish oil (E3) showed lower *TBA* (1.58 mg malonaldehyde kg^{-1}) value than E1 (9.85 mg malonaldehyde kg^{-1}) samples on the 7^{th} day. However, the levels of secondary lipid oxidation products were lower in the fish oil encapsulates prepared with addition of oregano essential oil than others (figure 1). It might be due to the protective effect of oregano essential oil incorporated in the fish oil. They react as effective protein cross-linkers, at the same time serve as a potent antioxidant for fish oil by acting as reducing agents or singlet oxygen scavengers. Binsi *et al*. (2017) found the lowest rate of increase in *TBARS* value in fish oil encapsulates containing sage polyphenols [34]. Jeyakumari *et al*. (2014) observed lower *TBARS* value in fish oil encapsulates prepared with fish gelatin and 0.25% ginger essential oil under room temperature [35].

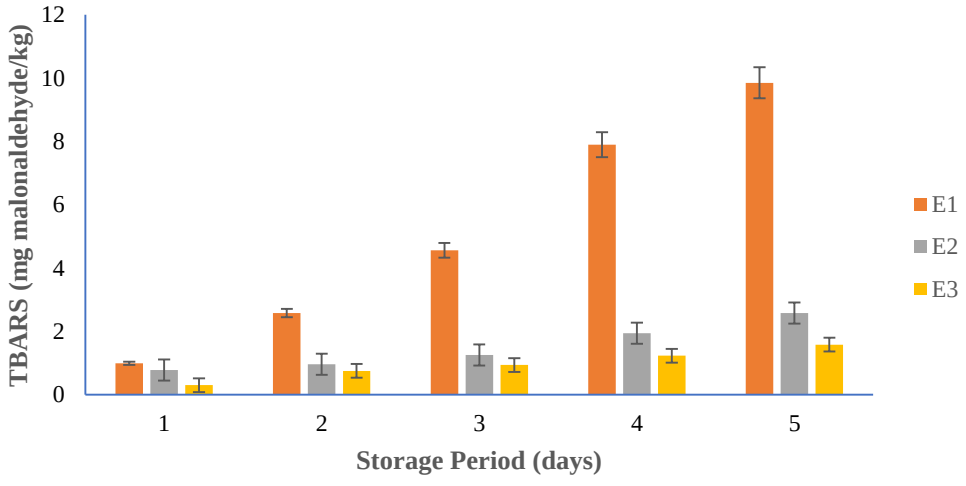

Figure 1: Changes in TBARS value of microencapsulated fish oil during storage

3.5 Formulation and evaluation of topical moisturizing cream

The parameters of the optimized encapsulated fish oil and ginger extract loaded cream were investigated and compared to those of the conventional ginger extract cream, as shown in Table 6. All formulations exhibited an appealing appearance with a smooth and uniform texture. The pH values were within an acceptable range, minimizing the risk of potential sensitivity or irritation. In addition, the viscosity of the formulations was investigated, and it was revealed that incorporating oil into cream resulted in significantly greater viscosity than cream ($p=0.006$); nonetheless, the viscosity of both preparations was within a good range, supporting topical application. The spreadability of the formulations was evaluated, and the results demonstrated their ability to distribute uniformly across the skin surface. Prepared formulation was pale green in colour. It has pleasant odor and smooth texture. The homogeneity of the formulated cream was evaluated based on its visual appearance and tactile properties. The cream exhibited a smooth texture and pleasant feel. Its physical appearance was pale white, a color attributed to the extract and its concentration, with only minor variations observed in the shade of pale white. All formulations had a strong distinctive smell of fish odors. Overall cosmetic acceptability was rated satisfactory for all formulations. Specifically, formulations containing wax had an advantage of providing an additional glossy appearance without having a negative impact on the homogeneity and greasiness. All creams were safe and did not develop any skin irritation, lesion or inflammation. All tested formulations were well tolerated. This result showed that all ingredients and concentrations of all ingredients were safe. The pH of the cream was found to be in range of 6.5 to 6.9 which is good for skin pH. The herbal formulation was shown pH nearer to skin required i.e. pH 7. The pH values of sample products were found to be neutral to basic. Viscosity of formulated cream was determined by Brookfield viscometer at 20 rpm using spindle no. LV-4.

Table 6: Characterization of the topical moisturizing cream

Sr. No.	Parameters	F1	F2	F3
1	Colour	Pale White	Slightly Pale White	Moderately Pale White
2	Odour	Aromatic	Pleasant	Pleasant
3	Visual inspection	Smooth	Smooth and homogenous	Smooth and homogenous
4	Immediate skin feels	Cooling sensation	Cooling sensation	Cooling sensation
5	Homogeneity	homogenous in nature without any clog	homogenous in nature without any clog	homogenous in nature without any clog
6	Type of smear	non-greasy in nature	non-greasy in nature	non-greasy in nature
7	Washability	Washable with water	Washable with water	Washable with water
8	Irritancy	Non-irritant	Non-irritant	Non-irritant
9	pH	6.5	6.8	6.9
10	Viscosity (cp)	47899	55970	79970

11	Spreadability (g.cm/s)	8.66±0.88	2.66±0.57	4.66±0.65
12	Tube Extrudability (g± SD)	4.84±0.79	3.95±0.49	3.32±0.07
13	Microbial growth	No	No	No
14	Saponification value	22.2	20.5	21.4
15	Acid value	4.5	4.2	4.3
16	Dye test	Colourless	Colourless	Colourless

The viscosity of cream was in the range of 47899 to 79970 cps which indicates that the cream is easily spreadable by small amount of shear. Cream spreadability was found to be inversely proportional to the wax concentration. As the wax amount was increased the creams became more viscous and, consequently, increasing the wax concentration from 0.5 % to 0.75% led to decreased spreadability. Formulations F1 exhibited the highest spreadability while formulations F2 displayed the lowest spreadability. The spreading values of these formulations were in the following order: F1 > F3 > F2.

Increasing the wax concentration reduced the extrudability of the creams. In most formulations, raising the wax content from 0.5% to 0.75% significantly lowered tube extrudability, except for formulation E3, where the decrease was less noticeable. As shown in Table 6, creams containing wax had the lowest extrudability, while those with beeswax were less viscous and exhibited the highest extrudability. The extrudability of the formulations ranked as F1 > F2 > F3. There were no signs of microbial growth after 24 hrs of incubation at 37ºC and it was comparable with the control. The saponification value and acid value results of formulated cream is shown in table 6 and showed satisfactorily values. The scarlet red dye is mixed with the cream. A small drop of the cream was placed on a microscopic slide, covered with a cover slip, and examined under a microscope. The dispersed globules appeared colorless against a red background, confirming the formulation as a water-in-oil (w/o) type cream.

3.6 *In-vitro* occlusivity test

The weight loss of water (water flux) depends on the occlusivity of membrane offered by the formulations tested. The results of occlusivity are shown in the figure 2. When values were compared to that of uncovered filter, all the creams showed significantly lower percentage of water loss than that of the uncovered filter. The better occlusivity of creams could be attributed to the lipid nature of the developed formulations, preventing water evaporation to a greater extent.

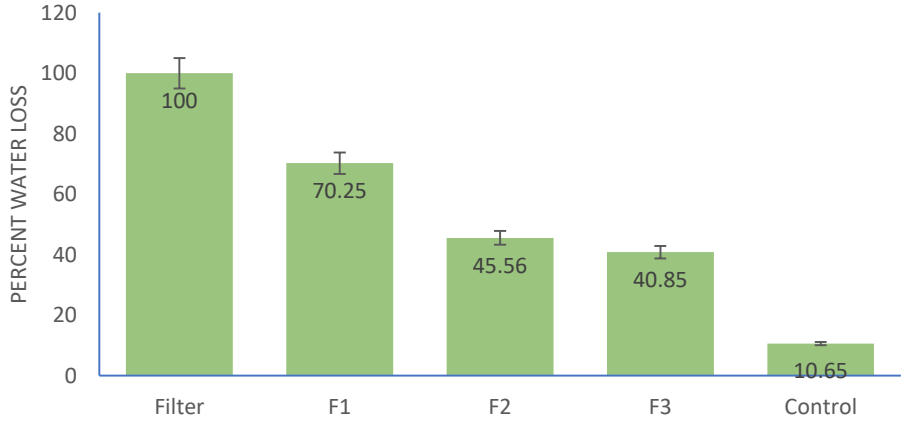

Figure 2: Comparative *in-vitro* occlusivity in terms of percent water loss of various formulations

The results of the peroxide value (PV) assay represent how stable the PUFAs were during storage for six months at ambient temperature (i.e., 22–23ºC) and for three months under accelerated conditions (i.e., 40ºC). The content of the primary lipid oxidation products measured as the PV of the extract was in the range 1.073–1.804 mmol/kg O_2 at the end of three months of accelerated testing and 0.824–1.298 mmol/kg O_2 at the end of six months of storage at room temperature. In both cases no statistically significant differences in the PV values were observed when the wax content was increased.

Fish oil is highly susceptible to rancidity because of its rich content of omega-3 polyunsaturated fatty acids (PUFAs), which are easily oxidized. Monitoring the peroxide value (PV) of the creams enabled the assessment of fatty acid oxidation and rancidification. As can be seen in figure 3, no correlation between PV and presence of wax was found, and the increased wax concentration did not alter the PV of any of the creams tested. In all cases, except in formulation F1, there was a rapid increase in the peroxide value during the first month of storage at 40ºC.

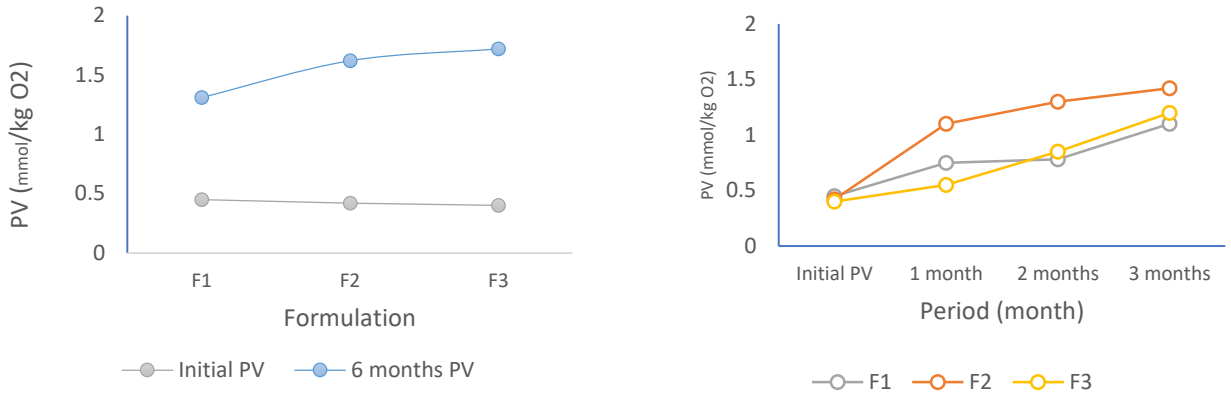

Figure 3: Lipid oxidation measured as peroxide: (a) real-time tested period; (b) accelerated tested period

A possible explanation for the observed results is the presence of residual oxygen in the headspace of the sealed container. Subsequently, the peroxide value (PV) showed only a slight increase over time under both ambient and accelerated conditions (40°C). It was evident that oxidation of PUFAs occurred under both ambient and accelerated storage conditions; however, it was not influenced by the cream composition. Nevertheless, the extent of lipid peroxidation was minimal, as the peroxide values (PVs) for all creams remained below the recommended limits for the acceptability and quality of fish oils intended for human consumption. All formulations tested met the quality standards of the European Pharmacopeia [36]. Stability studies of cream (F3) were further supported by the evaluation of sensory parameters. All creams were stable during the six months of storage at room temperature and displayed no change in texture, color or smell (Table 7).

Table 7: Stability studies of optimized topical moisturizing cream (F3)

Sr. No.	Parameter	Observations		
		Initial	Room temperature (6 months)	40±2°C, 75% RH± 5% RH (3 months)
1.	Colour	Moderately Pale White	Moderately Pale White	Pale White

2	Odor	Pleasant	Pleasant	Pleasant
3	Homogeneity	Homogenous	Homogenous	More homogenous
4	Type of Smear	Non-greasy	Non-greasy	Non-greasy
5	pH	6.9	6.9	6.7
6	Viscosity (cp)	79970	79971	79968
7	Spreadability g.cm/s)	4.66±0.65	4.64±0.5	4.62±0.25
8	Irritancy test	No irritation	No irritation	No irritation

A deviation from initial sensory parameters was observed in the case of F3 after six months of storage under ambient conditions as well as at 40ºC. A slight change in color in the formulations at 40ºC and "bleeding" after 90 days was observed. The phenomenon of bleeding was attributed to the storage at high temperature which, in fact, is a well-known change in consistency that typically appears on the top of a filled tube and is frequently referred to as the main stability problem of creams [37]. According to the results obtained from a short-duration stability study, formulated cream was slightly different in homogeneity than the original samples. However, besides the textural and sensory changes, phase separation was not observed. Further studies on long-term stability, sensory evaluation in volunteers, and scale-up feasibility are recommended to support clinical application.

4. Conclusion

The optimized formulation (F3) enriched with microencapsulated fish oil and ginger extract exhibited enhanced hydration efficiency and desirable cosmetic characteristics, demonstrated excellent skin moisturization properties. The inclusion of the fish oil and extract enhanced skin hydration, indicating its potential as an effective moisturizing agent in the cream formulation. The optimized cream also showed good *in-vitro* occlusivity and spreadability, suggesting that it can effectively incorporate higher amounts of microencapsulated fish oil and ginger extract to provide enhanced cosmetic and skin care benefits. These findings suggest that the formulation has potential for commercialization as a natural, antioxidant-rich topical product for skin hydration and protection.

5. References

1. Goldberg I. Functional foods: designer foods, pharmafoods, nutraceuticals. Springer Science and Business Media; 2012 Dec 6.
2. Ateya AM et al. Effect of omega-3 fatty acids on serum lipid profile and oxidative stress in pediatric patients on regular hemodialysis: a randomized placebo-controlled study. Journal of Renal Nutrition. 2017;27(3):169-74.
3. Saboori S et al. Beneficial effects of omega-3 and vitamin E coadministration on gene expression of SIRT1 and PGC1α and serum antioxidant enzymes in patients with coronary artery disease. Nutrition, Metabolism and Cardiovascular Diseases. 2016;26(6):489-94.
4. EM B. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: A systematic review. Atherosclerosis. 2006; 189:19-30.
5. Manuhara GJ et al. Aqueous extract composition of spent ginger (Zingiber officinale var. Amarum) from essential oil distillation. InIOP Conference Series: Materials Science and Engineering 2018; 333(1): 012069).

6. Haniadka R et al. A review of the gastroprotective effects of ginger (Zingiber officinale Roscoe). Food & function. 2013;4(6):845-55.
7. Govindarajan VS, Connell DW. Ginger—chemistry, technology, and quality evaluation: part 1. CRC critical reviews in food science and nutrition. 1983;17(1):1-96.
8. Sioen IA et al. Dietary intakes and food sources of fatty acids for Belgian women, focused on n-6 and n-3 polyunsaturated fatty acids. Lipids. 2006;41(5):415-22.
9. Maru AD, Lahoti SR. Formulation and evaluation of moisturizing cream containing sunflower wax. Int J Pharm Pharm Sci. 2018;10(11):54-9.
10. Green BK, Lowell S, inventors; NCR Corp, assignee. Oil-containing microscopic capsules and method of making them. United States patent US 2,800,457. 1957 Jul 23.
11. AOAC. 2005. Official Methods of Analysis. 17th Ed., Association of Analytical Chemists, Washington, DC 18.
12. Tan LH et al. Effect of oil loading on microspheres produced by spray drying. Journal of Microencapsulation. 2005;22(3):253-9.
13. McCord JM, Fridovich I. The utility of superoxide dismutase in studying free radical reactions: I. Radicals generated by the interaction of sulfite, dimethyl sulfoxide, and oxygen. Journal of biological chemistry. 1969;244(22):6056-63.
14. Ohkawa H et al. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry. 1979;95(2):351-8.
15. Mcdonald RE, Hultin HO. Some characteristics of the enzymic lipid peroxidation system in the microsomal fraction of flounder skeletal muscle. Journal of Food Science. 1987;52(1):15-21.
16. Karemore MNet al. Formulation and evaluation of a moisturizing cream using almond butter. International Journal of Pharmaceutical Sciences and Drug Research. 2017;9(3):105-12.
17. Saheb SU et al. Formulation and evaluation of cream from naturally containing plant extract. World J Pharm Sci. 2018; 7:851-61.
18. Ilievska B et al. Topical formulation comprising fatty acid extract from cod liver oil: development, evaluation and stability studies. Marine Drugs. 2016;14(6):105.
19. Klaypradit W, Huang YW. Fish oil encapsulation with chitosan using ultrasonic atomizer. LWT-Food Science and Technology. 2008;41(6):1133-9.
20. Sharma A et al. Functionality of milk powders and milk-based powders for end use applications—a review. Comprehensive reviews in food science and food safety. 2012;11(5):518-28.
21. Encina C et al. Conventional spray-drying and future trends for the microencapsulation of fish oil. Trends in food science & technology. 2016; 56:46-60.
22. Baik MY et al. Effects of antioxidants and humidity on the oxidative stability of microencapsulated fish oil. Journal of the American Oil Chemists' Society. 2004;81(4):355-60.
23. Hogan SA et al. Emulsification and microencapsulation properties of sodium caseinate/carbohydrate blends. International Dairy Journal. 2001;11(3):137-44.
24. Linke A et al. Contribution of the internal and external oxygen to the oxidation of microencapsulated fish oil. European journal of lipid science and technology. 2020;122(8):1900381.
25. De Melo Ramos F, Júnior VS, Prata AS. Impact of vacuum spray drying on encapsulation of fish oil: Oxidative stability and encapsulation efficiency. Food research international. 2021; 143:110283.
26. Kolanowski W et al. Microencapsulation of fish oil by spray drying--impact on oxidative stability. Part 1. European Food Research and Technology. 2006 Feb;222(3):336-42.

27. Shaw LA et al. Spray-dried multilayered emulsions as a delivery method for ω-3 fatty acids into food systems. Journal of agricultural and food chemistry. 2007;55(8):3112-9.
28. Labuza TP et al. Effect of temperature on the moisture sorption isotherms and water activity shift of two dehydrated foods. Journal of Food science. 1985;50(2):385-92.
29. Lavanya MN et al. Influence of spray-drying conditions on microencapsulation of fish oil and chia oil. Drying Technology. 2020; 38 (3): 279–292.
30. Hermes-Lima M. Oxygen in biology and biochemistry: role of free radicals. Functional metabolism: regulation and adaptation. 2004; 1:319-66.
31. Halliwell B, Gutteridge JM. The antioxidants of human extracellular fluids. Archives of biochemistry and biophysics. 1990;280(1):1-8.
32. Alves CQ et al. Methods for determination of in vitro antioxidant activity for extracts and organic compounds. 2010;33(10):2202-10.
33. Shahidi and Wanasundara UN. Chemistry, Nutrition and Biotechnology. In: Food Lipids, (Eds.): Akoh, C. C. and Min, D. B. Marcel Dekker, Inc., New York, NY; 2002, p.465–487.
34. Binsi PK et al. Structural, functional and in vitro digestion characteristics of spray dried fish roe powder stabilised with gum arabic. Food chemistry. 2017; 221:1698-708.
35. Annamalai J et al. Oxidative stability of microencapsulated fish oil during refrigerated storage. Journal of food processing and preservation. 2015;39(6):1944-55.
36. EFSA Panel on Biological Hazards (BIOHAZ). Scientific opinion on fish oil for human consumption. Food hygiene, including rancidity. EFSA Journal. 2010;8(10):1874.
37. Loyd VA. Remington: An Introduction to Pharmacy; Pharmaceutical Press: London, UK, 2013; p. 157.