Effect of using various amounts of patin (*Pangasianodon hypophthalmus*) fish oil on physical and chemical properties of moisturizing hand cream

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Abstract
Hand cream is used for rehydrating and improving skin properties. The skin is the first point of contact with the environment. Thus having healthy skin with proper barrier properties is quite important. The beneficial effects of fish oil on skin make it a favorable ingredient to be used in hand care products. In the present study, the efficacy and stability of various hand cream formulations using 0, 1.0, 2.5 and 5.0% of fish oil were investigated and compared. The appearance, odor, texture, and pH of formulations containing 1 and 2.5% of fish oil remained stable in very good qualities during the storage, whereas the related values to the appearance and texture of formulation containing 5.0% of fish oil at the end of 6 months of storage at 45°C were significantly ($p<0.05$) lower than other formulations and previous evaluations of this formulation. Weight loss was not observed in all formulations under study.

Keywords: Fish oil, Hand cream, Formulation, Cosmetic, Stability

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Introduction
Nowadays, consumers are more interested in products which are made from natural ingredients. The cosmetic industry is one example whereby small amounts of oils or hydro alcoholic extract from plants and animals can be used (Abamba, 1993). Common lipids which are used in the cosmetic industry are mono, di and triglycerides, fatty acids, waxes, lanolin, long chain esters, and mineral oils (Lodén, 2005). Health benefits of topical application of fish oil are desirable to be employed in cosmaceutical products (Puglia et al., 2005; Zulfakar et al., 2010). Topical use of fish oil is favorable for the treatment of localized inflammatory disease such as arthritis rather than using dietary supplementation with fish oil (Heard et al., 2003). Puglia et al. (2005) reported that topical application of extracted oil from some Mediterranean fishes such as mackerel (Scomber scombrus), sardine (Sardina pilchardus) and horse mackerel (Trachurus mediterraneus) produced positive effects on the treatment of UV induced erythema. Zulfakar et al. (2010) reported that the efficacy of fish oil in an ointment for the treatment of psoriasis. It has been thought that the anti-inflammatory effect of fish oil is more related to the polyunsaturated fatty acids (Gil, 2002; Calder, 2004). Patin catfish (Pangasianodon hypophthalmus) oil contains good amount of polyunsaturated fatty acids (Shabanikakroodi et al., 2014). Moreover, analysis of the physical and chemical properties of the patin catfish oil indicated the suitability of this oil to be used in the formulation of hand cream (Shabanikakroodi et al., 2015).

In the field of cosmetics, the term cream refers to a solid or semi-solid emulsion. Creams are emulsions of two immiscible phases including oil phase and water phase. Most of hand creams are oil in water (O/W) emulsions. The ratio between oil and water, the amount and type of ingredients, emulsifiers, and preservatives are the main factors that determine the aesthetic properties, efficacy, and stability of final product (Cannell, 1993). In a cream, appropriate emulsifiers should be able to combine the hydrophilic and lipophilic components in one molecule. This takes place by turning the polar ends into the water phase and nonpolar hydrocarbon ends into the oil phase and embedding the droplets. The size of these droplets is often between 1 and 100 μm which provide white color for a formulation. pH is an important factor in the stability of a cream which is usually between 3 and 8 (Alikhan et al., 2014).

Hand creams could be applied to rehydrate, smooth and leave a non-tacky protective film on the skin (Abamba, 1993). There are several ingredients with a moisturizing effect which can be used in formulation of a hand cream such as lipids. Lipids produce skin hydration by several mechanisms. Lipids can reduce loss of water from the outer layer of skin (Lodén and Lindberg, 1991). Moreover, they can penetrate into the skin and form an inert layer (Feingold et al., 1986; Escobar et al., 1992). All types of skin need to be moisturized at least twice a day. Moisturizers are the most...
recommended products in dermatology (Lodén and Lindberg, 1991). The basic components of moisturizing and nourishing products are moisturizers, water, emulsifier system, and preservatives. Moisturizing and nourishing products are available in the form of creams, lotions, milks, aerosol mousses, and gels.

There are a few examples of available cosmetic skin care products containing fish oil in the market but it is too hard to find a scholarly study presenting data on formula and procedure suitable for production of these kinds of products. Therefore, this study can provide vital information on choosing hand cream ingredients, their suitable amounts, and processes for formulation of hand cream which can contain fish oil and can be stable and effective. Stability tests can provide useful information which can be used for modification of a formulation or manufacturing process to optimize the product to retain its properties during its shelf life. However, many important properties of a product which is assessed subjectively such as appearance, odor, texture and color cannot readily be recorded in numerical terms (Cannell, 1993).

Generally, small amounts of fish oil are used in cosmetic products as compared to topical therapeutic products (Abamba, 1993). Unfortunately, there is a lack of data for determining the suitable and functional amounts of fish oil in a cosmetic skin care product and specifically in hand cream. This study attempted to find the suitable amount of fish oil which does not adversely affect the stability of hand cream during storage. Therefore, formulating and producing hand cream using various amounts of refined patin catfish oil and evaluating the appearance, odor, texture, pH, weight loss, and preservative efficacy of various formulations of hand cream during six months of storage at 4, 25, 37, and 45°C are the objectives of this study.

Materials and methods

Procedure of hand cream production

A modified method of Abamba (1993) was used to produce hand cream. Butylated hydroxy anisol (175 ppm) was added to the refined catfish oil to inhibit oxidation of unsaturated fatty acids. For the production of hand cream, oil phase and water phase were heated to 75°C, separately. Water phase was added to oil phase and mixed with a magnetic stirrer for 15 minutes. Then, the mixture was cooled at room temperature while mixing. At 40-45°C, heat sensitive materials such as perfumes were added. Mixing was continued until the temperature of the product reached room temperature. Then, the product was filled in special containers and weighed. The components in each formulation under study are shown in Table 1. The components were chosen according to the commercial cream formulation of Mero (Tehran, Iran).
Table 1: Components in each formulation under study.

<table>
<thead>
<tr>
<th>Components (%)</th>
<th>Formulation 1</th>
<th>Formulation 2</th>
<th>Formulation 3</th>
<th>Formulation 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stearic acid</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Cetearyl alcohol</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Cetyl alcohol</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Glycerin monostearate</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>Anhydrous lanolin</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Petrolatum</td>
<td>7.00</td>
<td>7.00</td>
<td>7.00</td>
<td>7.00</td>
</tr>
<tr>
<td>Paraffin wax</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Liquid paraffin</td>
<td>22.00</td>
<td>21.00</td>
<td>19.50</td>
<td>17.00</td>
</tr>
<tr>
<td>Fish oil</td>
<td>0.00</td>
<td>1.00</td>
<td>2.50</td>
<td>5.00</td>
</tr>
<tr>
<td>Propyl paraben</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Triethanol amine</td>
<td>0.70</td>
<td>0.70</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>Glycerin</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Perfume</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Water</td>
<td>54.20</td>
<td>54.20</td>
<td>54.20</td>
<td>54.20</td>
</tr>
</tbody>
</table>

Note: Fish oil containing butylated hydroxy anisol (BHA)

Hand cream sampling
Hand cream samples without, 1.00%, 2.50% and 5.00% of fish oil were stored at 4, 25, 37, and 45°C. Five samples were stored for each formulation at each temperature. Stability tests were carried out according to the examination time schedule suggested by Cannell (1993). Stability tests were performed upon the production of the hand creams, after two weeks, one, two, three, four, five, and six months of storage of various formulations at different temperatures.

Appearance, odor and texture evaluation
The study was conducted following the method of Cannell (1993). Twenty panelists evaluated these properties (appearance indicates the way the cream looks, odor indicates cream smell, and texture indicates the consistency of the cream). Panelists were cosmetic factory personnel, Iranians, non-smoking people including ten males (age: 25-58) and ten females (age: 20-56). Results were recorded in terms of the panelists’ satisfaction on a numerical scale of 1. Very poor, 2. Poor, 3. Fair, 4. Good, and 5. Very good.

pH determination
Each sample (2 g) was diluted in 18 g distilled water. The pH value was determined using a digital pH meter (744 pH meter, Metrohm, Swiss) at 25°C. The experiment was repeated three times for each sample.

Weight loss determination
Weight loss of samples was calculated by regular weighing of each sample by standard lab analytical balance with readability of 0.0001 g (ED2245 Sartorius Extend Series, Sartorius, Germany). The experiment was repeated two times for each sample.

Statistical analysis
All data were statistically analyzed using a SPSS program (version 16.0). Analysis of variance (ANOVA) was
performed for the results of the values related to the appearance, odor, and texture of the hand cream formulations under study (F1: without fish oil, F2: 1.00% fish oil, F3: 2.50% fish oil, and F4: 5.00% fish oil). POSThoc Tukey’s test was carried out in conjunction with ANOVA to determine significant difference ($p<0.05$) between the means of the results.

**Results**

The good values which were related to the appearance, odor, and texture obtained from all formulations were stored at 4, 25, and 37°C and remained unchanged during six months of storage. The values which were related to the appearance, odor, and texture of various formulations incubated at 45°C are shown in Figs. 1, 2, and 3. The appearance, odor, and texture of F2 (1.00% fish oil) and F3 (2.50% fish oil) remained in very good quality during the six months of storage at 45°C in an incubator as well as the formulation without the fish oil. The F4 (5.00% fish oil) showed a slight degree of separation at the end of six months storage at 45°C and the values which were related to the appearance and texture of F4 (5.00% fish oil) at this time were significantly ($p<0.05$) less than those related to the other formulations and previous evaluations of this formulation.

![Figure 1: The values related to the appearance of the hand cream formulations under study (F1: without fish oil, F2: 1.00% fish oil, F3: 2.50% fish oil, and F4: 5.00% fish oil). The symbol (*) indicates statistically significant difference between treatments ($p<0.05$).](image1)

![Figure 2: The values related to the odor of the hand cream formulations under study (F1: without fish oil, F2: 1.00% fish oil, F3: 2.50% fish oil, and F4: 5.00% fish oil). The symbol (*) indicates statistically significant difference between treatments ($p<0.05$).](image2)
The pH values of F1, F2, F3, and F4 were 7.15, 7.16, 7.19, and 7.21, respectively. The pH values of all formulations remained constant during six months of storage at different temperatures. In all tested formulations, sample weight loss was not observed during six months of storage at different temperatures.

**Discussion**

Every skin care product undergoes changes during the time. The types of changes that may occur include appearance, odor, texture, pH, loss of weight, and microbial spoilage. In stability testing, using temperatures higher than room temperature (20-25°C) at which cosmetic cream samples are stored accelerates the rate of these changes. Generally, the rate of changes doubles with a rise of 10°C. Therefore, a product which is stable at high temperatures is very likely to be stable under normal conditions on long-term storage (Cannell, 1993).

The F4 (5.00% fish oil) showed a slight degree of separation at the end of six months storage at 45°C and the values which were related to the appearance and texture of F4 (5.00% fish oil) at this time were significantly ($p<0.05$) less than those related to the other formulations and previous evaluations of this formulation. However, Cannell (1993) indicated that a product which shows instability after long time storage at a high temperature may not necessarily be unstable under normal conditions. Aubourg et al. (1996) showed that fish oil is very sensitive to heat. Therefore, the physical and chemical specifications of fish oil can be changed during storage for a long period of time at increased temperature (45°C) which resulted in emulsion instability in the cream containing 5.00% of fish oil, whereas other formulations containing 1.00 and 2.50% of fish oil as well as the formulation without the fish oil could tolerate these conditions and remained unchanged.

The formulation without the fish oil contains higher amount of liquid paraffin. Liquid paraffin is a highly refined mineral oil and colorless liquid which has a low molecular weight. It
has straight chain hydrocarbon of C16 to C32 which consist of paraffin and naphthene. It is stable in normal conditions of use and is nonirritant. Liquid paraffin provides an oily and moisturized feeling on human skin (Iwata and Shimada, 2013).

The beneficial effects of saturated (Man et al., 1996), monounsaturated (Badiu et al., 2010), and especially polyunsaturated fatty acids (Rhodes, 2000) on skin makes it a favorable ingredient to be used in the hand cream. High amounts of polyunsaturated fatty acid is the characteristic of fish oil which makes it different from other plant and animal oils. Moreover, fish oil is a natural product, whereas paraffin oil is a synthetic mineral oil with limited topical effects.

Determining the pH value is the important part of testing the chemical stability of pharmaceutical products. This test can provide useful information for drug formulation, storage, and degradation mechanisms (Some et al., 2000). A stable pH indicated the chemical stability of various hand cream formulations under study. The human skin pH value is within a broad range from 4.0 to 7.0 (Lambers et al., 2006). According to Lodén (2005) the pH value of skin care creams is usually between 3 and 8, in which the cream shows good stability. Therefore, the pH values of our product were near the range of normal human skin pH and within the best range of skin care creams which also showed good stability.

The stable weight of various formulations during six months of storage indicated that volatile constituents were not lost.

References
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