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Université de Béjaïa

Réf :……..

Laboratoire de Biochimie Appliquée Alimentaire

Mémoire de Fin de Cycle
En vue de l'obtention du diplôme

MASTER

Thème

Formulation cosmétique : Incorporation du safran (*Crocus sativus)* dans une émulsion

Présenté par :
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Soutenu le : **15 septembre 2022**

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First of all, we thank ALLAH, the master of the heavens and the earth who has given us the will, the power, and the health to realize and accomplish this modest work

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Who did us the honour of carrying out this work under her guidance by excellence. We would also like to thank her for the freedom she gave us and the great trust she placed in us from the beginning to the end of the work

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Thanks to all

Sorya / Wissam

Dedication

In the name of Allah the merciful the most merciful
First of all, I thank Allah who has enlightened my path and brought me to the moment I expected.

All love and respect to my dear parents and I wish God to keep and protect them INCHAA ALLAH.

I dedicate my modest work

To my dearest parents: Mum and Dad who did everything possible for me, who were always with me, who suffered during all my years of study, frankly I can't express around all that made me from my childhood until this moment.

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To my dear sisters: Hakima, Souhila, Mebarka, Tassaadit

To all my family: Bouzid Belkacemi, Kamel Belkacemi and…….. etc. who have always helped to give me courage

To my dearest friends: Amina, Ines, Yasmina, Nadjat, Yasmina, and Sonia, Ines, with whom I spent beautiful and unforgettable moments.

To all my colleagues and assistants who know me

Sorya

Dedication

I dedicate this work to the dearest beings who have sacrificed their lives for my happiness, who have always been by my side, in joy and sadness, my parents Boughouaou Noura and Salah whom I love very much.

To my dear sisters Mounia, Sabiha and Habiba

To my dear brothers Mounir, Amine, Abdalhak and his wife Hassiba and their two young sons, Wanis and Iyad

.

To my fiancé Bilal, who gives me self-confidence and courage and advises me not to fail and to continue until the end, I wish him a happy life full of happiness, as well as his whole family.

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Wissam

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Introduction

Introduction

Brown spots, or by their scientific name solar lentigo or senile lentigo, are indeed a pigmentation disorder which manifests itself in the form of hyper-pigmented macules of brown color, medium dark to very dark, and of different shapes - round , oval or polycyclic - with regular or slightly irregular borders. They are harmless and can even sometimes be confused with freckles, although they differ from the latter mainly in their size, being generally larger, ranging from 5 to 15 mm in diameter. They occur especially in the areas most exposed to the sun such as the back of the hands, the face, the forearms, and the feet (Ruiz-Maldonado et al, 1997).

Brown spots are not dangerous. The only reason to have them treated is cosmetic because we think it will improve our appearance, make us look younger and ultimately improve our self-esteem. There are over-the-counter skins lightening creams of various emulsion types, such as kojic acid serums, vitamin C serums, lactic acid, azelaic and glycolic acid preparations, which are relatively safe. There are also creams based plant extracts such saffron stigmas which is well-known for the bioactivities of its micro-constituent (Agarwal et al, 2017).

Saffron is a slow growing perennial. It is a bulbous plant that produces 6 to 9 sessile leaves surrounded by the lower part. 9 sessile leaves surrounded in their lower part by 4 to 5 leaves. Many chemicals are present in saffron such as carotenoids, flavonoids, carbohydrates, mucilage, monoterpenoids, and 31 compounds have been isolated from saffron petals (Shiva et al., 2009). Saffron also produces anti-ageing effects by decreasing melanogenesis by inhibiting tyrosinase activity (Welss et al., 2004). The site of antioxidant activity of Crocus sativus is due to the presence of Carotenoids, phenolic compounds and flavonoids (Jen et al., 2008), (Farzin et al., 2003).

Today, water-in-oil emulsions are increasingly used in the processing of emollient applications. Water-in-oil (W/O) emulsions offer a number of important advantages over oil-in-water (O/W) emulsions. By forming an occlusive layer on the skin, they effectively reduce water loss from the skin through evaporation. For this reason, they are widely used in formulations targeting the needs of consumers with dry skin.

In this context the purpose of our research consisting on the formulation of a cosmetic cream based on saffron extract. This study was devised in two parts:

- The first part is devoted to a bibliographical study, which includes two main chapters (Chapter I: Generalities on the saffron, chapter II: Cosmetic creams technology).
- The second part concerns the experimental part to formulate our cosmetic cream and evaluate its quality parameter.

  Finally, we ended our work with a conclusion that will summarise the whole study.

Bibliographic synthesis

Chapter I: Generalities on the saffron

I.1. History

Saffron is a spice used since more than 3 000 years. Crocus sativus L. As for the word saffron, it has a Latin origin: «safranal ", from the Arabo-Persian «za'faran» deriving from «asfar " meaning yellow. The term "sativus" means "cultivated," as Crocus sativus is not known to grow wild, but has been cultivated for a very long time for its stigmas (Crozet and al., 2012). The Arabic word zaʿfarān is assumed to derive from a Persian zar-parân, properly "golden-feathered," composed of zar, "gold" and by, "feather" (Boskabady et al., 2008); We can also cite some vernacular names to describe saffron such as autumn saffron, medicinal saffron, cultivated saffron, officinal saffron or saffron of the Gâtinais and given its high price, it bears the nickname of "red gold" (Fournier, 2010).

In 1719, saffron was used by Leeuwenhoek to color animal tissue sections and simplify microscopic analysis. In the 20th century, Conn applied it to detect liver lesions and obtain differential staining of glandular cells in the stomach (Ceccopieri et al., 2021)

Saffron is a geophytic herbaceous plant, whose stigmas have been used since ancient times as a spice in foods, as a coloring agent, in the preparation of perfumes and cosmetics, and for medicinal purposes (Basker and Negbi, 1983); Since then, saffron has been used to treat over 90 diseases (Al, 2015).

Crocus is grown in India, Iran, Spain, Greece, and Italy. The manufacturing process involves a large amount of manual labor and cannot be fully mechanized (Vignolini et al., 2008); it is grown in a wide range of environments with mild to dry climates. For a long time, saffron was neglected by researchers and farmers since it was considered a minor crop used only for agricultural diversification (Gresta et al., 2008).

It is from the IXth century that the culture of saffron appears in Western Europe; indeed it is the Arabs who bring it in North Africa, then the Moorish civilization which spreads it in Muslim Spain (Cardon, 2003).

I.2. Definition

Saffron is a prized food spice derived from the flowers of Crocus sativus (Iridaceae). Saffron has long been known as a medicinal plant (Mousavi et al., 2011), (Khorasany et al., 2016). Saffron is now expected to be an agent that has attracted the interest of physicians (Zhong et al 2020). At present, modern pharmacological research has shown that saffron and its ingredients have many therapeutic effects, such as anticancer (Abdullaev., 2002). anti-microbial (Vahidi., 2010), antineural (Safakhah et al., 2016), hypolipidemic (Asdaq et al., 2010), antidiabetic (Bajerska et al., 2013), anxiolytic (Pitsikas., 2016), antitussive (Hosseinzadeh., 2006), antiobesity (Mashmoul et al., 2013), antitremor (Amin et al., 2015) hypotensive (Imenshahidi et al.,2010), anticonvulsant (Hosseinzadeh et al.,2002), antidepressant (Lopresti et al.,2014),and antiarthritic (W. Liu et al.,2018) .In addition, healing of gastric ulcers (Tamaddonfard et al.,2019), improvement of memory (Finley et al.,2017) and management of metabolic syndrome (Razavi et al.,2017) have been reported for this medicinal plant.

Figure 01: Illustration of saffron flower and stigma (Vol et al. 2021).

I.3. Botanical classification

Crocus sativus is the only Crocus species producing saffron; its taxonomic classification is as follows (Winter halter and Straubinger, 2000) table I:

Table I: Botanical classification of Crocus sativus (G.Dupont, 2007).

Kingdom: Plant
Phylum : Spermatophyte
Sub-branch : Angiosperms (Magnoliophyat)
Class : Monocotyledons (Liliopsida)
Subclass : Liliidae
Order : Liliales
Family : Iridaceae
Subfamily : Crocoïdeae
Genus : Crocus
Species : C.sativus L

I.4.Description of the plant

The saffron is a small perennial plant which grows 10 to 30 cm high. From the center of the bulb, several leaves end in 2 to 3 flowers. The color depends on the level of carotenoid and lycopene inside a stigma, and its size varies rather than the branches, which is always worth 3 (Moshiri et al., 2014), the flowers of Crocus sativus are large, fragrant, blue, purple or lavender. At their appearance, the flowers are protected by whitish membranous bracts. The pistil is composed of an inferior ovary from which a thin style emerges. The style ends with a single stigma composed of three filaments of intense red color whose length exceeds that of the tepals, which are the part of the plant that is interesting for humans (Zubor et al., 2004) figure 02.

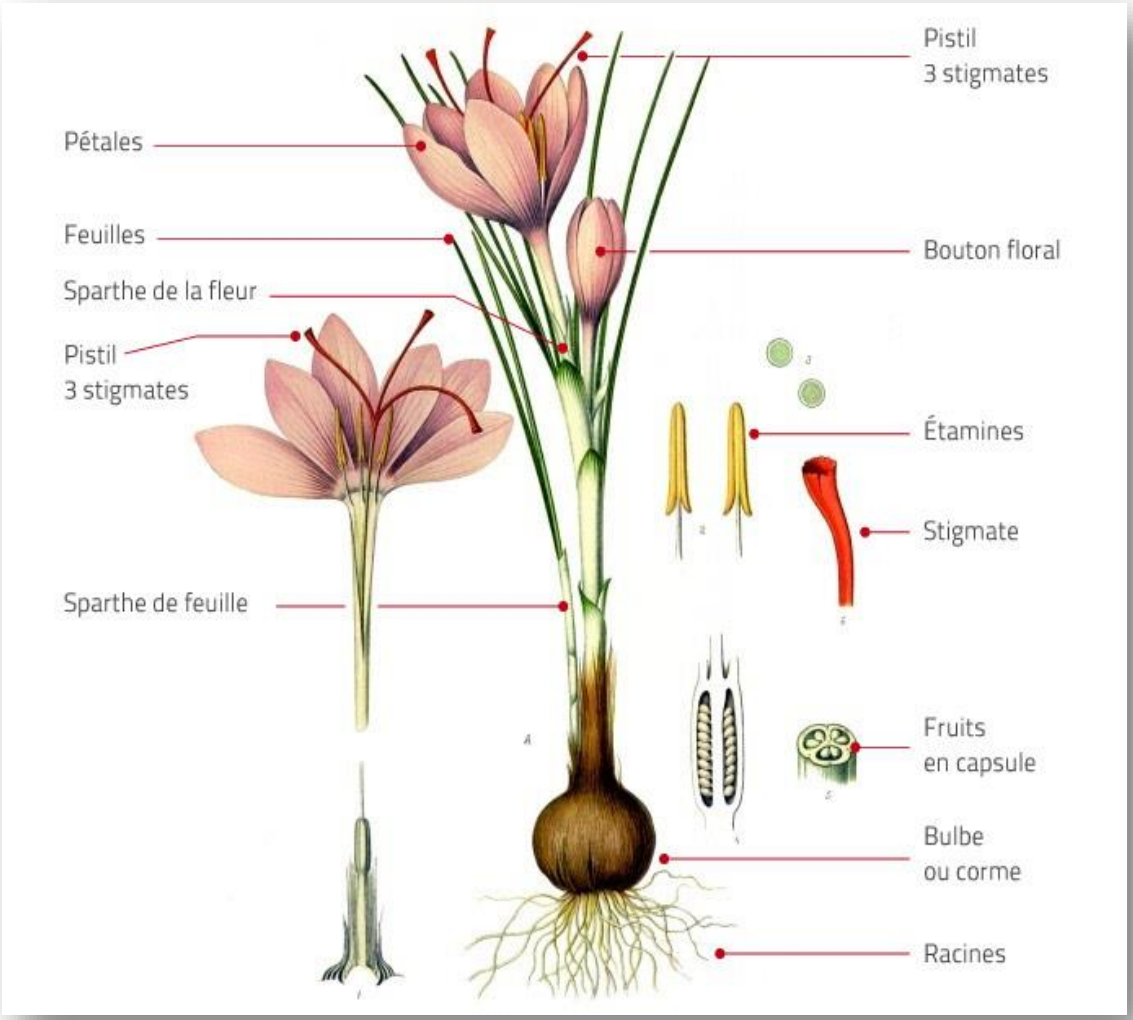


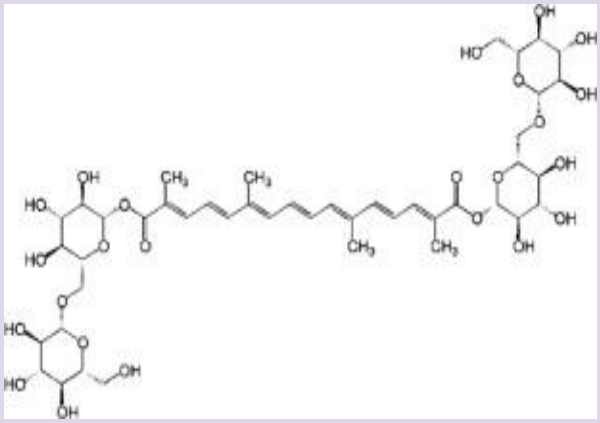
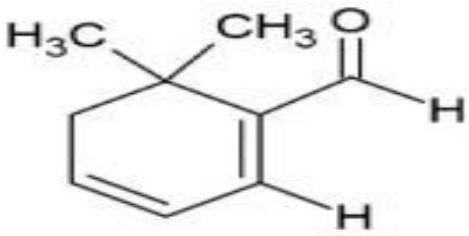
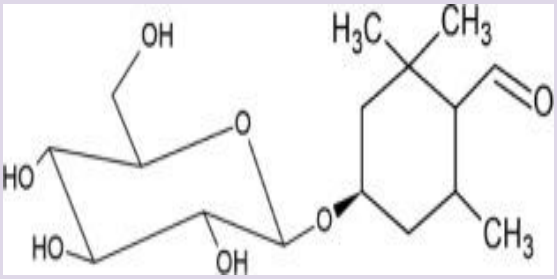
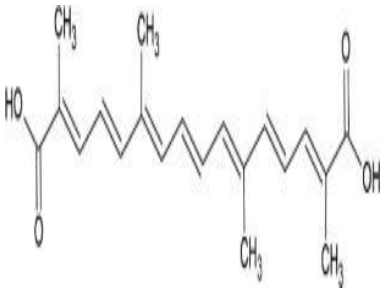
Figure 02: Different parts of the saffron crocus plant (Madan et al., 1996).

I.5.Main bioactive components of saffron

I.5.1.Carotenoids:

Carotenoids are the red, orange and yellow tetraterpenes pigments found in plants, algae and microorganisms, and they are considered important antioxidants and functional ingredients in foods (Gharibzahedi et al. 2013); the impact of saffron carotenoids on human health is due to their high antioxidant capacity (Makhlouf et al.2011); the main carotenoids found in saffron such as crocetin and crocin are actually derived from the metabolism of zeaxanthin (Melnyk et al. 2010) table II.

Table II: Chemical Structure and characteristic properties of Nonvolatile metabolites of Saffron (Palomares, 2015).

Metabolite	Chemical Structure	Characteristic Property	Refs
Crocin		Crocin ($C_{44}H_{64}O_{24}$) is a carotenoid chemical compound primarily responsible for the characteristic color of saffron.	(Palomares, 2015)
Safranal		Safranal ($C_{10}H_{14}O$) is a chemical compound isolated from saffron responsible for the saffron aroma.	(Palomares, 2015) (Eirini et al., 2015)
Picrocrocin		Picrocrocin ($C_{16}H_{26}O_7$) is responsible for the characteristic bitter taste of saffron. it is a monoterpene glycoside precursor of safranal.	(Nikolaos, 2016) (Palomares, 2015) (Rahimi, 2015) (Eirini et al., 2015).
Crocetin		Crocetin ($C_{20}H_{24}O_4$) is a natural apocarotenoid dicarboxylic acid present in the flower of *Crocus sativus* and *Gardenia jasminoides.*	(Rodel and Petrzika, 1991).

I.5.2.The phenolic components

The main mode of aromatic ring formation is via shikimic acid, among which are esters, coumarins, lignans (C6-C3 compounds), flavonoids (C6-C3-C6 compounds) and tannins (Harborne and Swain, 1969).

I.5.3.Flavonoids:

Glycosylation of flavonols at the 3, 7 and 4-OH positions in Crocus sativus results in the formation of flavonols chain .Three glucosides of kaemperol nature have been reported:

- Kaempferol 7-O sophoroside (K7OS),
- Kaempferol 3,7,4 triglucoside (K374T),
- Kaempferol 3-O sophroside 7-O glucopyranoside (K3OS7OG).

Each of these increases with the development of the stigma of Crocus sativus. Gallic acid and pyrogallol are two flavonoids that have been isolated from saffron stigma, while galangin, quercetin and kaempferol have been isolated from fresh saffron petals (Abert et al., 2013).

I.6.Fields of application of saffron

I.6.1. Food area

From ancient times to nowadays, most of the saffron produced is used in the culinary field, for the preparation of fish, rice and different traditional dishes (Joukar et al., 2010) due to its composition as: crocines, picrocrocine and safranal, which contribute to the colour, flavour and aroma respectively (Palomares, 2015).On the other hand it is used as :

- **Colouring power** to improve the appearance of food and eventually make it safer and healthier. Saffron stigmas, even in small quantities, produce a translucent yellow colour. The greater the amount of saffron, the more red the colour tends to be. The compound responsible for the colour of saffron is its bioactive molecule, crocin (cis and Trans) (Archivio et al., 2016).

- **Antioxidant power** that can be used as food additives, are widely acclaimed, not only for their free radical scavenging properties, but also because of the claim of natural origin. Crocins, the major carotenoids in saffron, and safranal, a monoterpene aldehyde, play an important role in antioxidant activity (Assimopoulou and Sinakos, 2005).
- **Flavouring power** used as a flavouring dye. However, during the dehydration process, significant changes in colour, taste and aroma take place in saffron. Safranal (2, 6, 6-trimethyl-1, 3-cyclohexadiene-1- carboxaldehyde) being the main volatile compound responsible for the characteristic aroma of saffron (Urbani et al., 2015).

I.6.2.Therapeutic area

Various pharmacological studies have described that saffron and its constituents (crocin, crocetin and safranal) have several beneficial properties for human health. Saffron extracts containing crocin and crocetin may be useful for the treatment of neurodegenerative diseases and to improve learning capacity and memory (Abek, 2000). Also improves blood flow to the eye, facilitating recovery of retinal function for which it could be used to treat ischaemic retinopathy and/or age-related macular degeneration (Xuan, 1999).

Saffron is not only a spice, but also a very popular medicinal plant used in traditional medicine for cramps, asthma and bronchospasm, menstrual disorders, liver disease and pain, with a soothing and invigorating effect on the gastrointestinal tract (Wintherhalter and Straubinger, 2000).

I.6.3.Cosmetic area

Saffron is used for beauty purposes, absorbed as an infusion, or applied to the skin, mixed with fat or macerated in milks for its beauty properties. In traditional Iranian medicine, saffron can improve skin tone and can be used to treat erysipelas. In traditional Greek medicine, it cools the skin of the face and is used to treat acne, skin diseases and wounds. In addition, the body will look younger and brighter (Mir, 2004). In addition to the anti-oxidant properties, saffron has multiple interests for cosmetic applications. The most promising activities are listed below:

- Anti-UV agent ;
- Perfumery ;
- Saffron as natural pigments in cosmetics;
- Anti-stain agent.

Chapter II: Cosmetic cream technology

I.1.CREAMS

I.1.2.Definition

Thickened creams or emulsions are multiphase preparations composed of a lipophilic phase and a hydrophilic phase. To keep the two phases of a cream stable, it is necessary to add one or more surfactants and some thickening or viscosity agents (Wouessi Djawe, 2015).

I.1.3.Different Types of Cream

There are two types of creams.

I.1.3.1.The hydrophobic creams

Lipophilic creams have a continuous phase (the most important component), the lipophilic phase. They usually contain water-in-oil emulsifying agents such as lanolin, sorbitan esters and monoglycerids (Coulibay Sie Adama, 2018).

I.1.3.2.The hydrophilic creams

The external phase is the water phase. These formulations contain oil-in-water emulsifiers such as sodium or triethanolamine soaps, as well as sulfated fatty alcohols, polysorbates and esters of polyoxyethylene fatty acids and alcohols, optionally in combination with water-in-oil emulsifiers (Coulibay Sie Adama, 2018).

I.2. Emulsion preparation

I.2.1.Preparation of multiple emulsions

The formulation is done in two steps.

- In a first step the classical preparation of the primary emulsion is carried out under rapid agitation. Ex: for O/W/O emulsions it is the O/W emulsion which is prepared first (Lebi Liewa Carine Larissa, 2017).

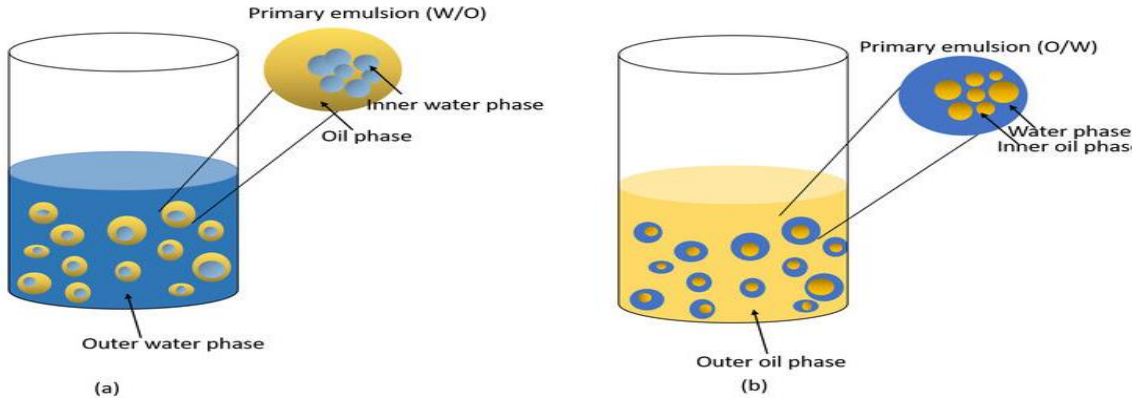


Figure 03: Schema of a) W/O/W and b) O/W/O emulsions (Fraj et al., 2021).

- In a second step, the primary emulsion is prepared in a hydrophilic TA solution with slow agitation. The inner water is separated from the outer water by an oil membrane. In order to stabilize the preparation, it is often necessary to gel one part of the emulsion in order to avoid leakage of the inner water into the outer water (Lebi Liewa Carine Larissa, 2017).

I.2.2.Preparation of simple emulsions

The components are measured in two different beakers according to their hydrophilic or lipophilic character and then heated in a water bath (70°C for W/O emulsions and 90°C for O/W emulsions). The beakers are then removed after complete melting of the fatty phase. Mixing is then carried out using a micro vortex by pouring a thin stream of the hydrophilic phase into the lipophilic phase along a glass rod and keeping the whole under agitation for 5 minutes. The mixing is done under rapid agitation, only when the emulsifier is a self-emulsifying base (Lebi Liewa Carine Larissa, 2017).

We obtain:

- A direct emulsification in the case of O/W emulsions.
- Emulsification by inversion of phase in the case of W/O emulsions.

The cooling is controlled and is carried out with a slow agitation.

The active thermolabile ingredients are introduced at a temperature of 40°C or less and the perfume is added at 30°C (Coulibay Sie Adama, 2018).

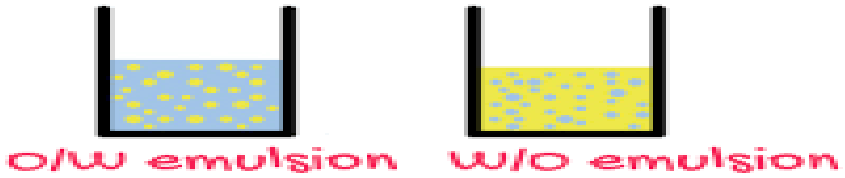


Figure 04: simple emulsions (Adam et al., 2013).

I.3.Physical instability of an emulsion

I.3.1.Particle Migration

➢ Creaming and settling

Creaming is the upward migration of droplets from the dispersed phase, while sedimentation is the downward migration of droplets (Martini, 2011).

➢ Flocculation

During agitation, the droplets dispersed in the continuous phase can aggregate to form agglomerates. This phenomenon then leads to coalescence of the droplets and thus to phase break-up (Martini, 2011).

A. Change in size

➢ Coalescence

The dispersed droplets merge to form larger droplets. This is the opposite of emulsification, that is to say the fragmentation of large droplets into small droplets. This phenomenons result in a phase break.

Most of the phenomena that involve bringing the dispersed droplets closer together lead to coalescence and, eventually, to the breakdown of the emulsion (phase shift) (Le Hir, 2006).

➢ Ostwald ripening

When the solubility between the ingredients of the dispersed and dispersing phases is not zero, the finest droplets of the dispersed phase diffuse into the dispersing phase, hence the term ripening (Le Hir, 2006).

B. Phase reversal

A phase inversion is the change from an O/W emulsion to a W/O emulsion, or vice versa. This phenomenon changes the properties of the product (Wouessi Djewe, 2015).

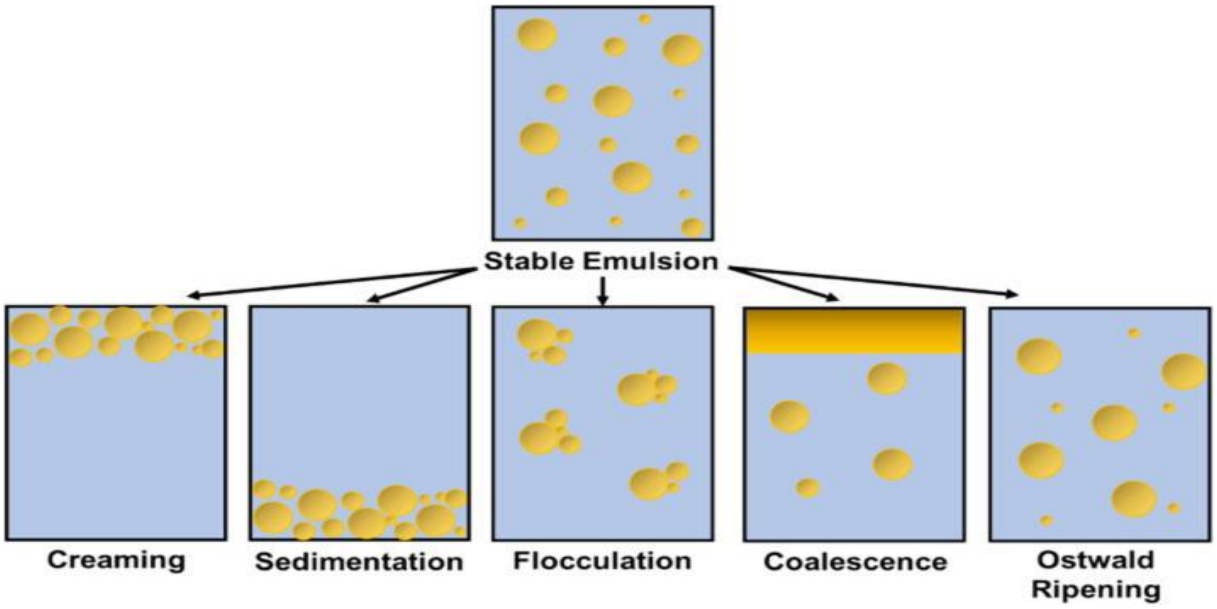


Figure 05: Instability phenomena in emulsions (Ping et al., 2020).

I.4. General consideration on stability of the cosmetic creams

I.4.1.Factors that infleunce stability

I.4.1.1. Extrinsic factors

These are related to external factors to which the product is exposed, such as:

a) Time

The drawing near of the expiry date of the product can lead to alterations in the organoleptic, physical-chemical, microbiological and toxicological characteristics (ISAAC et al., 2012).

b) Temperature

High temperatures accelerate physical-chemical and chemical reactions, generating alterations in: component activity, viscosity, appearance, color and odor of the product (Dutcosky, 2011). Low temperatures accelerate possible physical reactions such as turbidity, precipitation and crystallization. Problems created by high or very low temperatures can also derive from non-conformity during the manufacturing process, storage or transport of the product (Anvisa, 2005).

c) Light and Oxygen

Ultraviolet light along with the oxygen leads to the formation of free radicals and sets in motion oxidation-reduction reactions (Tubarão, 2020). Light-sensitive products should be stored away from light in opaque or dark bottles, and antioxidants should also be added to the formulation to delay oxidation processes (Anvisa, 2005).

d) Humidity

This mainly affects solid cosmetic forms, such as powder, soap bar, eye shadow, bath salts, among others (Anvisa, 2005). Some modifications may occur in the physical aspect of the product, making it soft or sticky or changing the weight or volume, as well as leading to microbiological contamination (ISAAC et al., 2008).

e) Containing Material

The materials used for the container of cosmetic products, such as glass, metal and plastic, can influence their stability (Anvisa, 2005).Suitability tests should be carried out between the container material and the formulation, in other words to determine the best relationship between them.

f) Microorganisms

The cosmetic formulations most likely to be contaminated are those that contain water in their formulation, such as emulsions, gels, suspensions and solutions. The proper and tested use of additive substances (Challenge Test of the additive system) as well as the compliance with Good Manufacturing Practices is necessary for the proper preservation of the formulation (Anvisa, 2005).

g) Vibration

Vibrations during transport can affect the stability of formulations, provoking a phase division of emulsions, solidification of suspensions, and alteration of viscosity, among others. A factor that aggravates the vibration factor is the modification of temperatures during the transport of the product (Anvisa, 2005).

I.4.1.2.Intrinsic factors

These are factors related to nature of the formulations themselves and above all to the interaction of the ingredients among themselves or with the containing material. They result in physical or chemical incompatibilities which may, or may not be apparent to the consumer.

- **Physical incompatibility**

Alterations occur in the formulation's physical appearance, visible as: precipitation, phase separation, crystallization, and formation of cracks, among others (Anvisa, 2005).

- **Chemical incompatibility**

a) pH

Three different aspects connected to pH values must be compatible: the stability of the ingredients in the formulation, the efficacy and the safety of the product (BRASIL, 2004).

b) Reactions of oxidation-reduction

Oxidation or reduction phenomena occur, leading to alterations in the activity of the active ingredients and the organoleptic and physical characteristics of the formulations (Anvisa, 2005).

c) Hydrolysis reactions

These occur through interaction with water. Esters and Amines are more susceptible to them. The higher the percentage of water in the formulation, the more likely the occurrence of this kind of reaction becomes (Anvisa, 2005).

d) Interactions among formulation ingredients

Undesirable chemical reactions that may occur between the ingredients of the formulation, reversing or changing its activity (Anvisa, 2005).

e) Interaction between formulation ingredients and the containing material

Chemical modifications that can lead to physical or chemical changes in the components of the container material and the formulation ingredients (ISAAC et al., 2008).

I.4.2.Aspects to be considered in relation to stability

- **Physical:** the original physical properties must be conserved, such as appearance, color, odor and uniformity, among others (IWAMOTO et al., 2016);
- **Chemical:** properties such as the integrity of the chemical structure, the percentages of the ingredients and other parameters must be maintained within specified limits;
- **Microbiological:** microbiological characteristics must be maintained in accordance with specifications. The accomplishment of good Manufacturing practices and the preservative systems used in the formulation can guarantee these characteristics (Anvisa, 2005).

Aside from these appearances, it is also necessary to consider the maintenance of the product's characteristics in the questions of:

- ✓ **Functionality:** the attributes of the products must be maintained unaltered in relation to the effects originally proposed.
- ✓ **Safety:** significant alterations must not occur that may influence the safety of product use.

I.4.3.When can stability tests be done?

- During the development of new formulations and of laboratory and factory pilot-batches.
- When significant changes occur in the manufacturing process.
- To validate new equipment or production processes.
- When significant changes occur in the raw material being used.
- When significant changes occur in the containing material that comes into direct contact with the product (Anvisa, 2005).

Materials and methods

Materials and methods

The formulation and characterizations, carried out from June 2022 to July 2022, were performed in the food biochemistry laboratory and sensory analysis laboratory. Microscopic observations were carried out on the technical platforms of physico-chemical analyses (PTAPC) of the University of Bejaia.

I. Plant material

The plant material consists of the stigmas of the saffron flower Crocus sativus (Figure 6), the saffron is grown and harvested in Constantine, it was provided by the company Safran-Tariki. The harvest was carried out in 2018 and the saffron has been stored at (-20°C) since that date.

Figure 6: Saffron stigmas photography.

I.1.Saffron extract

the preparation of saffron extract is presented in Figure 07.

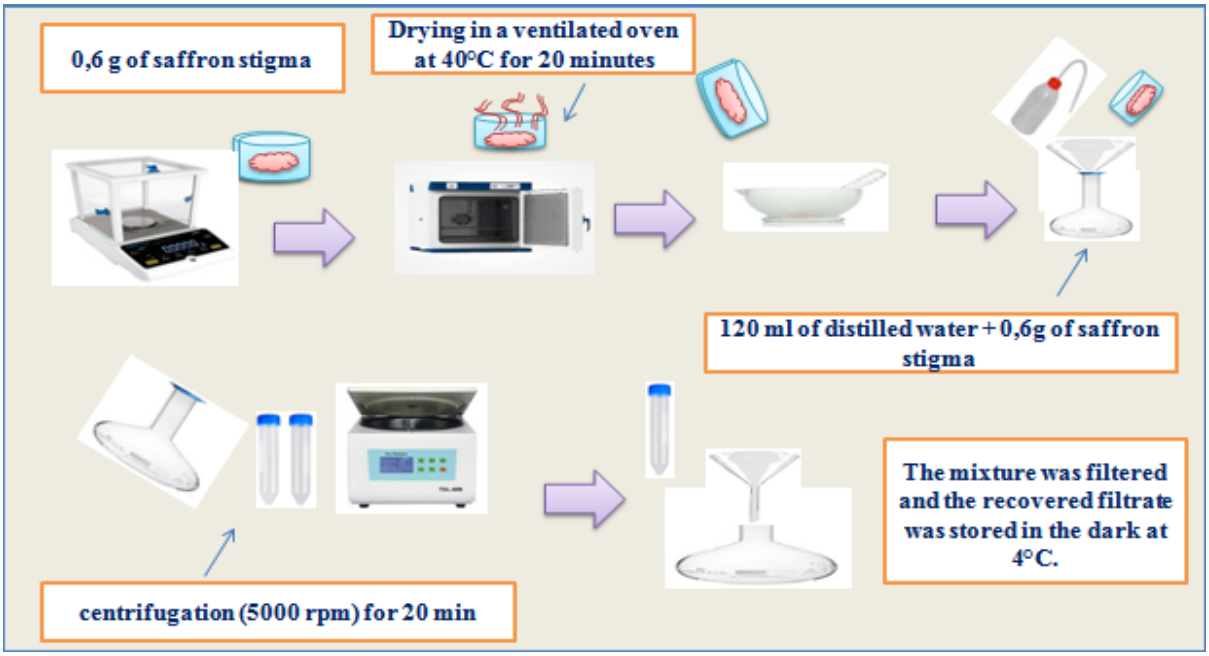


Figure 07 : The saffron extraction.

II.Other raw materials

They are divided into two categories: excipients of the hydrophilic phase and those of the lipophilic phase (**Table III).**

Table III: Hydrophilic and lipophilic excipients.

Excipients of the lipophilic phase	Excipients of the hydrophilic phase
• **Paraffin oil ;**	• Distilled water ;
• **Sesame oil ;**	• Glycerol ;
• **Black cumin oil ;**	• Agar ;
• **Copra oil ;**	• Tween 80 ;
• **Glycerin ;**	• Lactic acid ;
• **Beeswax ;**	• Ascorbic acid;
• **Stearic acid;**	• Benzoic acid.
• **Soap.**	

The roles s of the raw materials used for the preparation of the creams are summarized in the following table:

Table IV: Characteristics of raw materials used in creams and their roles.

Raw materials	Properties	Roles	References
Beeswax	**Solid**	**Stabilising the W/O emulsion**	**Rowe, 2006**
Saffron stigma	**Solid**	**Fight against brown spots**	**Akhtar et al., 2014**
Copra oil	**Semi-solid**	**Anti-ageing and nourishing Action**	**Pavan Kumar et al ., 2018**
Paraffin oil	**Liquid**	**hydrated and plumped up skin**	**Mehrnia et al., 2015**
Black cumin oil	**Liquid**	**Fights against the signs of skin Ageing**	**Tariq,2008**
Sesame oil	**Liquid**	**Protect the epidermis**	**Lin , 2007**
Glycerin	**Liquid**	**Hydrating**	**Vandeputte, 2012**
Stearic acid	**Solid**	**Thickening emulsions**	**Kuzdzal, 1971**
Agar	**Solid**	**Emulsifiant**	**Dalmazzone, 2000**
Glycerol	**Liquid**	**Prevents skin from drying out**	**Jean –marie, 2005**
Tween 80	**Liquid**	**Surfactants**	**Hasenhuettel, 2008**
Lactic acid	**Liquid**	**To stimulate collagen synthesis**	**Kerdudo, 2014**
Benzoic acid	**Solid**	**Anti-microbial preservative**	**Raymond et al., 2009**
Ascorbic acid	**Solid**	**Bleaching agent**	**Reginald et al., 2000**
Vitamin E	**Liquid**	**Anti-aging**	**Khazdair et al., 2021**
Rose water	**Liquid**	**Fragrance**	**Elkassouani,2013**
Lemon essential oil	**Liquid**	**Fresh smell for the cream**	**Misharina et al., 2007**
Mandarin essential oil	**Liquid**	**Fresh scent**	**Marie, 2020**
Distilled water	**Liquid**	**/**	**Mehrnia et al., 2015**

II.1. Preparation of creams

The ingredients used for the preparation of the creams are mentioned in the Tables (V and VI). All formulations are prepared by using the water-in-oil (W/O) emulsion; this method allows the preparation of creams with short production times and low energy costs (Hua et al., 2019; Moldoven et al., 2021) with some modifications.

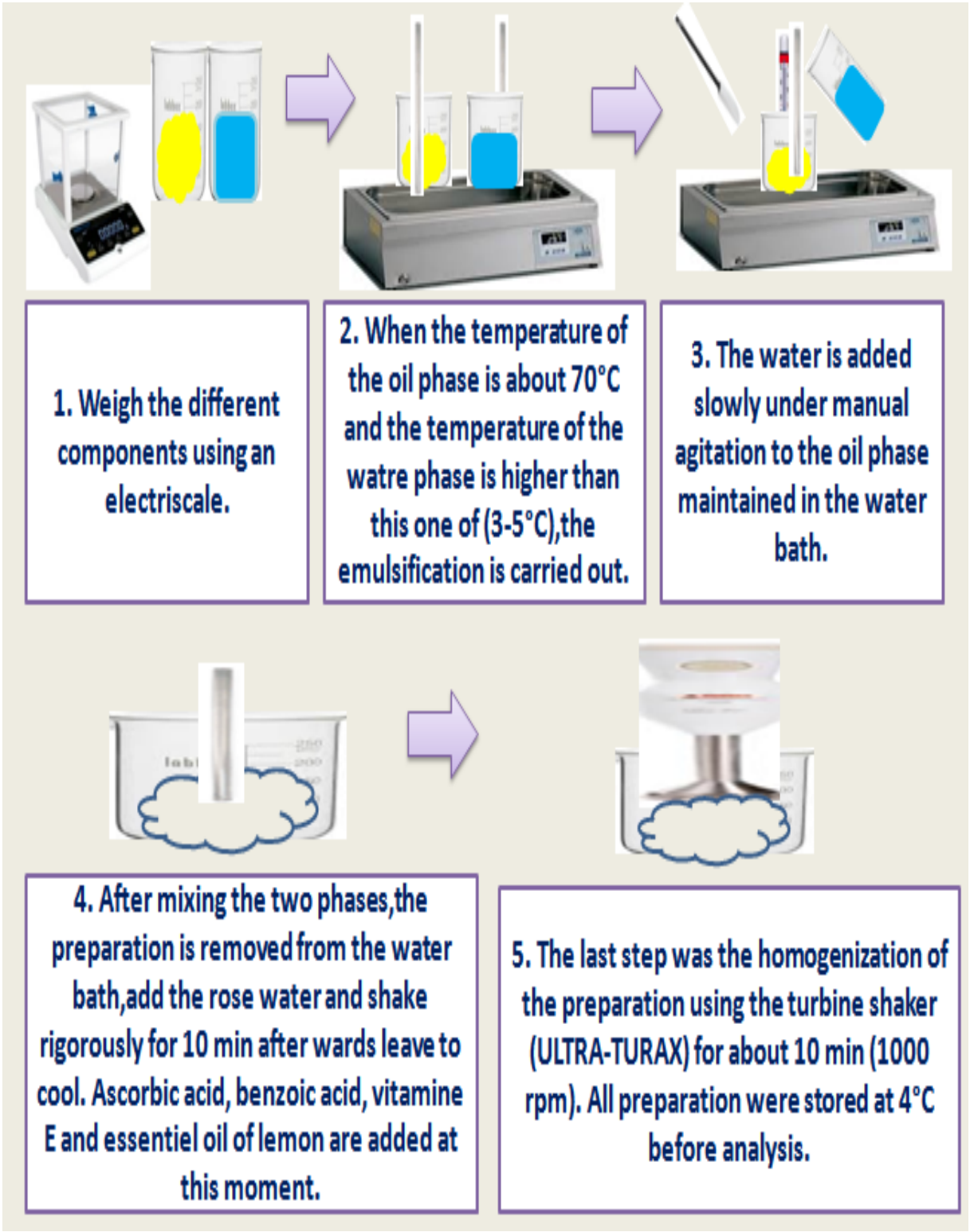


Figure 08: Schematic of the preparation of cosmetic creams (Hua et *al.*, 2019; Moldovan et *al.*, 2021).

➢ **The basic formula of our cream**

After a series of researches undergo some modification the following formula was tried to be realized: (Akhtar et al., 2011; Vyas et al., 2010).

Table V: Basic formula of the prepared creams.

Lipophilic phase excipients	Hydrophilic phase excipients	Additives (for 100g of cream)
Paraffin oil (10%) ; Sesame oil (1%) ; Black cumin oil (1%) ; Copra oil (3%); Glycerin (1%); Beeswax (2%); Stearic acid (2%); Soap (4%).	Distilled water (60%); Glycerol (2%); Agar (3%); Tween 80 (3%); Lactic acid (2%); Benzoic acid (1%).	Ascorbic acid (1%); Lemon essential oil (3 drops); Mandarin essential oil (1 drop); Vitamin E (2 capsules); Rose water (3 drops).

Table VI: The prepared extractfor saffron creams.

Creams	Preparation((for 100g of cream)
Cream 1	0% saffron extracts (60ml distilled water).
Cream 2	0,075 %(45ml distilled water + 15ml saffron extract).
Cream 3	0.15% (30ml distilled water + 30ml saffron extract).
Cream 4	0, 3% (60ml saffron extract).

II.2. Preparation of creams extracts

Dissolve 0.5g of each prepared cream (0.3%; 0.15%, 0.075% saffron extract creams and control cream) in 9.5ml of distilled water. Centrifuge the extracts at 3000Ts/30mn then recover the supernatant (Gani et al., 2020). As illustrated in following Figure 09:

Figure 09: Extract creams protocol (Gani et al., 2020).

III. Quantification of phenolic compounds in creams and saffron

III.1.Determination of total polyphenols and flavonoids

III.1.1. Total polyphenols

- **principe**

Polyphenols are a wide range of plant molecules, whose chemical nature and content are extremely variable from one species to another. Several analytical methods can be used for the quantification of total phenols (Singleton et al., 1999). The amount of total phenolic compounds in the saffron extract was determined using the Folin-Ciocalteu reagent according to (Halici *et al.*, 2005).

- **protocol**

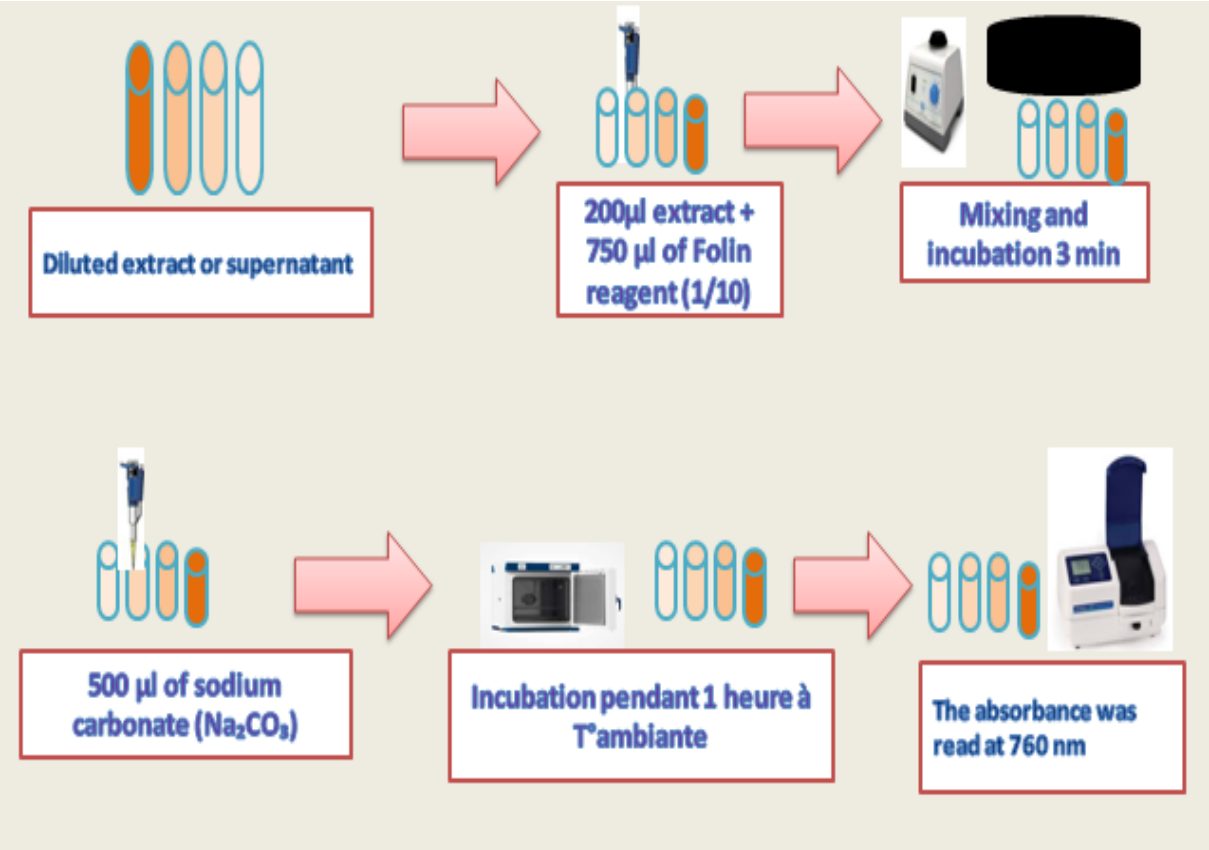


Figure 10: The total polyphenols protocol (Nickavar et al., 2008).

III.1.2. Determination of total flavonoids

❖ Principe

Quantification of total flavonoids in the extracts was carried out using the aluminium trichloride (AlCl3) method (YIM.L et al., 2008).

❖ protocol

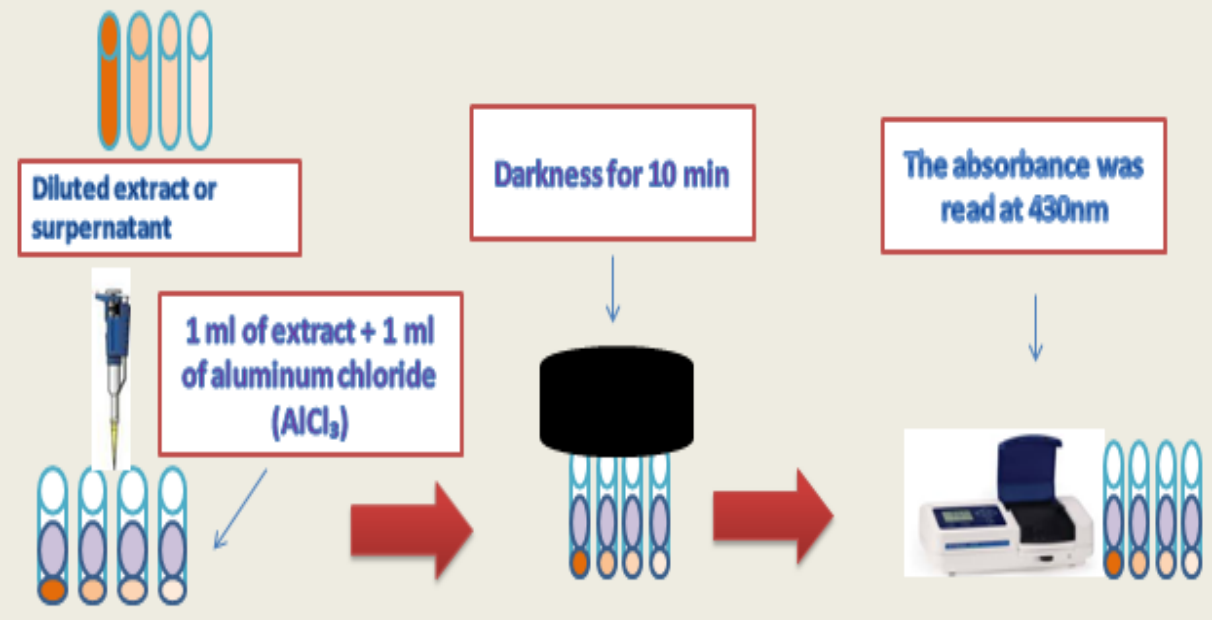


Figure 11: Determination of the total flavonoids (Nickavar et al., 2008).

VI.Evaluation of the antioxidant activity of creams and saffron

VI.1. DPPH radical scavenging activity

❖ Principe

The method is based on the reduction of the stable free radical DPPH in the presence of a hydrogen-donating antioxidant, and the formation of the non-radical form DPPH-H as a result of the reaction (Lahmass et al., 2018).

❖ protocol

The measurement of DPPH radical trapping was carried out according to the method described by (Brand-Williams et al.1995).The method wers illustrated in the following Figure 12:

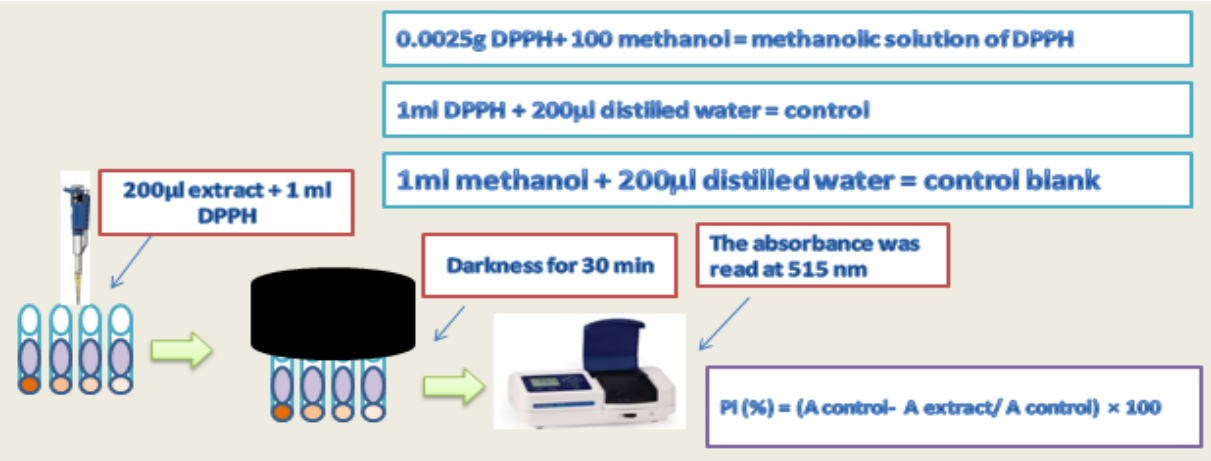


Figure 12: Evaluation of antioxidant activity (DPPH test) (Brand-Williams et al., 1995).

VI.1. Reducing power test

❖ Principe

The reducing power determination is based on the reduction of Fe^{3+} to Fe^{2+} by giving electron from sample or antioxidant. Increased absorbance indicates increased ability of the extracts to act as antioxidants by donating electrons (Lahmass et al., 2018). The method wers illustrated in the following Figure 13:

Protocol

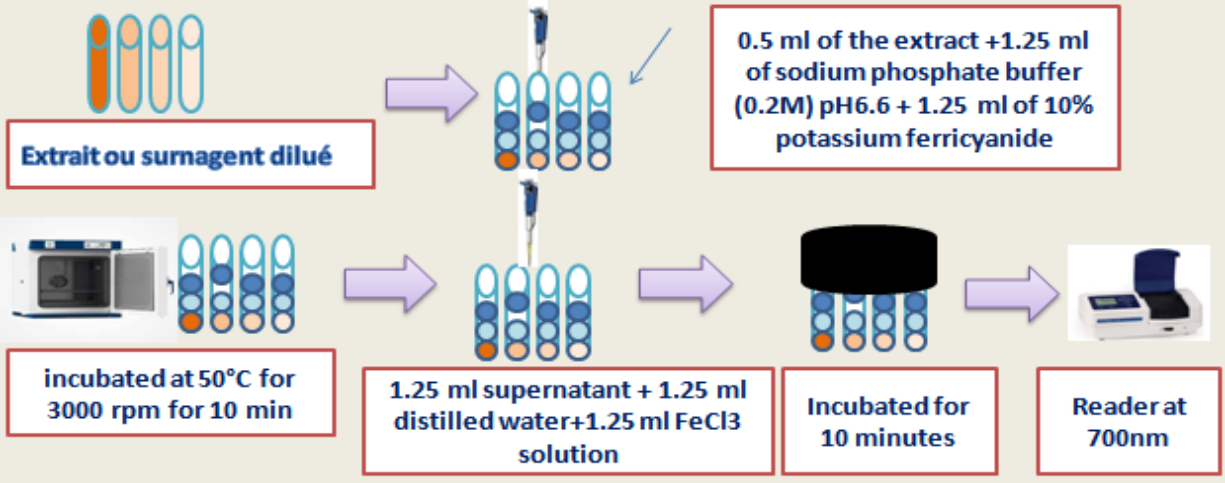


Figure 13: Reducing power test (Lahmass et al., 2018).

V. Stability test for cosmetic creams

Several analysis were performed for testing the stability of cosmetic creams: such as centrifugation, mechanical vibration, and light tests; pH, density, and viscosity determination; spectrophotometric assays, in addition, the accelerated and microbial stability tests (Baby et al., 2007).

V.1. Nature of emulsion (Dyeing method)

❖ Principe

The principle of this method (Figure 14) is based on the fact that a drop of hydrophilic or lipophilic dye mixed with a drop of the emulsion may or may not dissolve in its external phase, coloring the latter homogeneously or not. The dye used is methylene blue (Le Hir, 2009).

❖ protocol

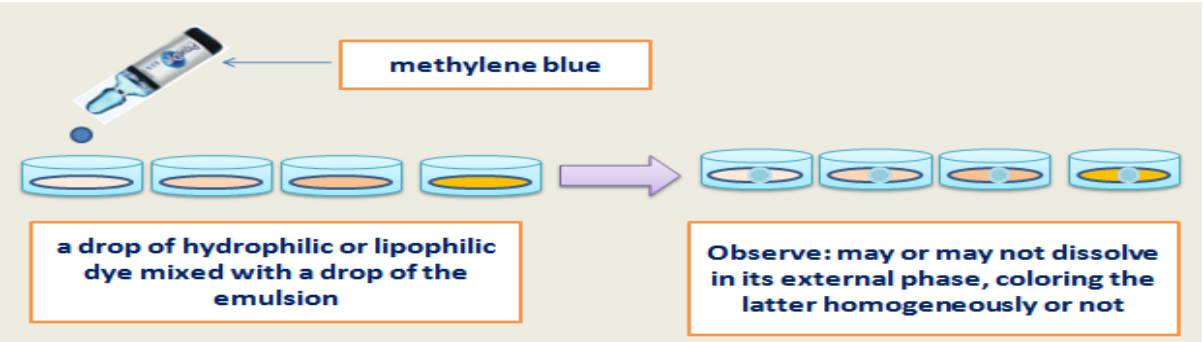


Figure 14: The Dyeing method protocol (Le Hir, 2009).

V.2. Determination of pH

It consisted of measuring the pH of creams , dipping the electrode of the pH meter in them and taking the reading (Monique et al., 1996).

- ✓ After calibration, wash the electrodes first with distilled water.
- ✓ Homogenized the sample, add a sufficient volume to the measuring vessel and immerse the electrodes.
- ✓ Check that the indication given by the pH meter is stable after one minute.
- ✓ Then read the PH.

V.3.Humidity test

This test consists of determining the water content in creams, according to (Tovar et al., 2002).

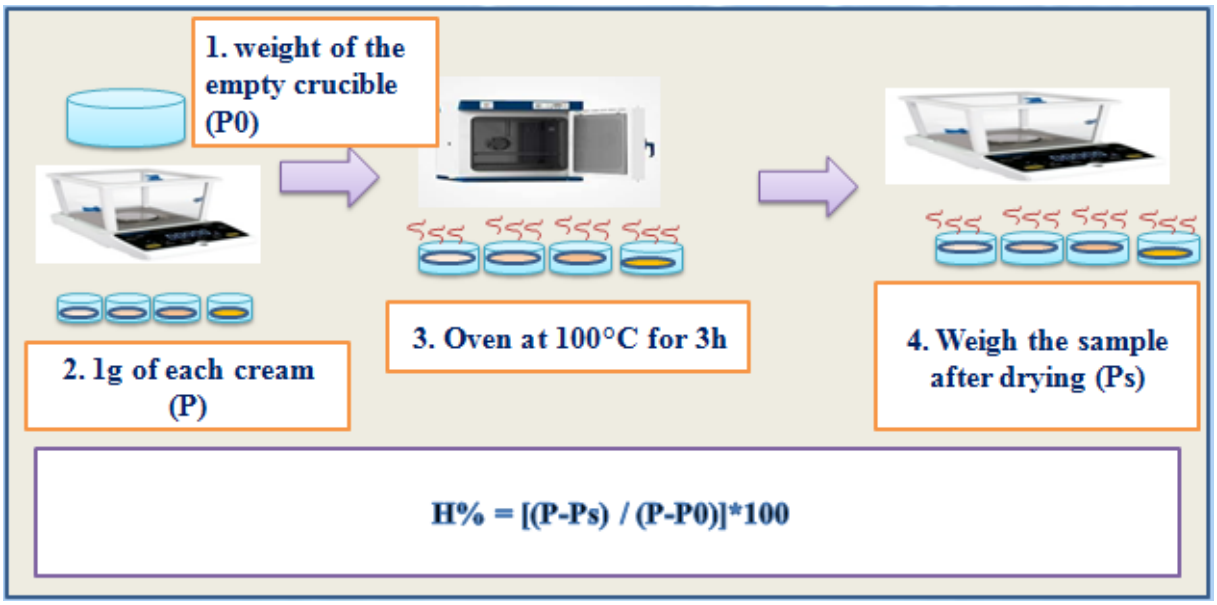


Figure 15: Humidity test (Tovar et al., 2002).

H%: moisture of the creams expressed as a percentage.

P and Ps: weight of the cup and sample before and after drying respectively.

P0: weight of empty crucible.

V.4. Determination of density

❖ Principe

The density is the ratio of the mass of a substance to the volume it occupies, and generally for liquids or semi-solids, this parameter can indicate the incorporation of air or the loss of volatile ingredients (Anvisa, 2005).

❖ protocol

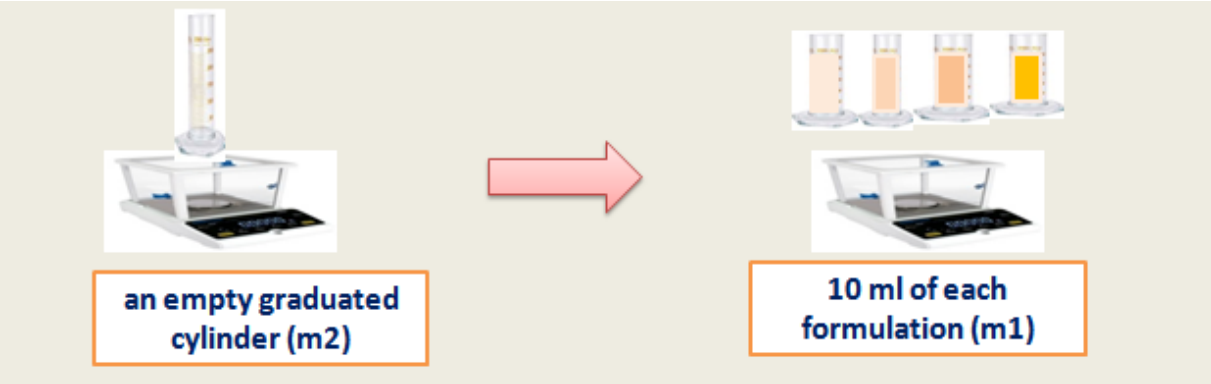


Figure 16: Density test of creams (Anvisa, 2005).

V.5.Assessment of particle size

❖ Principe

It is used to assess the homogeneity of the globules in the emulsion. The size (average diameter) of the particles in the dispersed phase is measured using a micrometer scale. The pharmacopoeia recommends examining at least 300 globules. Theoretically, the globules of an emulsion have a size between 0.5 and 50 µm (Table VI) and should be approximately identical (Liewa and Larissa, 2017).

Table VII: Appearance of emulsions according to globule size (Lebi, 2017)

Aspect of the emulsion	Globule size
Rough emulsions ± stable	>5µm
Medium milky white emulsion	1-5µm
Fine emulsions with bluish highlights Slightly translucent emulsions	0,1-1µm
Translucent microemulsions Micellar solutions	<0,1µm

❖ **protocol**

The size of the globules was determined using an optical microscope with a camera (x40 objective) available at the technical platforms for physico-chemical analysis. A drop of emulsion (neither diluted nor colored) the size of a pinhead was placed between the slide and the slat. The examination was carried out immediately. The homogeneity or heterogeneity of the emulsion and the shape of the dispersed globules were noted (Pierat, 2010).

V.6.Centrifugation test

❖ **Principe**

The centrifuging test produces stress in the sample, simulating an increase in the force of gravity and increasing the mobility of the particles thus anticipating possible instabilities. These changes may appear in the form of precipitation, separation of phases, caking, or coalescence among others. The sample is centrifuged at a standardized temperature, time and speed. Afterwards, the sample is visually evaluated (Anvisa, 2004).

The centrifugation period, the cosmetic formulations were examined for phase separation which is an indication of cosmetic formulation instability (Amira., 2017).

❖ **Protocol**

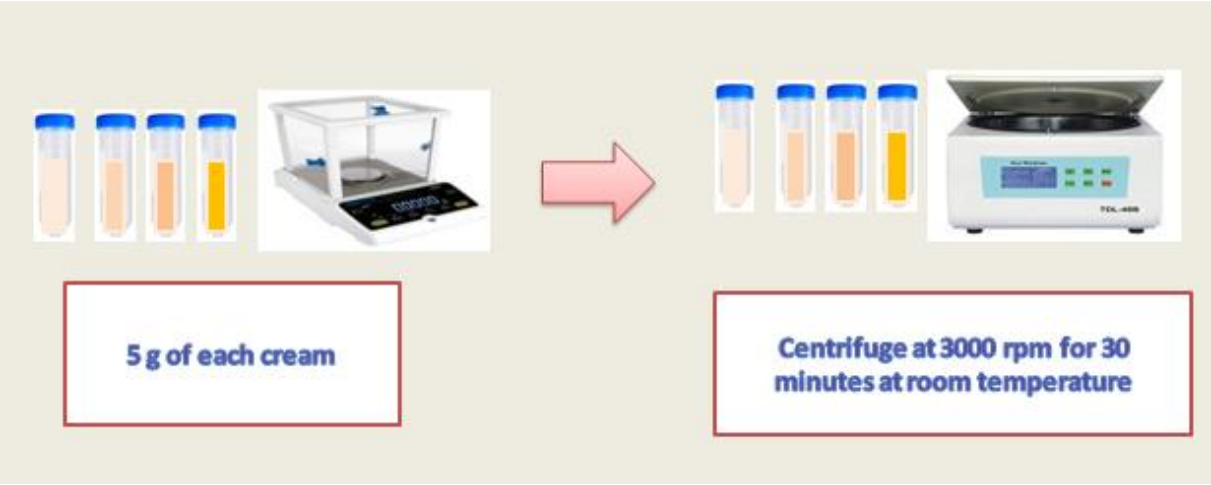


Figure 17: Centrifugation test (Anvisa, 2004).

V.7. Mechanical vibration test

❖ Principe

This test evaluates the stability of the cosmetic formulation when submitted to mechanical vibration movement, which may cause instability detected as phase separation.

❖ protocol

Figure 18: Vibration test of creams (Anvisa, 2004).

V.8. Microbial stability

❖ Principe

The microbial stability of the cosmetic formulations was evaluated through the microbial contamination test. After being prepared (Appendix 04) the culture media, they were autoclaved at 125°C for 20 minutes and then 20 mL of the culture medium was poured into a sterile petri dish (Bouranen, 2017).

❖ protocol

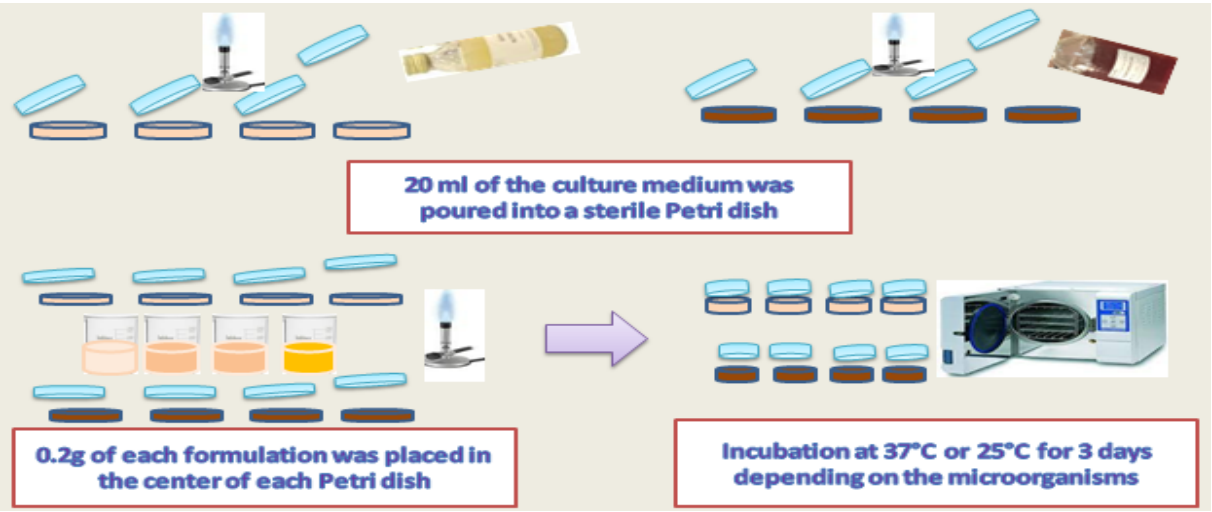


Figure 19: Microbial stability (Bouranen, 2017).

VI. Preliminary test of the depigmentation effect of creams

Two women volunteers were selected, one aged 26 and the other 49. They were examined for skin disease, particularly on the cheeks and forearms.They was not informed about the content of the formulations. Prior to the application of the formulation, a patch test was performed on the forearms of the volunteers for 48 hours to exclude any allergic reaction to any of the components of the formulation. On the second day, each volunteer received the formulation and were given appropriate instructions on the application of the formulation (Naveed Akhtar et al., 2014).

Figure 20: Depigmentation test protocol (Naveed et al., 2014).

VII. Sensory evaluation of creams

The sensory evaluation was carried out at the sensory analysis laboratory of the University of Bejaia**.** Macroscopic observation of emulsions was performed with the naked eye directly on the preserved emulsions. The main characteristics observed are: colour, physical aspects, consistency and homogeneity of the preparation (Savic et al., 2021).

Four samples of creams were presented for each taster with a questionnaire to be completed see (Appendix 07).The cream samples presented were coded as follows:

- Cream 145 (0% saffron);
- Cream 238 (0,75% saffron);
- Cream 396 (0,15% saffron);
- Cream 456(0, 3% de saffron).

The data collected from the questionnaires distributed to the judges were processed using the software XL STAT version 2014, which is a complete tool for data analysis and statistics, involved in marketing studies and consumer behavior analysis. This software uses Microsoft Excel as an interface for data recovery and display of results. However, all mathematical calculations are performed outside of Excel. Access to the various modules is possible through menus and toolbars (Addinsoft, 2013).

The main features of this software used to interpret the results of the sensory evaluation performed are: Product characterization, principal component analysis (PCA) and grouping the panelists according to their preferences by performing agglomerative hierarchical clustering (AHC) and Preference Mapping (PREFMAP).

Figure 21: Sensory properties protocol (Savic et al., 2021).

Results and discussion

I. Preparation of creams and extract

In this study we prepared four cosmetic creams (Figure24):

1-Cream 1 (0% saffron extract).

2- Cream 2 (0,075% saffron extract).

3- Cream 3 (0, 15% saffron extract).

4- Cream 4 (0, 3% saffron extract).

Figure 22: Creams prepared.

II. Determination of phenolic compounds

II.1. Phenolic and flavonoids compounds of creams

The results obtained are presented in Figure 25, which show that the creams contain a high content of polyphenols and flavonoids. The cream with 0.3% saffron is the richest in phenolic compounds compared to the other creams. Thus the phenolic content of the creams is proportional to the amount of saffron added.

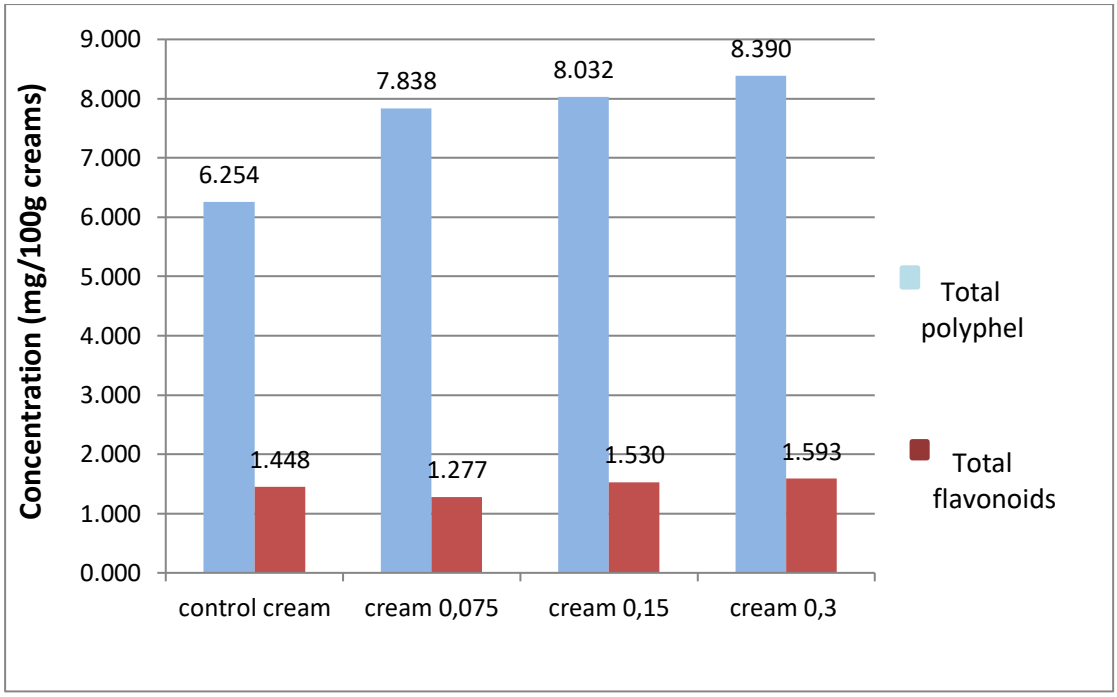


Figure 23: Phenolic contents of creams.

II.2. Determination of saffron phenolic compounds

The content of phenolic compounds in saffron extract was calculated from the calibration curves (Appendix 05). The results obtained are 197.287± 0.274 mg gallic acid equivalents/g of saffron for the total polyphenolic and 11.949 ± 0.133 mg quercetin (Appendix 06) equivalents /g saffron for the flavonoids.

III.Results of the evaluation of antioxidant activities

III.1. Results of the evaluation of antioxidant activities of creams prepared

➢ DPPH test

The results obtained are presented in the Figures 26 and 27. The Monitoring of DPPH inhibition by cream extracts over a period of 15 days; shows that antioxidant activity decreases over time and the cream with 0.3% saffron has a better antioxidant activity.

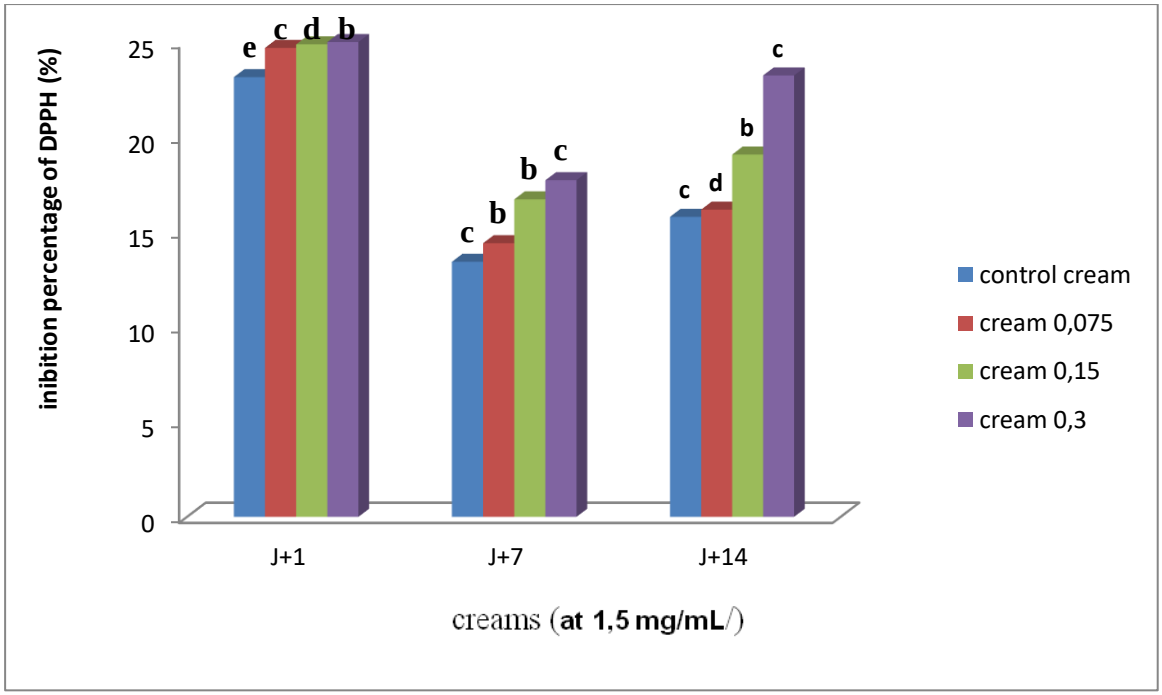


Figure 24: DPPH inhibition of prepared formulations during 15 days.

The histogram of inhibition of DPPH according to the concentration of extract of saffron present in the creams during 15 days, the results of antioxidants (DPPH) of the formulations present a strong percentage (%) of inhibition of DPPH in the D+1, and they are weak in the D+7 and D+14.in concluded that the percentage (%) of antioxidants (DPPH) decreases during the 15 days.

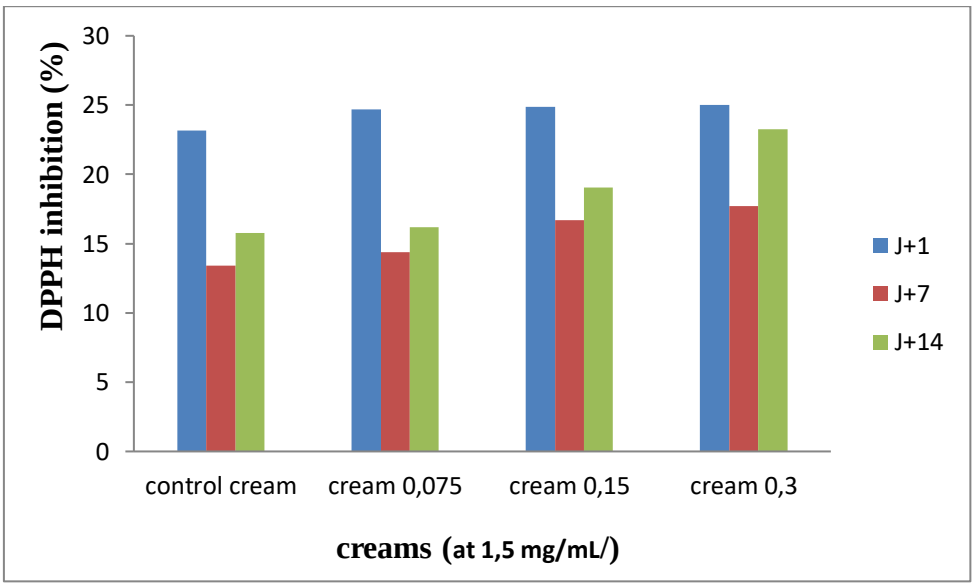


Figure 25: DPPH inhibition of prepared formulations.

➢ Reducing power

The evaluation of the reducing power is based on the reduction of the ferric complex (Fe+3) of the ferricyanide complex to ferrous iron (Fe+2), in the presence of reducing antioxidants, whose colour is green which is proportional to the reducing power of the extract (Gülçin et al., 2003). Our data in the figure 26 showed that the reducing power activity of different creams (control cream; 0.075% cream; 0.15% cream; 0.3% cream). The reducing power activity increases with the increase of the saffron extract concentration in the cream. These results could be due to its high content of phenolic compounds (polyphenols, flavonoids, tannins...).

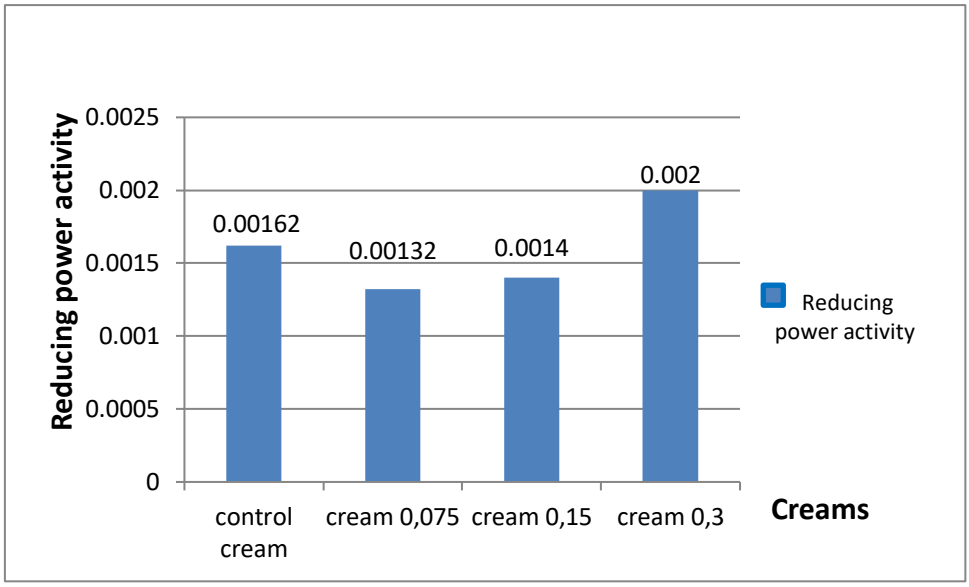


Figure 26: Reducing power results of prepared formulation.

III.2. Evaluation of antioxidant activation of saffron extracts

➢ Antioxidant activity assessment (DPPH test)

The data corresponding to the incubations of DPPH with different concentrations of saffron extracts are presented in Figure 29 which shows that our results with IC50=2,060 of mg/ml (IC50: the median inhibition concentration).

Possesses a stronger anti-free radical activity compared to the extract Moroccan saffron extract has an antioxidant power corresponding to an IC50 = 0.32 ± 0.059 mg/ml (Jadouali et al., 2019).

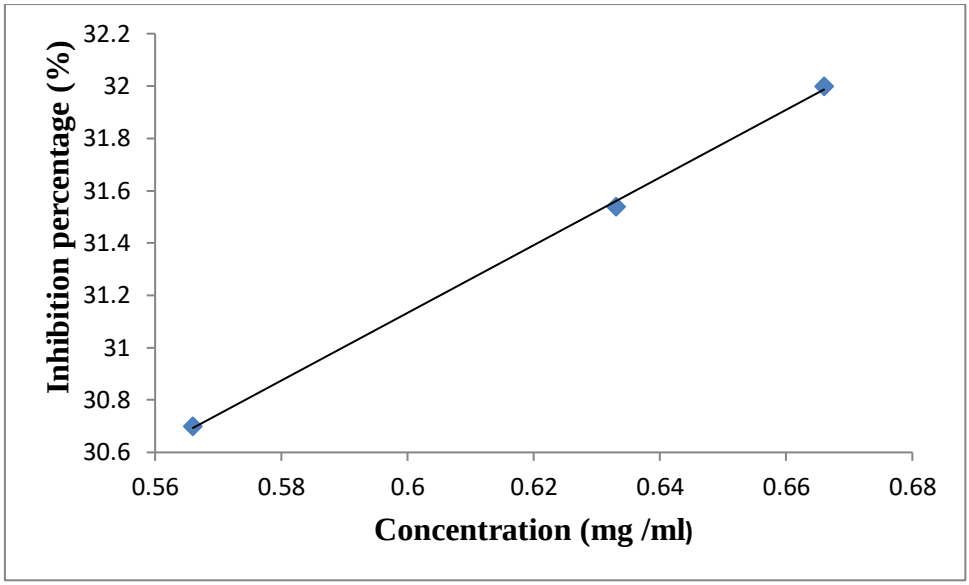


Figure 27: DPPH results in percentage of inhibition achieved on the saffron extract.

➢ Evaluation of reducing power

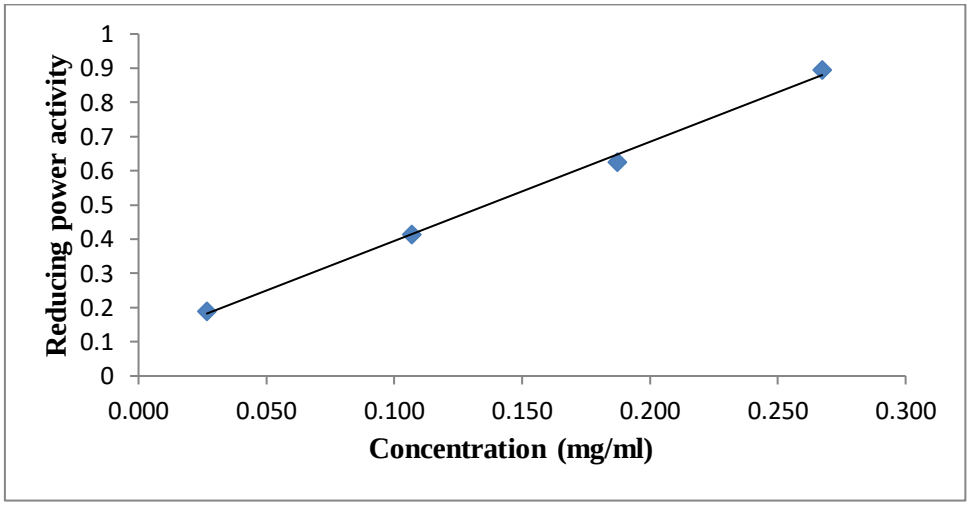


Figure 28: Reducing power results of saffron stigma extracts.

Our data in Figure 30 showed that the reducing power activity of different concentrations of saffron stigma extracts. Our saffron extracts possess higher reducing power activity (IC 50= 0,136 mg/ml) due to the presence of higher total phenolic and flavonoids, which play a major role in reducing power activity (Siddhuraju et al., 2003). For this reason, our saffron extracts can stop the chain reaction of radicals by giving electrons to free radicals and reacting with them. Thus converting them into more stable products.

IV.Stability tests on the Cream

IV.1.The meaning of emulsion (Dyeing method)

In the dye method, good diffusion was observed when methylene blue was added to the creams (Figure 31), which shows that our formulation is a oil-in-water type. In addition, water-in-oil emulsions are the most preferable (Abels et al., 2009).

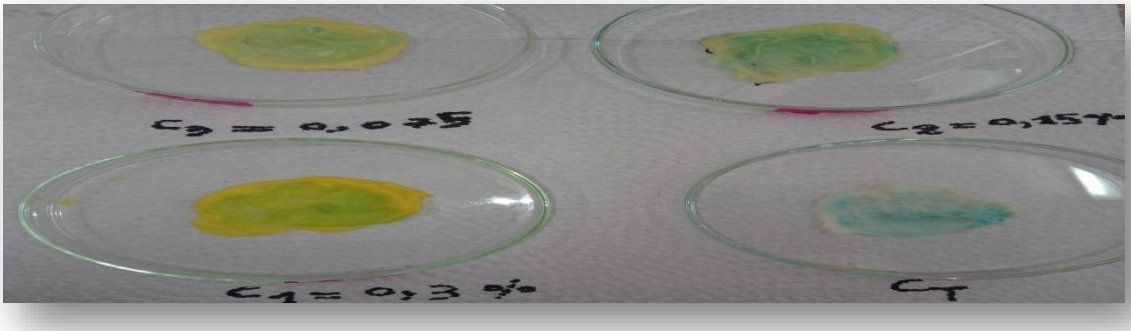


Figure 29: Emulsion sense results by the dye method.

VI.2.Determination of pH

The results obtained for cream stored at different temperatures (Table IX). The storage conditions did not influence the stability of the pH of our formulation, which remained compatible with that of the skin, according to the literature (Rosso et al, 1995).

The pH of our cream was the pH of cosmetic formulas, which is generally between 5 and 7. These preparations are thus adapted to a cutaneous application since they are compatible with the pH of the skin included between 5 and 7.

Table VIII: pH of creams under different conditions.

Creams	T= 6°C	T=27°C	T=40°C
Control cream	5,595	5,501	5,460
Cream 0,075	5,739	5,655	5,609
Cream 0,15	5,494	5,389	5,319
Cream 0,3	5,508	5,491	5,450

VI.3. Determination of humidity

The water content of creams guarantees the hydration of the epidermal layer (Palma et al., 2015). For example, a cream may contain 60-80% water, so its proportional importance makes its quality paramount. Based on this data, the water content of our creams is about 61%, which guarantees the hydration of the skin.

Table IX: The moisture content of creams.

creams	Control cream	Cream 0,075	Cream 0,15	Cream 0,3
Humidity (%)	64	62	60	60

VI.4. Determination of density

The density can give an indication of the incorporation of air or the loss of volatile ingredients in the case of liquids or semi-solids (Anvisa, 2005). The apparent density was determined by calculating the ratio between the mass of the formulation and the volume it occupies.

Results obtained are listed on Table XI. The density of our formulation is 0.91g/ml, which is near to the values recorded for the other formulations which are between 1.0 and 1.2 g/mL (Amira., 2017). Thus, we can underline that the compounds (saffron extract, sesame oil and lemon essential oil…) incorporated in the cosmetic formulations did not affect the density of the base formulations.

Table X: Density results.

Empty test tube (g)	Test tube + cream (g)	Volume of cream (ml)	Cream (g)	Density (g/ml)
21,603	26,171	5	4,568	**0,91**

VI.5. Assessment of particle size

Optical microscopy usually combines the power of a microscope with the image acquisition capability of a digital camera. It provides a direct estimate of the size, shape and quality of the dispersion of particles (Merkus, H.G., 2009). The creams presented a dominance of droplets are of different sizes between 4 and 19 µm, the sample is therefore polydisperse. We notice from the table that our creams are Rough emulsions ± stable. So the creams were coarse (Aka et al, 2013).

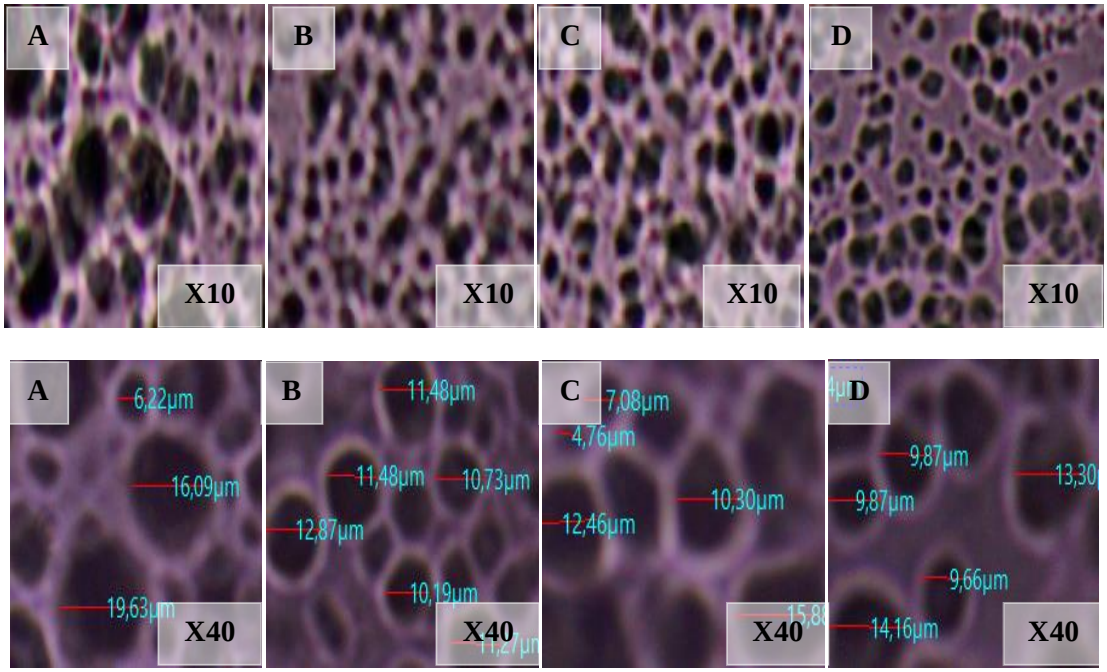


Figure 30: Evaluation of the granulometry of creams: (A) control cream, (B) cream 0, 75 %,(C) cream 0,15%,(D) cream 0,3%.

VI.6.Centrifugation test

The centrifugation test causes stress in the sample, simulating an increase in the force of gravity and increasing the mobility of the particles, thus anticipating possible instabilities. These changes can appear as a precipitation, phase separation, caking or coalescence, among others (Anvisa, 2005).

The formulations were centrifuged for 30 minutes. Then, they were visually evaluated for each formulation, as shown in the Figure 33. On the basis of these results, we can highlight that the phenomenon of sedimentation was observed (figure) which shows that our creams are unstable.

Figure 31: Physical evaluation (centrifugation) of aspect formulations.

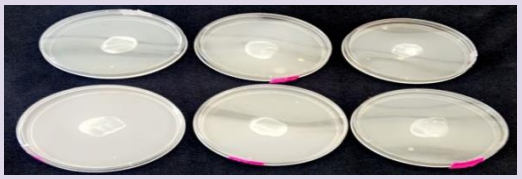
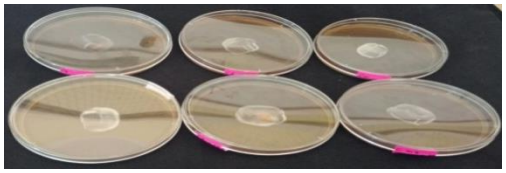
VI.7.Mechanical vibration test

Vibration during transportation may affect the stability of the formulations, causing a separation of the phases of emulsions, solidification of suspensions, alteration of viscosity, among others (Anvisa, 2005). At the end of the vibration test, none of the cosmetic formulations showed any phase separation, which is an indication of their physical stability.

VI.8. Microbial stability

The microbial contamination poses a risk to the safety of consumers and to the brand image of cosmetics (Amira., 2017). Therefore, it is crucial to assess the microbial stability of products in order to ensure their safety. For this purpose, in the Saffron creams (0.075%, 0.15% and 0.3%) no microbial and bacterial growth was observed in the incorporated formulations, but bacterial and microbial growth was observed in the control cream. For the Oxytetracycline-glucose-yeast agar (OGA) results, the creams showed no growth of yeasts or moulds, as shown in Figure. The results obtained confirmed the microbial stability of our formulations.

Table XI: Microbial stability evaluation.

Desired germs	Temperature	Duration	Results
bacteria and micro-organisms	125°C	01 day	
yeasts and moulds	125°C	03 days	

V. Preliminary test of the depigmentation effect of creams

The Figure 35 shows two volunteers (a woman aged 49 and another aged 26), who applied the 0.3% cream for 20 days as a night cream, taking photos before and after applying the cream every week under the same conditions. The results of our formulation were significant due to the presence of antioxidants. The antioxidant activity is mainly presented by monoterpenoids, crocin, quercetin, kaempferol and other phenolic components of Crocus sativus. The site of action of these compounds in the reduction of melanin in the skin is the inhibition of tyrosinase activity (Li CY et al., 2002). It was concluded that tyrosinase is a copper-containing enzyme that has various functions. It is glycosylated and found exclusively in melanocytes (Balakrishnan et al., 2011). It catalyses the conversion of Tyrosine to L-DOPA which is then converted to dopaquinone and then dopachrome (Shirota S et al., 1994). Dopachrome polymerises to form melanin. Inhibition of the tyrosinase enzyme inhibits the production of melanin which helps to eliminate hyperpigmentation of the skin. (Prity Rathee et al., 2021).

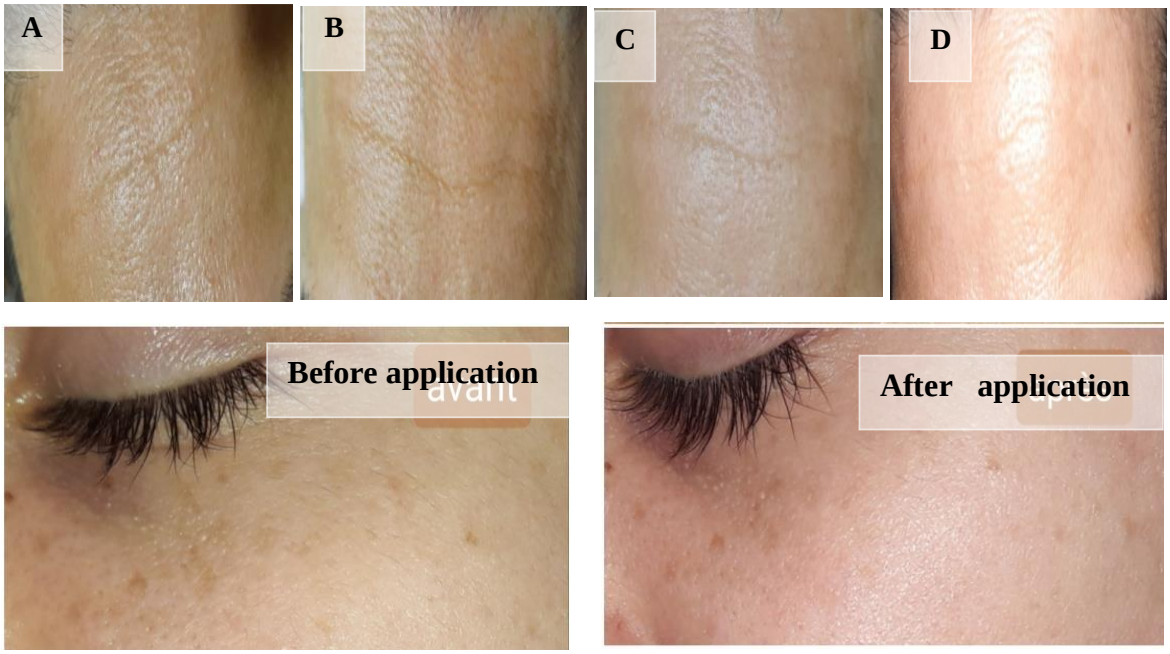


Figure 32: Depigmentation results before and after application of the formulation: (A) Before application, (B) After one week,(C) After two week,(D) Afler three week.

VI. Sensory evaluation of creams

VI.1.Product characterization

The objective of this analysis is to determine the descriptors that best discriminate the creams analysed and to determine their organoleptic characteristics.

VI.1.1 Discriminating power by descriptor

This test displays the descriptors which are ordered from the one with the highest discriminating power on products to the one with the lowest (from left to right). The results are presented in the Figure 35 below:

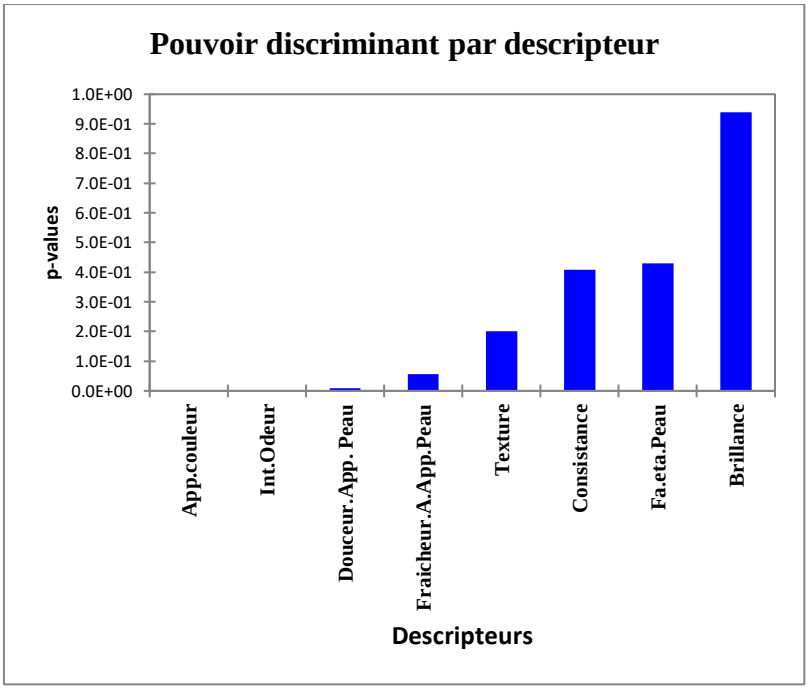


Figure 33: Discriminating power by descriptor.

From the results presented in (Figure 35), we notice that softness and freshness after application on the skin are the descriptors with the highest discriminating power. This means that the experts found differences between the previous characteristics on the four products of the creams analyzed. The discriminating powers of the characteristics: consistency and spreadability on the skin are average.

However, the descriptor of shine has the lowest discriminating power, so this characteristic does not change much between the four samples tested.

VI.1.2.Model coefficients

This test shows for each product of the creams, the coefficients of the connected model .Four histograms are obtained, each histogram corresponding to a product.

Figure 36 shows the histograms which correspond to cream 456 and cream 145. The following figure allows to see at a glance what defines the product (here cheese 401 and 404). In blue, we see the characteristics whose coefficient is significantly positive and in red those whose coefficient is significantly negative, in white the characteristics whose coefficients are not significant.

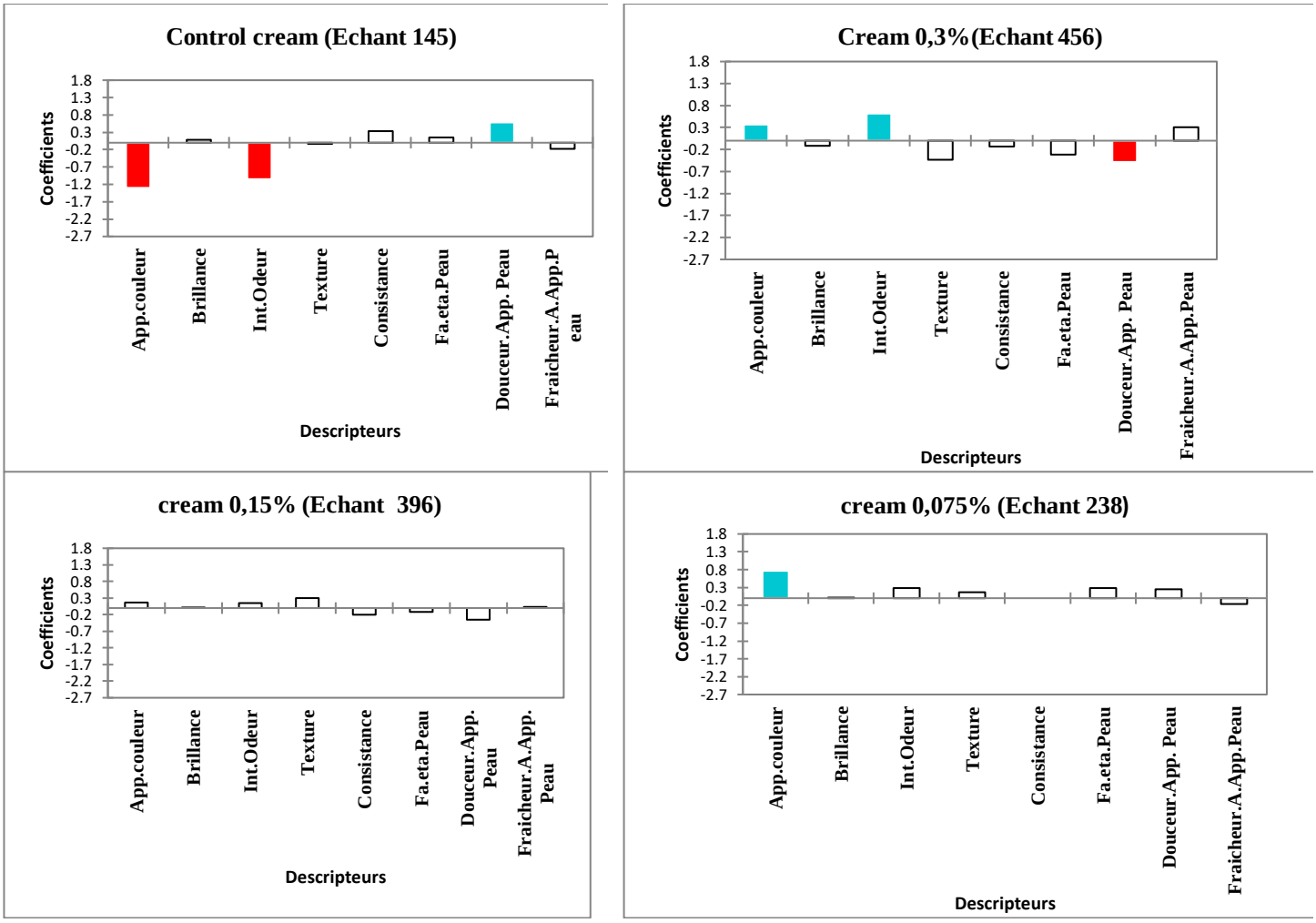


Figure 34: Coefficients of the cheese models 145, 238,396 and 456.

- Cream 145 (control cream 0%)
- Cream 456 (0, 3%)
- Cream 238 (0,075%)
- Cream 396 (0, 15%)

VI.2. Preference Mapping (PREFMAP)

VI.2.1. Principal Component analysis (PCA)

The map displayed in (Figure 37) allows the correlations between variables and factors to be presented by PCA.

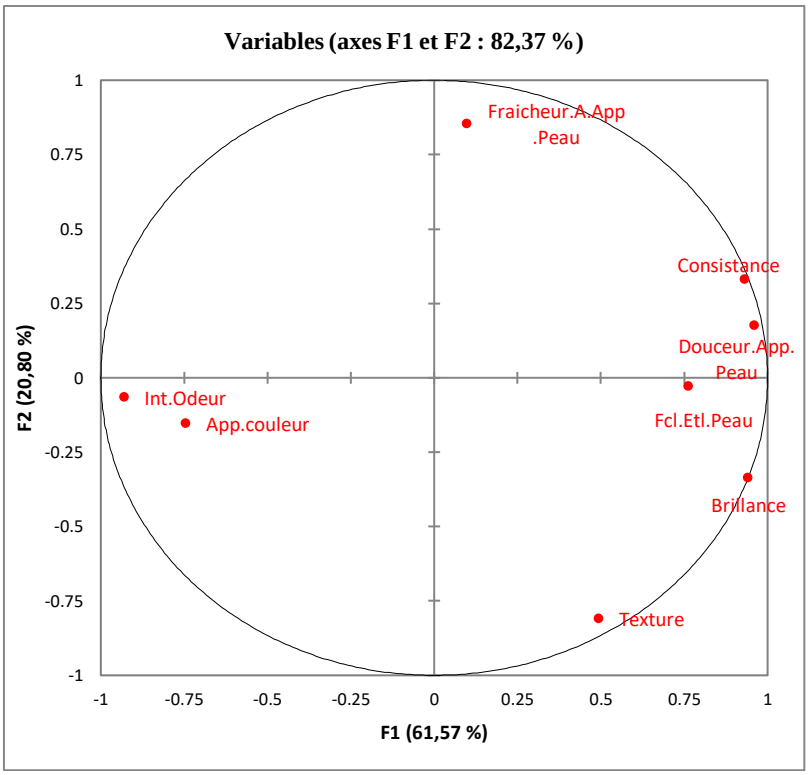


Figure 35: Correlations between variables/factors.

This figure shows that all the descriptors are presented in the graph. The descriptors texture, odor intensity, ease of spreading on the skin and color are significantly positively correlated since they are close to each other, the same for softness after application on the skin and consistency as well as freshness after application on the skin.

VI-2-2. Hierarchical ascending classification (HAC)

The graph in (figure 38) allows to represent the profile of the different classes created.

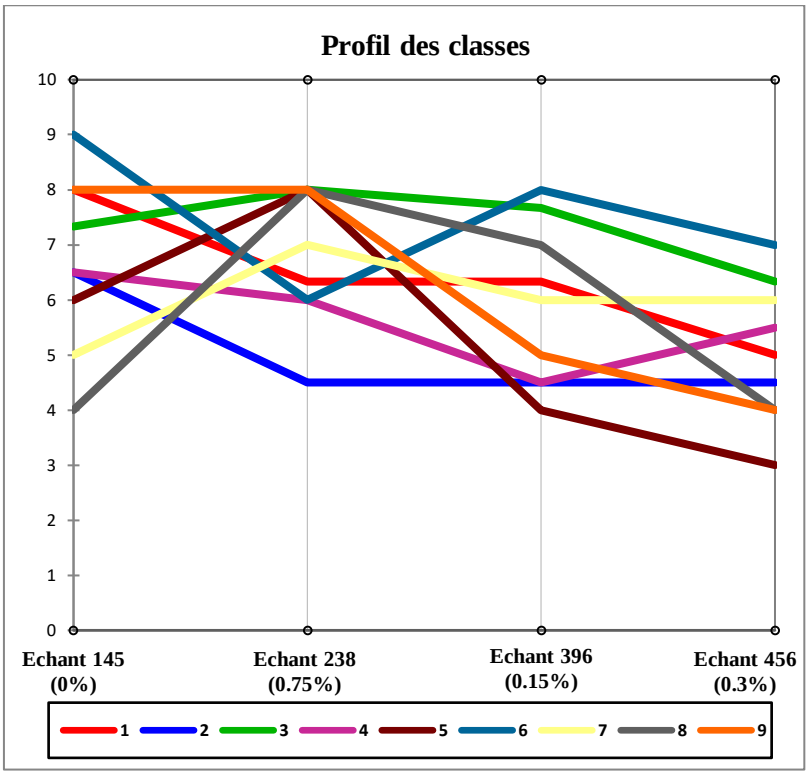


Figure 36: Profile of the created classes.

The class profile graph (made from the preference data) allows a visual comparison of the averages of the different classes created:

- **Class 1:** prefers in first position the cream 145 followed by the creams 238,396 and 456 successively.
- **Class 2:** prefers in first position the cream 145 followed by the creams 238,396 and 456 successively.
- **Class 3:** prefers in first position the cream 238 followed by the creams ,396 ,145 and 456 successively
- **Class 4:** prefers in first position the cream 145 followed by the creams 238,396 and 456 successively
- **Class 5:** prefers 238 cream in first position followed by 145, 396 and 456 creams successively

- **Class 6 :** prefers in first position the cream 145 followed by the creams 396,238 and 456 successively.
- **Class 7:** prefers in first position the cream 238 followed by the creams 145,396 and 456 successively.
- **Class 8:** prefers in first position the cream 238 followed by the creams 145,396 and 456 successively
- **Class 9:** prefers both creams 145 and 238 in first position followed by creams 396, 456 in second position.

VI.2.3. Preference mapping

According to figure 39, cream 145 (0% saffron extract) and cream 238 (0,075% saffron extract) are the most preferred (60 to 80% of consumers) by their consistency and softness when applied to the skin. Followed by cream 396 (0,15%). Finally the cream 456 is the less appreciated (0 to 20% of consumers) because of the intensity of the smell and the color. From the results obtained, the panel of 15 tasters did not appreciate the creams with a yellow color (due to the addition of saffron). They preferred the cream with 0% saffron extract which has a white color.

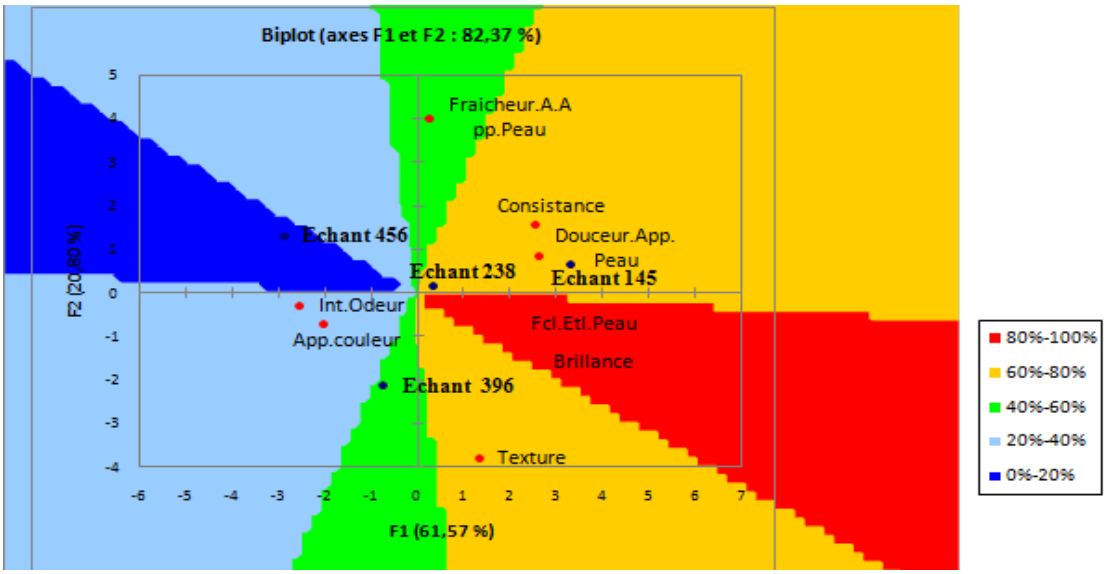


Figure 37: Contour lines and preference MAP.

Conclusion

Conclusion

Since ancient times, plants have been part of human life; in their food or their therapeutic use. Medicinal plants are a very important source of molecules with interesting biological activities.

In recent years, extensive studies have been carried out on compounds of plant origin, which are of particular interest in pharmacological and cosmetic terms...

In this work, the extract of saffron stigmas was chosen to determine its benefits in the cosmetic field.

The extraction of the stigmas of Crocus sativus which was done allowing the determination of total polyphenols, total flavonoids, reducing power, and antioxidants are all high which show the effectiveness of the extract.

In our creams, we used different concentrations of the extract under the same conditions (control cream, 0.075% cream, 0.15% cream, 0.3% cream). The homogeneity of the prepared creams is good, their texture is thick and smooth, and they spread easily. No apparent difference. They also show good stability with no difference between the creams at different concentrations.

The pH of the creams is adjusted towards neutrality (with a range of 5.4 and 5.6) to make it compatible with the skin pH and to avoid skin irritation. They have a high moisture content of about 61% which helps to moisturise the skin.

Stability tests, which we used to evaluate the antioxidant activity (DPPH) for 21 days showed a strong antioxidant activity proportional to the concentration of the extract. Saffron cream therefore has a strong antioxidant power and could be an anti-stain agent (lightening) by inhibiting the activity of the tyrosinase enzyme responsible for melanin biosynthesis by blocking the oxidation reaction chain.

The results of the tests on the candidates show that the cream has a good and effective anti-stain agent on the skin.

From the results of this study it is concluded that the extract of the stigmas of crocus sativus is a very good natural source of antioxidant.

This work can be complemented by further work to evaluate certain rheological tests such as flow.

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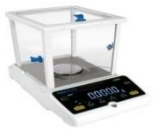
Appendixes

Appendixes



Analytic balance

Ventilated oven

Mortar and pestle in glass

Vial judged

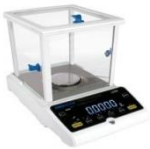
Funnel

Pissette

Centrifuge tubes

Centrifuge

Appendix 01: Materials for extract preparation.



Analytic balance

Thermometer

beakers

A spatula

glass stems

glass crystalliser

Appendix 02: Equipment for preparing cosmetic creams.

PH-meter

Centrifuge

Centrifuge tubes

Optical microscope

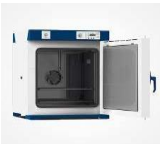
vortex

Test tubes

Racks

Test tubes

Spectrophotometer, micropipette and cuvette



ventilated oven

Appendixes

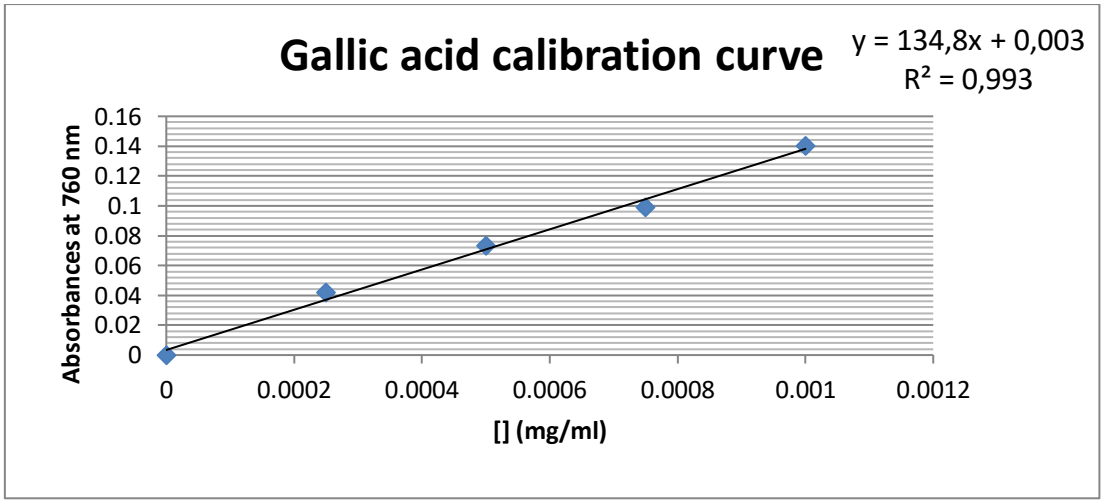
Autoclave

petri dish

Appendix 03: Equipment for Stability tests on the Cream.

Appendix 04: Culture medium of bacteria and yeast (Bouranen, 2017).

Nutrient agar culture medium (GN)	Quantity (g/L)	Oxytetracycline-glucose-yeast agar extract culture medium (OGA)	Quantity (g/L)
Tryptone	5g	Yeast extract	5g
Meat extract	1g	Glucose	20g
Yeast extract	2g		
Sodium chloride	5g	Agar	12g
Agar	12g		



Appendix 05: Gallic acid curve.

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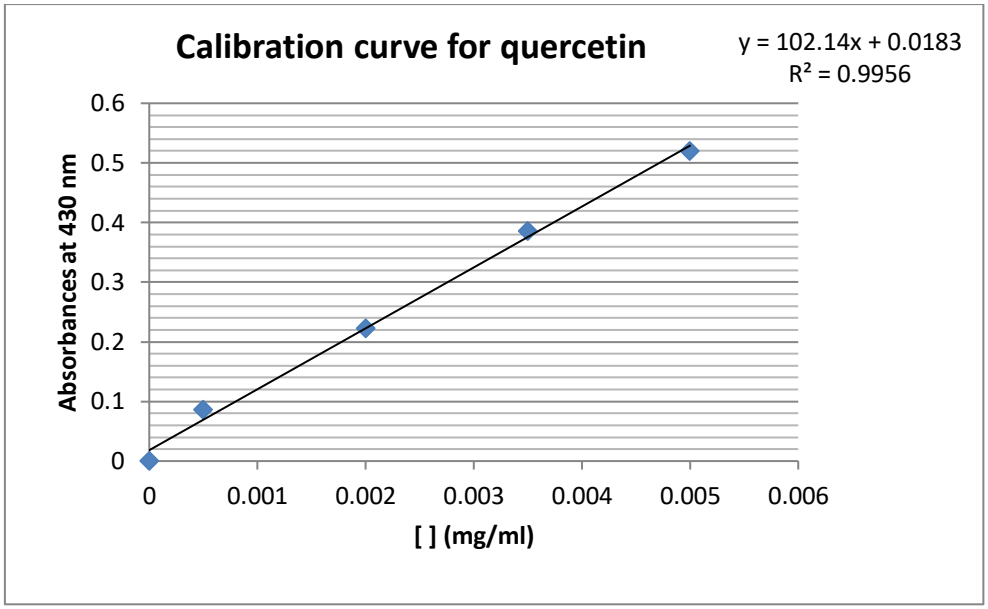

Appendix 06: Calibration curve for quercetin.

Sensory analysis questionnaires for cosmetic creams (expert panel)

First name : .....
Sex : M or F Age : ..... Job number :..... Date :.....

4 samples of coded cosmetic creams are presented to you. You are asked to evaluate the different organoleptic characteristics by giving a mark between 1 and 5 according to the scale presented.

1. Color:

1. White
2. Beige
3. Yellow
4. Yellow-green
5. green

Sample 145	Sample 238	Sample 396	Sample 456

2. Brilliance

1.no gloss
2.low
3.medium
4.intense
5.very intense

Sample 145	Sample 238	Sample 396	Sample 456

2. Odor intensity :

1 . Very weakly intense
2. Slightly intense
3. Medium intense
4. Intense
5. Very intense

Sample 145	Sample 238	Sample 396	Sample 456

Appendixes

3. texture

1. Granular
2. Slightly granular
3. Medium (neither granular nor smooth)
4. Smooth
5. Very smooth

Sample 145	Sample 238	Sample 396	Sample 456

4. Consistency

1. Very soft
2. Mole
3. Average
4. Firm
5. Very firm

Sample 145	Sample 238	Sample 396	Sample 456

5. Easy to spread on the skin

1.Very difficult
2.Difficult
3.Average
4.Easy
5.Very easy

Sample 145	Sample 238	Sample 396	Sample 456

6. Softness after application on the skin

1.absent
2.low
3.medium
4.mild
5.very soft

Sample 145	Sample 238	Sample 396	Sample 456

7. Freshness after application on the skin

1. Absence
2. Low
3. Medium
4. intense
5. Very intense

Sample 145	Sample 238	Sample 396	Sample 456

8. preferences :

Do you like the color?

1-Not liked
2-Little liked
3-Moderately liked
4-Appreciate it a lot
5-Very much appreciated

Sample 145	Sample 238	Sample 396	Sample 456

Appendixes

Do you like the smell?

1-Not appreciated
2-Somewhat liked
3-Moderately liked
4-Appreciate it a lot
5-Very much appreciated

Sample 145	Sample 238	Sample 396	Sample 456

Do you like the texture?

1-Not appreciated
2-Somewhat liked
3-Moderately liked
4-Appreciated well
5-Very much appreciated

Sample 145	Sample 238	Sample 396	Sample 456

Overall preference

Rate each sample on a scale of 1 to 9 according to your preference, with 1 being the least preferred sample and 9 being the most preferred. As presented in the scale below:

1. Extremely unpleasant
2. Very unpleasant
3. Unpleasant
4. Quite unpleasant
5. Neither pleasant nor unpleasant
6. Quite pleasant
7. Pleasant
8. Very pleasant
9. Extremely pleasant

Sample 145	Sample 238	Sample 396	Sample 456

Thank you for your contribution

Appendix 07 : Sensory analysis questionnaires for cosmetic creams (expert panel).

Abstract

Cosmetic formulation: Incorporation of saffron (Crocus sativus) in an emulsion.

Summary: Pigmentation spots are located on the face, hands and back. These spots are called in the common language brown spots. The solutions to remove them, natural treatments, effective creams. Like the lightening cream which contains plants with depigmenting effects like saffron stigmas. The general objective of our work was to characterize and evaluate the cream based on extract of saffron stigmas with anti-oxidant activity. The equipment used among others the oven, the microscope with camera, the spectrophotometer and the pH-meter etc.... allowed us to formulate the cream, to realize controls on this one, to analyze its texture, to study its stability. The controls carried out showed that cream was of white color (control cream) and creams saffroned was of young color of different concentration of the extract of the stigmas of saffron (0,075%; 0,15%; 0,3%). The evaluation of the antioxidant activity of the extract, its content of polyphenols, flavonoids and reducing power showed that the saffron used is of good quality. Our cream was soft, shiny, and W/O type. Its pH (5,5 at 27±°C), the moisture content is in accordance with the standards of the creams intended for this purpose. Moreover, the results of the tests on the candidates show that our preparation had a depigmenting effect. The evaluations included organoleptic controls and stability tests. In this study, our objective was to characterize and evaluate a cream based on saffron stigma extract. The evaluations including the organoleptic controls, the physicochemical controls, the textural analysis, showed that it remained not only stable whatever the temperature but also presented a good stability of the antioxidant activity measured by DPPH during 21 days highlighted a very low IC50 which informs on the effectiveness of the saffron incorporated in the cream to prevent pigmentary spots.

KEYWORDS: brown spots; saffron stigma; antioxidant; cream; depigmenting agent.

Formulation cosmétique : Incorporation du safran (*Crocus sativus)* dans une émulsion.

Résumé: Les pigmentaires sont localisées sur le visage, les mains et dans le dos. Ces taches sont appelées dans le langage courant taches brunes. Les solutions pour les enlever, traitements naturels, crèmes efficaces. *Comme la* crème éclaircissante *qui contient des plantes aux effets dépigmentant comme* les stigmates de safran. L'objectif général de notre travail a été de caractériser et d'évaluer la crème à base d'extrait des stigmates de safran à activité anti-oxydante. Le matériel utilisé entre autres l'étuve, le microscope avec appareil photo, le spectrophotomètre et le pH-mètre etc.… nous a permis de formuler la crème, de réaliser des contrôles sur celle-ci, d'analyser sa texture, d'étudier sa stabilité. Les contrôles effectués ont montré que crème était de couleur blanche (crème témoin) et des crèmes safranés était de couleur jeune de différentes concentration des d'extrait des stigmates de safran (0,075% ; 0,15% ; 0,3%). L'évaluation de l'activité antioxydante de l'extrait, sa teneur en polyphénols, flavonoïdes et de pouvoir réducteur ont démontré que le safran utilisé est de bonne qualité. Notre crème était douce, brillante, et de type E/H. Son pH (5 ,5 à 27±°C), Le taux d'humidité est conforme aux standards des crèmes destinées à cet effet. Par ailleurs, les résultats des tests sur les candidats montrent que notre préparation avait un effet *dépigmentant*. Les évaluations comprenant les contrôles organoleptiques, les testes de la stabilité. Dans cette étude, notre objectif était de caractériser et d'évaluer d'une crème à base d'extrait des stigmates de safran. Les évaluations comprenant les contrôles organoleptiques, les contrôles physico chimiques, l'analyse texturale, ont montré qu'elle demeurait non seulement stable quelque soit la température mais également présentait une bonne stabilité de L'activité antioxydante mesurée par DPPH pendant 21 jours a mis en évidence un IC50 très faible qui renseigne sur l'efficacité du safran incorporé dans la crème pour prévenir contre les taches pigmentaires.

MOTS CLES : taches brunes ; stigmate de safran ; anti-oxydante ; crème ; *dépigmentant.*