DELIVERABLES REPORT

MultiHemp

Multipurpose hemp for industrial bioproducts and biomass

(Ref n. 311849)

10.3 Demostrative production of cosmetic.

February 2017

Rosa de la Torre

ctaex centro tecnológico agroalimentario

INDEX

1. Introduction

The main objective of WP5 is the evaluation of the suitability of raw material obtained from the hemp biorefinery to industrial processing. Industrial partners involved in WP5 will provide indications on quality traits to be modified according to specific end use destination and will therefore demonstrate the effect that cultivation and use of selected and improved genotype has on final product characteristics.

Industrial partners will provide technical information to produce a comprehensive list of quality traits with a range of optimal values that hemp raw materials must have for specific end use destination. This will contribute to the realisation of a grading system.

Fibre from longitudinal and disordered system will be used to produce biobased composites, insulation and building materials. Oil from the seeds have been valuated for the production of cosmetics.

Hemp is grown for its primary products namely the oil, fibre, and shive, but we propose to develop this plant as a dedicated biorefinery crop from which every part is used to generate value. Secondary metabolites such as essential oils are not currently recovered but could add value in a whole crop processing scenario.

Other metabolites such as waxes, and phytosterol could be extracted from fibre processing wastes. We will optimise the extraction of these secondary metabolites and scale up production for commercial trials. Hemp fibre processing are mostly lignocellulosic and have a potential to be transformed into simple sugars and fermented to produce bioethanol.

2. Material and Methods

The raw material to work with belongs to the production of one of the partners of this project: Latgale Agricultural Research Centre Latvia, SME. We worked with seeds from FINOLA variety.

Several trial have been carried out in order to look for the best way to obtain hemp seeds oil

- Cold mechanical extraction
- CO2 supercritical fluid extraction

In mechanical extraction, ground or not, seeds pass a conditioner to obtain a homogeneous product which then goes to press where high pressure and in one step we proceed to the separation of oil cake residual protein, generally called pellets or residual cake.

Before the extraction is necessary, a previous phase of conditioning in that the initial optimum moisture for seeds are stablished, so this process improves the breaking of oil-rich cells, facilitating the expulsion to achieve the best yield.

3. Oil extraction.

The oil from hemp seeds have been extracted following two environmentally friendly processes: mechanical extraction and CO2 supercritical fluid extraction.

a) Mechanical extraction of hemp seeds oil has been carried out by cold-pressing; model KOMET SCREW OIL, CA CA59G- Expeller 5963, Oekotec, IBG Monforts, (Germany). (Fig. 1)

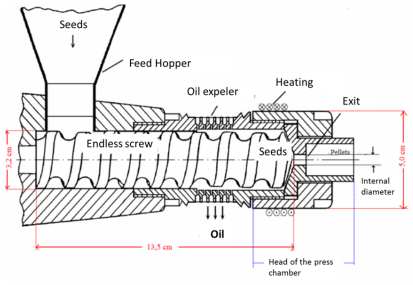

Fig. 1- Cold press for seeds

The yield of the extraction process can be seen in the next Figure 2:

Hemp seed oil mechanical extraction (Yield %)			
Fat content %	**Extraction Yield%**	**Yield after cleaning**	**Efficiency: clean oil**
32,1±0,2	31,5±0,7	28,1±0,5	87,5±0,5

Fig. 2- Cold press for seeds

b) CO2 Supercritical fluid extraction (SFE)

When a certain fluid is forced to a pressure and temperature higher than its critical point (see Figure 3), it becomes a supercritical fluid. Under these conditions, the different properties of the fluid are placed between those of a gas and those of a liquid. Although a supercritical fluid density is similar to a liquid and its viscosity is similar to a gas, its diffusivity is intermediate. Thus, the supercritical state of a fluid has been defined as a state in which liquid and gas are indistinguishable to each other, or as a state in which the fluid is compressible (i.e. similar behaviour to a gas) even though posses a density similar of a liquid and, therefore, has its solvating power.

Fig. 3 – Pilot CO2 SFE

Extraction tests were carried out on seeds of cannabis from Lithuania (5 kg) the same as in the previous trial.

3. Composition of oil.

1. Composition of oil.

The result of the quality of seed oil is shown depending on the extracting process used.

a) **mechanical cold extraction process**

The oil obtained presents the following chromatographic profile of fatty acids, obtained by gas chromatography analysis:

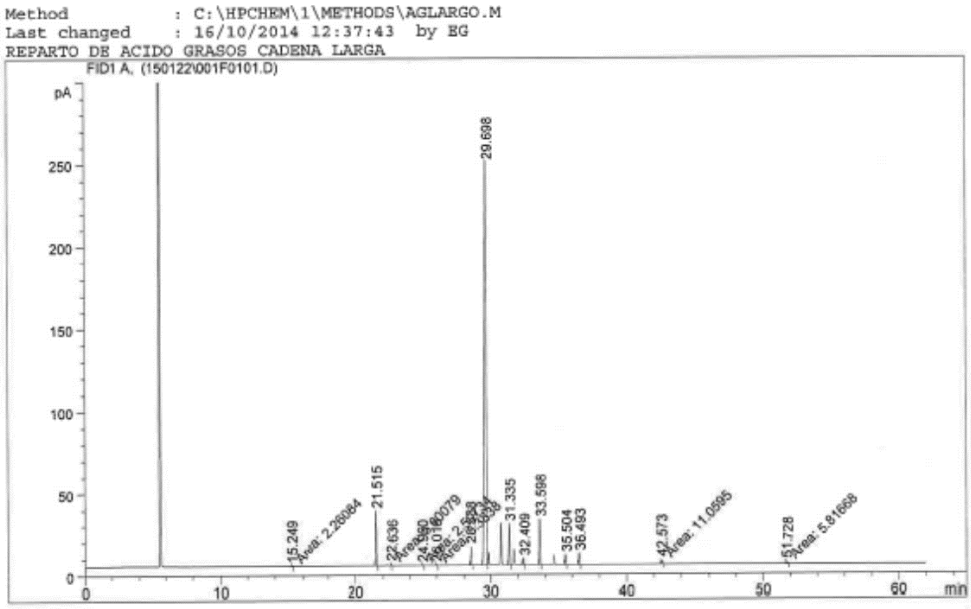

Fig. 4- Fatty acid chromatogram of hemp seed oil

FATTY ACIDS profile	%
Butirico acid (C4:0)	trace
Capronic acid (C6:0)	trace
Caprilic acid (C8:0)	trace
Capric acid (C10:0)	trace
Lauric acid (C12:0)	trace
Miristic acid (C14:0)	**0,07**
Miristoleic acid(C14:1)	trace
Palmitic acid(C16:0)	**5,09**
Palmitoleic acid (C16:1)	**0,18**
Margaric acid (C17:0)	**0,07**
Margaroleic acid (C17:1)	**0,10**
Estearic (C18:0)	**2,18**
Oleic acid (C18:1)	**80,02**
Linoleic (C18:2)	**4,12**
Linolenic acid (C18:3)	**4,71**
Araquic acid (C20:0)	**0,98**
Alfa Linolenic acid	**0,72**

Gadoleic acid (C20:1)	**1,11**
Behenic acid (C22:0)	**0,43**
EPA	trace
Lignoceric acid (C24:0)	**0,22**
DHA	trace

Fig. 5 Fatty acid composition of hemp seed oil

The phisical chemical parameters fixes by Legislation in vegetalbles oils have been alaysed too.

* (*Implementing regulations (UE) n° 29/2012 of the Commission, 13 of January 2012).*

	Peroxide index (mg/O_2 kg)	**Acidity (% oleic acid)**	**K232**	**K270**
Hemp seeds oil	2,51	0,17	1,349	0,217
Legislation*(Max.value allowed)	20,0	0,5	2,5	0,5

Fig. 6- Quality parameters of hemp seed oil

Cannabinoids profile was also determined analytically to check Δ9-Tetrahydrocannabinol (THC) content. The UE law admits until 0.02 % THC in hemp.

Cannabinoid profile (HPLC method) **(%)**	**FINOLA seed oil**
Cannabidiol (CBD)	0,03
Caannabigerol (CBG)	0,01
Cannabinol (CBN)	<0,01
Δ9-Tetrahydrocannabinol (THC)	0,02
Cannabichromene (CBC)	0,01
Cannabidiol acid (CBDA) (%)	<0,01
Δ9-Tetrahydrocannabinol acid (THCA)	<0,01
Cannabidiol total (CBDT)	0,03

Fig. 7- Cannabinoids content of hemp seed oil

Cannabinoids chromatogram by Gas Chromatography is shown as follows:

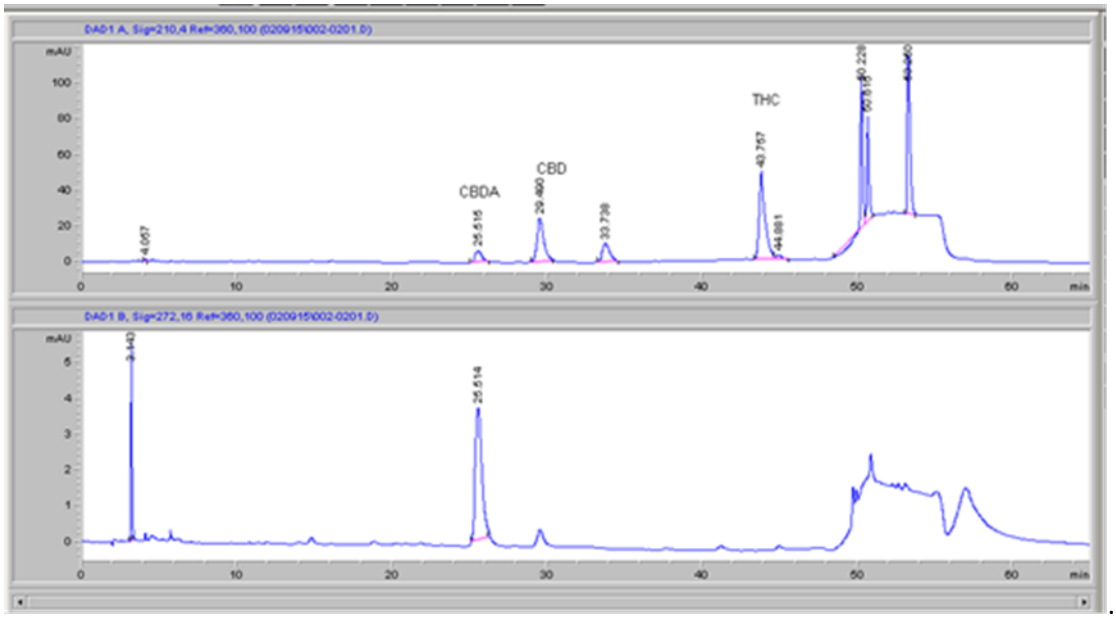

Fig. 7- Cannabinoids chromatogram HPLC of hemp seed oil

Some cosmetic products from the wide range of them that exist in the market have been develop:

- Shower gel
- Shampoo
- Face cream
- Hand cream
- Body milk.
- Sunscreen.
- After-sun Cream

A suitable supplier and specialist in these ingredients have provided the raw materials used for developing the cosmetics products.

The packaging material is also very important factor for the future market of these products, as well as the quality of ingredients.

Cosmetic products can be divided into three groups, depending on the process of preparing the same:

- GROUP 1: emulsion products: gel and shampoo.

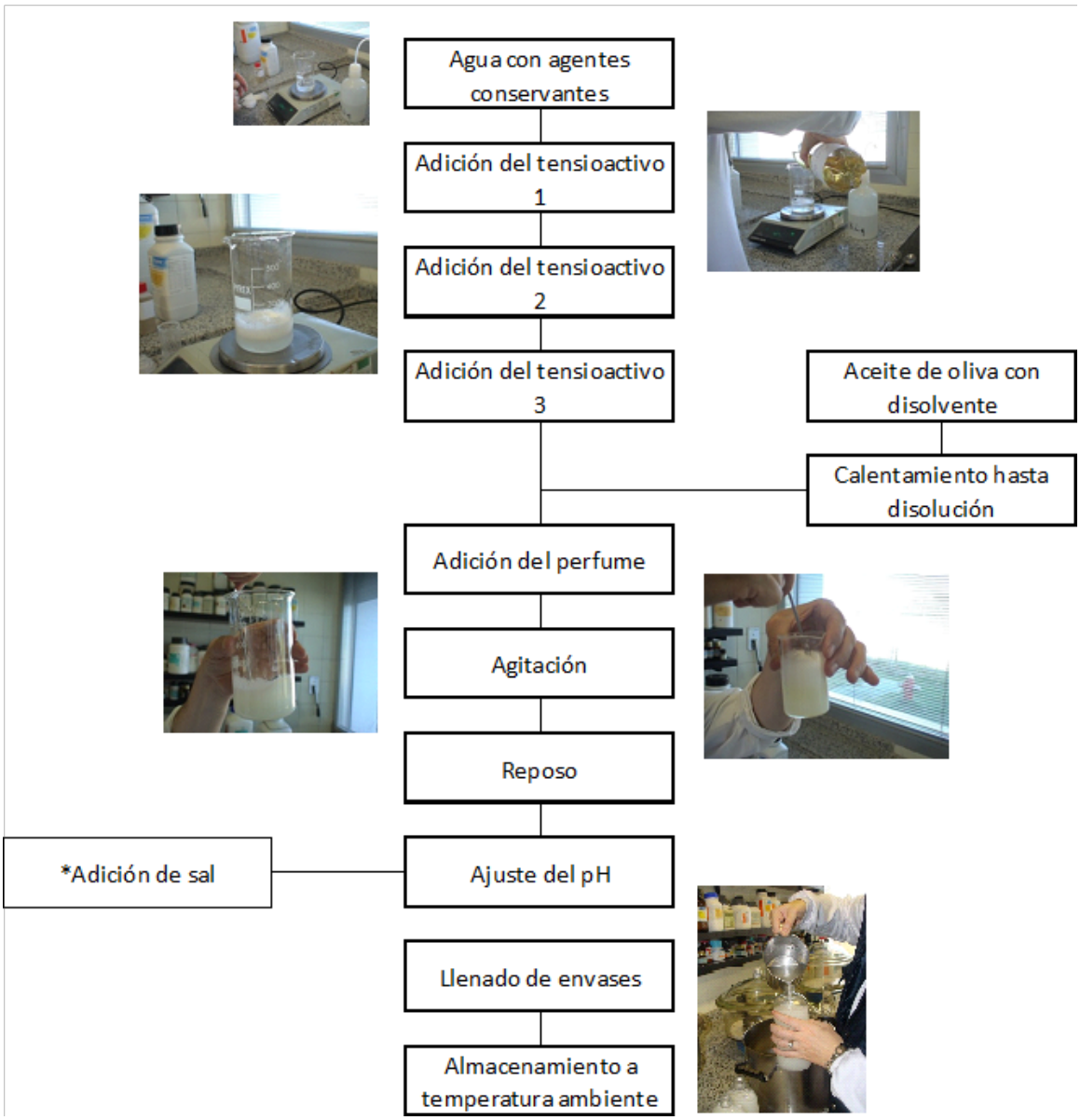

Fig. 8-Flow sheet of elaborating cosmetic products. Group 1

- GROUP 2: fat emulsion products in water: body milk; face cream, hand cream, after sun.

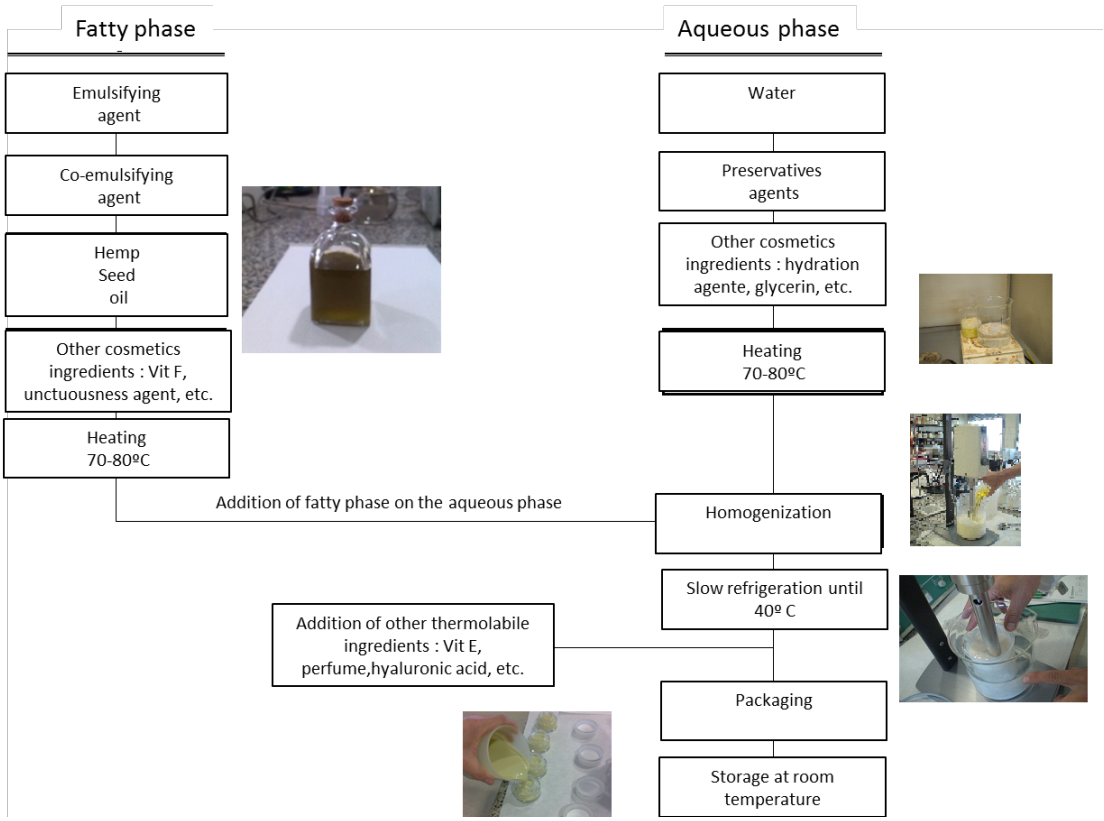

Fig. 9-Flow sheet of elaborating cosmetic products. Group 2

- GROUP 3: water emulsion products fat: sunscreen.

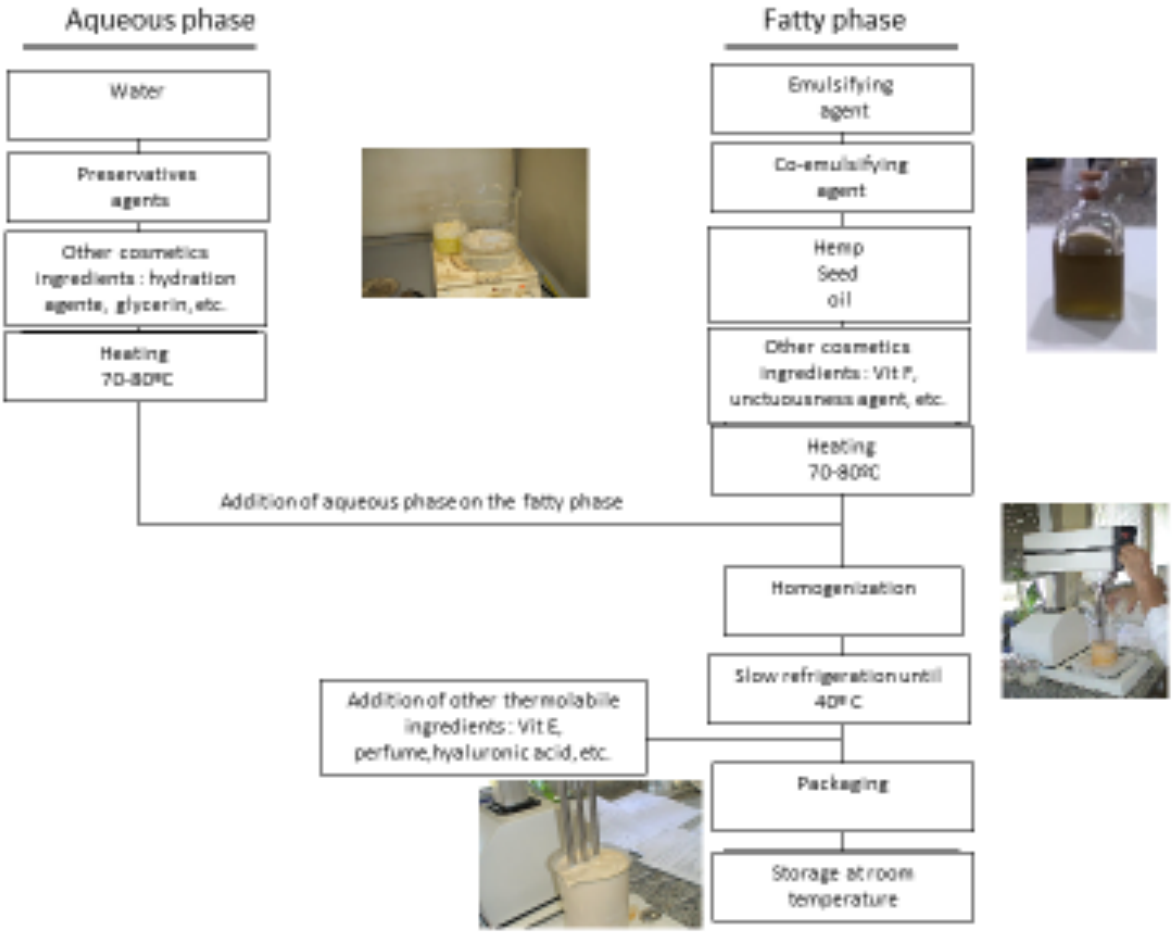

Fig. 10-Flow sheet of elaborating cosmetic products. Group 3

4. Storage test/ Shelf life stability.

The study of the stability of the processed products will provide us with information on the degree of relative stability of a product in the varied conditions to which it can be subject from its manufacture to its expiration.

This stability is relative, since it varies with time and in function of factors that accelerate or delay alterations in the parameters of the product.

The aspects considered in the stability tests are:

• Physical: the original physical properties must be preserved as appearance, color, smell, uniformity, among others;

• Chemicals: must be kept within the specified limits for the integrity of the chemical structure, ingredient content and other parameters;

• Microbiological: the microbiological characteristics must be preserved, according to the specified requirements. Compliance with Good Manufacturing Practices and the preservative systems used in the formulation can guarantee these characteristics.

Conservation tests have been performed on products made this latest milestone has been conducted under conditions that allow provide information on the stability of the product in less time possible. For that, samples must be stored under conditions that accelerate plausible changes occur during the period of validity.

- **Sample preparation**

For stability evaluation was used for final packaging material each product, with cover, ensuring a good seal and avoiding outgassing or steam to the medium; thus assessing the compatibility between the formulation and packaging is anticipated.

It has been estimated, on the other hand, the amount of product needed to produce the necessary assessments in the periodic sampling.

It has avoided incorporating air into the product during the packaging container samples participating in the conservation test. It is important not to complete the total volume of the container allowing an empty space (head space) of about one third of the capacity of the bottle for gas exchange possible.

- **Storage conditions**

In order to establish the conservation test, the European and Spanish regulations have been followed:

REGULATION (EC) No 1223/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL. This Directive was reevaluated in 2013 to enable further harmonization and a EU-wide Cosmetics Products Regulation entered into force in July 2013.

Before starting Stability Studies, it is recommended to subject the product to the centrifugation test. A sample is suggested centrifuging at 3,000 rpm for 30 minutes. The product must remain stable and any sign of instability indicates the need for reformulation. If the result is positive, the product can be subjected to stability tests.

In this storage test has followed the protocol of the Stability Preliminary Studies, also known as Screening Test, Accelerated Stability or short-term, which aims to guide the choice of the formulations.

The preliminary stability study consists of performing the test in the initial phase of product development, using different formulations laboratory and short duration.

It uses extreme temperature conditions in order to accelerate possible reactions between components and the emergence of signs to be observed and analysed according to the specific characteristics of each type of product. Because the conditions in which it is conducted, this study aims to estimate the lifetime of the product, but help in the selection of the formulations

The most common storage conditions of the samples are temperature (ambient, high, low), exposure to light and cycles of thermal stress.

- Ambient Temperature

Samples have been stored at room temperature.

- High Temperatures

The limits of temperature practiced during the development of the test have been:

Stove: T = 37 ± 20 ° C

Stove: T = 40 ± 20 ° C

- Low Temperatures

The temperature limits used were:

Refrigerator: T = 5 ± 20 ° C

- Exposure to Light Radiation

It can significantly alter the color and odor of the product and lead to degradation of formulation ingredients. For conducting the study, the source was the usual solar radiation.

- Thermal stress cycles

In this condition, the samples are stored in alternated temperatures at regular intervals.

- Cycles of 24 hours at 40 ± 2º C, and 24 hours at 5 ± 2º C, for 12 days (6 cycles).

- **Evaluation parameters**

The cosmetic products formulated, representative of the types of emulsions were submited to storage test: shampoo, shower gel, face cream, body milk, hand cream, after sun cream and sun scremcream

The evaluated parameters depend on the characteristics of the test product and the ingredients used in the formulation. Generally, they are evaluated from three point of view:

- Organoleptic Parameters: appearance, color, odor and taste
- Physical-Chemical Parameters: pH value and viscosity
- Microbiological Parameters: various microbial counts

In the centrifugation test, the products from GROUP 1 (shampoo and shower gel) GROUP 2 (face cream, body milk, hand cream and after sun cream) and GROUP 3 (sunscreen cream) did not show phase separation or any degree of syneresis, so the conservation test was established according to the methodology explained above.

Microbiological evaluation of cosmetics

Cosmetic samples	Aerobic mesophilic bacteria (ISO 21149) (cfu/g)	Molds and yeasts (ISO 16212) (cfu/g)
Shower gel	<10	<10
Shampoo	<10	<10
Body milk	<10	<10
Hand cream	<10	<10
Face cream	<10	<10
After sun cream	<10	<10
Sunscreen cream	<10	<10

Once the cosmetic products prepared were submitted to Protocol of the Stability Preliminary Studies (Screening Test, Accelerated Stability), all the parameters studied did not show significant changes throughout the storage period, except the rancidity, expressed as peroxide index due to the richness in polyunsaturated fatty acids (PUFA) especially linoleic and gamma-linoleic acids, of hemp seed oil

The rest of the parameters, organoleptic, physical-chemical and microbiological, remain constant during the storage test period.

New cosmetic formulas were prepared adding more quantity of alpha-tocopherol, as antioxidant agent (vitamin E)

Dermatological evaluation of cosmetic products:

The tested cosmetics products do not contain any substance, which is forbidden by the EEC legislation. (CENTRO DI COSMETOLOGIA. UNIVERSITA' DI FERRARA)

As far as the use of cosmetic and personal hygiene products is concerned, that the preservatives in the formula are in the list of the accepted components approved by the EEC and are used in a concentration provided for by the law.

These products are NOT IRRITATING if applied to human skin, after overcome successfully Patch test, Mean index of irritation analysis and the application of Applying Finn Chambers.

- Patch test: Number of irritative reactions (erythematous and/or oedematous) encountered at 15 minutes and at 24 hours after the removal of the patch. Erythematous reactions have been sorted out into three groups according to the reaction degree: light, clearly visible and moderate/serious erythema.
- Mean index of irritation analysis:
    - The scores are based on lightness, clearly visible and moderate/serious erythematous reactions (including the associated oedema) are shown in blue, purple and black, respectively
    - The dashed line (0,50) indicates the threshold above which the product is to be classified as slightly irritating
- Applying Finn Chambers:
    - Skin should be clean, healthy, and free of ointments, lotions, powders, acne, dermatitis, scars, hair or any other condition that might interfere
    - The patient should stand or sit in a relaxed position with the back bent slightly forward
    - Apply prepared patches to the upper back adjacent to the vertebrae. An alternative application site is the outer surface of the upper arm
    - Application on the healthy skin of 20 volunteers

After the different analyses on the skin, the results of dermatological study are as follows:

Group 1: applied diluted with distilled water (1:10) on the skin

Mean index of irritation	Time after the removal of the Finn Chamber		
	15 min	24 hours	Result
Shower gel	0,10	0,15	NOT IRRITATING if applied to human skin
Shampoo	0,10	0,15	

Group 2: applied directly on the skin

Mean index of irritation	Time after the removal of the Finn Chamber		
	15 min	24 hours	Result
Body milk	0,05	0,05	NOT IRRITATING if applied to human skin
Hand cream	0,05	0,05	
Face cream	0,05	0,05	
After sun cream	0,15	0,15	

Group 3: applied directly on the skin

Mean index of irritation	Time after the removal of the Finn Chamber		
	15 min	24 hours	Result
Sunscreen cream	0,20	0,20	NOT IRRITATING if applied to human skin