I. Rigano*, G. Giammarrusti*, F. Rastrelli**

Vegetable Oils – The Base of New Active Principles

Keywords:

Abstract

Current cosmetic research has been steered towards the employment of vegetable ingredients by both the consumer's preferences and the scientific research on the properties of natural origin raw materials. Olive oil with its prominent role in the 'Mediterranean diet' has become a starting point in the formulation of functional ingredients for cosmetic purposes with various potential activities which could be performed on the different epidermal layers. Preferred to the other vegetable oils for its high amount of mono-unsaturated fatty acids, it exhibits the well-known properties of integrating with the human physiology. Olive oil has the undoubted advantage of a lipid fraction with a millenary history of contact with vital cells, which thus allows olive oil to boast a high safety standard. If its lipid chains are chemically combined with active molecules whose role has been scientifically proven, functional ingredients that can successfully be included in innumerable cosmetic formulations are made up. Thanks to their biological structure, olive oil derivatives can also efficiently carry complex active molecules to which the epidermal barrier normally creates an obstacle. Therefore, this allows easier penetration and the performance of the designed activity in the skin areas involved.

Introduction

In current cosmetic science, the role of natural origin ingredients is increasingly important due to the influence of two new essential forces in the wellness strategy. On the one hand, there is a need to have finished products as compatible as possible with the human physiology, without toxic effects or even minor allergies. On the other hand, there is a strong interest in the (protective and reactivating) properties which are beneficial to the skin and are provided by the complex mixtures of natural origin ingredients. Olive oil is one of these. As a long-standing food ingredient, olive oil is a basic ingredient in the Mediterranean diet. Termed as 'mono-unsaturated oil' par excellence, olive oil is the only vegetable lipid which can be eaten after an exclusively mechanical process, which means extraction without using other substances. In fact, all the other oils must undergo different production processes, that is extraction by means of solvents or steam, rectification, fractioning, etc. Moreover, olive oil is different from many common oils for its high content of mono-unsaturated fatty acids and the relatively low content of unsaturated and poly-unsaturated fatty acids. **Table 1** shows the average percentage of fatty acids contained in the most used vegetable oils.

The high content of mono-unsaturated fatty acids makes olive oil able to reduce the rate of cardiovascular disorders and some tumours thanks also to the abundant presence of antioxidant agents, such as tocopherols, tocotrienols, squa-

Oils	Saturated Lipidic Chains	Mono-Unsaturated Chains	Poli-Unsaturated Chains
Olive Oil	16%	75%	9%
Peanut Oil	19%	53%	28%
Sunflower Oil	11%	33%	50%
Maize Oil	5%	31%	50%
Soya Oil	4%	23%	59%
Coconut Oil	87%	6%	2%

Table 1 Fatty acids contained in the most used vegetable oils

lene). In cosmetics, good polarity, miscibility with many oils, good stability and high presence of oleic chains are good reasons in themselves to employ olive oil as a source of lipid moieties with special effects.

Typical chemical composition of fatty acids in olive oil:

Oleic	(C18:1)	76,9%
Palmitic	(C16:0)	10,5%
Linoleic	(C18:2)	7,6%
Stearic	(C18:0)	2,6%
Palmitoleic	(C16:1)	0,6%
Linolenic	(C18:1)	0,6%
Arachidonic	(C20:0)	0,3%
Behenic	(C22:0)	0,2%
Misc.		0,7%

Moreover, an excellent unsaponifiable fraction (0,6-1,5%) is present. This is mostly made up of squalene (over 80%) in addition to other hydrocarbons, triterpenes and triterpenic alcohols, sterols (beta-sitosterol, stigmasterol, campesterol), tocopherols and carotenoids. The unsaponifiable fraction of olive oil is believed to perform an important emollient and sebum-controlling property, thanks to its affinity with the physiological composition of the sebum and the horny layer surface.

■ Advantages of Olive Oil

Nowadays, olive oil is a key ingredient to formulate ingredients and functional actives for cosmetic purposes. It offers the formulator a mixture of natural triglycerides rich in mono-unsaturated chains with high physiological skin-compatibility. This is strongly proved by the age-long employment of olive oil in the European and Mediterranean area. In other words, it offers the undoubted advantage of a lipid fraction with a long history of contacts with vital cells. Its lipid structure is able to integrate with the horny layer, thus helping to restore the structural balance of the hydro-lipidic structure needed to perform the barrier function. The peculiar structure of the main fatty acid may also act as a vehicle to carry the different active principles of cosmetic interest in-depth. It is known that mono-unsaturated chains are means of increased permeability compared to both saturated and poly-unsaturated chains.
As stated above, the other particularly interesting aspect of olive oil concerns its unsaponifiable fraction. The simultaneous presence of the above-mentioned substances with antioxidant power as well as emollient and sebum-controlling effect contributes to skin normalization and its protection from the effects of free radicals.
Finally, being highly compatible and combining well with a lot of lipids, either natural or not, it allows to achieve emulsions with homogeneous fatty phases.

■ Functional Actives

Olivoyl PCA (INCI: Potassium Oliovoil Pyrrolidon-carboxylate) and CLA Carnitine (INCI: Carnitine Conjugate-Linoleate) are two functional actives that clearly exhibit the typical advantageous features of olive oil derivatives. In fact, they combine the special composition of the lipid chains used for their synthesis with two substances characterised by different and specific properties, concerning on one side their activity and on the other the specific level of performance in the skin layers.

■ Emollient Lipoproteins

The first derivative is achieved by condensation of all fatty acids in olive oil and the potassium salt of pyroglutamic acid, hence the acronym PCA. It is a kind of amphiphilic molecule made up of a hydrophobic part, represented by the mixture of the fatty acids of the olive and the hydrophilic head of PCA. PCA, of vegetable origin, is obtained from wheat gluten. Glutamic acid is separated by hydrolysis and is then forced into cyclization by pyrolysis. Well-known for its moisturising properties, pyrrolidon-carboxylic acid plays an important role in natural skin moisturization. The advantage of combining these two substances comes from the simultaneous presence of the lipidic oleic fraction and the hydrophilic non-ionic one, with limited spatial expansion and with functional groups similar to those of allontoin.
Under standard conditions, the degree of moisturization of the horny layer is approximately about 25-30%. The permanence in it of water molecules, even under conditions that may tend to reduce this concentration, is essential to provide skin softness, plasticity and suppleness. The factor of natural skin moisturization (NMF, of which PCA is 12%) has the capability to make simultaneous bonds with both water and the horny layer. The hydrolytic enzymes in the skin have the capability to hydrolize the amide bond and to separate the active agent in both constituting molecules: fatty acids and PCA. Thus, PCA becomes part of the NMF, while fatty acids, especially oleic and palmitoleic, thanks to their high skin compatibility, enrich the composition of the cell membranes in a direct way. Linoleic and linolenic acids (or Vitamin F) control important skin functions at the same time. These functions include forming and preserving the skin barrier and prostaglandin synthesis. This is important for the immune skin responses cascade where linoleic acid is the key precursor. Deficiency in linoleic acid can cause skin dryness and scaling off in children and old people.
On designing Olivoyl PCA, the following hypotheses on the prospective functions were considered:

- to reduce the amount of trans-dermal water loss by evaporation;
- to protect the skin barrier by controlling homeostasis;
- to restore the balance and stimulate keratinisation;
- to prevent scaling-off and fissures
- to increase skin resistance at low temperatures.

Moreover, the peculiar amphiphilic structure might impart a potential activity as co-surfactant/cleanser in combination with common surfactants. This may result in a predictable increase in skin tolerability due to anti-irritant effects.

Technical and Sensory Evaluations

After synthesising this derivative, successive in-use tests was carried out, so as to prove the expected properties/functions. The tests allowed to make a direct comparison between the two formulations, one of which was a placebo, by simulating standard conditions of use. The tested product were applied symmetrically by the same subject in a fixed skin area (face) over a fixed time interval.

- Evaluation of sensory parameters (gel)
- Evaluation of tolerability and cosmetic acceptability (cleanser)
- Capability to stabilize foam in a cleansing system

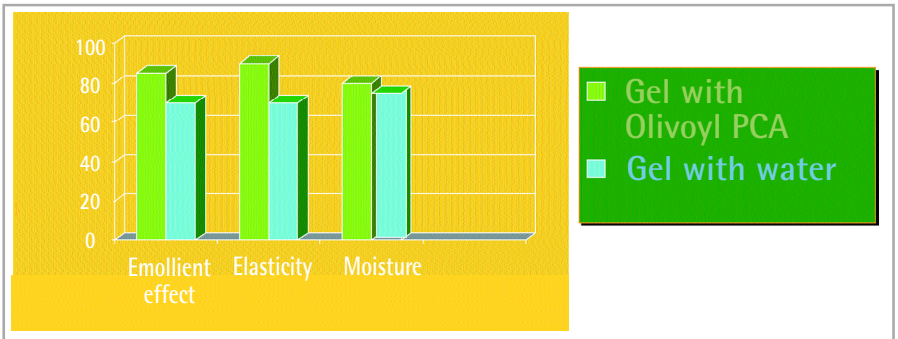

Fig. 1 Evaluation of the sensory properties of a gel containing Potassium Oliovoil Pyrrolidon-carboxylate

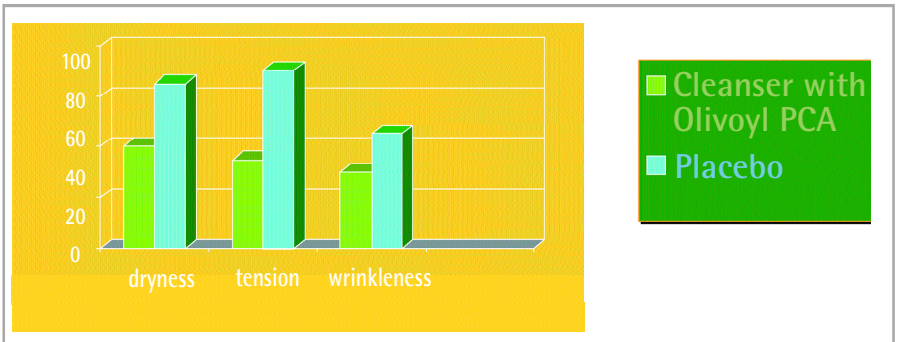

Fig. 2 Sensory evaluation of a cleanser containing Potassium Oliovoil Pyrrolidon-carboxylate

Evaluation of sensory parameters (gel)
Aim of the test was to evaluate the sensory perception of the skin parameters concerning the emollient property, moisture and elasticity perception (skin softness), after using a gel containing 5% Potassium Oliovoil Pyrrolidon-carboxylate in comparison with an inert aqueous gel, where the active principle had been replaced with the same water percentage.
Twenty subjects applied each gel on each half of their face twice a day for 5 consecutive days, by softly rubbing the product in. At the end of the day, the subjects answered a set of questions designed to assess the following skin parameters: emollient effect, elasticity and moisture effect (softness). The results are shown in **Fig. 1**.
With relation to the three parameters considered, the gel containing Olivoyl PCA yielded significantly better results than the placebo.

Tolerability and sensory perception of a cleanser
The same protocol described above was applied. Twenty subjects were selected and each of them applied two cleansers at 12% SLES. 5% Potassium Oliovoil Pyrrolidon-carboxylate was added to the tested sample while water replaced it in the placebo. The subjects applied each sample on half of their face washing it twice a day for 5 consecutive days. At the end of each day the subjects answered some questions designed to assess the perceived differences in skin dryness, tension and roughness. The test results are shown in **Fig. 2.**
The cleanser yielded lower values in the three tested parameters.

Foam stabilization
A comparison with Cocamide DEA was made. For this purpose, two solutions at 10% SLES were prepared. One containing 1% Olivoyl PCA (active substance) and the other Cocamide DEA at the same concentration. The Ross Miles method was applied at 20°C, using water at 15°F hardness and at the surfactant concentration of 1g/l. The results are shown in **Fig. 3**.
The active ingredient induced foam stabilization over time. Indeed, when the PCA derivative was present, the foam appearance was creamy and compact.

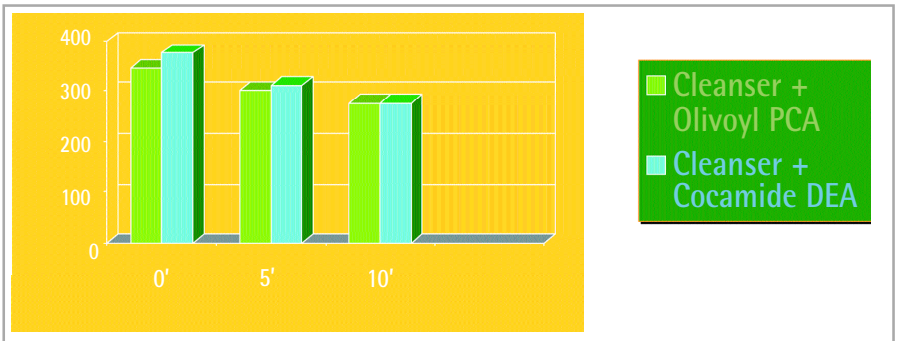

Fig. 3 Foam stabilization (height in mm at increasing time intervals)

Carnitine Conjugate-Linoleate: A Functional Active Against Akin Blemishes

A cosmetic field of biologic interest is represented by emulsions and other formulations aimed at preventing or balancing the cellular processes which may damage the skin appearance. These are regarded as skin blemishes, for example cellulite. It is due to excessive fat deposit in the cytoplasm of adipocytes, the cells of the fatty tissue, and gives the typical 'orange peel' appearance to the skin. Cellulite leads to visibly increased subcutaneous pads, oedema and a swelling sensation of the lower limbs.
Several ingredients and formulations claiming to have effective functional properties against this type of blemish can be found on the market. They have sometimes a base of vegetable extracts which have not been properly named or studied, or agents whose activity is unknown or lacks scientific documentary evidence.
An active ingredient which is of vegetable origin and has a provable activity reported in the literature is Carnitine Linoleate. This is a functional substance which combines L-Carnitine, well-known substance used in different scientific fields, with Linoleic acid in an innovative way through the ester bond.

Linoleic acid

It is an essential fatty acid, that is not synthesised in our body, that is deficient in the enzymes providing unsaturations at fixed points of the fatty chains. Linoleic acid can be found in nature in many vegetables and some oils (especially sunflower and safflower) and belongs to the acid fraction of olive oil. For this reason, olive oil is a fine source of an excellent chemical fraction to be employed for cosmetic purposes. In particular, a group of isomers of linoleic acid in a conjugate form and represented by the acronym CLA (Conjugate Linoleic Acid) is the key fraction. The chemical structure of CLA is constituted by 18 carbon atoms, two unsaturations at point 9 and 11 or 10 and 12 in all possible cis- and trans- combinations. Recent studies indicate that CLA induces the reduction in body fat and the increase in lean body mass. A double-blind study concerning the intake of CLA for 90 days showed a 20% average decrease in lean body mass without any significant change in body fat. In-vivo studies of CLA activity conducted on animals proved that this effect is due to a reduction in size of the adipocytes rather than to their decrease in number. Medium to high CLA doses on rats were able to decrease VLDL (very low-density lipoproteins) concentration in the blood and cholesterol in the liver. Therefore, CLA is an adjuvant in body weight control. However, the intake of CLA must always be in connection with a balanced diet and regular physical exercise so as to let CLA perform its function in the best possible way. Moreover, CLA also plays another important role as a carrier of Carnitine structure in CLA Carnitine.

Carnitine

Carnitine is a substance which aids in penetrating the skin barrier and the cellular membrane structures, since the adipocytes and the fat deposits are located in a deep skin layer, the dermis. Carnitine was firstly found in the muscular tissues, where it is present as is or esterified with an acyl group. L-Carnitine is a natural constituent of the body cells. Additional intake of L-Carnitine facilitates its natural stimulating role in the use of fatty acids which results in the increase of the metabolism and reduction of skin lipidic substrates. Moreover, Carnitine reduces glucose consumption in microcirculation, protects the endothelium, the smooth musculature of the surface blood vessel. In particular, peripheral vascularization is improved thanks to its structural similarity to acetylcholine, which is an endogenous neurotransmitter. This function makes it especially effective in treating cellulites.

CLA Carnitine

Once described the biological activities of both reaction partners, it is simple to foresee the effect achieved in the combination with each of them. The association of both molecular structures is able to provide better properties than every single ingredient. Moreover, this gives the advantage of a higher concentration as well as an increased amount of Carnitine in the cells. The expected cosmetic functions of CLA Carnitine can be summarised as follows:

- remarkable decrease in surface lipid deposit;
- increased metabolic efficiency in terms of use of subcutaneous lipids;
- increased enzymes activity in cell respiration and consequent increased energy production of the cells;
- decreased formation of free radicals during physical exertion.

Carnitine Conjugate-Linoleate: Hypotheses on Functions and Tests

In-use tests proved the efficacy in controlling cellulitis when Carnitine Conjugate-Linoleate is present in body emul-

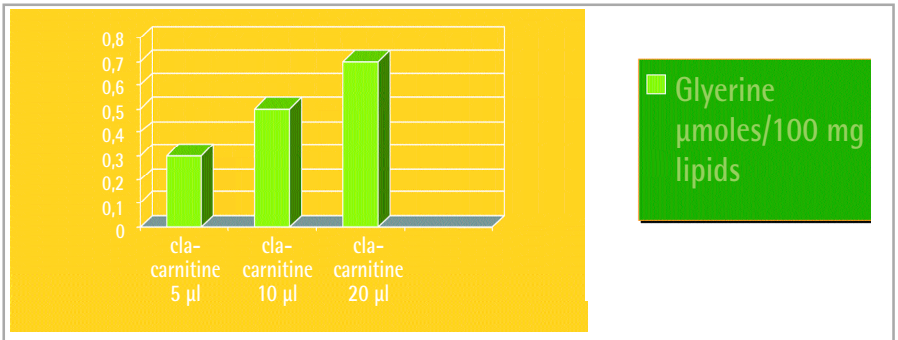

Fig. 4 Lipolitic effect of Carnitine Conjugate-Linoleate expressed in µmoles of glycerine produced

sions aimed at reducing the 'orange peel' appearance of the skin. In addition, the active ingredient can also be used in products designed to treat skin relaxation and elasticity loss of the connective tissue. These symptoms usually anticipate the appearance of stretch marks. Moreover, the active ingredient is suitable for very sensitive and seborrhoeic skin. The simultaneous presence of both Carnitine and its linoleic derivative creates a potential sebum-controlling property, to counteract the 'greasy' appearance of this skin type.

Lipolitic effect on adipocytes

The study involved a number of cells taken out from the perineal adipose tissue by means of collagenase. It was thus obtained a homogeneous suspension of isolated adipocytes which are able to keep their functionality for several hours after the treatment and can be kept either in an isotonic buffer solution (isotonic Krebs Ringer bicarbonate containing 3g/100ml albumin and 6mM glucose; pH 7,4 – KRBA) or in a culture medium commonly used for cells added with albumin (type DMEM/HAM F12).
Some culture samples were taken and either the amount of glycerine or the released free fatty acids were determined. Each trial was repeated for every fixed concentration of active ingredient. The lipolitic activity was measured by adding consecutive dilutions of the active ingredient (15 to µl) and is expressed in µmoles of glycerine or free fatty acids released for each weight unit of total cellular lipids after extraction **(Fig. 4)**.

2) In-use test (Half BODY).

An emulsion containing CLA Carnitine was tested for anti-cellulite and slimming efficacy in comparison with a placebo emulsion. Pleasantness and cosmetic acceptability assessments were also performed. The in-use test method was adopted to make an immediate and reliable comparison between the two cosmetic creams by simulating the standard use conditions. The subjects applied both emulsions on the same skin areas (thighs and gluteus muscles) over the same period of time. Differences among

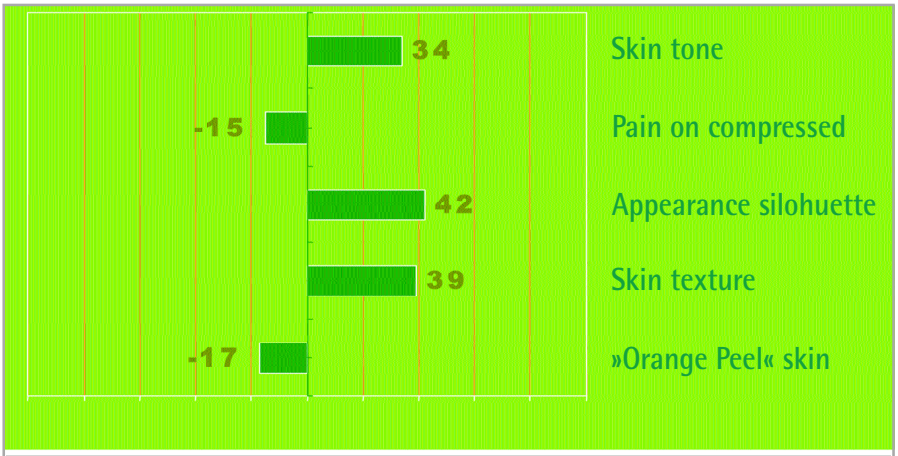

Fig. 5 In-USE test (Half BODY)

Formula 1 Refreshing Personal Hygiene Detergent

Formulated for a mild cleansing action, respecting skin physiology. The olive oil and wheat protein derived surfactant, re-establish the hydro-lipidic equilibrium. The moisturizing properties of Potassium Oliovoil Pyrrolidon-carboxylate fight the water loss form the skin cells. A refreshing action is provided by mint distilled water, while lactic acid buffers the pH of mucous membranes.

Phase	Ingredients/INCI-Name	%
A	Water Aqua	to 100
A1	Allantoin	0,10
A2	Panthenol	0.20
A3	Sodium Laureth Sulfate (27%)	21.50
A4	Didosium Laureth Sulfosuccinate (30%)	11.00
A5	Potassium Olivoyl PCA [1] Potassium Oliovoil Pyrrolidon-carboxylate	5.00
A6	Olivoyl Hydrolyzed Wheat Protein [1] (27%)	10.00
A7	Disodium Cocoamphodiacetate (27%)	7.0
A8	Parfum	0.20
A9	Phenoxyethanol	0.50
B	Water Aqua	26.00
C	DMDM Hydantoin	0.20
D	Mentha Piperita distilled water	0.50
E	CI 14700 0.1% aq	0.35
F	Lactic Acid	1.1

[1] Kalichem

Add in the main mixer A, A1and A2, while mixing until complete solution. Add A3, A4, A5, A6, A7, A8, A9 under maximum vacuum, mixing after each addition. Add slowly B while mixing. Always under vacuum and mixing, add C, D, E. Finally, add F, and mix some minutes. pH 5.0-5.5, viscosity (Brookfield RVT, 25°C, spindle #3, 5 rpm 1750 mPa.s

all the test were thus minimized. Fifteen female subjects in the 25-45 age group were selected. Each subject applied both the sample with CLA Carnitine and the placebo twice a day for 4 consecutive weeks. The creams were applied and gently rubbed in the skin areas of the thighs and gluteus muscles. Before applying the samples, the subjects washed their hands with a standard unscented detergent in order to remove substances which might bias the test. At the end of the test, the subjects were asked to fill in a self-assessment questionnaire whose results are reported in **Fig. 5**. Finally, thigh measurements were taken before and after the treatment.
The results show the perceived effect of CLA Carnitine activity. Moreover, the findings evidenced a decrease in the thigh measurements from 4 to 12 mm at the end of the treatment period (4 weeks).

■ Conclusions

Both functional actives in question proved to be interesting ingredients for the employment in a large number of cosmetic formulations, some of which are reported below as examples.
Besides their renewable vegetable sources, the main advantages offered by these ingredients are represented by their typical groups of lipid chains, high compat-

Formula 2 Mild Shampoo

Pearled mild shampoo, suitable for frequent cleansing and fragile hair. The vegetal-derived surfactant protects the scalp

Phase	Ingredients/INCI-Name	%
A	Water Aqua	34.60
	Panthenol	0.10
	Polyquaternium-39	1.00
B	Sodium Laureth Sulfate (27%)	28.00
	Sodium Methyl Cocoyl Taurate (45%)	14.00
	Olivoyl Hydrolyzed Wheat Protein [1] (27%)	10.00
	Cocamide DEA (85%)	3.00
C	Potassium Olivoyl PCA [1] Potassium Oliovoil Pyrrolidon-carboxylate	5.00
D	Phenoxyethanol	0.50
E	DMDM Hydantoin	0.20
F	Parfum	0.40
G	Glycol Distearate (and) Sodium Laureth Sulfate (and) Cocamide MEA (and) Laureth-10	2.00
H	CI 42051 (0.1%)	0.20
	CI 19140 (0.1%)	0.40
I	Lactic Acid (80%)	0.60

[1] Kalichem

Add A ingredients in the main mixer and mix until dissolved. Under vacuum, add B ingredients, then mix for 15' after last addition. Then, add C, D, E, F and G, while mixing after each addition. Finally, add H and I. Pearled green fluid, pH 6-6.5, viscosity Brookfield RVT Spindle#3, 5 rpm, 25°C, 1.560 mPa.s

ibility with cellular structures, their aid to keep the homeostatic balance of the skin metabolism. It must be underlined that both functional actives in question can be mixed in the same formulation. This allows the achievement of both synergistic effects and the strengthening of some properties, thanks mostly to their lipid fraction. Moreover, distinct activities on the different layers of the epidermal structure can be obtained. As a matter of fact, Olivoyl PCA acts on the surface levels of the skin (horny layer) providing the corneocytes barrier with a moisturizing and emollient effect; while, CLA Carnitine acts on a deeper layer, in the derma, where adipocytes are located.

Formula 3 Mild Cream Bath

Creamy emulsion, gently foaming, that cleanses skin without aggression. Formulated with mild surfactants, protects the hydro-lipidic film, for its olive oil lipids. Soothes skin and prevent dryness. Allantoine and betaine help maintain skin equilibrium and softness.

Phase	Ingredients/INCI-Name	%
A	Water Aqua	to 100
	Allantoin	0.10
	Panthenol	0.20
	Betaine	1.00
	Olivoyl Hydrolyzed Wheat Protein (27%)	10.00
	Zinc Coceth Sulfate (25%)	15.00
	Sodium C14-16 Olefin Sulfonate (27%)	18.00
	Disodium Cocoamphodiacetate (27%)	14.00
B	Isostearic Acid	2.00
	Olivoyl Hydrolyzed Wheat Protein and Cetearyl Alcohol and Glyceryl Oleate [1)]	3.00
C	Propylene Glycol	1.00
	Potassium Olivoyl PCA [1)] Potassium Oliovoil Pyrrolidon-carboxylate	5.00
D	Silica	2.00
E	Water Aqua	1.00
	Imidazolidinyl Urea	0.25
F	Phenoxyethanol	0.60
G	Parum	0.40

[1)] Kalichem

Add A ingredient into the main mixer, and mix while heating at 60°C under vacuum. Heat B at 65°C to complete solution, and add under vacuum into the main mixer, while homogenizing. Keep homogenizer for 15'. Cool down to 40°C under mixing. Add C and mix some minutes. Add D without mixing, put again under vacuum until lumps disappear. Add E, F and G while mixing after each addition. Creamy off-white emulsion, pH 6-6.5, viscosity Brookfield RVT Spindle#5, 5 rpm, 25°C, 41.600 mPa.s.

Literature

(1) *Watts R, Sousselier L*; Fruits of the gods; Soap, Perfumery & Cosmetics: 10 (2000), 63-8

(2) *Kiritsakis AK*; Virgin olive oil composition and its effect on human health; Inform, 13 (3) 2002, 237-41

(3) *Sousselier L*; Novel ingredients from Olive-nature's gift for beauty, p.171-76

(4) *Montgomery R, Dryer, Conway T, Spector A*; Biochimica – Aspetti medico biologici, Ed. Ermes, III ediz. 1981 (8), p. 429

(5) *Kruh J*; Biochimica-aspetti medici e biologici, Ed. scientifiche e tecniche Mondadori, I ed. 1976 (IV) 339

(6) *Rigano L, Sirigu S*; Analisi sensoriale: uno strumento della qualità in cosmetica; Cosmesi Dermatologica 47 (1990) 81-94

(7) *Stone H, Mc Dermott BJ, Sidel JL*; The importance of sensory analysis for the evaluation of quality"; Food Technology: 6 (1991)

(8) *Stone H, Sidel J*; Sensory evaluation practices- Ed. Academic Press Inc. (1995)

(9) *Hootman RC*; Manual on descriptive analysis testing for sensory evaluation; ASTM manual series: MNL 13 (1992)

(10) I.F.S.C.C. Monograph: Principle of Product Evaluation: Objective Sensory Methods; (1) 7

(11) Olivoil products- Brochure, by Kalichem Italia S.r.l.

(12) Technical file: Olivoil PCA, by Kalichem Italia S.r.l.

(13) Technical file: CLA-L-Carnitina, by Kalichem Italia S.r.l.

* *Author's address:*
Luigi Rigano
Via Bruschetti 1
20125 Milano
Italy
Email: rigano@thecosmetologist.com