PI-100-10

INKAKrameria

Rev 1: September 29th, 2014

INKA KRAMERIA is a Natural Ingredient preservative free, organic certifiable, based on the Rathany root (Krameria Triandra) widely used for centuries for its properties as astringent and anti-inflammatory. Moreover, the plant of Krameria triandra, also has photoprotective properties, so that its use is recommended as protection against of UV-B radiation.

INCI Denomination: Propanediol (and) Water (and) Krameria Triandra Root Extract

Description of the plant:

Family: Krameriaceae

Botanical Name: Krameria triandra Ruiz y Pavón

Synonyms: Krameria iluca Phil; Krameria lappaceae (Dombey) Burdet & B. Simpson[1,2] Krameria lappacea (syn.: Krameria, canescens Willd. ex Schultes, K. linearis Poiret, K., pentapetala R. et P., K. triandra var. humboldtiana Chodat, Landia lappacea Dombey)[3]

Other Names: Mapato (Spanish), Ratanhia (German), Rhatany (English); Antacushma, malapato, pumachucú, pumakachu (Aymara), ractania, rataña, sanyo; ratanya, Ratiñay (Quechua).[4,5,6,7]

Description:

Rhatany is a short shrub with a woody and branched stem. Oval and pointed leaves densely covered with silky hair and wide flowers with a pink to purple color. Fruits with the size of a pea, covered with fine and hard bristles containing one or two seeds. Root up to 50 cm long, cylindrical, flexible, reddish brown with dark scaly bark, astringent bark, wood almost tasteless.[8]

[1] DUKE (2009), page 398
[2] TAYLOR (2010)
[3] BRAKO et al. (1993) in HAMMOND G.B. et al. (1998) page 25
[4] DUKE (2009), idem
[5] SOUKUP (1970), page 178
[6] NETO C.C. et al. (2002), page 134
[7] H. DE-LA-CRUZ et al. (2007) page 290
[8] CULBRETH D. M., (1996) pages 299-300

Tnte. Enrique Delucchi 80. Barranco
Tel: +51 1 463 0350. Info@3qp.biz

Distribution:

Rhatany is Native to South America, particularly Bolivia and Peru[9]. It grows in rocky and dry areas over mountain slopes from Ecuador, Peru, Chile, and Bolivia to Argentina, at altitudes of 600-3600 meters above sea level.[10] In Peru, it is found in Inter-Andean valleys and coastal hills.[11]

Traditional Uses:

In 1784, Ruiz observed that the women of Huanuco and Lima used the Rhatany root to preserve the teeth and also as astringent. Tschudi mentioned that women of Lima used to clean their teeth with the "root of teeth" and that they always carried a piece of it in their pocket. It is used also as powerful astringent and hemostatic.[12]

The cooking of stems and roots is drunk to treat mouth and throat inflammations, and upset stomach.[13]

Phytochemicals:

The Rhatany root is rich in polyphenolic constituents: tannins, its most abundant constituents; neolignans of low molecular weight and oligomeric proanthocyanidins of medium-high molecular weight. The astringency characteristic of the extract is due to the proanthocyanidins with polymerization degree of 5-10.[14]

The tannins are phenolic polymeric compounds widely distributed in the plant kingdom that fulfill a role of chemical protection for the plant against predators and ultraviolet

[9] THOMSON W., (1980), page 76 in GODAY (1987) page 2
[10] SIMPSON (2004) in GRAZI D., 2008, page 2
[11] Brako, L. & J., Zarucchi. 1993. Catalogue of the Flowering Plants and Gymnosperms of Peru.
[12] SOUKUP (1970), page 178
[13] HAMMOND G. et al., (1998) page 25
[14] SCHOLZ E et al., Med. Plant (1989) in HAMMOND G. et al. (1998) page 25

radiation. They are classified as water-soluble tannins and condensed tannins.[15] This second category includes the proanthocyanidins; oligomers and polymers composed of flavonoid monomers, mainly (+)-catechin and (-)-epicatechin.[16] [17]

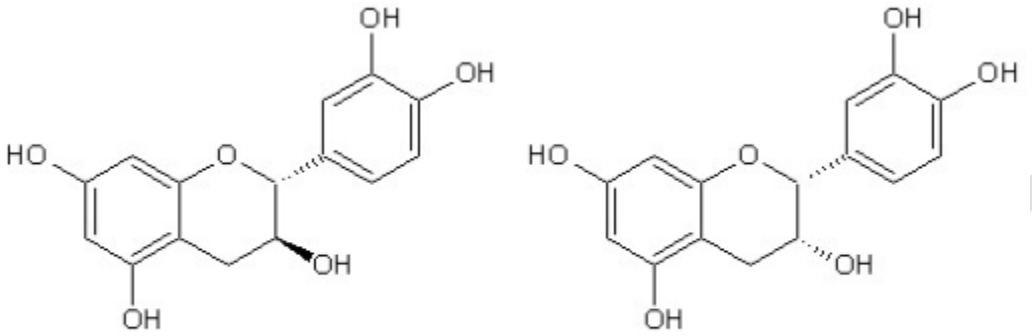


Fig 1: Chemical structure of catechin and epecatechin

COSMETIC BENEFIT:

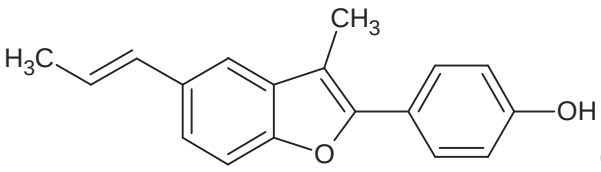
Due to its astringent action, the Rhatany root has been used for preparing hemostatic and antidiarrheal drugs and is part of the official abstracts of several countries: Japan, Germany, and France.[18]

Antibacterial and antifungal

The antifungal and antibacterial inhibiting activity of the proanthocyanidins is recognized and is explained by three mechanisms: the tannins cause inhibition of the extracellular microbial enzymes, exert direct action over the microbial metabolism and are capable of giving a complex of metallic ions, necessary for the microbial growth.[19]

[15] GALI-MUHTASIB HU et al., (1999)
[16] BEECHER G., page 2
[17] COS P. et al., 2003, pages 1345-1346
[18] KANSHIRO K. et al., US Pat 4,886,667
[19] COS P. et al., 2003, page 1349

The neolignans of the hydrophilic extract of Rhatany, particularly Eupomatenoid, have also shown antimicrobial activity against gram-positive bacteria, gram-negative bacteria, fungi and anaerobic strains.[20] [21]



eupomatenoid 6

Antioxidant activity

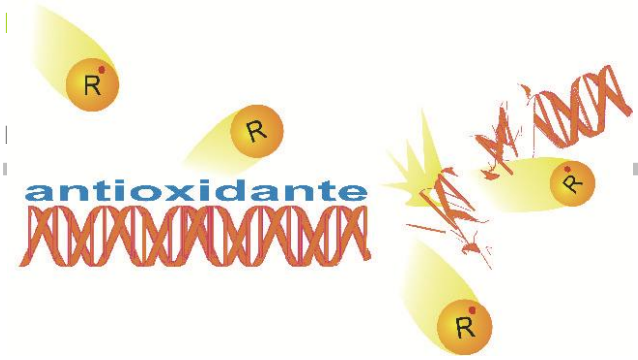
Plant polyphenols and particularly proanthocyanidins are potent antioxidants, comparable in *in vitro* assays to vitamins C and E[22,23] with anti-free radical effects, chelation of transition metals and inhibition of prooxidant enzymes.[24]

$$AH + R^{\bullet} \rightarrow A^{\bullet} + RH$$

The free radicals are molecules provided with a potent oxidant capacity. Normally, they occur during metabolism. Also, the immunological system of the body creates them to neutralize virus and bacteria. Other sources of free radicals and oxidative stress include environmental factors such as pollution, cigarette smoke and certain pesticides.[25]

Under normal conditions, our body is able to maintain a balance between the free radicals that are generated or that infiltrate from the outside, and the systems that neutralize them. When the antioxidant defense is not one hundred percent efficient, the formation of free radicals increases; this is called oxidative stress: the tissues become attacked, producing an accelerated aging.

[20] BOMBARDELLI E. et al., US 2001/0046525 A1
[21] ARNONE et al., 1988, 1990; DEBELLIS et al., 1994 in HAMMOND G. et al. (1998) page 25
[22] BEECHER G.R. 2004, page 5
[23] ARIGA T., 2004, page 198
[24] COS P. et al., 2003, page 1351
[25] BLAKE S., 2007.



The free radicals attack especially the cellular membranes causing it destruction. These membranes are the delicate support of the genetic map of the cells, which nucleus contains the DNA. The integrity of this membrane protects the DNA and the life of our cells.

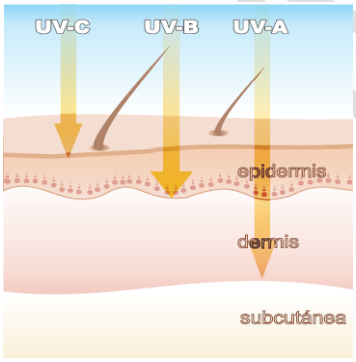
Assays with Rhatany extract have found protective effects against the damage induced by UB radiation in human keratinocytes.[26]

Photoprotection

The UV radiation is part of the electromagnetic spectrum between 200 nm and 400 nm. Its wavelength is considered: long UV-A (320–400 nm), medium UV-B (280–320 nm), and short UV-C (200–280 nm).

The UV-C radiation, extremely harmful, is absorbed almost completely by the atmosphere. However, due to the damage on the ozone layer, the amount of radiation that reaches the Earth's surface is increasing: 90% of the radiation that reaches the earth is UV-A; it penetrates the epidermis and reaches the dermis of the skin inducing oxidative processes in the cells: darkening reactions, loss of collagen, decrease in the amount of blood vessels, alteration of the connective tissue of the dermis and photosensitivity. In the UV-A range, the melanin and hemoglobin are the main absorbents.[27]

The UV-B radiation (5% of the UV radiation) acts on the epidermis and is 1000 times more powerful. It decreases the natural antioxidants of the skin, preventing its self-protection against the free radicals generated by the exposure to the sun.[28] It is the cause of suntan and immediate skin damage: erythema and sunburn.[29]



The exposure of the skin to UV radiation induces the generation of reactive oxygen species (ROS). They may react with the DNA, fatty acids and saccharides producing damage by oxidation. Many processes of modern life generate likewise free radicals and oxidative stress; in the long run, the accumulation of damages may lead to photoaging and eventually to the development of cancer.

[26] CARINI M. et al., (2002)
[27] DIFFEY, B.L. (2005) in JAIN S. K. et al. (2010), pages 89-90.
[28] SVOBODOVÁ A. et al., 2003, page 138
[29] DIFFEY, B.L. (2005) idem.

Although the human skin has a sophisticated system to protect itself from the damage caused by UV radiation, the prolonged and chronic exposure may surpass it; therefore, the use of photoprotective substances is recommended. The natural ingredients that contribute to this function have been receiving special attention, for example, the polyphenolic compounds (such as flavonoids and tannins) for their absorption in the UV region and their antioxidant activity. In trials on animals, administered as part of the diet or by topical application, the tannins showed protective effects for skin damage caused by UV exposure.[30] [31] In Vitro studies on cells of human keratinocytes concluded that the Rhatany root extract contains protective effects against the damage induced by UV-B radiation[32] and propose its use as herbal filter.[33] [34]

Elastin protection

The connective tissue of the skin is composed mostly of collagen and elastin. Collagen makes up 70-80% of the dry weight of the skin and gives the dermis its mechanical and structural integrity. Elastin is a minor component of the dermis (2 – 4%), but it has an important function in providing the elasticity, resilience and suppleness of the skin.

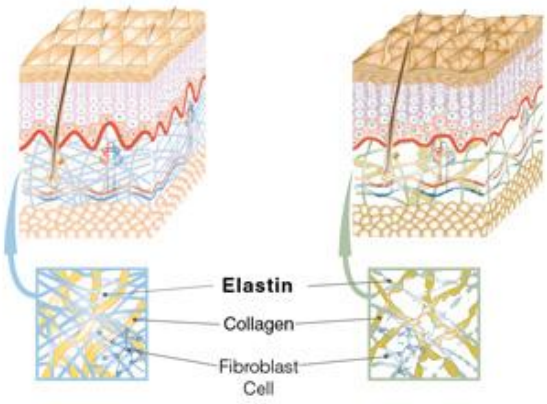


Fig 2: Model of the skin

Elastin is a protein that coil and recoils like a spring within the elastic fibers of connective tissue and accounts for the elasticity of structures such the skin, blood vessels, heart, lungs, intestines, tendons, and ligaments.

This connective network shows definite changes associated with aging. Sun exposed skin, is characterized by degeneration in the elastic fiber network. Trough different mechanisms, photoaging and intrinsic aging ultimately result in a deficiency of functional, structurally intact elastic fibers. These alterations largely account for the decrease in skin's physiological elasticity with increased age.

[30] GALI-MUHTASIB HU et al., 1999 in SVOBODOVÁ A. et al., 2003, page 142
[31] VAN WIJK, E et al., (2010)
[32] KHAZAELI P. et al., (2008) page 8
[33] JAIN S. K. et al., (2010), page 92.
[34] CARINI M. et al., (2002)

Plants containing a wide variety of compounds including polyphenols such as flavonoids, tocopherols, phenolic acids and tannins have been found to provide Collagenase and Elastase inhibitory activities.

INKA KRAMERIA rich in these antioxidant elements additionally has shown to have an important inhibition of Elastase enzyme, providing protection to the skin against oxidative stress.

Astringent and soothing

Due to their astringent and antimicrobial properties, as well as potentially anti-inflammatory, the Ratania extracts are included in several formulations for skin treatment (mild inflammation of the skin, acne).[35] [36] [37]

Efficacy Tests

EVALUATION OF ANTIOXIDANT ACTIVITY

Total Antioxidant Activity DPPH Assay

The general free radical scavenger activity of the INKA KRAMERIA was evaluated by its interaction with DPPH (2,2-diphenyl-1-picrylhydrazyl) in solution. DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. Because of its odd electron, the methanolic solution shows a strong absorption band at 517 nm (Blois, 1958), which decreases in the presence of free radical scavengers. DPPH scavenging activity has been largely used as a quick and reliable parameter to assess the in vitro general antioxidant activity of plant extracts, which has been assigned to phenolic compounds namely, phenolic acids and flavonoids.

[35] ISHII SUMIE et al., (1993), JP 5229930 (A)
[36] BOMBARDELLI E. et al., US 2001/0046525 A1
[37] TAKADA et al., (2001), JP2001278797 (A)

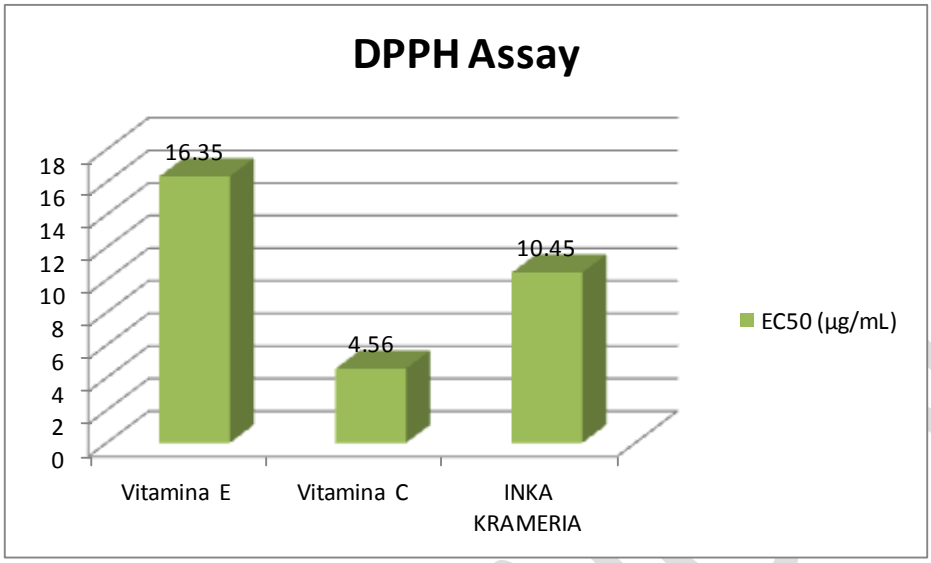


Fig 3: DPPH Assay for INKA KRAMERIA, Vitamine E and Vitamine C

INKA KRAMERIA has shown an important scavenging activity, with an EC50 of 10.45±0.48 µg/mL (n=3), superior than the one of Vitamin E (16.35±2.71µg/mL) and a little low than the one of Vitamin C (4.56±1.03 µg/mL).

TROLOX Assay

	TEAC average±
BHT	1.29 ± 0.04*
BHA	1.02 ± 0.04*
VITAMIN E	0.89 ± 0.01*
INKA KRAMERIA	0.71 ± 0.04

The TEAC (Trolox equivalent antioxidant capacity) assay is based on scavenging of 2,2′ -azinobis-(3-ethylbenzothiazoline-6-sulfonate) radical anions ($ABTS^{\bullet-}$) In this method, an antioxidant was added to a solution pre-formed out of the $ABTS^{+\bullet}$ radical-cation and, within a fixed range of time, the $ABTS^{+\bullet}$ residual radical-cation was spectro-photometrically quantified.

Reducing Power

The hydroxyl radical plays a significant role in the damage caused by the UV radiation and is more reactive towards the damage of the cellular constituents compared to the hydrogen superoxide and peroxide radicals.

The reducing potential of the INKA KRAMERIA was measured in its capacity to reduce the ion Fe^{3+} by the method of Hazra et al[38] and L-ascorbic acid was used as positive control. The results are showing in Figure 2.

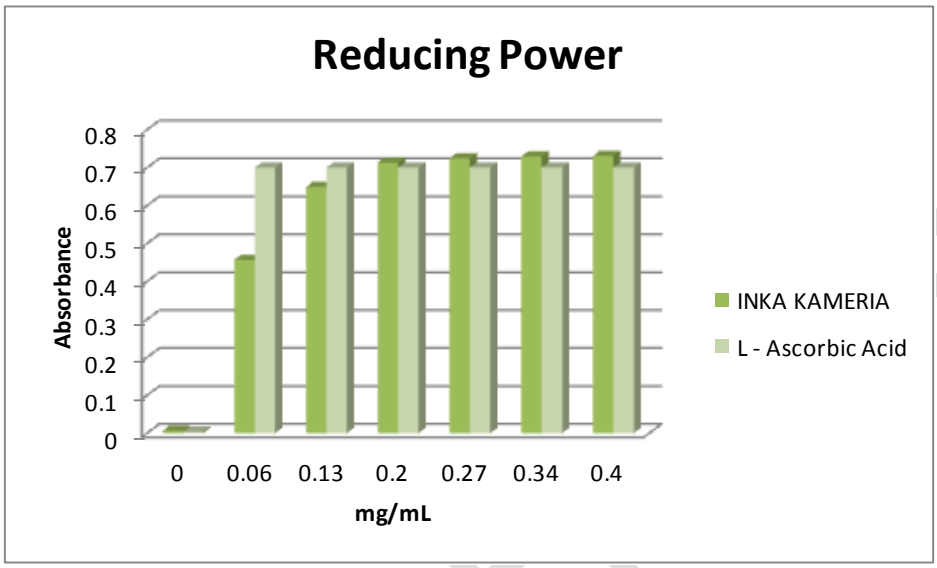


Fig 4: Reducing Power of INKA KRAMERIA

It is observed that from a concentration of 0.20 mg/mL, the reducing activity of the INKA KRAMERIA is equal to and even a little higher than the one of L-ascorbic acid.

INHIBITORY EFFECT ON MUSHROOM TYROSINASE

There are various causes for the darkening of skin color; UV rays are considered the primary source. Abnormal pigmentation such freckles or chloasma can be serious aesthetic problem. Tyrosinase plays the most important role in melanin synthesis.

Purified tyrosinase from mushroom was used in for this *in vitro* test according the method described by Yoshimura et al[39]. In table 1 is summarized the concentrations of various Tyrosinase-Inhibiting Agents that inhibit 50% of the enzyme activity (IC50).

Inhibitory effects on mushroom tyrosinase	
	EC50 (µM)

[38] Hazra B. et al., (2008)
[39] Yoshimura M. et al. *Biosci. Biotechnol. Biochem.,*69 (12),2368-2373, 2005

Arbutin	144.6*
Kojic Acid	34.7*
Oxyresveratrol	2.4*
INKA KRAMERIA	22.2 µg/ml

Table 1: Inhibitory effects on mushroom tyrosinase

*[40]

INKA KRAMERIA shows a very important inhibitory effect on mushroom tyrosinase assay suggesting that could be effective in protective role against UVB radiation.

ELASTASE ASSAY

The assay employed was based on methods from Tamsyn SA Thring (2009)[41]. This assay was performed using Porcine pancreatic elastase. As substrate N-Succinyl-Ala-Ala-Ala-*p*-nitroanilide was used. The test extract were incubated with the enzyme for 15 minutes before adding substrate to begin the reaction. EGCG (250 µM or 0.114 mg/mL) was used as a positive control. Negative controls were performed using water. Absorbance values between 381 and 402 nm (following pre-screen scans) were measured immediately following addition of the substrate and then continuously for 20 minutes using a Cary 50 Microplate Reader in Nunc 96 well microtitre plates. Result can be seen in figure 5.

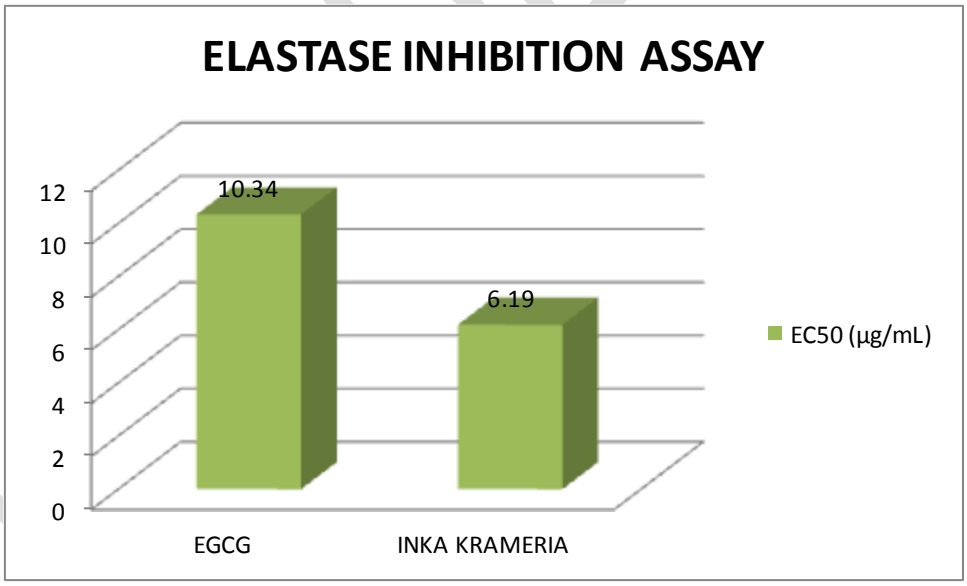


Fig 5. INKA KRAMERIA ELASTASE INHIBITION ASSAY

[40] Michelle Kim, *et al.* Cosmetics & Toiletries March 2006

[41] Thring Tamsyn SA, et al. Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. *BMC Complementary and Alternative Medicine* 2009, 9:27

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An outstanding Elastase Inhibition property shows **INKA KRAMERIA** extract; with an IC50 of 6.19 ±0.91 is 40% more potent than EGCG the positive control.

CONCLUSION

INKA KRAMERIA is a novel innovative **Natural Ingredient with Traditional Knowledge** of use and studies that confirmed its employ over centuries. INKA KRAMERIA could be **use in cosmetic products in a new way to defend the skin against cellular oxidation, UV radiation and elastin degradation, protecting the skin from premature aging. Additionally, INKA KRAMERIA could provide a good complementary action of the use of filters or anti-free radicals in sun care products.**

It is indicated for:

- Skin Care. Products for sensitive skins.
- Anti-aging treatments: Anti-free radical products. Protective products.
- Sun and after sun products.
- Body care products
- Capillary products (helps to maintain coloration)

Dose of use – Solubility – Preparation

3QP INKA KRAMERIA EXTRACT is manufacture **without preservatives, without GMO organism** and without petroleum or animal derivatives.

DOSE OF USE: From 1 to 5%.

SOLUBILITY: Water-soluble.

PREPARATION: The 3QP INKA KRAMERIA EXTRACT is a product sensitive to light, humidity and contact with iron. Preferably, it will be incorporated in the preparations at the end of the manufacturing process and below 35°C.

Analytical Information

Aspect:	homogeneous liquid
Odor:	characteristic
Color:	brown reddish
Solubility in water:	miscible
pH (20°C):	4.0 – 6.5

Tnte. Enrique Delucchi 80. Barranco
Tel: +51 1 463 0350. Info@3qp.biz

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Specific gravity, 20°C: 1.000 – 1.045

PRESERVATIVES: None

MICROBIOLOGY:

Total aerobic mesophilic count:	≤ 1000 ufc/ml
Total fungi and yeast count:	≤ 100 ufc/ml
Pathogens:	Absence

PRESERVATION: Store in airtight container, protected from light and humidity, at 15 25°C.
If the original container is opened, it should be handled with special care in order to avoid a secondary microbiological contamination.

We provide our best knowledge about the subject; however, the formulator will have the responsibility to ensure the stability of the formulation by performing the necessary tests.

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Tnte. Enrique Delucchi 80. Barranco
Tel: +51 1 463 0350. Info@3qp.biz

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