PI-100-03

INKACamuCamu

Rev1. June 23rd, 2014

INKA CAMU CAMU is a unique Natural Ingredient, preservative free; organic certifiable, extracted from Amazonian Camu camu fruit, one of the richest natural sources of Vitamin C, that provides to the skin an important antioxidant capacity against free radicals, one of the causes of its premature aging.

INCI Denomination: **Propanediol (and) Water (and) Myrciaria Dubia Fruit Extract**

Description of the Plant:

Family: Myrtaceae

Botanical Name: Myrciaria dubia (H.B.K.) McVaugh.

Synonyms: Myrciaria divaricata, M. paraensis, M. spruceana, Psidium dubium

Other Names: camo-camo, caçari o araçá-de-água (Brazil); guayabo (Colombia); guayabato (Venezuela)[1]

The camu-camu is a shrub or small tree, 4-8 m tall, without a detectable trunk, heavily branched from ground level. The spherical fruit is 2-3 cm in diameter, reddish-purple to purplish-black when mature. The pulp is acidic, edible, with an agreeable flavor.

Camu camu is a shrub or small tree 4-8 m tall with a spherical soft fruit with skin of pink to purple color and black fleshy pulp very acid in flavor and a pleasant aroma.

Distribution:

This species is widely distributed in the low Amazon Rainforest, mainly in the margins of the rivers and lakes of Peru, Colombia, Brazil and Venezuela[2]. It grows in humid tropical climate, in flood alluvial zones as wild and cultivated shrub[3].

[1] Elesbao R. et al., (2002). Dostert N. et al., (2009), p. 7.

Tnte. Enrique Delucchi 80. Lima 04. Peru
T: +51 1 463 0350
E-mail: info@3qp.biz

PI-100-03

INKACamuCamu

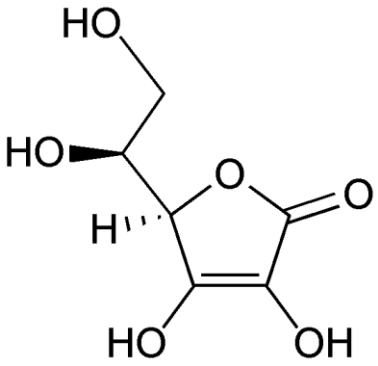
Rev1. June 23rd, 2014

In natural populations, the harvest is difficult: the plants are in flooded lands and only the fruits available over the water level are extracted with the help of canoes[4].

Traditional Uses:

The uses of camu camu are very diverse: as food, the ripe fruit is consumed in soft drinks and delicious ice cream. According to Dr. G. T. Prance, director of the Royal Botanical Gardens at Kew in London and one of the leading experts on Amazonian botanty, the fruit contains thirty times more Vitamin C than citrus[5]. Among the macronutrients, potassium was the most abundant mineral (711 mg kg^{-1}) and could be considered, like vitamin C, nutritionally significant.

Regarding the medicinal aspect, a poultice is made with the ground bark to heal wounds; also, the fruit is drank liquefied with water as flu remedy, laxative, to soothe gastro-intestinal discomfort and rheumatism[6].



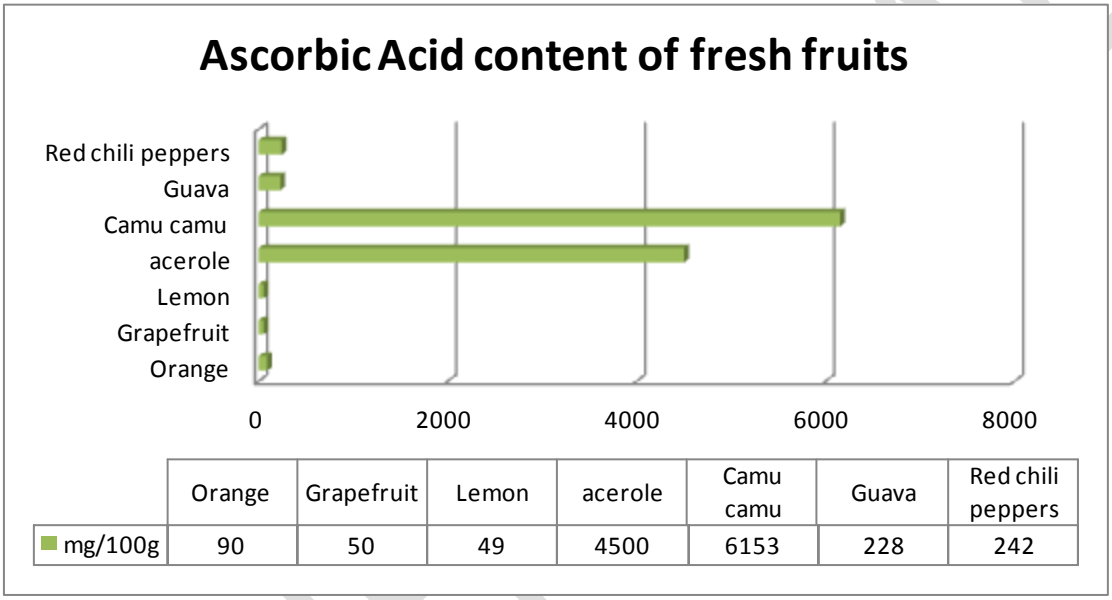
Phytochemicals:

The main characteristic of the camu camu fruit is its extremely high content of L-ascorbic acid (Vitamin C, natural antioxidant): 2,400 to 3,000mg/100g[7],[8], much higher than the one found in the acerole pulp (Malpighia marginata) or citrics, traditionally known as sources of this vitamin. Currently, it is recognized as one of the plants richest in Vitamin C.[9]

[2] Elesbao R. et al., (2002).
[3] Brack Egg A., (1999), p. 334
[4] Dostert N. et al., (2009), p. 7.
[5] Mark J. Plotkin. Tales of Shaman´s Apprentice. ISBN 0-670-83137-9. Penguin Group.
[6] Pierce S., (2009).
[7] Silva, (1998) en Elesbão R et al. (2002)
[8] Justi K. et al. (2000)
[9] Kenichi N et al. (2006)

Tnte. Enrique Delucchi 80. Lima 04. Peru
T: +51 1 463 0350
E-mail: info@3qp.biz

Fig 1: Ascorbic Acid



	Orange	Grapefruit	Lemon	acerole	Camu camu	Guava	Red chili peppers
mg/100g	90	50	49	4500	6153	228	242

Fig 1: Ascorbic Acid content of some fresh fruits

The peel, besides containing higher amount of L-ascorbic acid than the pulp 5,000mg/100g)[10] contains a phenolic fraction rich in anthocyanins, coloring compounds that provide the red to purple shade characteristic of the ripe fruit[11] and which also act as antioxidants.[12]

Other substances identified in the camu camu fruit include carotenoid compounds (trans-luteine, β-carotene, violaxantine and luteoxantine)[13], volatile terpenics (cis-β-ocimeno, caryophellene, α-pinene, d-limonene.[14]

[10] Villachica, H., (1996)
[11] Altenhofen Da Silva M., (2008)
[12] Chirinos R et al., (2000)
[13] Zanatta et al., (2007)

COSMETIC BENEFIT

1. ANTIOXIDANT ACTION

The free radicals are chemical agents provided with a potent oxidant capacity. The free radicals occur normally during the metabolism. The immunological system of the body also creates them to neutralize virus and bacteria. Other sources of free radicals and oxidative tensions include environmental factors such as pollution, cigarette smoke and certain pesticides.[15]

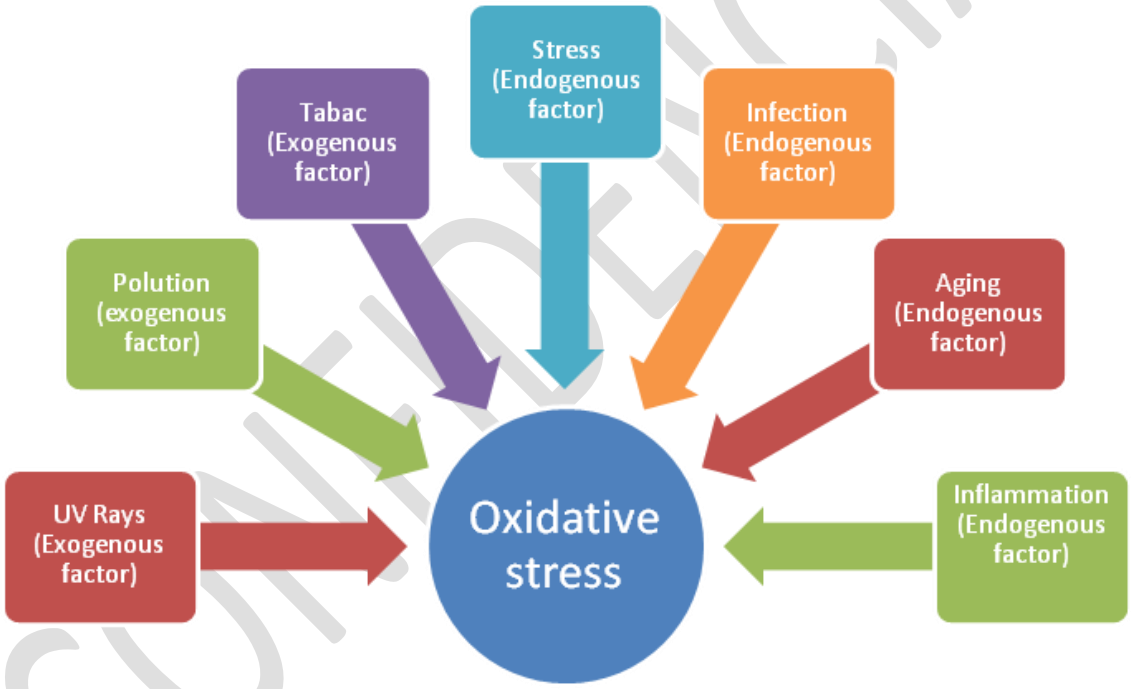


Fig 2: The Free Radicals and the Oxidative Stress

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

[14] Franco et al., (2000)
[15] Blake S., (2007)

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.

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

Vitamin C (L-ascorbic acid) is one of the most important antioxidants in the body fluids and the skin[16] since it safeguards many molecules essential in the body, such as proteins, fats, carbohydrates and nucleic acids (DNA and RNA) from the damage due to free radicals. The protection to the DNA from the oxidative damage is a way in which this vitamin may reduce the risk of cancer. Similarly, it plays a fundamental role in the regeneration of the Vitamin E and the beta-carotene after they have performed their antioxidant functions, whereby they may act synergically.[17]

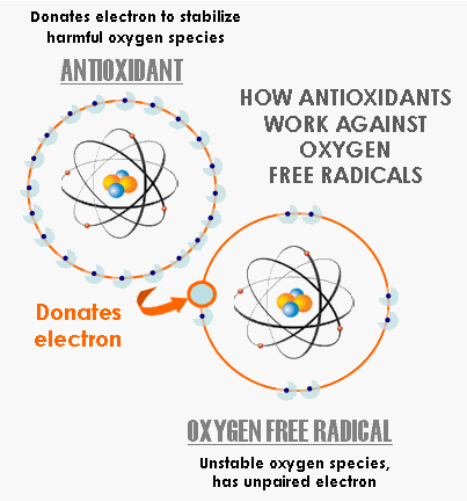


Fig 3: How antioxidants work

Studies carried out conclude that the Vitamin C and anthocyanins present in the camu camu fruit function as natural antioxidant even more efficiently than the synthetic L-ascorbic acid due to a joint action with other natural substances present in the fruit.[18]

2. VITAMINE C AND THE SKIN

Vitamin C is a normal skin constituent that is found at high levels in both the dermis and epidermis[19]. The vitamin C content of the epidermis is higher than the dermis, although the vitamin C concentrations in both layers are approximately equal to that of other water-soluble antioxidants. Aging, however, causes a decline

[16] Pinell S. et al.. (2001)
[17] Blake S. (2007)
[18] Teuro I. (2008)
[19] Shindo Y, et al. (1994)

in vitamin C content in both the epidermis and dermis[20]. Excessive exposures to UV light or pollutants (e.g., cigarette smoke and ozone) may also lower vitamin C content, primarily in the epidermis.

Functions in healthy skin:

Photoprotection:

Vitamin C limits the damage induced by ultraviolet (UV) light exposure. Vitamin C is not a "sunscreen" because it does not absorb light in the UVA or UVB spectrum. Rather, the antioxidant activity of vitamin C protects against UV-induced damage caused by free radicals[21].

UV light decreases vitamin C content of skin, an effect that is dependent on the intensity and duration of UV exposure[22]. In cultured keratinocytes, the addition of vitamin C reduces UV-related DNA damage and lipid peroxidation, limits the release of pro-inflammatory cytokines, and protects against apoptosis[23]. Vitamin C also modulates redox-sensitive cell signaling in cultured skin cells and consequently increases cell survival following UV exposure[24].

Role in the formation of collagen

Ascorbic acid also intervenes in the synthesis of collagen, protein that constitutes 70% of the dermis and provides to it strength and flexibility, stimulating its formation and strengthening the skin against external attacks.

Vitamin C (VitC) plays a critical role in the maintenance of a normal mature collagen network in humans (anti-scurvy properties) by preventing the auto-inactivation of lysyl and prolyl hyroxylase, two key enzymes in collagen biosynthesis. If you don't get the

[20] Rhie G, ET AL. (2001)
[21] Darr D, et al. (1992)
[22] Shindo Y, et al. (1994)
[23] Tebbe B, et al. (1997)
[24] Savini Iet al. (1999)

proper amount of vitamin C, collagen production will slow. A decline in collagen production will not only make skin more susceptible to wrinkles, but it might also make it more susceptible to bruising.

It is proposed that the topical application of Vitamin C protects the skin from sunburns and reverts the pigmentation and wrinkles caused by photoaging.[25], [26]

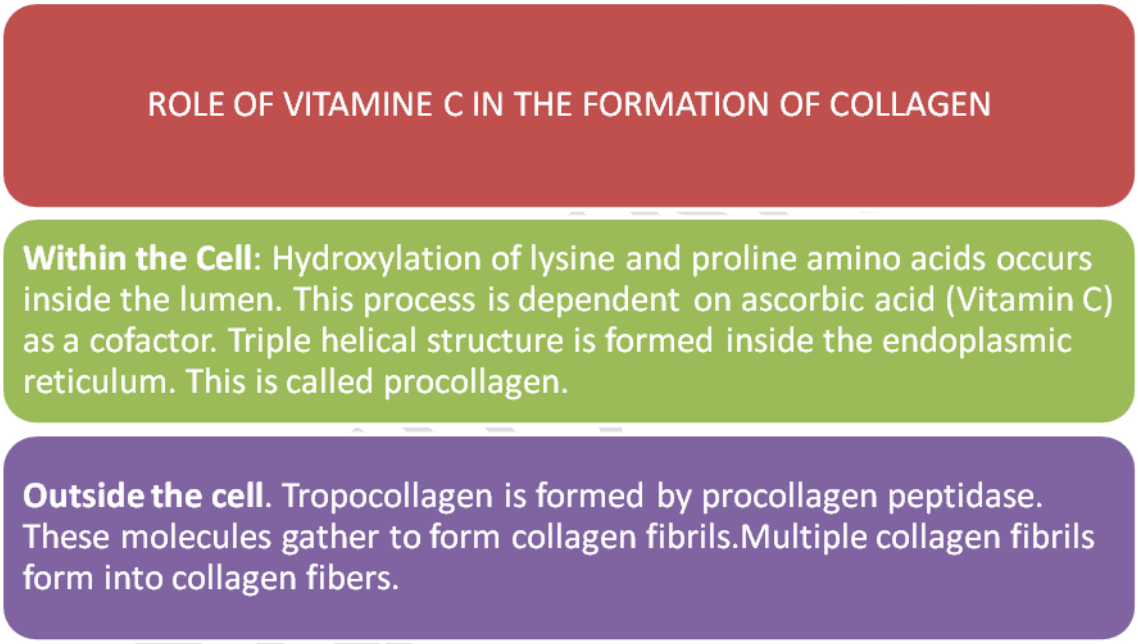


Fig 4: Vitamin C role in Collagen Synthesis

Efficacy Tests

ANTI-FREE RADICAL EFFECT

[25] Burke K. (2004)
[26] Tsai T. (2008)

The INKA CAMU CAMU was evaluated by a battery of chemical trials to determine its capacity to neutralize the free radicals.

Reducing Power

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activities of antioxidants have been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity and radical scavenging. As shown in figure 5, the reducing power of INKA CAMU CAMU was compared with the standard L- ascorbic acid and found to be superior. The reducing potential of the INKA CAMU CAMU was measured in its capacity to reduce the ion Fe^{3+} by the method of Hazra et al[27].

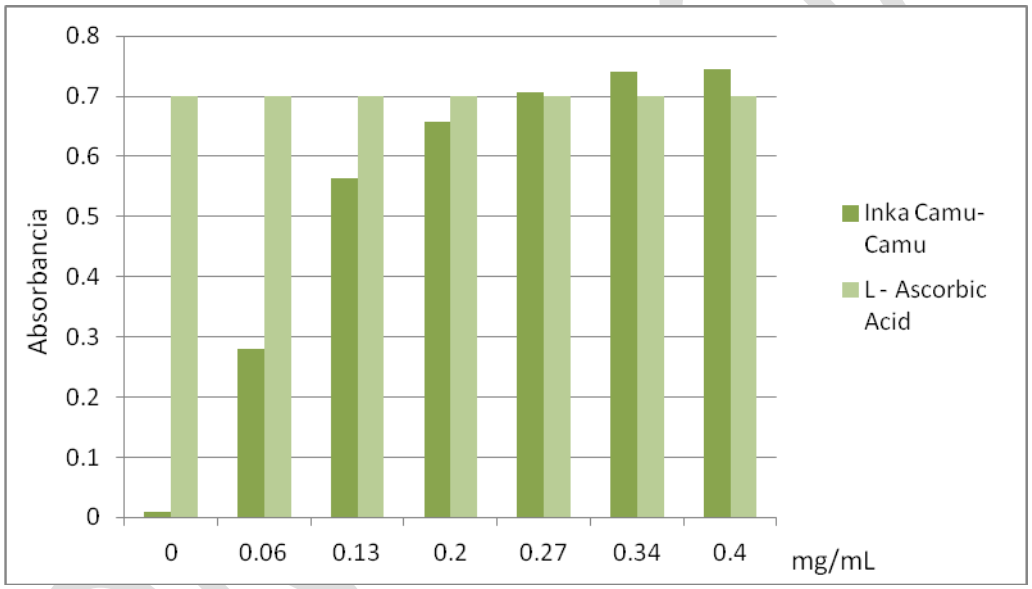


Fig 5: Reducing power of INKA CAMU CAMU AND L-ASCORBIC ACID

It is observed that from a concentration of 0.27 mg/mL, the reducing activity of the INKA CAMU CAMU is equal to and even a little higher than the one of L-ascorbic acid.

Hydroxyl radical scavenging activity

[27] Hazra B. et al., (2008)

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 antioxidant activity for the hydroxyl radical of the INKA CAMU CAMU was determined according to the method described by Apak et al[28] where the Mannitol was used as compound of reference. See Figure 6.

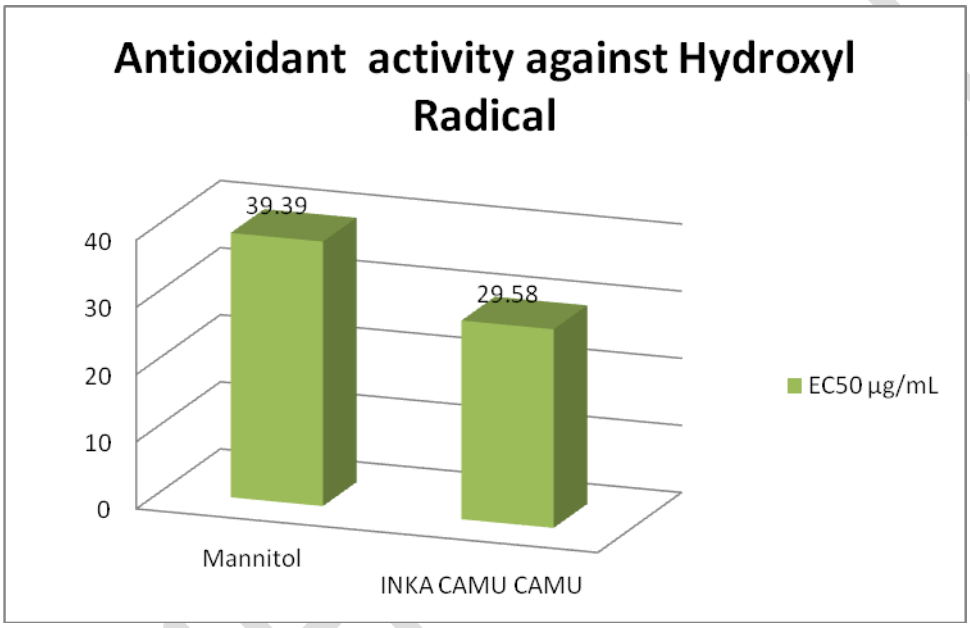


Fig 6: INKA CAMU CAMU Antioxidant activity against Hydroxyl Radical

The IC_{50} value indicates that INKA CAMU CAMU is 25% better hydroxyl radical scavenger than the standard mannitol.

Inhibition of Collagenase Activity

The inhibition of the collagenase activity was carried out using the method described by Thring et al[29]. It used the enzyme Collagenase of Clostrodium and the epigallocatechin gallate (EGCG) as compound of reference. See Figure 7.

[28] Apak R. et al., (2008)
[29] Thring T., (2009)

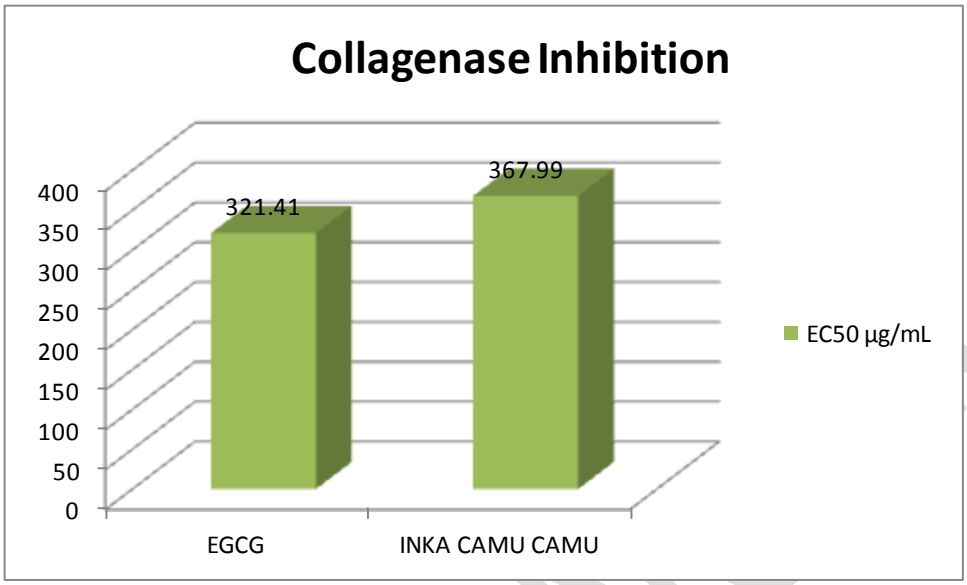


Fig 7: Collagenase Inhibition

The results show that the **INKA CAMU CAMU has an interesting anti collagenase activity** comparable to the one of the EGCG, used as control.

Inhibitory effect on viability of uv-b-exposed skin cells.

To examine cell viability in UV-B-irradiated 3T3 human dermal fibroblasts for 24 h, the MTT reactive analysis was conducted, according Ji-Young Bae *et al.* method[30]. The fibroblasts were inoculated in 24-well plates (5 x 105 cells / well) and cultured for 24 hours in Iscove's modified Dulbecco for 24 hours. Hydroalcoholic extracts at different concentrations were then added and incubated for another 24 hours, after which the supernatants were removed and fresh medium and re-incubated. After 24 hours the supernatants were collected and the amount of type I procollagen was measured using a kit from Takara (Takara Bio, Japan).

[30] Bae J-Y et al. *Exp. Dermatol.* 2010,19:e182-e190

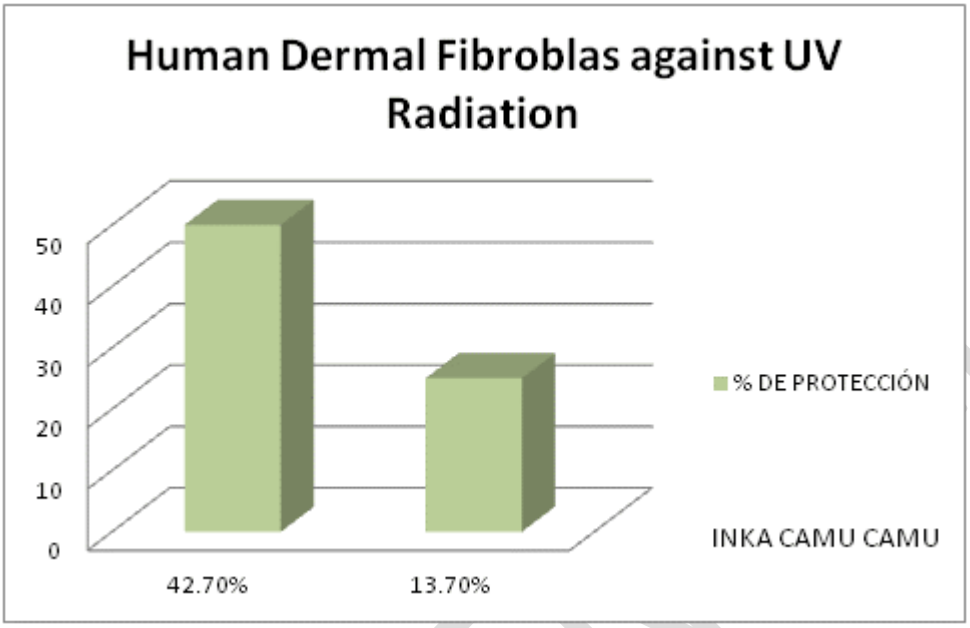


Fig 8: Inhibitory effect on viability of uv-b-exposed skin cells with Inka Camu Camu

When 3T3 fibroblast were pretreated with INKA CAMU CAMU at a concentration of **50 µg/ml, the product showed a high protective effect up to 42.7%, and at a concentration of 25 µg/ml showed a protective effect up to 13.7% against UV-B radiation**. (Fig 8)

CONCLUSIONS

INKA CAMU CAMU is an innovative Natural Ingredient with a very important antioxidant activity against the free radicals pernicious for the integrity of the skin, in addition protects against the degradation of the collagen fibers, responsible for the support tissue of the skin.

It is indicated for:

- Anti-aging products
- Sunscreen products
- After sun products

Dose of use – Solubility – Preparation

Tnte. Enrique Delucchi 80. Lima 04. Peru
T: +51 1 463 0350
E-mail: info@3qp.biz

PI-100-03

INKACamuCamu

Rev1. June 23rd, 2014

INKA CAMU CAMU is a NON GENETICALLY MODIFIED ingredient.

DOSE OF USE: From 1 to 10%.

SOLUBILITY: Water-soluble.

PREPARATION: INKA CAMU CAMU is sensitive to light, humidity and contact with iron. Preferably, it will be incorporated into the cosmetic preparations at acid pH, lower than 4.0, at the end of the preparation and below 35°C.

Analytical Information

Aspect:	Homogeneous liquid
Odor:	Characteristic
Color:	Yellow to orange
Solubility in water:	Soluble
pH (20°C):	3.8 – 5.0
Specific gravity, 20°C:	1.035 – 1.050

PRESERVATIVES: None

MICROBIOLOGY:

Total aerobic mesophilic count:	≤ 1000 cfu/gr
Total fungi and yeast count:	≤ 100 cfu/gr
Pathogens:	Absence

PRESERVATION: Store in airtight container, protected from light and humidity and between 15°C to 25°C.

If the original container is opened, it should be handled with special care in order to avoid a secondary microbiological contamination.

Tnte. Enrique Delucchi 80. Lima 04. Peru
T: +51 1 463 0350
E-mail: info@3qp.biz

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.

REFERENCES

- ALTENHOFEN DA SILVA Mariana, DO AMARAL SOBRAL Paulo J. , GUENTER KIECKBUSCH Theo, Phase Transitions of Frozen Camu camu (Myrciaria dubia (H.B.K.) McVaugh) Pulp: Effect of Cryostabilizer Addition, Food Biophysics (2008) 3:312–317
- BASABE T. Betsabe, Functions of Vitamin C in the metabolism of Collagen, Rev Cubana Alim Nutr; 2000, 14(1), 46-54, p.47, http://bvs.sld.cu/revistas/ali/vol14_1_00/f0107100.gif
- BLAKE S., Vitamins and Minerals Demystified. Blacklick, OH, USA: McGraw-Hill Professional Publishing, 2007, p 82.BRACK EGG, Antonio, 1999, Encyclopedic Dictionary of Useful Plants of Peru, Cuzco, Peru, page 334.
- BURKE K., 2004, Photodamage Of The Skin: Protection and Reversal with Topical Antioxidants, Blackwell Publishing Ltd., River Court, New York, USA.
- CASTAÑEDA C. B.1, RAMOS LL. E. 2, IBAÑEZ V. L. 3 Evaluation of the Antixoxidant Capacity of Seven Peruvian Medicinal Plants, Revista Horizonte Médico 56, Volume 8, N° 1, July 2008.
- CHIRINOS R. , GALARZA J, BETALLELUZ-PALLARDEL I, PEDRESCHI R, CAMPOS D, Antioxidant compounds and antioxidant capacity of Peruvian Camu Camu (*Myrciaria dubia* (H.B.K.) McVaugh) fruit at different maturity stages, Food Chemistry 120 (2010) 1019–1024.
- DARR D, Combs S, Dunston S, Manning T, Pinnell S. Topical vitamin C protects porcine skin from ultraviolet radiation-induced damage. Br J Dermatol 1992;127:247-253
- DOSTERT Nicolás, ROQUE José, BROKAMP Grischa, CANO Asunción, LA TORRE María, WEIGEND Maximilian, 2009, Botanical Leaves: Camu Camu, botconsult GmbH, Museum of Natural History UNMSM, Peru.
- ELESBÃO ALVES R, CUNHA FILGUEIRAS, H A HEBSTER MOURA Carlos Farley, COSTA ARAÚJO Nágela Cristina, SILVA ALMEIDA, Adriano, 2002, Camu camu

(Myrciaria dubia Mc Vaugh): A Rich Natural Source of Vitamin C Proc. Interamer. Soc. Trop. Hort. 46:11-13. Fruit/Frutales – Octubre.

- FLORES P. S. 1990, Old Fallow Management in Brillo Nuevo, pp 121-146 in DENEVAN W. M. y C. PADOCH 1990 Traditional Agroforestry in the Peruvian Amazon Rainforest, CIPA, Document II, Lima.
- FRANCO Maria Regina B. y SHIBAMOTO Takayushi, Volatile Composition of Some Brazilian Fruits: Umbu-caja (Spondias citherea), Camu camu (Myrciaria dubia), Araça-boi (Eugenia stipitata), and Cupuaçu (Theobroma grandiflorum) *J. Agric. Food Chem.*, 2000, 48 (4), pp 1263–1265
- BIBHABASU HAZRA, Antioxidant and free radical scavenging activity of *Spondias pinnata.* BMC Complement Altern Med. 2008; 8: 63
- JUSTI, Karin Cristiane, VISENTAINER, Jesuí Vergílio, EVELAZIO DE SOUZA, Nilson et al. Nutritional composition and Citamin C stability in stored Camu Camu (Myrciaria dubia) pulp. ALAN, Dic. 2000, vol.50, no.4, p.405-408. ISSN 0004-0622.
- NAGAMINE Kenichi, HAYASHI Miki, YAMASAKI Kaori, Compound Process For Producing The Same And Use Thereof, US Patent US 2006/0104927 A1, 2006.
- PIERCE S., 2009, Investigation of Plants, 3QP document
- PINELL S., HUANSHU Y, OMAR M., MONTEIRO N., DEBUYS H., WALKER L., WANG Y., LEVINE M., (2001), Topical L-Ascorbic Acid : Percutaneous Absorption Studies, Dermatol Surg 27 : 2 Febrero 2001, Pags 137-142
- RHIE G, Shin MH, Seo JY, et al. Aging- and photoaging-dependent changes of enzymic and nonenzymic antioxidants in the epidermis and dermis of human skin in vivo. J Invest Dermatol 2001;117:1212-1217
- RUFINO Maria do Socorro, ALVES Ricardo. DE BRITO Edy S, PÉREZ-JIMÉNEZ Jara, SAURA-CALIXTO Fulgencio, MANCINI-FILHO Jorge, Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil, Food Chemistry 121 (2010) 996–1002
- SAVINI I, D'Angelo I, Ranalli M, Melino G, Avigliano L. Ascorbic acid maintenance in HaCaT cells prevents radical formation and apoptosis by UV-B. Free Radic Biol Med 1999;26:1172-1180
- SILVA, J. F. 1998. Camu Camu: da flora Amazônica para você, a dose certa de vitamina C. Informativo SBF, Brasília. 17(2).

- SHINDO Y, Witt E, Han D, Epstein W, Packer L. Enzymic and non-enzymic antioxidants in epidermis and dermis of human skin. J Invest Dermatol 1994;102:122-124.

- TAMSYN SA THRING**,** PAULINE HILI AND DECLAN P NAUGHTON**.** Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. *BMC Complementary and Alternative Medicine* 2009, **9**:27

- TEBBE B, Wu S, Geilen CC, Eberle J, Kodelja V, Orfanos CE. L-ascorbic acid inhibits UVA-induced lipid peroxidation and secretion of IL-1alpha and IL-6 in cultured human keratinocytes in vitro. J Invest Dermatol 1997;108:302-306

- TERUO INOUE, HIROSHI KOMODA, TOSHIHIKO UCHIDA y KOICHI NODE, Tropical fruit camu camu (Myrciaria dubia) has anti-oxidative and anti-inflammatory properties, Journal of Cardiology Volumen 52, No. 2, Octubre 2008, Pags 127-132

- THOMAS C. TSAI, BS, BASIL M. HANTASH. Cosmeceutical Agents: A Comprehensive Review of the Literature, Clinical Medicine: Dermatology 2008:1 1–20

- TONG Szeto Yim, TOMLINSON Brian, BENZIE Iris F. F., Total antioxidant and ascorbic acid content of fresh fruits and vegetables: implications for dietary planning and food preservation, British Journal of Nutrition (2002), 87, p. 57.

- VILLACHICA, H. 1996, Frutales y Hortalizas promisorios de la Amazonia. TCA, Lima en ELESBÃO ALVES R, CUNHA FILGUEIRAS, H A HEBSTER MOURA Carlos Farley, COSTA ARAÚJO Nágela Cristina, SILVA ALMEIDA, Adriano, 2002, Camu Camu (Myrciaria dubia Mc Vaugh): A Rich Natural Source of Vitamin C Proc. Interamer. Soc. Trop. Hort. 46:11-13. Fruit/Frutales – October

- ZANATTA Cinthia Fernanda, MERCADANTE Adriana Z., Carotenoid composition from the Brazilian tropical fruit camu–camu (Myrciaria dubia) Food Chemistry, Volumen 101, No. 4, 2007, Pag 1526-1532.

- Hazra B, Biswas S, Mandal N: Antioxidant and free radical scavenging activity of Spondias pinnata. BCM Complement Altern. Med. 2008,8:63

- Apak Resat et al. Analytical chimica acta 616(2008)196-206

- Thring Tamsyn SA et al. BMC Complementary and Alternative Medicine 2009,9:27

PI-100-03

INKACamuCamu

Rev1. June 23rd, 2014

Tnte. Enrique Delucchi 80. Lima 04. Peru
T: +51 1 463 0350
E-mail: info@3qp.biz