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Article

Hydrolyzed collagen interferes with *in vitro* photoprotective effectiveness of sunscreens

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The chronological skin aging is a progressive and natural process with genetic and physiological changes. However, ultraviolet (UV) radiation may accelerate the oxidative stress, generating carcinogenesis and photoaging. Natural compounds and their applications are considered a trend in the cosmetic market. The protein-based film-forming compounds play an important role, once it collaborates for the better distribution of sunscreens on the skin. Here we investigated the *in vitro* photoprotective effectiveness of sunscreens containing the hydrolyzed collagen associated with UVA, UVB and/or inorganic filters. Sunscreens were developed with octocrylene (7.5%), butyl methoxydibenzoylmethane (avobenzone) (3.0%) and/or titanium dioxide (5.0%), associated or not with the hydrolyzed collagen (3.0%). *In vitro* photoprotective effectiveness was determined in a Labsphere® UV2000S by the establishment of the sun protection factor (SPF) and critical wavelength (nm) values. Physicochemical and organoleptic characteristics were also assayed. The hydrolyzed collagen subjectively improved the formulation sensory characteristics. However, this bioactive compound led to a decrease of the SPF values of the photoprotective formulations containing octocrylene alone and octocrylene + butyl methoxydibenzoylmethane + TiO_2. This inadequate interaction may be considered during the development of new sunscreens intended to contain protein-based components.

Uniterms: Hydrolyzed collagen/protective effects. Photoprotector/effectiveness. Photoprotector/*in vitro* study. Sun protection factor. Sunscreen/UV filter. Critical wavelength.

INTRODUCTION

Towards the global knowledge about skin cancer and premature aging, public health authorities and experts still consider that the daily use of sunscreens should increase. Additionally, a cumulative exposure to ultraviolet radiation (UVB: 290-320 nm and UVA: 320-400 nm) accelerates photoaging, leading to an imbalance between endogenous oxidants and antioxidant molecules (Bianchi, Antunes, 1999; Hirata, Sato, Santos, 2004; Bagatin, 2009; Rivas *et al.*, 2009; Brasil, 2014).

Brazil is one of the largest producers and exporters of meat, generating many sub-products such as pig skin, bovine hide, bones and others that become natural and sustainable sources of proteins, mainly collagen (Zucchi, Caixeta-Filho, 2010) that is regarded as one of the most useful biomaterials. The excellent biocompatibility and safety due to its biological characteristics, such as biodegradability and weak antigenicity, made collagen a resource for cosmetic use (Nehlyudov, 2003; Teixeira, 2006; Walrand *et al.*, 2008; Gomez-Guillén *et al.*, 2011; Wang, Lim, 2011; Wang *et al*., 2013; Comblain *et al.*, 2015).

The use of natural and sustainable compounds substances in skin care products has become a trend nowadays. In the past years, the cosmetic market has included the collagen in its formulas as a consequence of its multifunctional characteristics, such as moisturizing (Walrand *et al.*, 2008; Gómez-Guillén *et al.*, 2011; Silva, Penna, 2012). In this context, the properties of collagen can be divided in two categories. First, the properties associated with its gelling behaviour, i.e., gel formation, texturizing, thickening and water binding capacity; and

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second, the properties related to their surface behaviour, which include emulsion and foam formation and stabilization, adhesion and cohesion, protective colloid function, and film-forming capacity (Bordbar *et al.*, 2013; Wang, Lim, 2011; Wang *et al.*, 2013; Walrand *et al.*, 2008; Zague *et al.*, 2011).

Here we selected two well-established chemical and physical UV filters: butyl methoxydibenzoylmethane (avobenzone), octocrylene and titanium dioxide. Butyl methoxydibenzoylmethane, a potent UVA filter, was the first FDA-approved organic agent to filter UVA radiation. However, it is highly photolabile and after 1 h of sun exposure it decreases its photoprotective properties by half. Additionally, it can affect the stability of other sunscreen ingredients. Besides octocrylene ability to absorb the UVB radiation, it also improves photostability and it is a common choice for sunscreens available at the market. Titanium dioxide is a metallic oxide common used as inorganic UV filter by its higher refractive index, which provides a superior UVB protection and a whiter tone (Kockler *et al*., 2012; Sambandan, Ratner, 2011).

Oliveira and co-workers (2015), proved through *in vivo* studies the skin biocompatibility of samples containing butyl methoxydibenzoylmethane 3.0% w/w, associated or not to a natural compound (rutin 0.1% w/w), as well as, Peres and co-workers (2016), determined the cutaneous compatibility of sunscreens containing octocrylene 10.0% w/w, with or without rutin 0.1% w/w. Both *in vivo* protocols were performed with 17 volunteers and the samples were applied in theirs volar forearms using epicutaneous patches during 24h. Distinct bioengineering approaches were used to assess the biocompatibility and the variables found to be the most representative of skin tolerance were cutaneous redness, hydration and transepidermal water loss (TEWL). Butyl methoxydibenzoylmethane and octocrylene were well-tolerated by the skin, confirming their good biocompatibility.

Here we investigated the photoprotective effectiveness, obtained *in vitro*, of sunscreens (O/W emulsions) containing the hydrolyzed collagen (3.0% w/w) associated with UVA, UVB organic and inorganic filters.

MATERIAL AND METHODS

Material

The UV filters octocrylene (CAS number: 6197-30-4), butyl methoxydibenzoylmethane (CAS number: 70356-09-1) and titanium dioxide (CAS number: 13463-67-7) were purchased from BASF (São Paulo, Brazil). The hydrolyzed collagen (CAS number: 92113-31-0/73049-73-7) was kindly donated by Gelita Brazil (São Paulo, Brazil). All materials were used as received, without any further purification. Purified water was used for all experiments.

Development of the formulations

Eight O/W emulsions were developed, containing one or more UV filters, octocrylene (7.5% w/w), butyl methoxydibenzoylmethane (3.0% w/w) and titanium dioxide (5.0% w/w), in the collagen absence or presence (3.0% w/w). Samples were stored for 72 hours prior to physical, physicochemical and *in vitro* photoprotective efficacy evaluation. Also, two formulations without UV filters were used as controls (**F1** and **F2**). Table I shows the qualitative and quantitative composition % (w/w) of the formulations (FDA, 2007).

Samples were prepared as follows: first, oil phase emollients and the emulsifier were heated with octocrylene and butyl methoxydibenzoylmethane. After, water phase components (including the hydrolyzed collagen) were added, and the mixture was mechanic stirred (1,500 rpm). The water phase was added to the oil phase and the mixture was mechanic stirred (1,500 rpm) for 3min. At the end, titanium dioxide was added into the samples, previously dispersed in isopropyl myristate.

Apparent viscosity and pH values were evaluated in a Fungilab®, Visco Star R, viscometer, and in a Quimis® pH meter, respectively. Organoleptic characteristics were analysed subjectively by means of colour, odour and appearance. The results were obtained in triplicate (Anvisa, 2004; Sarruf *et al.*, 2013).

Photoprotection efficacy *in vitro*

The *in vitro* photoprotective effectiveness was performed using an UV-2000S Ultraviolet Transmittance Analyzer equipped with an integrating sphere (Labsphere®, USA). Samples were weighed and uniformly applied with a glove-coated finger on quartz plates with Transpore® tape (3M, Canada), as the substrate. Samples were then allowed to dry at room temperature for 20 min, protected from light. The analyses were carried out in replicates of three, and nine different points per plate were measured for each sample. The average of the obtained spectral absorbance values were used by the Labsphere® software UV2000 to calculate *in vitro* SPF and critical wavelength (nm) values (Velasco *et al.*, 2008; Diffey *et al.*, 2000; Cosmetics Europe, 2011; Wang, Lim, 2011; Oliveira *et al*., 2016).

TABLE I - Qualitative and quantitative composition (% w/w) of the O/W formulations

Composition	Concentration %(w/w)									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Cetearyl alcohol (and) PEG-150 stearate (and) polysorbate60 (and) steareth-20	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Isopropyl myristate	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Phenoxyethanol (and) methylparaben (and) ethylparaben (and) propylparaben (and) butylparaben (and) isobutylparaben	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Octocrylene**			7.5	7.5					7.5	7.5
Butyl methoxydibenzoylmethane**					3.0	3.0			3.0	3.0
Titanium dioxide**							5.0	5.0	5.0	5.0
Hydrolyzed collagen		3.0		3.0		3.0		3.0		3.0
Aqua	***	***	***	***	***	***	***	***	***	***

Legend: * INCI (International Nomenclature of Cosmetic Ingredient); ** UV filter; ***Enough to 100%

Statistical analysis

Statistical analysis was performed by the software Minitab® (Version 16), with a significance level of 5% (a = 0.05). Data were statistically treated using one-way ANOVA followed by the Tukey test for multiple comparisons and paired t-test. Regarding the formulations, they were divided into pairs according to the presence or absence of collagen in association with the UV filters. The p-values between each group were calculated by the two-sample t-test for equal means, with a confidence interval of 95%.

RESULTS AND DISCUSSION

Most sunscreens perform their efficacy through a combined chemical and physical UV protection and they should meet particular requirements like, for example, balanced UVA/UVB efficacy with high level of protection, water resistance, easy of application with film-forming property, uniform protection, high stability, and good skin biocompatibility (Balogh *et al.*, 2011; Stiefel, Schwack, 2014; Oliveira *et al*., 2016).

Formulations showed satisfactory stability with high viscosity (14400 to 33500 cP), possibly due to the water retention capacity of the medium, in addition to the emulsifying and stabilizing properties of hydrolyzed collagen. The systems had pH values biocompatible with skin (4.84 to 7.71) (Walrand *et al.,* 2008; Gómez-Guillén *et al.*, 2011; Silva, Penna, 2012). Stratum corneum (SC) presents an acid mantle that contributes with the skin acidic surface, which is relevant for good tissue condition and homeostasis, considering the skin microflora control and support of physiological processes (Lambers *et al.*, 2006). The activities of the skin enzymes, for instance, are significantly reduced with the increasing of the SC pH. Likewise, SC proteases are more efficient at near-neutral or alkaline pH. Several skin disorders are consequences of disturbed enzyme activity, for example, Gaucher's disease is due to altering β-glucocerebrosidase activity, and a deficiency in acidic sphingomyelinase cases the Niemann-Pick disease (Natarajan *et al*., 2014). According to this scenario, a topical product projected to be applied in high extensions of the cutaneous tissue must have pH value compatible with the skin.

The presence of hydrolyzed collagen improved the organoleptic characteristics of the developed samples, subjectively, increasing their brightness and spreadability. In respect to the colour, a slight acceptable modification was observed, probably due to the dark yellow characteristic coloration of the incorporated active compounds.

F1 and F2 samples did not absorb the radiation with, approximately, 100% transmittance at the UVA and UVB regions, since they were UV filters-free. Regarding the *in vitro* photoprotective effectiveness, sunscreens containing the UVA filter (butyl methoxydibenzoylmethane) (F5 and F6), TiO_2 (F7 and F8) and octocrylene + UVA filter + TiO_2 (F9 and F10), containing or not the hydrolyzed collagen, developed indicative profiles of broad-spectrum action against UV radiation, with critical wavelength over 378 nm (Dominique, 2008; Rai, Shanmuga, Srinivas, 2012). This type of broad-spectrum absorption profile is of utmost importance for sun-care products, considering the harm

affects from radiation. Excess of UV radiation causes damages to the skin and hair, such as sunburn, photoaging, local dryness, and cancer, as well as, immunosuppression. UVB (mainly) and UVA may provoke direct cellular DNA harm, triggering an inflammatory response and melanin production. It has been confirmed by an explorative intra-individual study that a broad-spectrum sunscreen with a high protection factor is able to prevent UV-induced erythema and hyperpigmentation in health volunteers (Khun *et al.*, 2016). Actually, the formulation containing all the three UV filters and hydrolyzed collagen showed an UVA/UVB rate of 0.92 (data not shown), considered of high UVA protection (Baby *et al.*, 2009).

The UVB protection was more evidently achieved with the formulations containing the octocrylene, with SPF values above 10 (Kaimal, Abraham, 2011), although, the combination of hydrolyzed collagen and octocrylene decreased the SPF in 41% (F3 and F4), while the association of the three UV filters with the hydrolyzed collagen led to a SPF decay of 38% (F9 and F10). These results showed that this natural component contributed inadequately to the absorption on the UV range established *in vitro*. Table II shows the *in vitro* photoprotective effectiveness of the sunscreens.

Interactions among actives, bioactives from natural sources, excipients and vehicles of sunscreens may generate negative responses that compromise the efficacy of these systems, like active compound degradation with the respective formation of reactive species of oxygen. Also, studies on photochemical behaviour of UV filters are an important part of sunscreen development. Through interactions with sunlight, artificial light or several substances used to obtain the final product, it may provoke the photodegradation of UV filters, resulting in decreased protection ability and formation of new by-products with possibly different properties (Kockler *et al.*, 2014; Stiefel, Schwack, 2014).

Considering the use of protein-based bioactives, Graziola and co-workers (2016), observed that gelatin at 5.0% w/w, as an amorphous raw-material, was able to improve the SPF of mineral oil-based dispersions containing 6.0% w/w of benzophenone-3 or 7.5% of ethylhexyl methoxycinnamate, from 2.8 to 4.1 and 6.4 to 8.4, respectively. The critical wavelength (nm) of those dispersions was not affected, being kept below 354.5 nm, even in the UVA filter (benzophenone-3) presence (Graziola *et al.*, 2016). Stiefel and Schwack (2014), identified UV spectra alterations of the octocrylene incubated at 37 °C with bovine serum albumin (BSA) compared to the UVB filter alone. The interaction between octocrylene and BSA, after incubation, was assumed to be a result of covalent bound of the UVB filter to BSA through Michael addition followed by ethylhexyl cyanoacetate elimination (Stiefel, Schwack, 2014). This reaction has led to a maximum wavelength displacement from 310 to 280 nm, approximately, and a reduction in absorbance intensity. Stiefel and Schwack (2014) also detected interactions between octocrylene and gelatin after simulated irradiation. These behaviours could possibly impact the SPF value, regarding its parameter property of defence mainly against UVB radiation, as observed in our research, when the SPF values of the samples containing octocrylene associated with the hydrolyzed collagen have diminished.

TABLE II - Sunscreens *in vitro* photoprotective effectiveness

Samples	Estimated Sun Protection Factor (SPF)		Critical wavelength (nm)	
	Mean ± StDev	*p*-value	Mean ± StDev	*p*-value
F1	N.A.	N.A.	N.A.	N.A.
F2	N.A.		N.A.	
F3	17.3 ± 0.6	<0.001	362.0 ± 1.0	0.288
F4	10.7 ± 0.6		363.0 ± 1.0	
F5	4.0 ± 1.0	0.146	381.3 ± 0.6	0.059
F6	5.5 ± 0.5		380.0 ± 0.1	
F7	6.0 ± 1.0	0.288	379.0 ± 1.0	0.288
F8	5.0 ± 1.0		378.0 ± 1.0	
F9	66.3 ± 2.5	0.004	380.3 ± 0.6	0.519
F10	41.0 ± 1.0		380.7 ± 0.6	

Legend: N.A.: not applicable. Values reported as mean ± standard deviation. The *p*-values between each group were calculated by the two sample *t*-test for equal means, with a confidence interval of 95%.

CONCLUSION

The hydrolyzed collagen moisturizing and texturizing properties subjectively improved the formulation sensory characteristics. However, this bioactive compound at 3.0% w/w did not influence the critical wavelength (nm) of the sunscreens and it led to a decrease of the SPF values, especially, of the high UVB photoprotective formulations containing octocrylene at 7.5% w/w (alone) and octocrylene (7.5% w/w) + butyl methoxydibenzoylmethane (3.0% w/w) + TiO_2 (5.0% w/w). This identified interaction, according to the *in vitro* established efficacy, may be considered by the Research & Development professionals during the elaboration of new sunscreen products intended to contain protein-based components.

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REFERENCES

AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA. ANVISA. *Guia de estabilidade de produtos cosméticos*. Brasília: Ministério da Saúde, 2004. v.1, 45p.

BABY, A.R.; BALOGH, T.S.; PEDRIALI, C.A.; KANEKO, T.M.; VELASCO, M.V.R. UVA I-protection effectiveness of bioactive compound and organic UV filters: an in vitro assessment. *Quim. Nova*, v.32, n.5, p.1321-1323, 2009.

BAGATIN, E. Envelhecimento cutâneo e o papel dos dermocosméticos. *Bol. Derm*., v.5, p.1-4, 2008.

BALOGH, T.S.; VELASCO, M.V.R.; PEDRIALI, C.A.; KANEKO, T.M.; BABY, A.R. Proteção à radiação ultravioleta: recursos disponíveis na atualidade em fotoproteção. *An. Bras. Dermatol.*, v.86, n.4, p.732-742, 2011.

BIANCHI, M.L.G.; ANTUNES, L.M.G. Radicais livres e os principais antioxidantes da Dieta. *Rev. Nutr.*, v.12, n.2, p.123-130, 1999.

BORDBAR, S.; EBRAHIMPOUR, A.; HMID, A.A.; MANAP, M.Y.A.; ANWAR, F.; SAARI, N. The improvement of the endogenous antioxidant property of stone fish (*Actinopyga lecanora*) tissue using enzymatic proteolysis. *Biomed. Res. Int*., ID 849529, 2013. DOI:10.1155/2013/849529.

BRASIL. MINISTÉRIO DO MEIO AMBIENTE. *Ações brasileiras para a proteção da camada de ozônio.* Brasília: Ministério do Meio Ambiente, 2014. 144p.

COMBLAIN, F.; SANCHEZ, C.; LESPONNE, I.; BALLIGAND, M.; SERISIER, S.; HENROTIN, Y. Curcuminoids extract, hydrolyzed collagen and green tea extract synergically inhibit inflammatory and catabolic mediator's synthesis by normal bovine and osteoarthritic human chondrocytes in monolayer. *Plos One*, v.10, e0121654, 2015.

COSMETICS EUROPE. *COLIPA Guidelines*. *In vitro* method for the determination of the UVA protection factor and ''critical wavelength'' values of sunscreen products. 2011.

DIFFEY, B.L.; TANNER, P.R.; MATTS, P.J.; NASH, J.F. *In vitro* assessment of the broad-spectrum ultravioleta protection of sunscreen products. *J. Am. Acad. Dermatol.,* v.43, n.6, p.1024-1035, 2000.

DOMINIQUE, M. How to measure UVA protection afforded by sunscreen products. *Expert Rev. Dermatol*., v.3, n.3, p.307-313, 2008.

FOOD AND DRUG ADMINISTRATION. FDA. Department of Health and Human Services. 21 CRF Parts 347 and 352. *Sunscreen drug products for over-the-counter human use*. Proposed amendment of final monograph, proposed rule, Silver Spring: FDA, 2007. pt.3, p.54.

GÓMEZ-GUILLÉN, M.C.; GIMÉNEZ, B.; LÓPEZ-CABALLERO, M.E.; MONTERO, M.P. Functional and bioactive properties of collagen and gelatin from alternative sources: a review. *Food Hydrocoll*., v.25, n.8, p.1813-1827, 2011.

GRAZIOLA, F.; CANDIDO, T.M.; OLIVEIRA, C.A.; PERES, D.A.; ISSA, M.G.; MOTA, J.; ROSADO, C.; CONSIGLIERI, V.O.; KANEKO, T.M.; VELASCO, M.V.R.; BABY, A.R. Gelatin-based microspheres crosslinked with glutaraldehyde and rutin oriented to cosmetics. *Braz. J. Pharm. Sci.*, v.52, n.4, p.603-612, 2016.

HIRATA, L.L.; SATO, M.E.O.; SANTOS, C.A.M. Radicais livres e o envelhecimento cutâneo. *Acta Farm. Bonaerense*, v.23, n.3, p.418-424, 2004.

KAIMAL, S.; ABRAHAM, A. Sunscreens. *Indian J. Dermatol. Venereol.*, v.77, n.2, p.238-243, 2011.

KOCKLER, J.; OELGEMÖLLER, M.; ROBERTSON, S.; GLASS, B.D. Influence of Titanium Dioxide particle size on the photostability of the chemical UV- filters Butyl Methoxy Dibenzoylmethane and Octocrylene in a microemulsion. *Cosmetics,* v.1, p.128-139, 2014.

KOCKLER, J.; OELGEMÖLLER, M.; ROBERTSON, S.; GLASS, B.D. Photostability of sunscreens. *J. Photochem. Photobiol. C: Photochem. Rev.*, v.13, n.1, p.91-110, 2012.

KHUN, A.; ZAHN, S.; PATSINAKIDIS, N.; LANDMANN, A.; GRAEF, M.; SAUERLAND, C.; SURBER, C.; WENZEL, J. Resistance to water and abrasion of a broad-spectrum sunscreen: a prospective, open-label study. *Exp. Dermatol.*, v.25, n.2, p.151-164, 2016.

LAMBERS, H.; PIESSENS, S.; BLOEM, A.; PRONK, H.; FINKEL, P. Natural skin surface pH is on average below 5, which is beneficial for its resident flora. *Int. J. Cosmet. Sci.*, v.28, n.5, p.359-370, 2006.

NATARAJAN, V.T.; GANJU, P.; RAMKUMAR, A.; GROVER, R.; GOKHALE, R.S. Multifaceted pathways protect human skin from UV radiation. *Nat. Chem. Biol*., v.10, n.7, p.542-551, 2014.

NEHLYUDOV, A.D. Nutritive fibers of animal origin: collagen and its fractions as essential components of new and useful food products. *Appl. Biochem. Microbiol*., v.39, n.3, p.229-238, 2003.

OLIVEIRA, C.A.; PERES, D.D.; GRAZIOLA, F.; CHACRA, N.A.B.; ARAÚJO, G.L.B.D.; FLÓRIDO, A.C.; MOTA, J.; ROSADO, C.; VELASCO, M.V.R.; RODRIGUES, L.M.; FERNANDES, A.S.; BABY, A.R. Cutaneous biocompatible rutin-loaded gelatin-based nanoparticles increase the SPF of the association of UVA and UVB filters. *Eur. J. Pharm. Sci.*, v.81, p.1-9, 2016.

OLIVEIRA, C.A.; PERES, D.D.; RUGNO, C.M.; KOJIMA, M.; PINTO, C.A.S.O.; CONSIGLIERI, V.O.; KANEKO, T.M.; ROSADO, C.; MOTA, J.; VELASCO, M.V.R.; BABY, A.R. Functional photostability and cutaneous compatibility of bioactive UVA sun care products. *J. Photochem. Photobiol. B-Biol.*, v.148, p.154-159, 2015.

PERES, D.A.; OLIVEIRA, C.A.; COSTA, M.S.; TOKUNAGA, V.K.; MOTA, J.P.; ROSADO, C.; CONSIGLIERI, V.O.; KANEKO, T.M.; VELASCO, M.V.; BABY, A.R. Rutin increases critical wavelength of systems containing a single UV filter and with good skin compatibility. *Skin Res. Technol.*, v.22, n.3, p.325-333, 2016.

RAI, R.; SHANMUGA, S.C.; SRINIVAS, C.R. Update on photoprotection. *Indian J. Dermatol.*, v.57, n.5, p.335-342, 2012.

RIVAS, M.; ARAYA, M.; DURÁN, V.; ROJAS, E.; CORTES, J.; CALAF, G.M. Ultraviolet light exposure and skin cancer in the city of Africa. *Mol. Med. Rep*., v.2, p.567-572, 2009.

SAMBANDAN, D.R.; RATNER, D. Sunscreens: an overview and update. *J. Am. Acad. Dermatol*., v.64, n.4, p.748-758, 2011.

SARRUF, F. D.; PERES, D. A.; NISHIKAWA, D. O.; CONSIGLIERI, V. O.; KANEKO, T. M.; VELASCO, M. V. R.; BABY, A. R. Assessment of in vitro Sun Protection Factor (SPF) and rheological profile of commercial infant sunscreens. *Rev. Cienc. Farm. Basica Apl.*, v.34, n.1, p.33-36, 2013.

SILVA, T.F.; PENNA, A.L.B. Colágeno: características químicas e propriedades funcionais. *Rev. Inst. Adolfo Lutz*, v.71, n.3, p.530-539, 2012.

STIEFEL, C.; SCHWACK, W. Reactivity of cosmetic UV filters towards skin proteins: Model studies with Boc-lysine, Boc-Gly-Phe-Gly-Lys-OH, BSA and gelatin. *Int. J. Cosmet. Sci.*, v.36, n.6, p.561-570, 2014.

TEIXEIRA, E.C. *Injeção de gel de polimetilmetacrilato em músculo estriado de rato: estudo da biocompatibilidade e da resposta fibrosa.* 2006. Tese (Doutorado) - Faculdade de Medicina, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2006.

VELASCO, M. V. R.; BALOGH, T. S.; PEDRIALI, C. A.; SARRUF, F. D.; PINTO, C. A. S. O.; KANEKO, T. M.; BABY, AR. Associação da rutina com *p*-metoxicinamato de octila e benzofenona-3: avaliação in vitro da eficácia fotoprotetora por espectrofotometria de refletância. *Lat. Am. J. Pharm*., v.27, n.1, p.23-27, 2008.

WALRAND, S.; CHIOTELLI, E.; NOIRT, F.; MWEWA, S.; LASSEL, T. Consumption of a functional fermented milk containing collagen hydrolysate improves the concentration of collagen-specific amino acids in plasma. *J. Agric. Food Chem*., v.56, n.17, p.7790-7795, 2008.

WANG, B.; WANG, Y.M.; CHI, C.F.; LUO, H.Y.; DENG, S.G.; MA, J.Y. Isolation and characterization of collagen and antioxidant collagen peptides from scales of croceine croaker (Pseudosciaena crocea). *Mar. Drugs*, v.11, n.11, p.4641-4661, 2013.

WANG, S.; LIM, H. Current status of sunscreen regulation in the United States: 2011 food and drug adminitration's final rule on labeling and effectiveness testing. *J. Am. Acad. Dermatol*., v.65, n.4, p.863-869, 2011.

ZAGUE, V.; FREITAS, V.; ROSA, M.C.; CASTRO, G.A.; JAEGER, R.G.; MACHADO-SANTELLI, G.M. Collagen hydrolysate intake increases skin collagen expression and suppresses matrix metalloproteinase 2 activity. *J. Med. Food*, v.14, n.6, p.618-24, 2011.

ZUCCHI, J.D.; CAIXETA-FILHO, J.V. Panorama dos principais elos da cadeia agroindustrial da carne bovina brasileira. *Inf. Econ.*, v.40, n.1, p.18-33, 2010.

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