INTRODUCTION

The knowledge of the effects of UVB and UVA radiation on human skin has increased significantly\(^1,^2\) and the authentic necessity of human skin protection against solar radiation has led to the concerning and upgrading of the development of broad spectrum sunscreens highly effective over the UVB-UVA absorbing range.

Sunscreens are mainly used to prevent erythema formation from sun exposure and solar radiation at the Earth’s surface is approximately 90-99% UVA and 1-10% UVB.\(^3,^4\) UVB (290-320 nm) radiation primarily causes photocarcinogenesis due its direct interaction with cellular DNA but there are several reasons why investigation of the role of UVA is also relevant. Major consequence of cumulative UVA (320-400 nm) radiation is the generation of reactive oxygen species and the alteration of tumor suppressor genes, like p53. UVA radiation is additional subdivided into UVA II (320-340 nm) and UVA I (340-400 nm).\(^5,^6\)

UVA radiation directly affects the dermal compartment and is thought to be the major factor responsible for photoaging of human skin. It had been shown that the UVA I accounts for damaging effects in human dermal fibroblasts, as induction of cytokines, matrix metalloproteinases, and mtDNA mutations. Of these, the induction of matrix metalloproteinase-1 which degrades collagen type I is of particular significance since the extent of collagen I reduction correlates with photocarcinogenesis due its direct interaction with cellular DNA.

This research work aimed at determining the in vitro effectiveness of sunscreens developed with a bioactive substance, the rutin, associating or not with organic UVB-UVA filters incorporated at a phosphate-base O/W emulsion. Sunscreens provided conflicting and unpredictable results concerning the anti-UVA protection, specially, at the UVA I region. Possible interactions among the organic UV filters and the polyphenolic bioactive substance may have accounted with improvement or reduction of UV protection by a complex and not yet elucidated mechanism, probably regarding wavelength delocalization to superior or inferior values, by resonant molecule stabilization or destabilization.

EXPERIMENTAL

Isolated rutin (99.1%, Henrifarma, Brazil), ethylhexyl methoxy-cinnamate (EHMC) (UVB organic filter, Uvinul® MC 80, Basf, Brazil) and benzophenone-3 (BZP) (UVA organic filter, Uvinul® M 40, Basf, Brazil) were incorporated into emulsified systems in accordance with the following associations: \(CB\) – no active substances; \(CR\) – 0.1% w/w rutin; \(CMF\) – 3.5% w/w EHMC + 1.0% w/w BZP; \(CMFR\) – 0.1% w/w rutin + 3.5% w/w EHMC + 1.0% w/w BZP; \(CF\) – 7.0% w/w EHMC + 2.0% w/w BZP; and \(CFR\) – 0.1% w/w rutin + 7.0% w/w EHMC + 2.0% w/w BZP.

Emulsified system was previously developed by Velasco and co-workers\(^8\) as a phosphate-base O/W emulsion, containing: cetaryl alcohol (and) dicetyl phosphate (and) ceteth-10 phosphate (Crodafos® CES); disodium EDTA (Uniquelan® NA2S); dimethicone (DC® 200/350); propylene glycol; paraben-type preservatives (Phenova®) and aqua (distilled water).

Absorbance spectra of the samples were measured by diffuse transmittance analysis (UV1000S Ultraviolet Transmittance Analyzer coupling to an integrating sphere, Labsphere®) in 5 nm increments from 290 to 400 nm. Prior to the UVA I effectiveness assessment, the substrate (Vitro-Skin®) composed of collagen was hydrated (24 h) and, then, 70.0 mg of the samples were homogeneously spread over it with circular movements, edges to center, by a saturated gloved finger. Samples were allowed to rest and to dry at room temperature until absorbance recording.\(^9,^10\)

UVA I/UV ratio was calculated according to the following equations, as described by US Food and Drug Administration:\(^11\)

\[
aUVA/\lambda_1 = \frac{5}{3} \times \left[\frac{A_{290} + A_{400} + 4(A_{345} + \ldots + A_{395}) + 2(A_{350} + A_{360} + \ldots + A_{390})}{60}\right]
\]

(1)

\[
UVA \text{ I area per unit wavelength (aUVA/}\lambda)\text{. } A: \text{ absorbance.}
\]

\[
aUVA/\lambda_1 = \frac{5}{3} \times \left[\frac{A_{290} + A_{400} + 4(A_{295} + A_{305} + A_{315} + \ldots + A_{395}) + 2(A_{300} + A_{310} + \ldots + A_{390})}{110}\right]
\]

(2)

\[
UV \text{ area per unit wavelength (aUV/}\lambda)\text{. } A: \text{ absorbance.}
\]

\[
aUV/\lambda_1 = aUVA/\lambda
\]

(3)

UVA I/UV ratio.
RESULTS AND DISCUSSION

Bioactive compounds, such as isolated rutin, are an increasing trend toward to the development of sunscreen cosmetic products, like sunscreens, effectively active against UV radiation that are composed of decreasing organic UV filter proportions and, yet, appreciating high absorbing properties ranging from UBV to UVA.

Highest values of UVA I/UV ratio were obtained for CB (1.37) and CR (1.59). Table 1 summarizes the UVA I/UV ratios. Rutin alone at the emulsified system and the vehicle active-free reached values in the order of 2 times superior than the systems containing the other associations of the bioactive compound and the organic UV filters (CMF, CMFR, CF and CFR).

Table 1. UVA I/UV ratio values for the sunscreen emulsified systems (n = 5)

|       | CB     | CR     | CMF    | CMFR   | CF     | CFR     |
|-------|--------|--------|--------|--------|--------|---------|
| aUVA I| 0.05   | 0.15   | 0.54   | 0.71   | 0.71   |         |
| aUV   | 0.04   | 0.10   | 0.57   | 0.68   | 0.84   | 0.84    |
| UVA I/UV ratio | 1.37 | 1.59 | 0.73   | 0.79   | 0.84   | 0.84    |

CR – no active substances; CR – 0.1% w/w rutin; CMF – 3.5% w/w EHM C + 1.0% w/w BZP; CMFR – 0.1% w/w rutin + 3.5% w/w EHM C + 1.0% w/w BZP; CF – 7.0% w/w EHM C + 2.0% w/w BZP; CFR – 0.1% w/w rutin + 7.0% w/w EHM C + 2.0% w/w BZP

Organic UV filters, from CMF and CF, acquired UVA I/UV ratios of 0.73 and 0.79, respectively. These sunscreens differentiated from each other by the proportions of EHM C and BZP. It was observed that these organic UV filter associations were not as effective against UV A I region. Possible interactions among the organic UV filters and the polyphenolic bioactive substance may have accounted for the observed decreases of UV A I defense in which CMFR and CFR had not intrinsically provided the respective improvement of the UV A protection. Furthermore, the bioactive association with the organic UV filters decreased the rutin UV A I defense in which CMFR and CFR did not intrinsically provide the respective improvement of the UV A protection. Additionally, the bioactive compound and the organic UV filters associations were not as effective against UV A as CB and CF. It was also verified that the 2-fold increase of decreasing organic UV filter proportions and, yet, appreciating high absorbing properties ranging from UBV to UVA.

Highest values of UVA I/UV ratio were obtained for CB (1.37) and CR (1.59). Table 1 summarizes the UVA I/UV ratios. Rutin alone at the emulsified system and the vehicle active-free reached values in the order of 2 times superior than the systems containing the other associations of the bioactive compound and the organic UV filters (CMF, CMFR, CF and CFR).

Table 2. Categories of anti-UVA effectiveness in reference to US Food and Drug Administration, with some modification, based on UVA I/UV ratio, critical wavelength (λc, nm) and UV A I/UV ratio.

|       | Low  | Medium | High | Highest |
|-------|------|--------|------|---------|
| UVA I/UV ratio | 0.20 to 0.39 | 0.40 to 0.69 | 0.70 to 0.95 | greater than 0.95 |
| λc (nm) | 325 to 335 | 335 to 350 | 350 to 370 | 370 |
| UV A rating | 0.20 to 0.39 | 0.40 to 0.69 | 0.70 to 0.95 | greater than 0.95 |

CONCLUSIONS

In summary, sunscreen emulsified systems containing bioactive compounds provided conflicting and unpredictable results, through in vitro assessment, concerning the anti-UVA protection, specially, at the UV A I region. Possible interactions among the organic UV filters and the polyphenolic bioactive substance may have accounted for the improvement or reduction of UV protection by a complex and not yet elucidated mechanism, probably regarding wavelength delocalization to superior or inferior values, by resonant molecule stabilization or destabilization.

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