Journal of Photochemistry & Photobiology, B: Biology 185 (2018) 46–49

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Ferulic acid photoprotective properties in association with UV filters: multifunctional sunscreen with improved SPF and UVA-PF

Daniela D'Almeida Peres[a], Fernanda Daud Sarruf[b], Camila Areias de Oliveira[c], Maria Valéria Robles Velasco[a], André Rolim Baby[a,*]

[a] Department of Pharmacy, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, SP, Brazil
[b] IPclin, Pesquisa Integrada, Clinical research company, Jundiaí, SP, Brazil
[c] Department of Biochemical and Pharmaceutical Technology, School of Pharmaceutical Sciences, University of São Paulo, SP, Brazil

ARTICLE INFO

Keywords:
SPF
UVA-PF
Ethylhexyl triazone
Bis-ethylhexyloxyphenol methoxyphenyl triazine
Ferulic acid

ABSTRACT

Ultraviolet (UV) radiation stimulates several injurious biological effects on cutaneous tissue, causing, for instance, photocarcinogenesis. Sunscreens are topical products designed to protect the skin against these harmful effects and their use must be encouraged. The addition of antioxidants, as ferulic acid (FA), a phenolic compound from the class of the hydroxycinnamic acids, in sunscreens could improve their sun protection factor (SPF) and prevent inflammatory reactions. Here, the clinical safety and efficacy of an association of ethylhexyl triazone and bis-ethylhexyloxyphenol methoxyphenyl triazine (UV filters) with ferulic acid were assessed. Samples had good skin biocompatibility and presented satisfactory safety profile, even in a sun-exposed condition. A synergic effect between the natural polyphenol and the UV filters was evidenced, as well as, FA increased *in vivo* SPF in 37% and the UVA protection factor (UVA-PF) in 26%. The *in vivo* data indicated that FA reinforced the broad-spectrum characteristic of the photoprotective formulations. Additionally, according to the results from the *ex vivo* antioxidant test, it is plausible to recommend adjustments on the *ex vivo* protocol to explicitly determine the positive effects of topical antioxidant ingredients applied over the skin. These results provided a new perspective for the development of multifunctional bioactive sunscreens using FA as a new platform.

1. Introduction

The ferulic acid (FA) is a phenolic compound from the class of the hydroxycinnamic acids that can be found in several natural sources. This substance has proven results in the treatment of various diseases, such as cancer and diabetes, as well as antimicrobial action, anti-inflammatory and, mainly, antioxidant activity, responsible for its main benefits and applications [1,2].

FA exhibits marked antioxidant activity based on four structural features: (i) the hydroxyl group, electron donor, attached to the benzene ring, responsible for neutralizing the reactive oxygen species; (ii) the side vinyl chain, which connects the carboxyl group to benzene ring and increases the stability of the molecule; (iii) the methoxyl substituent capable of forming a hydrogen bond with the hydroxyl group and provide additional stability to the molecule; and (iv) the carboxylic group that provides protection against lipid peroxidation [2,3]. Furthermore, as may be seen in Fig. 1, the double bound in the side chain of FA is subjected to *cis-trans* isomerization [4]. *Cis*-FA is found as yellow oil, with maximum UV absorption at 316 nm, whereas *trans*-FA has two maximum absorption peaks at 284 and 307 nm. In previous research, we demonstrated, through *in vitro* estimated sun protection factor (SPF), critical wavelength (nm) and ultraviolet UV transmittances, that interactions occurred between FA and UV filters, being the FA an ingredient that positively affected the functional profile of the sunscreen system. Ferulic acid influenced the results for *in vitro* antioxidant activity, providing a 90% increase in the antioxidant potential. Conclusively, the analysis of the experimental design demonstrated the synergy between UV filters and FA [5].

Regarding the *in vivo* application, researchers have demonstrated the effectiveness of FA against the harmful effects of UV radiation, such as erythema, photoaging and skin cancer. In three similar *in vivo* studies, using a solar simulator to induce an inflammatory reaction, the FA incorporation in topical solutions containing vitamins increased the chemical stability of the vitamins and also enhanced the photoprotective effect, reducing the levels of erythema and apoptosis of corneocytes [1,6,7].

In the present study, we examined the association of ethylhexyl triazone and bis-ethylhexyloxyphenol methoxyphenyl triazine with

* Corresponding author at: Department of Pharmacy, School of Pharmaceutical Sciences, University of São Paulo, 580 Prof. Lineu Prestes Av., Bl. 15, 05508-900 São Paulo, SP, Brazil.
E-mail address: andrerb@usp.br (A.R. Baby).

https://doi.org/10.1016/j.jphotobiol.2018.05.026
Received 28 March 2018; Received in revised form 15 May 2018; Accepted 25 May 2018
Available online 26 May 2018
1011-1344/

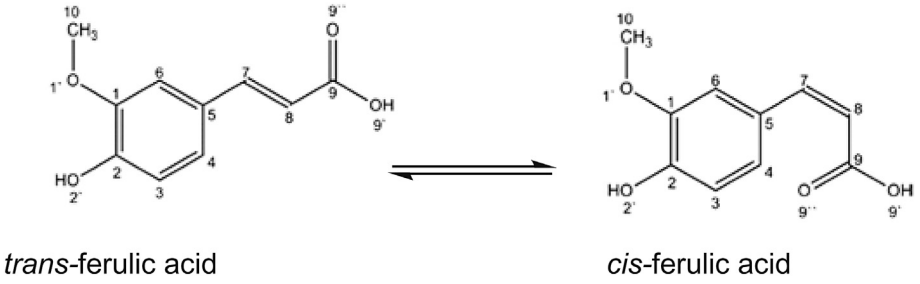

Fig. 1. Chemical structure of *trans*- and *cis*-ferulic acid [4].

ferulic acid, in order to obtain multifunctional sunscreens with antioxidant efficacy. Both UV filters are photostabilized molecules with low skin permeation and high efficacy at low concentrations, ideal characteristics for the preparation of photoprotectors. Here we investigated the clinical safety of the bioactive sunscreens and evaluated the effect of FA in improving the photoprotective and antioxidant efficacy of the samples.

2. Material and Methods

2.1. Reagents, Solvents and Active Ingredients

2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (Brazil). Analytical grade methyl alcohol was acquired from Merck (São Paulo, Brazil). *Trans*- Ferulic acid was purchased from Henrifarma (Brazil). Ethylhexyl triazone was acquired from D'Altomare (Brazil) and bis-ethylhexyloxyphenol methoxyphenyl triazine was purchased from Brasquim (Brazil). All materials were used as received, without any further purification. Purified water was used for all experiments.

2.2. Formulations

Oil-in-water emulsions associating or not FA and the UV filters were developed based on an anionic self-emulsifying agent. Table 1 describes the qualitative and quantitative composition (% w/w) of the samples.

2.3. Clinical Assays

Procedures were in accordance with the ethical standards on human experimentation and with the Helsinki Declaration. All protocols were approved by the Human Experimentation Committee of the School of Pharmaceutical Sciences of the University of São Paulo (protocol number: 735.493). For all subjects, oral informed and written consent were previously provided.

2.4. Human Repeat Insult Patch Test (HRIPT) and Phototoxic/Photosensitivity Potential

A six-week HRIPT assay was performed in 55 male and female volunteers. Subjects were 18–60 years old with skin phototypes of II to IV. Epicutaneous semi-oclusive patches were applied to volunteers´ backs for 48 h, three times a week. Each patch had three chambers, two that contained the sunscreen samples (F1 and F2) and, another, containing purified water, as negative control. The skin was scored 30 min later and new material was applied for two more weeks. The next two weeks were the rest period (no samples applied) and, after that, new patches with the samples and negative control were applied for one last week, *i.e.*, the challenge phase. The scores used were 0 for no erythema, 1 for well-defined erythema, 2 for erythema and induration, and 3 for vesiculation and bullous reaction [8–10].

A phototoxic and photosensitivity potential assays were performed in 27 male and female volunteers aged between 18 and 52 years old and with skin phototypes of II to IV. Epicutaneous patches were applied to the volunteers´ backs for 48 h, twice a week (patches contained F1, F2 and purified water in separate chambers). After 48 h, sites were exposed to an UVA simulated irradiation dose of 4.0 J/cm^2 for 7 min. The skin was scored 30 min later, aiming to evaluate any phototoxic reaction. The formulations were, then, reapplied and the sites were irradiated for one more week. The next two weeks were the rest period and, subsequently, new patches with the samples were applied and irradiated for the challenge phase, targeting to evaluate photosensitivity reactions. The scores used were: 0 for no erythema; 1 for well-defined erythema; 2 for erythema and induration; 3 for vesiculation and bullous reaction [10,11].

Table 1
Qualitative and quantitative (% *w*/w) composition of the sunscreen samples.

Ingredients		Concentration (% w/w)	
		F1	F2
Oil phase	Ethylhexyl triazone	5.0	5.0
	Bis-ethylhexyloxyphenol methoxyphenyl triazine	10.0	10.0
	C12-C15 alkyl benzoate	9.0	9.0
	Butylene glycol cocoate	6.75	6.75
	Isopropyl myristate	6.75	6.75
	Hydroxyethyl acrylate (and) sodium acryloyldimethyl taurate copolymer (and) isohexadecane (and) polysorbate 60	4.00	4.00
	Cyclomethicone	1.75	1.75
	Cyclomethicone (and) dimethicone crosspolymer	1.25	1.25
Water phase	Glycerin	5.00	5.00
	Phenoxyethanol (and) methylparaben (and) ethylparaben (and) butylparaben (and) propylparaben (and) isobutylparaben	0.75	0.75
	Disodium EDTA	0.30	0.30
	Acrylates (and) C10–30 alkyl acrylate crosspolymer	0.10	0.10
	Ferulic acid	–	1.0
	Triethanolamine	*	*
	Purified water	**	**

* Sufficient to adjust the pH value.
** Sufficient to complete to 100%.

2.5. Ex vivo Antioxidant Activity Assay

F1 and F2 (2.0 mg/cm^2) were applied over the volar forearm of 10 subjects in randomized areas of 9.0 cm^2 previously outlined. Two consecutive applications of each sample were carried out with an interval of 2 h between them [12]. Two hours after the last application, a tape stripping technique was performed. Twenty tapes (2.0 × 2.0 cm, 3 M®) were taken for each area and, after, the tapes were exposed for 2 h in a solar simulator (Suntest® CPS +, Atlas, Germany) equipped with a xenon lamp, an optical filter to cut off wavelengths shorter than

290 nm and an IR-block filter to avoid thermal effects. The irradiation device has 1500 W xenon lamp and a benchtop design of 90x35x35 cm with 28 × 20 cm (560 cm^2) exposure area. The solar simulator emission was maintained at 580.08 W/m^2 (300–800 nm), corresponding to an UV irradiance of 55.0 W/m^2 (irradiation dose of 396.0 kJ/m^2) [13]. Tapes were placed and rested in 15.0 mL of methanol for 17 h. Further, the samples were immersed in an ultrasound bath for 60 min in order to extract the *stratum corneum*.

The spectrophotometric (UV–Vis Evolution® 300, Thermo Scientific, USA) readings were obtained using methanolic solutions with 140.0 μM DPPH solution at a ratio of 1:1. Samples rested for 60 min at room temperature (24.0 ± 2.0 °C) and protected from light. All samples were prepared and analyzed in triplicate. The absorbance values were measured at 515.0 nm and converted into percentage of free radical scavenging using the **Eq.(1)** [14]:

$$\%FRS = \frac{(Abs_{control} - Abs_{stratum\ corneum}) \times 100}{Abs_{control}} \quad (1)$$

Percentage of free radical scavenging. % FRS: Percentage of free radical scavenging; $Abs_{control}$: Absorbance of negative control sample; $Abs_{stratum\ corneum}$: Absorbance of the tapes samples.

2.6. In vivo SPF and in vitro UVA-PF

In vivo SPF testing was performed according to the International Sun Protection Factor Test Method [15]. The *in vivo* test involved 10 subjects with skin phototypes I to III, using a Multiport® 601 (Solar Light Company, USA) solar UV simulators [16]. The SPF of each sample (F1 and F2) on each subject was calculated from the individual minimum erythemal dose [MED] on unprotected skin and the individual MED on product protected skin. *In vitro* UVA-PF was performed according to the determination of the UVA protection factor and critical wavelength guideline [17]. Three PMMA plates were prepared for each product to be tested. Transmission measurements between 290 and 400 nm were carried out using a spectrophotometer equipped with an integrating sphere (UV Transmittance Analyzer UV-2000S, Labsphere®, USA) [18]. The coefficient of the variation for the individual UVA-PF values after irradiation was evaluated and did not exceed 20%.

2.7. Statistical Analysis

Statistical treatment was performed using a Minitab® (Version 17) software. The results were expressed as mean ± standard deviation and subjected to One-way Analysis of Variance (ANOVA) followed by Tukey post-test, adopting a confidence level of 95% (α = 0.05) to determine significant results.

3. Results

Clinical safety analysis proved that the sunscreen samples had a safe profile, even under sun-exposed conditions. After the phases of the tests (induction, rest and challenge), no erythema was detected, in comparison with the negative control. The FA, through the *ex vivo* antioxidant test, presented no tendency to increase the antioxidant activity on the skin after treatment with the formulation containing it (F2) (Table 2). *In vivo* SPF and UVA-PF are summarized in Table 3.

4. Discussion

UV radiation is one of the major exogenous agents responsible for the generation of free radicals. While UVB causes direct damage to cellular DNA, UVA radiation instigates indirect ones, leading to mutations and cancer [19]. UVA radiation was, likewise, associated with the matrix metalloproteinase-1 activation (proteolytic enzyme produced by skin cells that damages and degrades collagen) through depletion of the skin antioxidant defenses and/or an excessive ROS production [20]. It has been shown that UV exposure for short periods of time was already able to generate hydroxyl free radicals and hydrogen peroxide. Also, ROS production can lead to degradation of endogenous antioxidants, exacerbating the oxidative damage caused by free radicals. The endogenous antioxidant system efficiency is required in adverse conditions, including exposure to UV radiation; however, such contribution may be depleted, leading to oxidative stress. In these cases, supplementation with exogenous antioxidant becomes crucial [19].

In this research, the synergy between FA and UV filters led to the development of multifunctional sunscreens with photoprotective and antioxidant activities. We tested the clinical safety of the formulations, which presented good skin biocompatibility and no phototoxicity nor photosensitivity. The results evidenced that the sunscreens had safe profile under sun-exposed conditions, containing or not the FA.

The *ex vivo* antioxidant assessment was performed based on a tape stripping technique and data showed that there was no tendency to increase the antioxidant activity on the skin after treatment with the formulation containing FA (F2), when compared to F1. Peres and coworkers [5] have established the *in vitro* antioxidant profile of the systems “ethylhexyl triazone + bis-ethylhexyloxyphenol methoxyphenyl triazine” and “FA + ethylhexyl triazone + bis-ethylhexyloxyphenol methoxyphenyl triazine” and found that the best antioxidant activity occurred in the presence of the FA, even though this property was not reproduced on the subjects in this present study, through the *ex vivo* test. Additionally, the distribution of the formulations (active ingredients) on/across the *stratum corneum* (tapes 2 to 10 and 11 to 20) did not show apparent differences on the antioxidant efficacy establishment.

Our results could be explained due to a relatively low skin penetration/permeation of FA across the subjects' skin and, also, the characteristics of the sample application protocol of this study, the tape stripping technique or the used amount of FA (% *w*/w) into the F2. Chen and coworkers [19] evaluated the use of liposomes and ethosomes as delivery systems for FA. These researchers confirmed that the vesicular particles improved the FA skin permeation and retention, especially the ethosomes, which were capable of increasing the FA permeation in 75 times compared to its free form. Zhang and coworkers

Table 2
Antioxidant activity of the tape-stripped *stratum corneum expressed in* % percentage of free radical scavenging.

Samples	Irradiation profile	Tapes 2 to 10	Tapes 11 to 20
Skin (control)	Not irradiated	37.43 ± 9.61 ^{A}	37.90 ± 7.77 ^{A}
	Irradiated	30.72 ± 8.93 ^{A}	32.88 ± 7.41 ^{A}
F1	Not irradiated	38.20 ± 7.98 ^{A}	39.87 ± 10.44 ^{A}
	Irradiated	31.44 ± 6.84 ^{A}	34.50 ± 6.75 ^{A}
F2	Not irradiated	43.07 ± 8.97 ^{A}	43.00 ± 6.43 ^{A}
	Irradiated	39.67 ± 7.86 ^{A}	35.92 ± 9.45 ^{A}

The letter indicates the results of ANOVA statistical analysis. Same letter for the same parameter indicate statistically significant equivalences among samples (Control, F1 and F2) or between the pool of tapes tested (Tapes 2 to 10 and Tapes 11 to 20), with a confidence value of 95% ($p < .05$). Ten volunteers were investigated for the antioxidant activity of the tape stripped stratum corneum.

Table 3
In vivo SPF and *in vitro* UVA-PF.

Sunscreens	*In vivo* SPF	*In vitro* UVA- PF
F1	19.7 ± 2.9 ^{A}	11.3 ± 0.6 ^{C}
F2	26.0 ± 1.6 ^{B}	14.0 ± 0.0 ^{D}

The letters indicate the results of ANOVA statistical analysis. Different letters for the same parameter indicate statistically significant differences between samples (F1 and F2), with a confidence value of 95% ($p < .05$). UVA-PF results were analyzed in triplicate. Ten volunteers were investigated for the SPF test.

[21] have also assessed the skin permeation of a ferulic acid ester, the ethyl ferulate. They concluded that the structural modification of the compound provided an increase in its skin permeation; however, the ester had a decrease in its antioxidant activity compared with FA. Saija and coworkers [22] investigated the *in vitro* cutaneous permeation of FA in excised human skin obtained from breast reduction operation and they observed that FA permeated through the *stratum corneum*, however, the sample was composed of a saturated aqueous solution of FA and the application protocol involved the period of time of 24 h.

In summary, no significant dissimilarities were observed for the epidermis antioxidant activity, regardless the treatment applied (formulations or control). Nevertheless, strategies could be used to increase the penetration of FA through skin for topical, multifunctional application of this compound. Our results also underscore the importance to consider adjustments on the *ex vivo* assay protocol, aiming a more accurate analytical quantification of the bioactive compound and a possible correlation with the antioxidant efficacy of the sunscreens on the epidermis.

Based on the obtained SPF values, the samples were considered sunscreens, contemplating that they had SPF ≥ 6. Formulations F1 and F2 had, also, a broad spectrum protection profile, since they showed SPF values higher than 15 and a level of UVA-PF higher than 1/3 of the SPF, meeting the requirements for the registration of sunscreen formulations. Additionally, both formulations generated critical wavelength values over 370 nm (data not shown) that corroborated with broad spectrum protection [15,17,23,24].

The photoprotective profile is justified not only by the broad defense provided by the UV filters used but, in particular, by the FA antioxidant characteristics against the erythema establishment [25]. Data from F2 attested the photoprotective potential of this bioactive compound in synergism with the UV filters, since the presence of FA promoted a 32% increase on the *in vivo* SPF value and a 24% enhancement on the amount of UVA-PF, both meaningful. Such response could be corroborated by the UV filtering capacity from bis-ethylhexyloxyphenol methoxyphenyl triazine, that is able to absorb both UVB and UVA radiations. Besides, the UVA-PF increase provided by the FA represented a noteworthy outcome, since literature reported its photoprotective efficacy prominently against UVB radiation [7].

The combination of antioxidants from several sources with UV filters has been presented as a promising platform for the development of multifunctional sunscreens, since these compounds would act synergistically by different and complementary mechanisms. First, they would work as filters over the skin surface, absorbing or reflecting UV radiation and, second, the antioxidants may act both on the surface and into deeper skin layers, combating oxidative stress, providing, therefore, a complement and more robust sun protection [1].

5. Conclusions

These results provided a new perception for the development of bioactive sun care products and allowed to characterize FA as a particular platform for bioactive multifunctional sunscreens. The non-irritant profile of the sunscreens, products that are recommended to be used under sun-exposed conditions, qualified the samples as safe. The *in vivo* data indicated that FA increased the SPF and, also, the UVA-PF of the developed sunscreens, reinforcing the broad-spectrum characteristic of the photoprotective formulations. Additionally, according to the results from the *ex vivo* antioxidant test, it is plausible to recommend adjustments on the *ex vivo* protocol to explicitly determine the positive effects of topical antioxidant ingredients applied over the skin.

Acknowledgments

Authors greatly acknowledge FAPESP (São Paulo Research Foundation) for the financial support (process 2016/24360-4) and CNPq (Universal 14/2013 process 470649/2013-8). We also thank FAPESP, CNPq and CAPES for the scholarships.

References

[1] F.H. Lin, J.Y. Lin, R.D. Gupta, J.A. Tournas, J.A. Burch, M.A. Selim, N.A. Monteiro-Riviere, J.M. Grichnik, J. Zielinski, S.R. Pinnell, J. Invest. Dermatol. 125 (2005) 826.
[2] E. Graf, Free Radic. Biol. Med. 13 (1992) 435.
[3] J. Teixeira, A. Gaspar, E.M. Garrido, J. Garrido, F. Borges, Biomed. Res. Int. 2013 (2013) 1.
[4] A. Urbaniak, M. Szelag, M. Molski, Comput. Theor. Chem. 1012 (2013) 33.
[5] D.D. Peres, M.B. Ariede, T.M. Candido, T.S. de Almeida, F.R. Lourenço, V.O. Consiglieri, Kaneko TM, Velasco MVR, Drug Dev. Ind. Pharm. 43 (2017) 246.
[6] C. Oresajo, T. Stephens, P.D. Hino, R.M. Law, M. Yatskayer, P. Foltis, P. Sreekumar, Pinnell SR, J. Cosmet. Dermatol. 7 (2008) 290.
[7] A. Saija, A. Tomaino, D. Trombetta, A. De Pasquale, N. Uccella, T. Barbuzzi, D. Paolino, F. Bonina, Int. J. Pharm. 199 (2000) 39.
[8] Marzulli FN, Maibach HI, Food Cosmet. Toxicol. 13 (1975) 535.
[9] Kligman AM, Wooding WM, J. Invest. Dermatol. 49 (1967) 78.
[10] C.A. de Oliveira, M.F. Dario, F.D. Sarruf, I.F.A. Mariz, M.V.R. Velasco, C. Rosado, Baby AR, Colloids Surf. B 140 (2015) 531.
[11] C.W. Stott, J. Stasse, R. Bonomo, A.H. Campbell, J. Invest. Dermatol. 55 (1970) 335–338.
[12] D.R. Sambandan, D. Ratner, J. Am. Acad. Dermatol. 64 (2011) 748–758.
[13] C. Alonso, C. Barba, L. Rubio, S. Scott, A. Kilimnik, L. Coderch, J. Notario, J.L. Parra, J. Photochem. Photobiol. B Biol. 97 (2009) 71.
[14] V. Bondet, W. Brand-Williams, C. Berset, LWT Food Sci. Technol. 30 (1997) 609.
[15] COLIPA, Methods for Testing Efficacy of Sunscreen Products, (2006).
[16] E.P. Santos, Z.M. Freitas, K.R. Souza, S. Garcia, A. Vergnanini, Int. J. Cosmet. Sci. 21 (1999) 1.
[17] COLIPA, In Vitro Method for the Determination of the Uva Protection Factor and "Critical Wavelength" Values of Sunscreen Products, (2011).
[18] C. Couteau, E. Paparis, C. Chauvet, L. Coiffard, Int. J. Pharm. 487 (2015) 120–123.
[19] L. Chen, J.Y. Hu, S.Q. Wang, J. Am. Acad. Dermatol. 67 (2012) 1013.
[20] T. Pluemsamran, T. Onkoksoong, U. Panich, Photochem. Photobiol. 88 (2012) 961.
[21] L.W. Zhang, S.A. Ael-Suwayeh, P.W. Hsieh, J.Y. Fang, Int. J. Pharm. 399 (2010) 44–51.
[22] A. Saija, A. Tomaino, R. Cascio, D. Trombetta, A. Proteggente, A. Pasquale, N. Uccella, F. Bonina, J. Sci. Food Agric. 79 (1999) 476.
[23] Food and Drug Administration, Guidance for Industry Labeling and Effectiveness Testing: Sunscreen Drug Products for over the Counter Human Use - Small Entity Compliance Guide, (2012).
[24] Brasil, Ministério da Saúde, Conselho Nacional de Saúde. Resolução n° 466, de 12 de dezembro de 2012. Diário Oficial da República Federativa do Brasil, Poder Executivo, Brasília, 2012.
[25] M. Srinivasan, A.R. Sudheer, V.P. Menon, J. Clin. Biochem. Nutr. 40 (2007) 92.