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# INFLUENCE OF THE INCORPORATION OF ANNATTO OIL BIXA ORELLANA L. IN THE SUN PROTECTION FACTOR BY VISIBLE SPECTROSCOPY OF PHOTOPROTECTIVE FORMULATIONS

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**ABSTRACT**

The cancer of skin type no melanoma is the most frequent cancer in Brazil and accounts for 25% of all malignant tumors registered in the country. It has high rates of cure if detected early. Among the tumors of the skin, non-melanoma type is the higher incidence and lower mortality. Test bioproducts with protective solar activity is crucial in the prevention of public health and promotes technological development. The study evaluated the influence of *Bixa orellana L.* oil on SPF (Sun Protection Factor) six emulsions containing chemical filters Eusolex 232 and Neo Heliopan MBC. The physicochemical analysis of oil (acid value and saponification) preceded the spectrophotometric reading tests of each formulation. Then, centrifuge tests and thermal stress were performed and preceded the accelerated stability study (2 months) in the refrigerator (10°C), environment temperature (32°C) and oven (40°C) with three formulations. Acidity and saponification indices showed values slightly above those permitted. The spectrophotometric analysis showed that the oil enhances the effect of solar protection of Eusolex 232 chemical filters and Neo Heliopan MBC. The pH test showed satisfactory results for formulations containing the chemical filter Neo Heliopan MBC®. The spin test showed satisfactory results for 7 of the 9 formulations. The thermal stress test showed positive results only for three of the nine formulations. The SPF analysis has verified that the oil annatto of *Bixa Orellana L.* 5% and 10% when incorporated in photoprotective formulations with chemical filters Eusolex 232® and Neo Heliopan MBC® potentiates the effect of solar protection.



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## INTRODUCTION

The therapeutic use of medicinal plants is an ancient practice that has basis in popular wisdom. This knowledge can be applied in the treatment and prevention of various diseases and also in the use of topical preparations in order to combat an infection in the skin, or aesthetic care in different cultures around the world (Serafini *et al*., 2015; Costa, 2007; Vilar Dde *et al*., 2014). Regarding the Annatto (*Bixa orellana* L.) this fact can be evidenced from ancient times when the Indians who inhabited the lands of Central America (especially Mexico and Puerto Rico) and South America (Brazil and Peru) used the extracted pigment from its seeds to paint the skin not only as an ornament, but also to treat burns and insect bites, as well as sunscreen (Diniz *et al*., 2015) See Figure 1.

The chemical constituents of greater importance in *Bixa orellana L.* are the bixin and norbixin carotenoid responsible for the red color of the seeds and have photoprotective and antioxidante action. Antioxidants are substances that in minimum concentration, delay or inhibit effectively oxidation process (Souza, 2011; Shami, 2004). As protection against the sun's rays, there are sunscreens that protect the skin by filtering or blocking solar radiation. So now consumers demand cosmetics products that have SPF (Sun Protection Factor), a measure used to quantify the protection capacity of sunscreens. Thus, the purpose of this study is to investigate the annatto oil as a new possibility of raw material to increase the sun protection factor of cosmetic formulations. It is now clear that the incorporation of plant extracts with protective action against sun rays and antioxidant action adds value to the product making it more appealing to customers.

**Photo taken from the Medicinal Garden of Liceu Maranhense, São Luís, Maranhão, Brazil. Phytotherapy Program of the Federal University of Maranhão; Diniz, Roseane Costa *et al*, 2015 [4]**

## MATERIALS AND METHODS

The *Bixa orellana L.* oil was provided by Via Farma Importadora LTDA. The report provided by the manufacturer was parsing parameters of appearance, color, odor, saponification number, acid value and peroxide value. A line of study consisted of eight parts:

**Physico-chemical analysis:** The acidity indices and oil saponification were analyzed according to the methodology recommended of the Adolf Lutz Institute (1985), which is based on the methods of the AMERICAN OIL CHEMISTS' SOCIETY (AOCS) (Adolf Lutz Institute-Brasil, 2008). The acid value indicates the amount in milligrams of potassium hydroxide required to neutralize the fatty acids in one gram of sample. The test was performed in duplicate and also a blank test. The index calculation was carried out based on the following formula:

$$\frac{V \times CF \times 40}{S}$$

V = volume de NaOH 0.1M gasto na titulação
CF = correction factor
S = sample weight in grams

The saponification number indicates the required amount of alkali to saponify a defined amount of sample. This method is used for all oils and fats and expresses the number of milligrams of potassium hydroxide required to saponify one gram of sample. The test was performed in duplicate and was also carried out a blank test.

The calculation of the index was based on the following formula:

$$\frac{28 \times CF \times (B-A)}{P}$$

A = volume spent on the titration of the sample
B = volume spent on white titration
CF = correction factor
P = number of grams of the sample

**Obtaining cosmetic formulations (sunscreens):** Nine formulations were developed and the first three formulations composed of only chemical filter Eusolex 232® (formulation I) - Phenylbenzimidazole sulfonic acid, Neo Heliopan MBC® - Camphor Metilbenzidileno (formulation II) and a formulation containing the two filters (formulation III). The other six formulations were developed with the addition of annatto oil at concentrations of 5% and 10%. The composition of the formulations is described in Tables 1, 2 and 3. The pharmaceutical form of choice was emulsion, formulations that provide greater FPS's, accept more easily the incorporation of sunscreens and oily products, besides having good spreadability forming a uniform film on the skin and also provide good value for money (Ferrari *et al*., 2003).

**Table 1. Standard photoprotective cosmetic formulations with Eusolex 232® and Neo Heliopan MBC®**

| Components of the oil phase | Percentage of components in each formulation | | |
|---|---|---|---|
| | Formula I | Formula II | Formula III |
| Mineral Oil | 2.0% | 2.0% | 2.0% |
| Liquid Vaseline | 2.0% | 2.0% | 2.0% |
| Eusolex 232 | 8.0% | - | 8.0% |
| Neo Heliopan | - | 4.0% | 4.0% |
| Self emulsifying wax non-ionic | 4,0% | 4.0% | 4.0% |
| Components of the Aqueous Phase | Percentage of components in each formulation | | |
| | Fomula I | Fomula II | Formula III |
| Glycerin | 5.0% | 5.0% | 5.0% |
| Nipagin | 0.5% | 0.5% | 0.5% |
| q.s.p. distilled water | 100ml | 100ml | 100ml |

*Formula I: Eusolex 232®; Formula II: Neo Heliopan MBC®; Formula III: Eusolex 232® + Neo Heliopan MBC®; quantity sufficient to: q.s.p.*

**Table 2. Photoprotective cosmetic formulations with Eusolex 232® and Neo Heliopan MBC® and 5% annatto oil**

| Components of the oil phase | Percentage of components in each formulation | | |
|---|---|---|---|
| | Formula IV | Formula V | Formula VI |
| Mineral Oil | 2.0% | 2.0% | 2.0% |
| Liquid Vaseline | 2.0% | 2.0% | 2.0% |
| Eusolex 232 | 8.0% | - | 8.0% |
| Neo Heliopan | - | 4.0% | 4.0% |
| Cera autoemulsificante não iônica | 4.0% | 4.0% | 4.0% |
| Self emulsifying wax non-ionic | 5.0% | 5.0% | 5.0% |
| Components of the Aqueous Phase | Percentage of components in each formulation | | |
| | Fomula IV | Fomula V | Formula VI |
| Glycerin | 5.0% | 5.0% | 5.0% |
| Nipagin | 0.5% | 0.5% | 0.5% |
| q.s.p. distilled water | 100ml | 100ml | 100ml |

*Formula IV: Eusolex 232 + 5% annatto oil; Fórmula V: Neo Heliopan MBC + 5% annatto oil; Formula VI: Eusolex 232 + Neo Heliopan MBC + 5% annatto oil; Quantity sufficient to: q.s.p.*

**Table 3. Photoprotective cosmetic formulations with Eusolex 232® and Neo Heliopan MBC® and 10% annatto oil**

| Components of the oil phase | Percentage of components in each formulation | | |
|---|---|---|---|
| | Formula VII | Formula VIII | Formula IX |
| Mineral Oil | 2.0% | 2.0% | 2.0% |
| Liquid Vaseline | 2.0% | 2.0% | 2.0% |
| Eusolex 232 | 8.0% | - | 8.0% |
| Neo Heliopan | - | 4.0% | 4.0% |
| Self emulsifying wax non-ionic | 4.0% | 4.0% | 4.0% |
| annatto oil | 10.0% | 10.0% | 5.0% |
| Components of the Aqueous Phase | Percentage of components in each formulation | | |
| | Fomula VII | Fomula VIII | Formula IX |
| Glycerin | 5.0% | 5.0% | 5.0% |
| Nipagin | 0.5% | 0.5% | 0.5% |
| q.s.p. distilled water | 100ml | 100ml | 100ml |

*Formula VII: Eusolex 232® + 10% annatto oil; Formula VIII: Neo Heliopan MBC® + 10% annatto oil; Formula IX: Eusolex 232® + Neo Heliopan MBC® + 10% annatto oil; quantity sufficient to: q.s.p.*

**Stability evaluation of cosmetic formulations:** The accelerated stability studies were performed using macroscopic analysis forty-eight hours after the manipulation of the samples, in order to visualize possible variations in the organoleptic and homogeneity characteristics (Ferrari *et al*., 2003; Roland *et al*., 2003).

**pH Determination:** The pH determination was made preparing solutions with 5.0g of each formulation diluted in 100 mL of freshly distilled water and homogenized with the aid `` mixer`` and then inserted into the electrode (Analion pH metro-Mod. PM608). The test was performed in duplicate (Moreira, 2012).

**Centrifuge Test:** In graduated conical centrifuge tube for assay were added 5.0 g of each sample and subjected to 1000 cycles, 2500 and 3500 rpm corresponding to 70, 440 and 863 G respectively (Fanem Ltd. - Mod R 206, Excelsa BABY II-440 watts) for a fifteen-minute time slot in rotation at room temperature. After each cycle, samples were analyzed for their respective observation homogeneity or phase separation signals (Masson, 2005; Ferrari *et al*., 2003; ANVISA, 2004).

**Thermal Stress Test:** The nine formulations were stored in closed polyethylene plastic bottles subjected to heating in termoestabilizado bath (Nova Técnica Ltda-Mod. 281 NT) at temperatures between 40 ± 2 to 80 ± 2 ° C. the temperature was increased by 5 at 5 ° C and kept for thirty minutes each temperature. Readings were perfomed at interval increased temperature and between one another. An specific nomenclature was used for macroscopic analysis tests, centrifugation and thermal stress it is: N = Normal, no change; ML = Mildly Modified; M = Modified (ANVISA, 2004; Morais *et al*., 2005).

**Analysis of the formulations spectrophotometric profile:** The spectrophotometric (Perk elmer UV/Visible Spectroscopy) readings were conducted by weighing 0.002g of each formulation that were diluted in 100ml ethanol alcohol P.A. from each dilution were taken three aliquots to the test in triplicate. The wavelength during analysis was maintained between 200 and 400nm, corresponding to spectrum band of ultraviolet light. How white was used ethanol alcohol P.A. The Mansour equation was used to convert the absorbance values of the formulations and the pure oil in SPF.

**Mansour Equation**

$$SPF = CF \sum_{290}^{320} x\ EE(\lambda)\ x\ I(\lambda)\ x\ Abs(\lambda$$

Where CF is the correction factor = 10; EE (λ) is the erythematogenic effect of solar radiation λ wavelength; I (λ) is the intensity of sunlight at a particular wavelength (λ) and Abs (λ) it is the spectrophotometric reading of the sample absorbance at each wavelength. Being that of EE values (λ) x I (λ) were calculated and are in the table 4 (Nascimento *et al*., 2009; Franzen, 2011; Mansour *et al*., 1986).

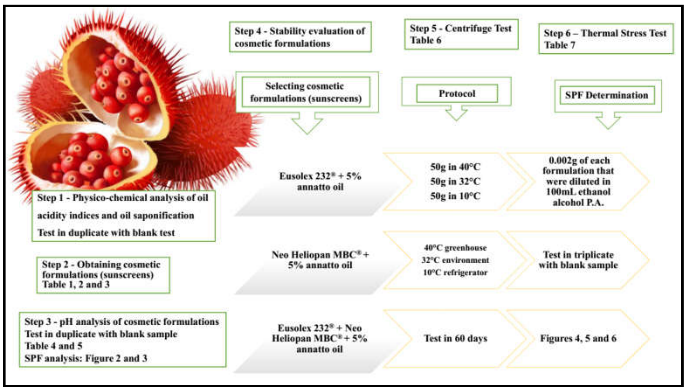
**Table 4. Weighting used in the calculation of the sun protection factor by spectrophotometry**

| Wave-length (nm) | EE(λ) x I(λ) (normalized) relative values |
|---|---|
| 290 | 0.0150 |
| 295 | 0.0817 |
| 300 | 0.2874 |
| 305 | 0.3278 |
| 310 | 0.1864 |
| 315 | 0.0839 |
| 320 | 0.0180 |
| ∑ | 1,0000 |

**Preliminary accelerated stability study**

After the thermal stress test the formulations less macroscopically altered were:

Eusolex232® + 5% urucum oil (formulation IV)
Neo Heliopan MBC® + 5% urucum oil (formulation V)
Eusolex 232® + Neo Heliopan MBC® + 5% urucum oil (formulation VI) and with them the study was continued for two months as explicitly stated in the graphical abstract below.




## RESULTS AND DISCUSSION

The value found for the acid number (4.8mg NaOH) was slightly higher than allowed according to the Option Phoenix Distributor Petrochemicals Ltda. technical literature, which cites the acid value of the oil as at most 3. However, should take into consideration that this technical information is in the case of use of Potassium Hydroxide (KOH) and the acid value of oil varies according to the extraction method (Moreira, 2012). In this case, for the *Bixa orellana L.* oil obtained by Via Farma Importadora Ltda, despite the oily extract characteristics, there was no information about the acid value of the extract obtained by extraction with oily solvent. The saponification value obtained (224.78KOH/g) was slightly above the amount recorded in the report of the analysis technical literature of the Option Phoenix Distributor Petrochemicals Ltda. (2011) which is 170-200mg KOH/g. Before we insert a cosmetic product on the market, it is necessary to analyze its stability assuring consumers of a safe and quality product. The stability study guides the development of the formulation, providing subsidies for the improvement of the of it, besides estimating the validity and assist in monitoring the organoleptic, physicochemical and microbiological stability (ANVISA, 2008; Diogo *et al*., 2015). The *Bixa orellana L.* oil was purely analyzed and its SPF (Sun Protection Factor) was insignificant compared with the SPF of the formulations containing it. The patterns formulations, i.e., those in which no oil incorporation, obtained in its entirety a lower SPF that the same formulation containing 5% oil and 10%. Among all formulations, the one that showed more satisfactory result was composed of Eusolex 232® + Neo Heliopan MBC®. Neo Heliopan MBC® + oil 10% (formulation IX) which was 8.82, almost twice of the same formulation 5% (formulation VI) and more than triple of the same formulation without added oil of the photoprotective formulations and pure annatto oil SPF can be seen in Figure 2.

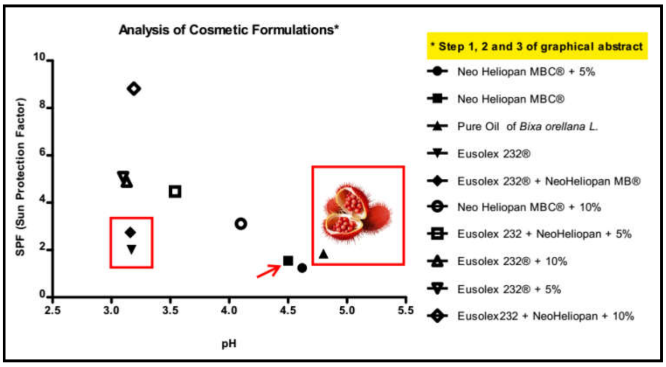



**Figure 2. SPF (Sun Protection Factor) × pH of cosmetic formulations and pure annatto oil before of Thermal Stress Test**

The results can be explained due to the vegetable oils that have photoprotective action due to the presence of substances such as flavonoids, antraquinônicos derivatives and carotenoids tend to be combined with chemical and physical filters increasing photoprotection, so its use is relevant (Alamo *et al*., 2012). Moreover, oils and fats help solubilize the chemical filters are mostly soluble as Neo Heliopan MBC® (camphor Metilbenzidileno) is ideal for formulations waterproof and if Eusolex 232®, which has solubility in water 0.012g / L oil may have benefited from this filter solubility increasing the SPF of formulations that contained (Mishra *et al*., 2012). Cosmetics in general should have a pH similar to the pH of human skin, this in turn is slightly acid ranging from 4.6 a 5.8 or 4.5 a 5.5 and which may vary each skin region. The skin acid pH combat fungi and bacteria as well as infections and rashes, so it is important to maintain the physiological value even with the use of chemicals (Lambers *et al*., 2006; Leonardi *et al*., 2002). The formulations analyzed that more approached the skin pH values described in the literature, were the ones containing the pure chemical pure filter Neo Heliopan MBC® and incorporated into the oil to 5% and to 10%, respectively Formulation II, Formulation V and Formulation VIII. The formulations with the chemical filter Eusolex 232® is not well behaved front oil incorporation seen that the results were below 4.0, thus, more acid than the skin pH. In this case it is suggested that there is incorporation of a buffering agent to stabilize the pH of these formulations. It is noteworthy that the oil acid value is above the limit accepted (max. 3) then this factor may also be taken into consideration for all formulations that did not reach the expected pH value. The values found in the analysis are in Table 5.

**Table 5. SPF and pH analysis of cosmetic formulations**

| Formulations | pH (*x*) | SPF (*y*) |
|---|---|---|
| Eusolex 232® | 3.17 | 2.01 |
| Neo Heliopan MBC® | 4.50 | 1.54 |
| Eusolex 232® + Neo Heliopan MBC® | 3.16 | 2.74 |
| Eusolex 232® + 5% annatto oil | 3.10 | 5.08 |
| Neo Heliopan MBC® + 5% annatto oil | 4.62 | 1.24 |
| Eusolex 232® + Neo Heliopan MBC® + 5% annatto oil | 3.54 | 4.47 |
| Eusolex 232® + 10% annatto oil | 3.13 | 4.89 |
| Neo Heliopan MBC® + 10% annatto oil | 4.10 | 3.11 |
| Eusolex 232® + Neo Heliopan MBC® + 10% annatto oil | 3.19 | 8.82 |
| Pure oil of *Bixa orellana L.* | 4.80 | 1.84 |

The centrifuge test provides some future instability of the product, such as phase separation. However, no phase separation does not guarantee the stability, only the legitimate that the product may be subjected to prolonged stability tests (Diogo *et al*., 2015; Leonardi *et al*., 2002). The emulsion galenical forms are types of creams and / or lotions that have two immiscible phases with each other but with the addition of an emulsifying agent may become a homogeneous system containing polar and apolar components and therefore being considered the type of vehicle which has greater protection (Pineda-Sotomayor, 2015). In this case emulsions O/A were handled which allows the incorporation of hydrophilic and lipophilic filters which can act synergistically See Table 6.

**Table 6. Centrifuge Test**

| *Samples* | *1000rpm* | *2500rpm* | *3500rpm* |
|---|---|---|---|
| Eusolex 232® | N | LM | LM |
| Neo Heliopan MBC® | N | LM | LM |
| Eusolex 232® + Neo Heliopan MBC® | N | LM | LM |
| Eusolex 232® + 5% annatto oil | N | N | N |
| Neo Heliopan MBC® + 5% annatto oil | LM | LM | LM |
| *Samples* | *1000rpm* | *2500rpm* | *3500rpm* |
| Eusolex 232® + Neo Heliopan MBC® + 5% annatto oil | N | LM | LM |
| Eusolex 232® + 10% annatto oil | N | N | N |
| Neo Heliopan MBC® + 10% annatto oil | LM | LM | M |
| Eusolex 232® + Neo Heliopan MBC® + 10% annatto oil | LM | LM | M |
| - | - | - | - |

*N- Normal LM- Mildly Modified M- Modified*

Only Eusolex 232® + Neo Heliopan MBC® + 10% oil (formulation IX) and Neo Heliopan MBC® + 10% oil (formulation VII) among the analyzed samples showed phase separation after completion of the test. All other formulations developed from oils. Thus, it is questionable the ratio of the incorporated oil concentration to 10% when in contact with the chemical filter Neo Heliopan MBC® ensuring that it is necessary to use a different test chemical filter or annatto oil at lower concentrations to minorizar phase separation, making it a more stable emulsion. The thermal stress test provides signs of instability in the formulations analyzed in order to find changes in the same (ANVISA, 2008; Diogo *et al*., 2015; Leonardi *et al*., 2002). Thus, all the formulations analyzed showed no visible changes in the temperature interval between 40ºC and 50ºC, from 55ºC all formulations except those composed by Eusolex 232® + Neo Heliopan MBC® (Formulation III) showed slight changes, no longer consistent emulsions and became fluids, from 60ºC all modifications had

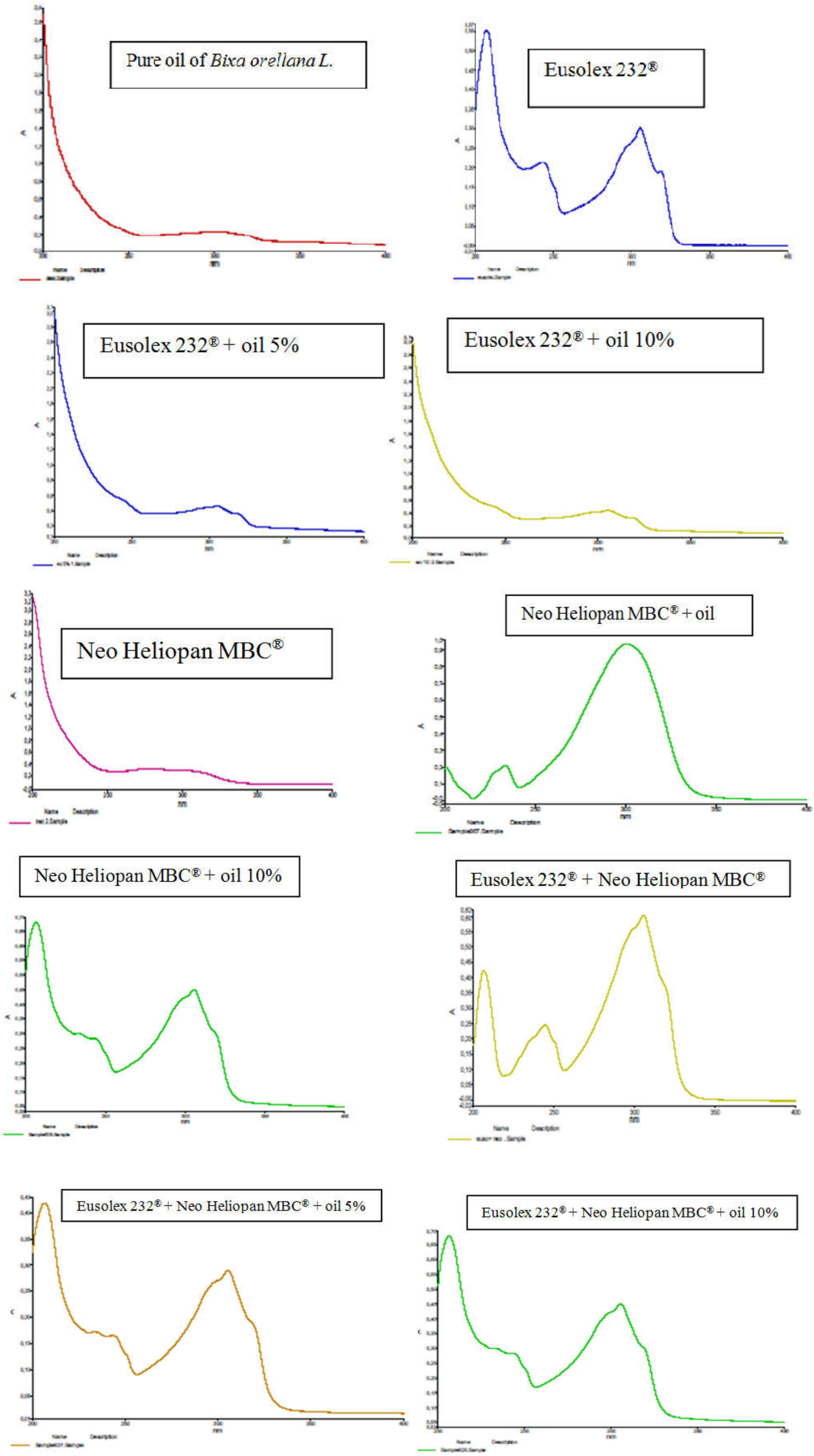



**Figure 3. Reading of the absorbances of the formulations of table 5**

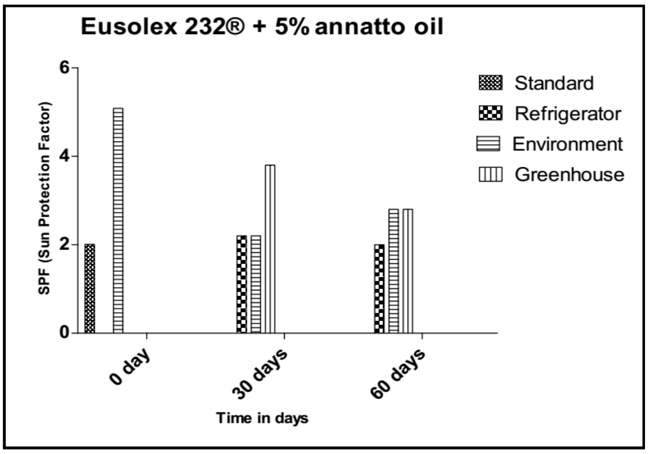
strong modifications and those containing oil, there was phase separation, while still maintaining only slightly modified formulation Eusolex 232® + Neo Heliopan MBC® (formulation III) that as all other become altered in the temperature range 65 ° C to 80 ° C. A specific nomenclature was used for macroscopic analysis tests, centrifugation and thermal stress it is: N = Normal, no change; MM = Mildly Modified; M = Modified (Masson *et al*., 2005; ANVISA, 2004; Pineda Sotomayor, 2015). The results are show in Table 7.

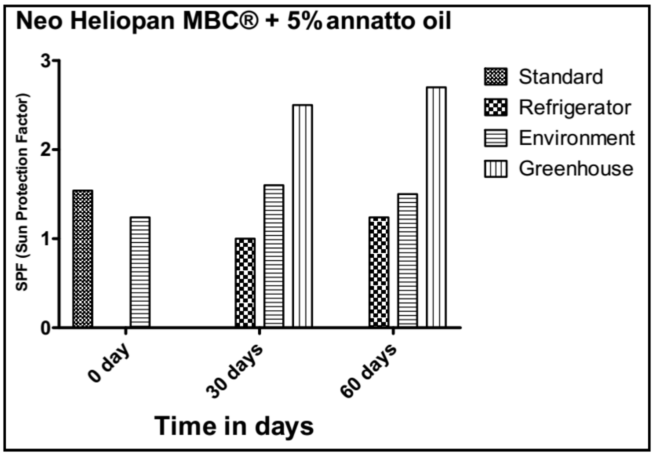
Heliopan MBC® + annatto oil 5% (formulation VI) formulations were analyzed for 2 months and statistical analysis proved that the refrigerator, stove and room temperature samples are not statistically different from the standard sample without oil annatto (Figures 4,5 and 6). The samples kept in the refrigerator and at room temperature remained close to the value of the standard sample. However, the samples kept in the oven remained distant from the standard value, showing that the temperature along with time are extrinsic factors that affect the value of the cosmetic

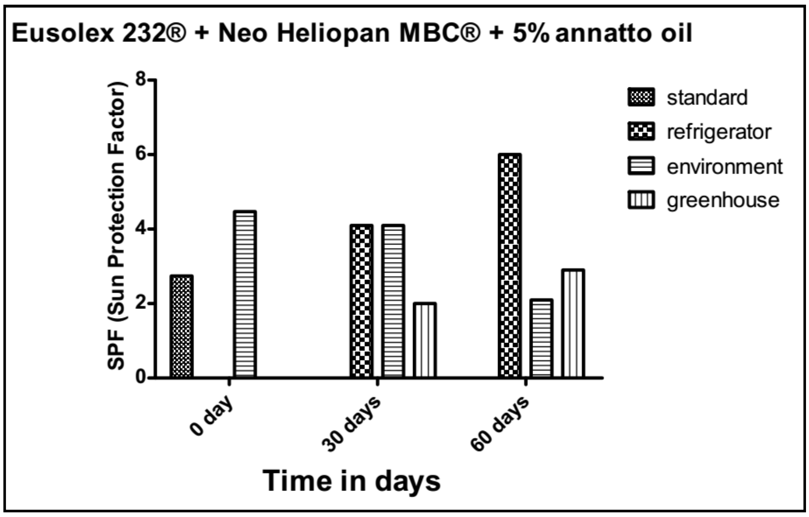
**Table 7. Thermal stress testing in cosmetic formulations**

| | Temperatures | | | |
|---|---|---|---|---|
| Formulations | 40°C - 50°C | 55°C | 60°C | 65°C – 80°C |
| I.Eusolex 232® | N | LM | M | M |
| II.Neo Heliopan MBC® | N | LM | M | M |
| III.Eusolex 232® + Neo Heliopan MBC® | N | N | LM | M |
| IV.Eusolex 232® + 5% annatto oil | N | LM | M | M |
| V.Neo Heliopan MBC® + 5% annatto oil | N | LM | M | M |
| VI.Eusolex 232® + Neo Heliopan MBC® + 5% annatto oil | N | LM | M | M |
| VII.Eusolex 232® + 10% annatto oil | N | LM | M | M |
| VIII.Neo Heliopan MBC® + 10% annatto oil | N | LM | M | M |
| IX.Eusolex 232® + Neo Heliopan MBC® + 10% annatto oil | N | LM | M | M |

*N- Normal, LM- Mildly Modified , M- Modified*




**Figures 4-6. Ratio analysis SPF / time respectively for the photoprotective formulations**

The least altered macroscopic formulations were Eusolex 232® + annatto oil 5% (formulation IV), Neo Heliopan MBC® + annatto oil 5% (formulation V) and Eusolex 232® + Neo Heliopan MBC® + annatto oil 5% (formulation VI) and with them the study was continued. The Eusolex 232® + annatto oil 5% (formulation IV), Neo Heliopan MBC® + annatto oil 5% (formulation V) and Eusolex 232® + Neo

formulations SPF. This fact can be understood when it is known that high or low temperatures favor in order to anticipate chemical and/or physical reactions and why certain changes are acceptable, what should not happen at room temperature (Diogo *et al*., 2015; Alamo *et al*., 2012; Pineda Sotomayor, 2015) See Figures 4, 5 and 6. *The time 0 is analysis before counting (see table 2). Standard sample not* *have oil and environment sample have oil of Bixa orellana L. In this case with the temporal evolution the increase of the temperature did not promote significant changes in the values of SPF. In this case with the temporal evolution the temperature did not promote the decrease of SPF. In this case with the temporal evolution the increase of the temperature promoted the reduction of SPF.*

**Conclusion**

The oil characteristics determined by the physico-chemical study, have a small difference from the results described in the literature, however the same cannot be regarded as bad. As for SPF analysis has verified that the oil annatto 5% and 10% when incorporated in photoprotective formulations with chemical filters Eusolex 232® (Sulfonic phenylbenzimidazole Acid) and Neo Heliopan MBC® (metilbenzidileno camphor) potentiates the effect of solar protection. As to accelerated stability testing (2 months) the statistical analysis demonstrated that the Eusolex232® + 5% annatto oil (formulation IV), Neo Heliopan MBC® + 5% annatto oil (formulation V) and Eusolex 232® Neo + Heliopan MBC® + 5% annatto oil (formulation VI) formulations kept in the refrigerator, stove and room temperature are not statistically different from the standard sample without oil annatto. Based on these results it is suggested that more research on new raw vegetable materials that can be incorporated in photoprotective formulations in order to increase the SPF of them and add value to the product.

**Author contributions**

L.H.A.C. conceived the project; D.C.S.C., R.C.D, M.C.A.B. performed the experiments of research; D.C.S.C., R.C.D., L.H.A.C., M.C.A.B., C.M.V. analyzed data and discussions; R.C.D. provided expert knowledge and revised; C.M.V. contributed with materials and equipaments; D.C.S.C. and R.C.D. wrote the paper.

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