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Reducing the impact of artificial blue light on the skin: A spectroscopic study

Silvia Martiniaková[1], Jarmila Hojerová[1], Zuzana Turányiová[1], Dana Dvoranová[2], Miriama Malček Šimunková[2]

[1]Institute of Food Science and Nutrition and
[2]Institute of Physical Chemistry and Chemical Physics, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, SK-812 37 Bratislava, Slovak Republic
silvia.martiniakova@stuba.sk, jarmila.hojerova@stuba.sk

Abstract: As people spend many hours looking at digital screens, the negative effects of artificial blue light are becoming more apparent. While most research has focused on its effects on eyes, less is known about the effects of blue light on the skin, where similar photoreceptors are located. Unlike the sunscreens against UVB and UVA radiation, there is no standard method for determining skin protection against blue light. The lipophilic complex Carotolino, a system combining carrot root extract, carrot seed oil, and β-carotene, was chosen as a model substance for this research. Spectrophotometric investigation demonstrated the ability of Carotolino to absorb radiation in the blue light region (400—500 nm). After a 60-minute LED@450 nm exposure, corresponding to the maximum wavelength of radiation from the displays of common smartphones, only small changes (1.4 %) in the optical spectra were observed. The spectra showed sufficient photostability of Carotolino and its stabilizing effect on the photolabile Ubiquinone. In the 415—455 nm wavelength range associated with oxidative stress, Carotolino (0.4 % wt.) reduced blue light by ~97.0 %. EPR spin trapping showed that blue light with a maximum wavelength of 450 nm causes significant formation of reactive free radicals, which can be partially eliminated by the application of Carotolino. The results confirmed the suitability of both methods to evaluate the effectiveness of substances to reduce physical impact of incident light on the skin. Further methods are needed to investigate biological protection of skin against blue light by promising substances.

Key words: blue light; carotenoids; EPR spin trapping; free radicals; light emitting diodes; skin care

Abbreviations: BL: blue light; DMPO: 5,5-dimethyl-1-pyrroline N-oxide; EPR: electron paramagnetic resonance; INCI: International Nomenclature of Cosmetic Ingredients; LED: light-emitting diode; ROS: reactive oxygen species; UV: ultraviolet; VIS: visible

Introduction

Since the creation of human life on Earth, humans have been exposed to varying levels of visible and invisible radiation from the sun. In general, blue light (BL) represents about 25 % of visible light (~380—780 nm), and its actual intensity depends on location, time of day, season, and various weather conditions and atmospheric pollution. Blue light is known as high-energy visible (HEV) light in the wavelength range of ~400—500 nm with maximum emission from 440 to 460 nm (Coats et al., 2021; Pourang et al., 2022). Over millions of years, specialized cells in the human eye have evolved to respond to natural BL with wavelengths between 470 and 490 nm, thus keeping track of time and regulating the body's biological functions. Adequate BL exposure is important for synchronizing the natural circadian rhythm, which can affect many processes including sleep quality, metabolism, immune function, and even mood. Sufficient BL, especially in the morning, induces wakefulness, while its deficiency in the evening triggers the production of the sleep hormone melatonin (Heiting, 2019; Françon, 2024).

Recently, due to modern life consequences, humans are not exposed to adequate levels of natural BL during the day but are overexposed to artificial BL outside daylight hours. Such light is emitted by the screens of digital devices such as smartphones, laptops, tablets, computers, and televisions illuminated by light emitting diodes (LEDs) as well as LED street, household, architectural, and security lighting. Unlike the continuous and nearly uniform visible sunlight, cold-white LEDs emit spectrally unbalanced light characterized by a high-energetic blue peak at 450 nm (~47.0 %), a low-energetic green-yellow peak, and a weak emission in the red region of the spectrum (Zhao et al., 2018; Heiting, 2019; Françon, 2024). Especially smartphones have become an integral part of our daily life. According to statistics, the average person spends 4 hours and 37 minutes on their smartphone every day (Howarth, 2024). Teran et al. (2018) evaluated the light emission spectrum of 36 smartphone displays from various producers using the integrating sphere method. When in the regular mode, the power spectra of the smartphones resembled a white LED source,

showing three characteristic peaks at about 450, 545, and 603 nm. For almost all of them, the blue peak (~450 nm) had twice as much power as the yellow (~545 nm) and red (~603 nm) ones. Ultraviolet (UV) and infrared regions were practically null compared to the three emission peaks in the visible range (Teran et al., 2018). Therefore, it is BL that is being investigated for its potentially harmful effects on humans.

Although the intensity of artificial BL is low compared to BL from the sun, its cumulative effect on the body represents serious health risk for people who are in front of LED devices for too long during the day and sometimes even at night, and the screen is too close to their eyes and skin. They are exposed to a relatively large intensity of blue wavelengths outside of normal daytime hours, so the evening and night-time production of the hormone melatonin in their bodies is suppressed. The consequence can be not only disruption of the circadian rhythm and sleep disorders, but also other problems, for example metabolic dysregulation (Zerbini et al., 2020; Haghani et al., 2024). In addition, particularly short-wave BL with wavelengths between 415 nm and 455 nm is closely related to oxidative and phototoxic damage to photoreceptors in human eyes, especially the retina (Marie et al., 2018; Zhao et al., 2018; Wang et al., 2024; Haghani et al., 2024).

While most of the research to date has focused on the effects of BL on eyes, less is known about its impact on skin, where similar photoreceptors are found. BL has been shown to cause a wide range of beneficial as well as harmful effects depending on the exposure dose, applied wavelength, and skin condition. Therapeutic effects of blue LED light with specific wavelengths are used in the treatment of dermatological diseases such as psoriasis, eczema, acne vulgaris, actinic keratosis, and skin malignancies, as well as in cosmetic treatments. Controlled short-term exposure to blue LED light is used in phototherapy in hyperbilirubinemic newborns (Sadowska et al., 2021; Olagbenro, 2022; Lebleu et al., 2023). LED emission doses from these sources are significantly lower than the minimal doses reported to induce skin damage within practical exposure times (Brown et al., 2024).

However, the results of *in vitro, in vivo,* and clinical studies show that repeated long-term exposure to BL causes direct and indirect harmful effects to the skin. Blue wavelengths, especially in the wavelength range of 415–455 nm, exert their direct oxidizing effect on the skin by interacting with chromophores (flavins, porphyrins, nitrosated proteins, and opsins) in which they trigger a change from ground state to activated state. Activation of these molecules leads to the release of reactive forms of nitrogen, i.e., nitric oxide (NO), and to the overproduction of reactive oxygen species (ROS), e.g., superoxide radical anion, while UVA light induces predominant formation of singlet oxygen (Sadowska et al., 2021; Suitthimeathegorn et al., 2022; Kumari et al., 2023). In an *in vitro study* on human dermal fibroblasts, Mann et al. (2020) found that the rate of ROS generation was highest between the wavelengths of 400–450 nm and then decreased continuously, with only minimal effects observed at wavelengths above 500 nm. Similarly, Mamalis et al. (2016) exposed dermal fibroblasts to various effects of blue LED light (415 nm; irradiance 5–80 $J \cdot cm^{-2}$) and demonstrated a dose-dependent increase in ROS. As BL penetrates the skin deeper than UV light (Suitthimeathegorn et al., 2022), the reactive free radicals can cause DNA damage of epidermal keratinocytes and dermal fibroblasts, reduction of normal cell proliferation and uneven melanogenesis leading to hyperpigmentation (Dong et al., 2019; Campiche et al., 2020; Pourang et al., 2022; Lebleu et al., 2023; Lago, 2024).

The skin, as the surface of the human body, is immediately exposed to radiation and chemical pollutants from external environment, including free radicals causing oxidative stress outside and inside the organism (Ácsová et al., 2019; Martiniaková et al., 2022). Therefore, consequences of free radicals from BL on the skin will be manifested not only by premature external photoaging (promotion of wrinkles), but also by accelerated internal aging of the organism with all the health consequences (Arjmandi et al., 2018). In addition, as excessive exposure to BL can disrupt the circadian rhythm, it can negatively affect skin renewal processes that take place during the night (Suitthimeathegorn et al., 2022).

From this point of view, measures should be taken to protect human skin and eyes during frequent and long-term exposure to blue LED light (Coats et al., 2021; Kumar et al., 2023). One of the possible ways to reduce the adverse light effects of blue LED light on the skin is the application of cosmetic products containing BL protective ingredients. However, unlike the evaluation of the effectiveness of sunscreen products against UVB and UVA radiation, there is currently no standardized method for determining skin protection against BL. Therefore, this research is oriented on verification of the spectrophotometric method for evaluating the ability of a model substance to reduce physical effect of BL in the 415–455 nm wavelength range, which is closely related to oxidative stress. The indirect technique of electron

paramagnetic resonance (EPR) spectroscopy, spin trapping, was applied to determine transient paramagnetic intermediates content formed upon BL exposure.

Materials and Methods

Chemicals and reagents

Based on our previous research (unpublished results), the lipophilic substance Carotolino (reddish-orange liquid) from Lipoid Kozmetik (Steinhausen, Switzerland) was selected for this research from several cosmetic substances currently presented as effective against the impact of blue light on the skin. This natural complex consists of *Canola Oil, Daucus Carota Sativa Seed Oil, Daucus Carota Sativa Root Extract, Helianthus Annuus Seed Oil, Tocopheryl Acetate, Beta-Carotene* (names according to the International Nomenclature of Cosmetic Ingredients, INCI). Ubiquinone (INCI: *Ubiquinone*), the oxidized form of coenzyme Q10 (orange powder), was obtained from Kaneka Corporation (Osaka, Japan) and was used as a model lipophilic excipient. Canola oil (INCI: *Canola Oil*) from Palma (Bratislava, Slovakia) was used as a lipophilic solvent and a reference sample. The 5,5-dimethyl-1-pyrroline N-oxide (DMPO) spin trapping agent was obtained from Sigma-Aldrich (Merck) and distilled prior to the application.

Spectrophotometric experiments

Electronic absorption spectra within the 400–550 nm wavelength range were recorded using a UV-1800 Shimadzu spectrophotometer (Kyoto, Japan) in a quartz cell (optical path length of 10 mm). Canola oil served as the reference. The reaction mixtures were irradiated by LED@450 nm in a Keva3 photochemical reactor (Brno, Czech Republic) (Figure 1) for defined time and the electronic absorption spectra were recorded. The wavelength was chosen considering the findings of Teran et al. (2018) regarding the power spectra emitted by common smartphone displays. To demonstrate the effect of Carotolino, a lipophilic substance that absorbs radiation in the blue light region but exhibits photolability was sought. The Ubiquinone substance (oxidized form of coenzyme Q10) in Canola oil (5 % wt.) was chosen. The investigated mixture (Carotolino (0.4 % wt.) in Canola oil or Carotolino (0.4 % wt.) plus Ubiquinone (5 % wt.) in Canola oil) were prepared and optical spectra before and after a 60-minute LED@450 nm exposure were recorded.

EPR spin trapping experiments

EPR experiments were carried out at room temperature using an EPR spectrometer EMX*Plus* (Bruker, Mannheim, Germany) operating at 100 kHz field modulation using a high sensitivity

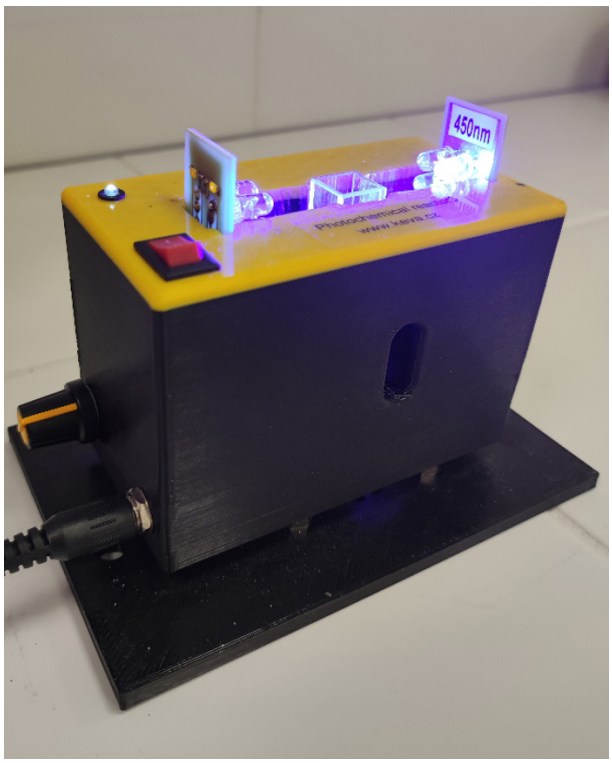

Fig. 1. Reaction system in Keva 3 photochemical reactor upon LED@450 nm exposure.

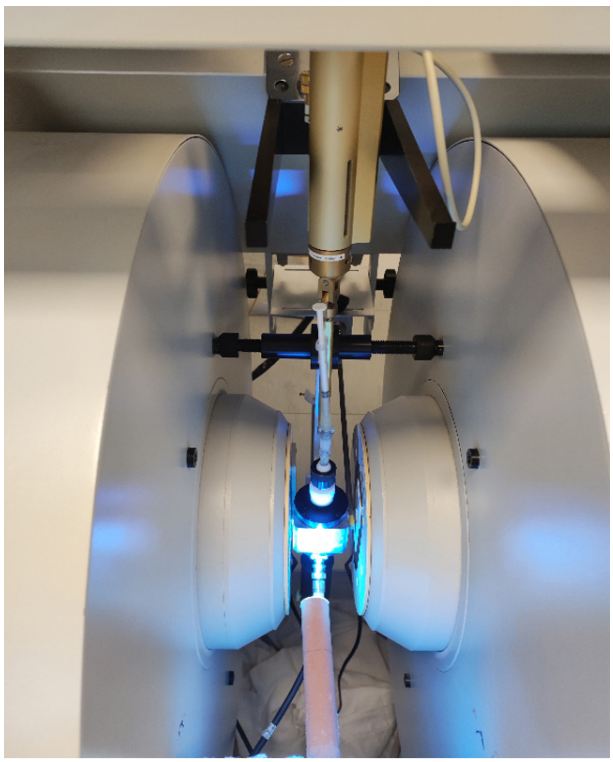

Fig. 2. Reaction system during blue light exposure in the cavity of EPR spectrometer.

probe-head in a small quartz flat cell (optical cell length of 0.45 mm, WG 808-Q, Wilmad-LabGlass, Vineland, USA). Cold-light source KL 1600 LED (T = 5600 K; Schott, Germany) was applied to provide the VIS-light irradiation directly in the EPR cavity, characterized by the illumination intensity of 160 klx measured by a digital lux meter (Metra Blansko, Czech Republic), and a blue filter was set to select blue light (λ_{max} = 450 nm). The 5,5-dimethyl-1-pyrriline N-oxide (DMPO) spin trapping agent was used to determine the content of transient paramagnetic species.

The reference system was prepared directly before the measurement by mixing 200 µL of Canola oil (reference sample) with 50 µL of DMPO (210 mM). The system was immediately transferred to a flat cell under air and inserted into the cavity of the EPR spectrometer (Figure 2). Acquisition of EPR spectra started 2 minutes after mixing the reaction system. The system was first analyzed before irradiation (10 minutes in the dark). Subsequently, it was irradiated with BL for 10 minutes, while EPR spectra were measured *in situ* during exposure (Figure 2). The reaction system prepared directly before the measurement consisted of 50 µL of a solution of Carotolino in Canola oil (1:1 vol.), 150 µL of Canola oil and 50 µL of DMPO. Thus, Carotolino (10 % wt.) solution in Canola oil was analyzed before and after the irradiation using the same procedure as for the reference sample. The experimental EPR spectra were analyzed using the WinEPR software (Bruker).

Results and Discussion

Figure 3 shows electronic absorption spectra of studied systems before and after LED@405 nm exposure. The absorption spectrum of Carotolino in Canola oil (red solid line) with absorption maxima in the blue region at 449 and 475 nm is characteristic for carotenoids with a π-bond conjugated system (Domenici at al., 2014). The absorbance value of Carotolino (0.4 % wt.) in Canola oil was $A_{450\ nm/0\ h}$ = 1.89 (Figure 3, solid red line) before exposure, and after a 60-minute irradiation, minor changes in optical spectra were observed with only negligible decrease (Figure 3, dashed red line), i.e., decrease of only 2.1 % was measured. The integrated area under the absorption curve over the entire BL wavelength range (400—500 nm) before and after irradiation was calculated. Decrease in the absorption capacity of the Carotolino solution was only 1.4 %, which means that Carotolino in Canola oil has sufficient photostability.

Due to known photodegradation of both forms of coenzyme Q10 (Ubiquinol and Ubiquinone) under visible light (Hojerová, 2012) and the absorption of radiation in the BL region, Ubiquinone was chosen as a model photolabile substance, as documented in Figure 3 (green lines). The absorbance of Ubiquinone (5 % wt.) in Canola oil dropped from $A_{450\ nm/0\ h}$ = 0.523 before irradiation (Figure 3, solid green line) to $A_{450\ nm/1\ h}$ = 0.201 after 60 minutes of irradiation (Figure 3, dashed green line), which represents a decrease of up to 61.7 %. The integrated area under the absorption curve in the 400—500 nm wavelength range before and after irradiation decreased by 57.0 %. The Ubiquinone solution can therefore be considered photounstable in the BL region and suitable for verifying the possible stabilizing effect of the Carotolino substance in this work.

After the addition of Carotolino (0.4 % wt.) to the solution of Ubiquinone (5 % wt.) in Canola oil, the absorbance values increased since the components absorb in the same region, but the shape of absorption curves before and after irradiation (Figure 3, solid and dashed black line) changed only slightly. In the Japanese patent JP 5344768B2 (JP, 2010), β-carotene in combination with ascorbic acid is used as a photodegradation inhibitor of coenzyme Q10. We assume that the substance Carotolino, composed of predominantly carotenoids, especially β-carotene, forms an active complex with Ubiquinone, which is resistant to blue light. However, these considerations require confirmation by analysis of the composition of the resulting complex. It can be concluded that Carotolino demonstrated not only stable absorption of BL before and after irradiation under the experimental conditions, but also photostabilizing effect on the photolabile substance Ubiquinone.

Furthermore, for a more comprehensible expression of the degree of BL protection by the evaluated substances, the BASF method (BASF, 2024) was applied. This method is currently used by BASF to evaluate the effectiveness of cosmetic UV filters against BL and is expressed as a percentage reduction in transmittance within the 400—450 nm wavelength range. However, the transmittance values in our work were calculated from the absorbance values in the wavelength range of 415—455 nm, which was reported to induce a considerable amount of reactive free radicals and oxidative stress (Marie et al., 2018; Zhao, 2018; Wang et al., 2023; Heiting, 2019; Pourang et al., 2022). The transmission dependencies are shown in Figure 4. The larger the area above the transmission curve, the more BL the substance blocks, i.e. the less BL is transmitted. Reciprocally, the larger the area under the transmission curve, the more BL is transmitted and the less is blocked. Finally, the percentage of BL reduction in the 415—455 nm wavelength range

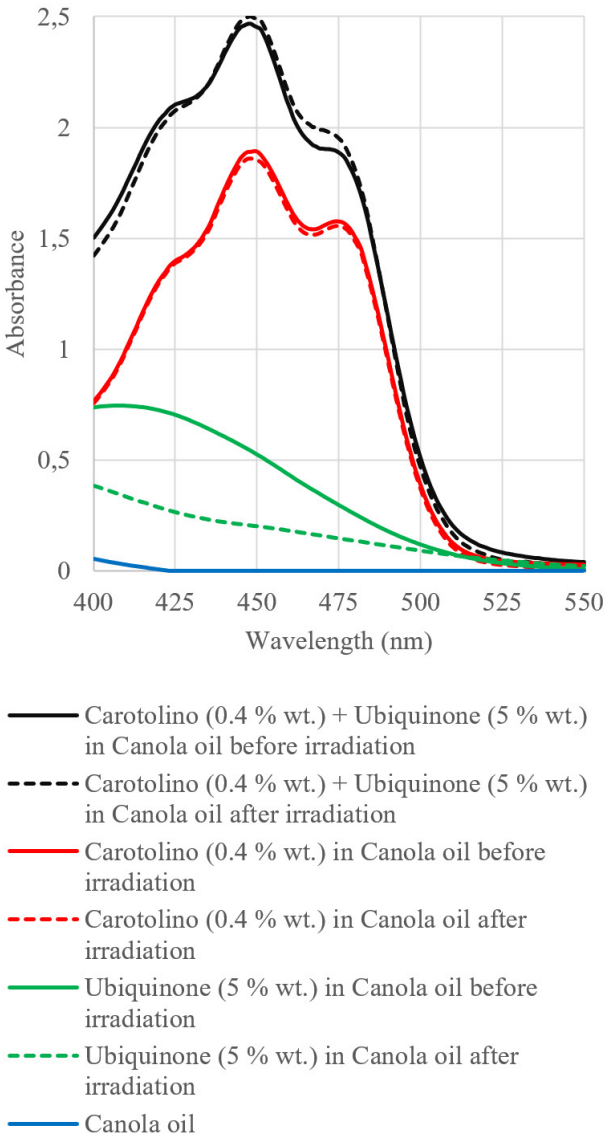

Fig. 3. Absorption spectra of studied substances in the region of 400–550 nm before and after a 60-minute exposure (LED@450 nm) in Keva 3 photochemical reactor.

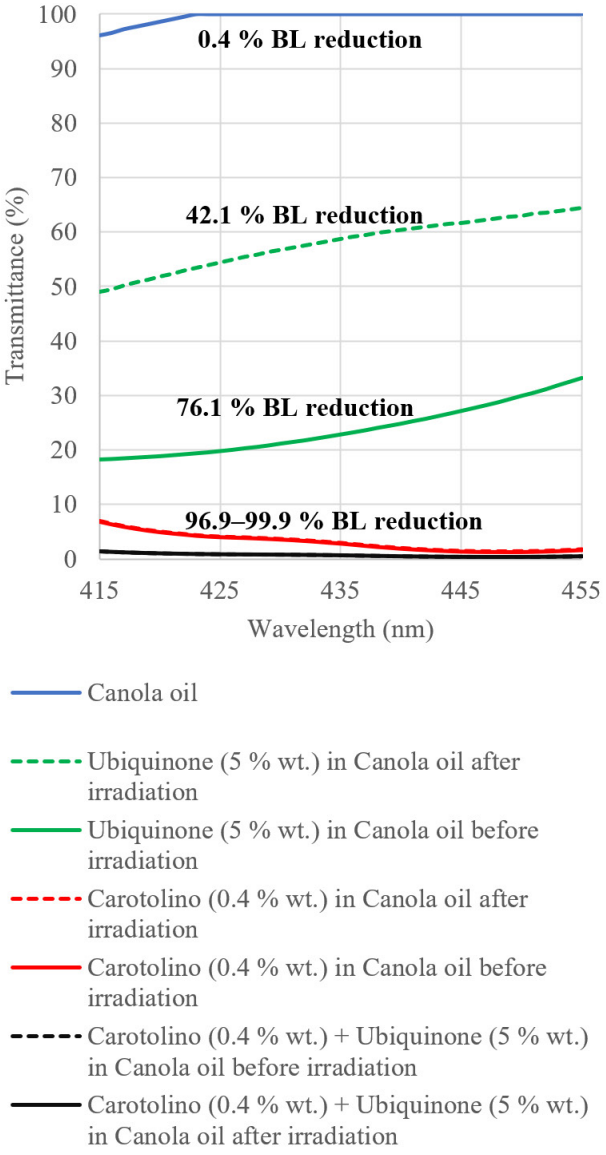

Fig. 4. Calculated transmission spectra of studied substances in the region of 415–455 nm before and after a 60-minute exposure (LED@450 nm) in Keva 3 photochemical reactor.

Tab. 1. Blue light reduction by the evaluated substances in the 415–455 nm wavelength range.

Substance	**Blue light reduction (%) range 415–455 nm**
Canola oil before irradiation	0.4
Ubiquinone (5 % wt.) in Canola oil before irradiation	76.1
Ubiquinone (5 % wt.) in Canola oil after irradiation	42.1
Carotolino (0.4 % wt.) in Canola oil before irradiation	97.0
Carotolino (0.4 % wt.) in Canola oil after irradiation	96.9
Carotolino (0.4 % wt.) + Ubiquinone (5 % wt.) in Canola oil before irradiation	98.6
Carotolino (0.4 % wt.) + Ubiquinone (5 % wt.) in Canola oil after irradiation	99.3

was calculated from the integrated area above the transmission curve using equation (1):

$$\text{Blue light reduction (\%)} = 100 \times \sum_{415\text{ nm}}^{455\text{ nm}} (1 - T) / 41 \quad (1),$$

where 41 is the number of wavelength steps in the 415—455 nm range with a step of 1 nm and T means transmittance.

As shown in Table 1, Carotolino (0.4 % wt.) in Canola oil reduces the potential exposure of skin to BL in the wavelength range of 415—455 nm (associated with oxidative stress) up to 97.0 %. Moreover, after a LED@450 nm exposure for 60 minutes, the Carotolino solution is still able to block up to 96.9 % of blue light at 415—455 nm.

The formation of reactive free radicals is one of the most harmful side effects of exposure to blue light. Free radicals generated by light exposure often possess a short lifetime and therefore cannot be detected by cw-EPR spectrometers, which requires the use of indirect techniques, such spin trapping, which utilize diamagnetic compounds to trap free radicals while forming a more stable spin-adduct (Dvoranová et al., 2014; Jomova et al., 2022). The amount of spin-adducts reflects the amount of trapped free radicals, which can be obtained by quantitative analysis of the EPR spectra. The determination of overall radical content in the studied system was performed in two parallels and the average of the double integrals values of EPR signal intensities was calculated. Double integral of the EPR signal intensity in non-irradiated Canola oil showed only insignificant amount of spin-adducts present in the reference system (Figure 5), thus indicating negligible amount of reactive free radicals. However, following a 10-minutes blue LED light exposure (λ_{max} = 450 nm) of Canola oil, the total amount of generated reactive free radicals, detected as spin-adducts, notably increased; expressed by the double integral of the EPR signal intensity of 8.03 × 10^9 (Figure 5, blue column). This observation indicates that the active substance Carotolino is able to eliminate some of the reactive free radicals generated upon blue LED light exposure due to its radical scavenging components, such as carotenoids. Another possible explanation is that the lower amount of spin-adducts in sample with Carotolino is due to its ability to absorb BL, which leads to less energy being absorbed by the components of Canola oil which are prone to form radicals.

Conclusions

As was mentioned, blue light occurs naturally as part of sunlight. Like all living things on Earth, humans have evolved to respond to the daily cycle of light and dark. In recent decades, because of modern lifestyle, people are not exposed to adequate levels of natural blue light during the day but are overexposed to relatively high levels of blue LED light at night. There is growing concern that the increased exposure to artificial sources of blue light from lighting and digital screens influences our health.

Although the full extent and exact nature of the biological effects of blue light on the skin are not yet fully understood, certain beneficial as well as harmful effects have already been confirmed. As the skin's

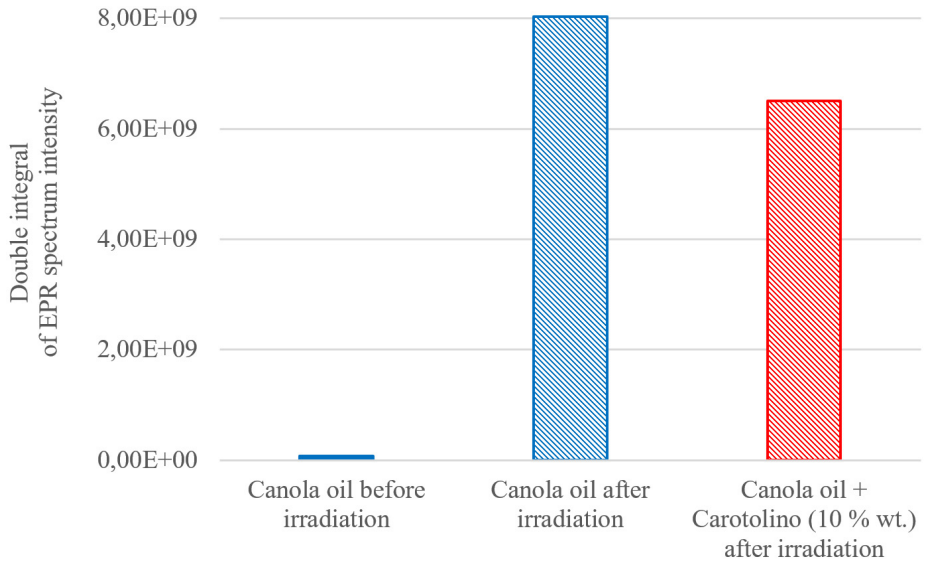

Fig. 5. Double integrated EPR spectra of studied systems before and after 10-minute blue LED light (λ_{max} = 450 nm) exposure in the cavity of EPR spectrometer.

exposure to blue light from artificial sources gradually increases, research into its protection is needed. When considering cosmetic interventions to protect the skin from blue light exposure, a combination of daytime protection and nighttime active repair strategy may be optimal. Ideal daily protection regimen includes reduced exposure to blue light by all available means and prevention or minimization of the formation of free radicals with skin care containing effective anti-blue light ingredients.

One such substance appears to be Carotolino, a stabilized complex of carotenoids and other components in Canola oil. In this research, the ability of Carotolino to *i)* absorb light in the wavelength range of 400—450 nm corresponding to blue light, *ii)* reduce the transmission of light at wavelengths from 415 to 455 nm, which generate reactive free radicals, and *iii)* maintain photostability in the entire evaluated range of wavelengths under irradiation conditions, was proven by absorption spectrophotometry.

Furthermore, an EPR spin trapping experiment showed that blue light with a maximum wavelength of 450 nm, which corresponds to the maximum wavelength of radiation from the displays of common smartphones, causes considerable formation of reactive free radicals which can be partially eliminated by applying the model cosmetic ingredient Carotolino.

Considering the results obtained, both methods seem to be suitable for the evaluation of the physical effectiveness of substances promising skin protection against blue light. It is highly possible that by appropriate *in vitro* or *in vivo* biological assays, for example by dermal fibroblast or epidermal keratinocyte cells viability evaluation, Carotolino substance will also demonstrate direct biological effectiveness in reducing skin damage caused by blue light. In our opinion, the future of topical products providing skin protection against free radical damage from digital screens is a combination of photostable blue light-reducing substances and photostable UVA filters effective even above the wavelength of 360 nm.

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