

CAROTENOID EXTRACT AND OIL FROM PUMPKIN (*CUCURBITTA SPP.*) BY-PRODUCTS FOR FACIAL CREAMS WITH HIGH ANTIOXIDANT ACTIVITY

*Daiga Konrade 

Riga Technical University, Latvia

*Corresponding author's e-mail: daiga.konrade@rtu.lv

Abstract

Nowadays, different sun protective creams are available in the market, but most of them contain harmful synthetic chemicals and minerals which can induce skin allergies and premature ageing. Usage of sunscreens and photoprotectors of natural origin and natural antioxidants can reduce skin damage caused by excessive sun exposure. The effectiveness of the use of β -carotene – vitamin's A precursor against excessive irradiation caused by oxidative stress cell damages has been proven of its very good antioxidative properties and leading to sooner regeneration of the skin after several environmental damages. Carotenoids and high value oil can be extracted from pumpkin (*Cucurbitta* spp.) by-products – peel and seeds with SC CO₂. The objective of this study was to incorporate pumpkin seed oil (PSO) and carotenoid extract (CE) from pumpkin by-products in to facial creams for UV protection. Two types of creams with PSO and CE were prepared emulsion o/w and w/o. A cream without extracts and natural ingredients was used as control sample. The sun protection factor values *in vitro* (SPF = 0.92 to 1.18), total content of carotenoids (TCC = 1.08–17.95 $\mu\text{g mL}^{-1}$), β -carotene content (0.64–0.72 $\mu\text{g mL}^{-1}$), total phenolics content (TPC = 62.64–95.82 mg GAE g⁻¹), antiradical scavenging activity (11.26–43.66 %) and rheological behaviour of facial creams were determined. Although creams with CE and PSO show comparatively low SPF values, β -carotene and phenolic compounds found in samples demonstrated very high antioxidant abilities valuable for skin protection.

Key words: antioxidants, SC CO₂, β -carotene, by-products, pumpkin, sun protection factor.

Introduction

Skin damage is induced in various ways – through environment, consumption of unhealthy food, drugs, alcohol and nutrient deficiency, exposure of solar radiation (Rasul *et al.*, 2011). The effects of excessive irradiation include the development of wrinkles, loss of skin firmness, phototoxic and photoallergic reactions, increased risks of developing various forms of skin cancer (Arct & Mieloch, 2020). Therefore, it is crucial to prevent photo ageing and protect human skin from sunburns and to use moisturizers in form of creams, tonics or gels (Rasul *et al.*, 2011). Additional value to cream formulations can be given by including active ingredients with specific cosmetic or dermatological effects, essential oils, antioxidants in form of carotenoids (Rodoplu *et al.*, 2021). For example, plant extracts, vegetable oils and essential oils are rich sources of provitamins, pigments, pyrazine (Sabir *et al.*, 2005), squalene (Idouraine, Kohlhepp, & Weber, 1996), saponins, phytosterols, triterpenoids (Černiauskiene *et al.*, 2014), phenolic compounds, unsaturated fatty acids (Sabir *et al.*, 2005), flavonoids and proteins (Akhtar, Iqbal, & Nawazish, 1980). Lutein, α -carotene and β -carotene, violaxanthin, neoxanthin, zeaxanthin are the main carotenoids in vegetables, and they are prone to isomerization and/ or oxidation due to their unsaturation (Murković *et al.*, 2004).

Carotenoids have been extensively studied because of their beneficial effect on human health. Carotenoids, such as β -carotene and lutein are transformed by the body into the important antioxidant - vitamin A, violaxanthin, neoxanthin, xanthophylls (Soengas *et al.*, 2011). Vitamin E, including both γ -tocopherol and α -tocopherol, has important antioxidant proper-

ties and health benefits (Henriques, Guiné, & Barroca, 2012; Rawson *et al.*, 2013; Secil & Berrin, 2011; Seo *et al.*, 2005). Carotenoids reduce the risk of different diseases (Berna *et al.*, 2011), act as singlet oxygen quenchers (Kuncewicz, 2008; Hughes, 2000) and function as chain-breaking antioxidants, protecting cells from free radical attack (Munarin, Tanzi, & Petrini, 2012). In epidermis, β -carotene is transformed into retinol and its esters, it is very well tolerated by the skin; therefore, these properties qualify this compound as a particularly valuable active ingredient in protective and anti-ageing cosmetics (Arct & Mieloch, 2020). Systematic sun screening agents used are β -carotene, α -tocopherols, retinol, green tea polyphenols (Latha *et al.*, 2013).

β -carotene is a structural analogue of vitamin A (Patil *et al.*, 2009) and is found in various fruits and vegetables mainly in dark orange and red coloured plants (Hughes, 2000). Pumpkins (*Cucurbitta* spp. L) are rich sources of carotenoids (Sook *et al.*, 2005), Total content of carotenoids (TCC) in the peel and pomace was 68.07 mg 100g⁻¹ to 91.28 mg 100g⁻¹ (Konrade *et al.*, 2018). Pumpkin seed oil is rich in unsaturated fatty acids (Seo *et al.*, 2005), such as oleic acid, linolic acid, palmitic and stearic acid. Under the influence of sunlight, extraction temperature and oxidation, the pigments can be destroyed and have less protection efficiency (Soengas *et al.*, 2011).

Carotenoids and high value oil can be extracted from pumpkin (*Cucurbitta* spp.) by-products – peel and seeds with SC CO₂.

The objective of this study was to incorporate pumpkin seed oil (PSO) and carotenoid extract (CE) from pumpkin by-products in to facial creams for UV protection and determination *in vitro* sun protective

factor values, total content of carotenoids, β -carotene, antioxidant scavenging activity, the total phenolic compounds content and rheological behaviour of facial cream samples.

Materials and Methods

Pumpkin (*Cucurbita pepo* L, Pink Banana Jumbo) by-products – peel and pomace were obtained from Lat Eko Food Ltd., 2021. For formulation of facial creams (Table 1) were used the following: pumpkin seed oil (PSO) and carotenoid extracts (CE) from

peel extracted with supercritical CO₂ (Konrade & Spalvins, 2022); bee wax; cacao seed butter (La Saponaria, L221879); shea butter (La Saponaria, 221451 06-24); calendula oil (Farfalla, NO1F000); cetyl, glycerol, Tween80 and Span80 (Sigma Aldrich, Germany).

Carotenoid extract is water-insoluble and thus was used as an oil phase in the test formulations (Akhtar, Ahmed, & Mahmood, 2008). Cetyl and water were used as aqueous phase in formulations (Korhonen *et al.*, 2001; Mbanga *et al.*, 2014).

Table 1

Formulation of facial creams

Ingredients	C1.1*	C1.2*	C2.1**	C2.2**	Control**
Span80, g	-	-	-	-	6
Tween80, g	-	-	-	-	6
Bee wax, g	6	6	6	6	6
Cetyl, g	-	-	2	2	2
Glycerol, g	-	-	3	3	3
Pumpkin seed oil, mL	-	1.5	-	1.5	-
Carotenoid extract, mL	-	2	-	2	-
Cacao seed butter, g	6	6	6	6	-
Shea Butter, g	6	6	6	6	-
Calendula oil, g	6	2.5	6	2.5	6
Water, mL	-	-	18	18	18

creams – *oil/ water; ** – water/oil

Determination of β -carotene and total content of carotenoids (TCC)

To determine β -carotene content and total content of carotenoids (TCC) 1 \pm 0.01g of each sample was separately homogenized with 10 mL of solvent for 2 minutes (acetone and hexane (2:3) were used for β -carotene determination, and cyclohexane was used to determine TCC). The samples were sonicated for 3 minutes (Ultrasound processor Hielscher Ultrasound Technology, Ultrasonic Processor UP200S), vortexed and absorption at different wavelength was read: for β -carotene absorption was read at $\lambda = 453; 505; 663$ and 645 nm; and for TCC at $\lambda = 456$ nm. The absorption coefficients ($A_{1\text{cm}}^{1\%}$) of common food carotenoids were used for calculations. (Braniša *et al.*, 2014; Konrade & Spalvins, 2022).

Calculation for β -carotene ($\mu\text{g mL}^{-1}$) (Equation 1).

$$\beta\text{-carotene} = C (0.216 A_{663} - 1.22 A_{645} - 0.304 A_{505} + 0.452 A_{453}), \text{ where:} \quad (1)$$

C = concentration of sample, mg mL^{-1} , A = absorbance, nm (Braniša *et al.*, 2014).

TCC was calculated from Equation 2.

$$\text{TCC} = \frac{A}{2505C}, \mu\text{g mL}^{-1} \quad (2)$$

TCC – total content of carotenoids;

2505 – coefficient of extinction ($E^{1\%}$);

A – absorbance at 456 nm;

C – concentration of sample solution, mg mL^{-1} .

Determination of total phenolic compounds (TPC)

Total phenolic compounds (TPC mg GAE g^{-1}) were determined according to the Folin-Ciocalteu Colorimetric Method with minor modifications (Kampuss *et al.*, 2009; Priečina & Karklina, 2014).

20 \pm 0.1 mg of the sample was extracted with acetone and ethanol (1:1) in a 100 mL volumetric flask in a US bath for 10 min. 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with water) was added to 0.5 mL of the extracted sample in test tubes and after 3 minutes 2.0 ml of 10% saturated sodium carbonate solution was added. The resulting solution was mixed and allowed to stand for 30 min at 20 ± 1 °C in the dark, afterwards, the absorbance was read at 765 nm.

Gallic acid (0–100 mg L⁻¹) was used to calibrate the standard curve.

The results were expressed as milligram gallic acid equivalent per 100 g of sample (mg GAE 100 g⁻¹).

Quantification of TPC was based on a standard curve (Equation 3).

$$y = 0.0875x + 0.0676, R^2 = 0.998, \quad (3)$$

y – total content of phenolics, TPC, mg GAE 100g⁻¹,

x – Absorption at λ = 765nm, R² – coefficient of determination.

Antiradical scavenging activity determination

Antiradical scavenging activity was determined with the method based on scavenging activities of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. 4 ± 0.01 mg DPPH reagent was dissolved in 100 mL MeOH (solution concentration – 40 μL mL⁻¹) (Kruma *et al.*, 2016; Tirzitis & Bartosz, 2010). 200 μL DPPH reagent was mixed with 200 μL of sample extract in a test tube, incubated at room temperature for 30 min, and, after incubation, the absorbance was measured at λ = 514 nm. The antiradical scavenging activity (A, %) of selected material was expressed (Equation 4).

$$A = \frac{(Abs1 - AbsS)}{Abs1} * 100, \% \quad (4)$$

A – antiradical scavenging activity, Abs1 – absorbance of blank, AbsS – absorbance of sample extract.

2,2-diphenyl-1-picrylhydrazyl reagent (Sigma-Aldrich (Germany), CAS Number 1898-66-4), Gallic acid monohydrate (3,4,5-trihydroxybenzoic acid monohydrate), Folin-Ciocalteu's phenol reagent (Sigma-Aldrich, Cat. No. F9252), sodium carbonate, (Merck, Cat No. 106392) were used for determination of TPC and antiradical scavenging activity.

Sun protection factor values determination in vitro

In *vitro* sun protection factor (SPF) values determination was performed with method described by Mbanga (2014) with minor modifications: 0.1±0.01g of sample was diluted with ethanol (96% concentration) to 100 mL in volumetric flask, sonicated for 5 min, filtered and the absorption spectra of samples in solution were measured every 5 nm in the range of 290 to 320 nm (Mbanga *et al.*, 2014).

Triplicate determinations were performed at each point using ethanol as a blank. SPF values were determined using the Mansur equation (Equation 5).

$$SPF = CF * \sum_{290}^{320} (EE(\lambda) * I(\lambda) * Abs(\lambda)), \quad (5)$$

where EE – erythemal effect spectrum; I – solar intensity spectrum; Abs – absorbance of sunscreen product (λ); CF – correction factor (CF = 10). EE * I are constants (Sayre *et al.*, 1979) (Table 2).

Table 2

Calculation of SPF (Mbanga *et al.*, 2014)

Wavelength (nm)	EE * I (normalized) (Sayre et al., 1979)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0837
320	0.0180

Antioxidant activity, sun protective factor values (SPF), β-carotene and total content of carotenoids (TCC) in *vitro* of cream samples were measured with UV/ Visible Spectrophotometer, M501 Single Beam (Campsec UV, United Kingdom), equipped with 1 cm quartz cell.

Rheological measurements of facial creams

Rheological properties of facial creams were determined with Modular Compact Rheometer Anton Paar, Smart Pave 102, Germany, the amplitude sweep mode, with constant oscillation, and angular frequency of 10 rad s⁻¹. In this mode, the shear deformation is changed. G' – the storage modulus, G'' – the loss modulus, and loss factor, tan(δ) = G''/ G' were deter-

mined with shear rate from 0.1 to 100 s⁻¹ at 25 °C (Dabbaghi *et al.*, 2021; Zhang *et al.*, 2016).

Data Analysis

All measurements were performed on three independent samples (n = 3) and results were expressed as mean values ± standard deviation (SD). A mathematical analysis of the data has been performed using MS Excel Data Analysis, ANOVA, a Single-factor, correlation and regression analysis were used. The proposed hypotheses had been tested with the p-value method and the factors had been evaluated as significant if p < α = 0.05. Analysis of variance used the Tukey and Friedman test to justify differences in results between the studied samples.

Results and Discussion

Antioxidants have important protective factor effects on skin (Mbanga *et al.*, 2014; Rasul *et al.*, 2011; Rawson *et al.*, 2013). Their main role is to scavenge free radicals such as peroxides that contribute to tyrosinase activation and melanin formation; therefore, facial creams were tested for total phenolic compounds (TPC) and antiradical scavenging activity. β -carotene content and its antioxidant activity in biological systems is one of the most valuable active ingredients used in cosmetics as β -carotene reveals potent antiradical activity properties. Furthermore, it is one of the few agents that effectively neutralizes the singlet form of oxygen (Arct & Mieloch, 2020). β -carotene content in the skin is low, and exposition to sunlight reduces the content of this compound in the skin. In our previous studies of carotenoid and chlorophylls content in extracts from pumpkin peel TCC reached $32.43 \pm 2.09 \mu\text{g mL}^{-1}$, β -carotene content was $5.37 \pm 1.12 \mu\text{g mL}^{-1}$. Moreover, these extracts contained lutein, zeaxanthin, Chl a, Chl b (Konrade & Spalvins, 2022). Though carotenoids are very sensitive to temperature changes and light exposure, incorporated into facial creams there was found higher sun protective factor values (SPF) for facial creams with carotenoid extract (CE) and pumpkin seed oil (PSO) than in Control from Tween80 and Span80. SPF values of prepared cream samples and control is attached in Figure 1.

Ultraviolet radiation (UVA (320 – 400 nm), UVB (290 – 320 nm) and UVC (200 – 290 nm) is the primary environmental factor that seriously affects human skin and β -carotene reveals strong antioxidative properties that enable it to effectively neutralize two most reactive oxygen species: molecular singlet oxygen and superoxide radicals. Moreover, β -carotene is nearly 50 times more effective than α -tocopherol (Arct & Mieloch, 2020).

There were significant differences of SPF values in all samples of creams ($p = 0.006 < 0.05$).

Highest SPF was determined for creams C1.2. with carotene extract and pumpkin seed oil, C2.2.

showed lower SPF, while Control was the lowest SPF (Figure 1).

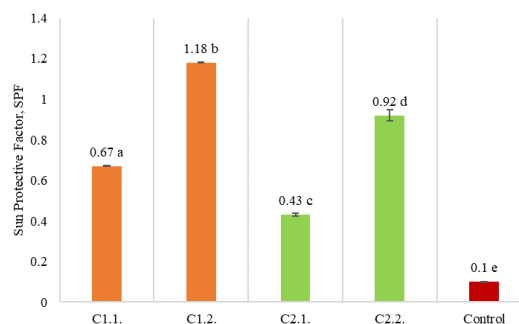


Figure 1. Sun protective factor values (SPF) for facial creams and Control sample. Different letters (a, b, c, d, e) indicate significant difference among samples ($p < 0.05$). The data is presented as a mean ($n = 3$).

Pumpkin seed oil (PSO) is valuable source of phenolic compounds as tyrosol, vanillic acid, vanillin, luteolin and sinapic acid having high antioxidant activities (Mala & Kurian, 2016). PSO showed antiradical scavenging activity (DPPH assay) 20.2% and high content of TCC ($47.67 \pm 2.09 \mu\text{g mL}^{-1}$), especially β -carotene and lutein (Konrade & Spalvins, 2022).

TCC, β -carotene, TPC content and antiradical scavenging activity of creams are summarized in Table 3.

There were significant differences in TCC and β -carotene content ($p < 0.05$) among samples; furthermore, β -carotene was not detected in Control and in samples C1.1., C2.1., though, showing little amount of TCC because of chemical composition of calendula oil (Miguel *et al.*, 2004) and bee wax. Phenolic compounds (TPC) were not detected in Control sample (Table 1) and samples without carotenoid extract and pumpkin seed oil (C1.1. and C2.1.), while in samples with carotenoid extract and pumpkin seed oil TPC were $95.82 \pm 1.18 \text{ mg GAE g}^{-1}$ for sample C1.2. (o/w) and $62.64 \pm 2.14 \text{ mg GAE g}^{-1}$ for C2.2. (w/o). According to researchers, Folin Ciocalteu reagent can react non-specifically with phenolic compounds;

Table 3

Total Content of Carotenoids, β -carotene, Total Phenolics Content and Antiradical Scavenging Activity of Facial Creams

	C1.1.	C1.2.	C2.1.	C2.2.	Control
TCC, $\mu\text{g mL}^{-1}$	3.04 ± 0.03^a	17.95 ± 1.14^c	1.08 ± 0.03^b	12.22 ± 0.03^d	n.d.
β -carotene, $\mu\text{g mL}^{-1}$	n.d.	0.72 ± 0.11	n.d.	0.64 ± 0.03	n.d.
TPC, mg GAE g^{-1}	n.d.	95.82 ± 1.18^a	n.d.	62.64 ± 2.14^b	n.d.
Antiradical scavenging activity, %	15.49 ± 1.06^a	43.66 ± 2.76^c	11.26 ± 0.82^b	36.62 ± 2.99^d	n.d.

Different letters in rows (a, b, c, d) indicate significant difference among samples ($p < 0.05$). Each sample was prepared and subsequently analysed in triplicate ($n = 3$), \pm standard deviation (SD), n.d. – not detected.

it can also be reduced by a number of nonphenolic compounds, e.g., vitamin C, Cu (II), etc. (Tomson, Kruma, & Galoburda, 2012).

Strong correlation between TPC and DPPH ($R^2 = 0.987$), TPC and SPF ($R^2 = 0.952$), DPPH and SPF ($R^2 = 0.985$) was found.

The rheological parameters can be influenced by several factors such as the chemical composition and temperature (Faustino & Pinheiro, 2021). Cosmetic products require different rheological behaviour for different testing: texture, spreadability, foamability, washability, texture (Yao & Patel, 2001); therefore, rheology can be applied to help in formulation on consistency; prediction of flow behaviour under manufacturing or production environment conditions. Furthermore, rheological analysis is more suitable for the storage stability as sensory (Korhonen *et al.*, 2001). Rheological properties of UV protecting facial creams and Control sample are attached in Figure 2.

Figure 2 a) shows plots of complex viscosity (CV), Figure 2 b) shows results of loss factor for samples – the behaviour of emulsions under similar conditions was different, sample w/o without carotene extracts was of the highest loss factor, when $\tan \delta > 1$ ($G'' > G'$). Therefore, viscous properties and a liquid-like or fluid state prevail (Simões *et al.*, 2020). There were no differences in complex viscosity and storage modulus or elasticity of creams. Behaviour was similar for all samples as all samples showed similar results. Figure 2 c) shows plots of shear stress – sample 2.1. without extracts (w/o) showed highest shear stress. Figure 2 d) gives information about storage modulus – elasticity of samples, samples without CE and PSO remained more elastic, though more studies and experiments are necessary for evaluation of rheological properties to obtain samples for good viscoelastic and sensory attributes.

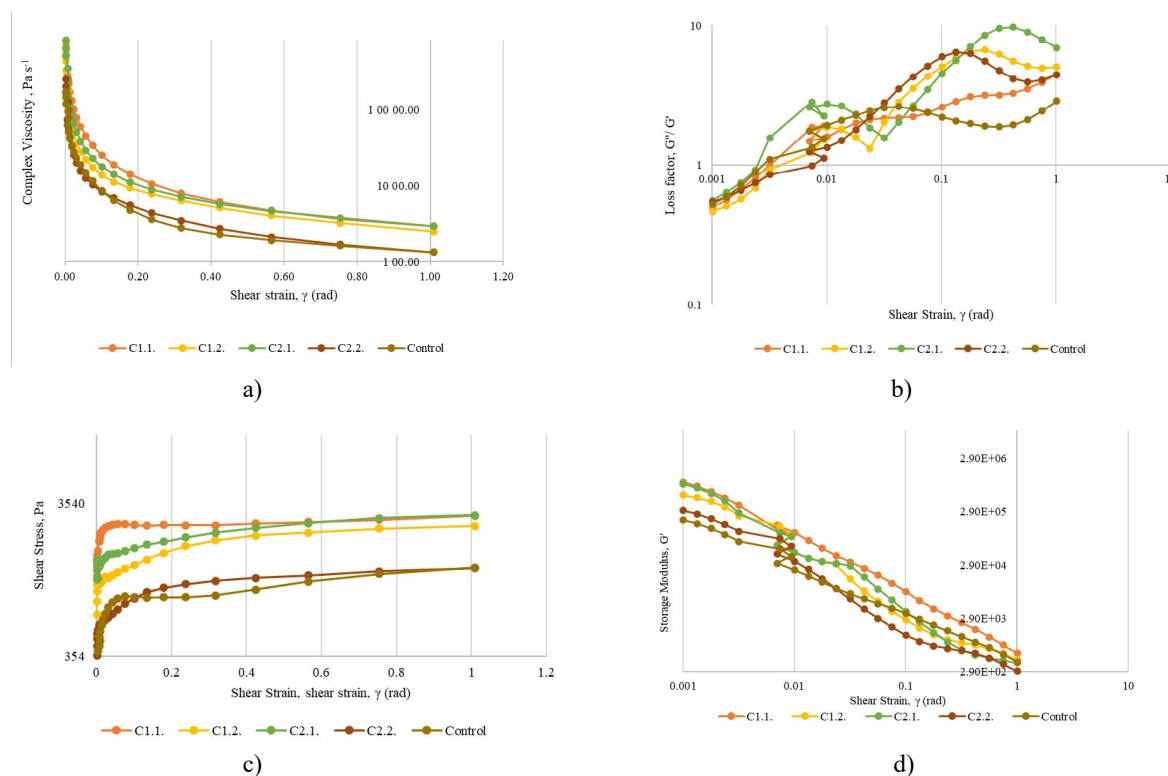


Figure 2. Rheological properties of creams: a) – Complex Viscosity; Pa s^{-1} , b) Loss Factor $\tan \delta = G''/G'$; c) Shear Stress, Pa; d) Storage Modulus G' .

Conclusions

Facial creams with added carotenoid extracts and pumpkin seed oil showed high antioxidative properties due to their total phenolic content. Sun protective factor value was high in comparison to those without carotenoid extracts and pumpkin seed oil addition. Effectiveness would be considerable when such creams are used for skin protection as the content of total carotenoids and especially β -carotene was high. In terms of rheological properties, there was

no significant difference between the control sample containing Tween80 and Span80 and between the samples containing natural origin raw materials with carotenoid extracts and pumpkin seed oil.

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