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EXTRACTION OF BIOACTIVES FROM PUMPKIN BY-PRODUCTS AND DETERMINATION OF THEIR ANTIOXIDANT ACTIVITY

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Abstract
Agro-industrial and food processing from pumpkins (*Cucurbitaceae*) produces a large number of by-products: bark, pomace, seeds still rich in bioactive compounds, especially carotenoids and green pigments (proto chlorophyll (a and b) and proto pheophytin (a and b)), which exhibit a broad spectrum of health-promoting effects and can be used as ingredients in functional food and cosmetics. For extraction of bioactive compounds from dried pumpkin by-products different methods were used: supercritical CO_2, Soxhlet extraction with n- hexane, ethanol. Vegetable oils (rapeseed, coconut, grapeseed and olive oil) were used as green solvent alternatives to conventional organic solvents for carotenoid extraction. Detection and analyses of chlorophylls and carotenoids was done with hexane/acetone, cyclohexane, ethanol as solvents. The aim of this study was to use pumpkin by-products for extraction of high-value bioactive compounds with different methods, to determine antioxidant´s content and profile – carotenoids (ß-carotene, lutein, lycopene, zeaxanthin), pigments (chlorophylls a, chlorophylls b) with different solvents and to find out what solvent can be used for detection of pigments and carotenoids; to determine antiradical scavenging activity of biologically active compounds in extracts from pumpkin by-products (peel and hulled seeds).
Key words: antiradical activity, carotenoids, chlorophylls, oil, supercritical CO_2.

Introduction

Family of *Cucurbitaceae* is rich in approximately 825 species including 26 species of pumpkins that are grown as vegetables and used for food production (Mala, 2016). 760 000 tonnes of pumpkin were harvested in the EU countries in 2020 (Eurostat, 2021). Production process generates large amount of by-products – seeds, peel, pomace that are rich in bioactive compounds such as – phenolic compounds and their derivatives, provitamins, pigments and carotenoids, pyrazine, squalene, saponins, phytosterols, triterpenoids, unsaturated fatty acids, flavonoids (Černiauskiene *et al*., 2014;coronary heart and other diseases. Dietary fiber (DF Konrade *et al.*, 2016; Fruhwirth *et al*., 2003; Sook *et al*., 2005). Carotenoids in pumpkin are lutein, α- and β-carotene, violaxanthin, neoxanthin and xanthophylls (Soengas *et al*., 2011). Pumpkin seed oil is rich in vitamin E – important antioxidant, including both ɣ-tocopherol and α-tocopherol. Pumpkin seed oil is rich in unsaturated fatty acids, such as linolic acid, oleic acid and saturated as palmitic acid and stearic acid (Konrade *et al*., 2016; Seo *et al*., 2005; Henriques, Guiné, & Barroca, 2012; Secil & Berrin, 2011; Rawson *et al*., 2013).

There is an increasing interest in natural antioxidants from plant origin instead of synthetic antioxidants – carotenoids as their radical scavenging potential is well-documented and their extracts can be used in food, cosmetic, and pharmaceutical industries (Jian *et al*., 2005). Therefore, extraction methods should be observed to obtain the highest recovery and quality (Soengas *et al*., 2011; Salami, Asefi, & Esmaeilzadeh, 2021).

Carotenoid content in dried pumpkin by-products was 91.28 mg 100 g^{-1} (Konrade *et al*., 2019) and ranged from 0.06 to 7.4 mg 100 g^{-1} for β-carotene, from 0 to 7.5 mg 100 g^{-1} for α-carotene and from 0 to 17 mg 100 g^{-1} for lutein (Murkovic & Mu, 2002). Carotenoids are free radical quenchers and liquid antioxidants, they can interact with reactive oxygen species and as singlet oxygen scavengers (Lu & Yeap Foo, 2000). Carotenoids and green pigments, chlorophylls, their derivatives are important health promoters as their role is important in reduction of risks of some types of cancer, diabetes, cardiovascular diseases; they are antimutagenic and with anti-inflammatory activities (Hsu *et al*., 2013; Gebregziabher *et al*., 2021).

Various physical and chemical barriers in the food matrixes can occur during carotenoid extraction; therefore, attention should be paid to methods and solvents polarity (Gebregziabher *et al*., 2021). Edible vegetable oils are used as green solvent alternatives to conventional organic solvents for carotenoid extraction (Borguini *et al*., 2020). Supercritical CO_2 extraction technology has been widely used as an alternative to conventional solvent extraction for the extraction of natural products, because it gives extracts free of organic solvents (Durante, Lenucci, & Mita, 2014).

The aim of this study was to use pumpkin by-products for extraction of high-value bioactive compounds with different methods – supercritical CO_2 ($SCCO_2$), Soxhlet extraction with n- hexane, ethanol and edible oils; to determine antioxidant´s content and profile – carotenoids (ß-carotene, lutein, lycopene, zeaxanthin), pigments (chlorophylls a, chlorophylls b) with different solvents, and antiradical scavenging activity of biologically active compounds in extracts from pumpkin by-products (peel and hulled seeds).

Materials and Methods

Materials

Pumpkin (*Cucurbita pepo* L, Pink Banana Jumbo and *Cucurbita* Moshata, Muscade de Provence) by-products peel and seeds were obtained from Lat Eko Food Ltd. and local farmers in Latvia, 2021.

By-products were washed, peels were ground with a single speed food processor – Robot-Coupe Blixer3, France. Ground peels and hulled seeds were dried with a conventional air dryer Essiccatori professionali B.Master, SR19390 *(*Italy), temperature +40 ℃, 36-48 h to moisture content 5-8%. Afterwords, seeds were ground. Drying, washing and grinding was done at the Institute of Horticulture, Latvia. Dried seed and peel samples were kept in dark plastic boxes in a dark place at temperature 20±2 ℃ till extraction and analyses.

Extraction

1) Supercritical CO_2 extraction of pumpkin by-products was performed with SFE 1000 FANEKS extractor with some modifications (Saini & Keum, 2017). Operating parameters: 252 bar pressure in extractor zone, 60.6-67.4 bar in Separator 1 and Separator 2 zones, CO_2 heater – +33.9 ℃, temperature control in separators 36.6 ℃ and 40.2 ℃, CO_2 flow rate was 8 kg h^{-1}. For extraction of peels, rapeseed oil was added to ground pumpkin by-products (28.4%) to obtain better mass flow in extractor. Seeds of two cultivars (*Cucurbita pepo* L, Pink Banana Jumbo and *Cucurbita* Moshata, Muscade de Provence) were extracted for obtaining the pumpkin seed oil.

2) Soxhlet extraction of pumpkin seeds at 60 ℃ was done with ethanol or hexane to determine the oil content in samples (Mustafa, Trevino, & Turner, 2012)about 25% of the annual production is regarded as by-products due to strict market policies. The aim of this study was to extract carotenoids from those by-products. Conventional carotenoid extraction methods require the use of organic solvents, which are costly, environmentally hazardous, and require expensive disposal procedures. Pressurized liquid extraction (PLE. Dried ground pumpkin seeds (PS) with coat/ hulled were pressed for oil recovery with Oil press Täby Press Type *20* (Sweden) (working conditions – hot single screw extruder type) (Navale, Swami, & Thakor, 2015; Jain, Devi, & Thakur, 2013) banana.

3) Extractions with some modifications with edible vegetable oils – rapeseed, coconut oil, olive oil, grapeseed oil (1:10 (w/v), w – weigh of the sample, v – volume of the oil), were carried out using pumpkin seeds (PS) and peel (PP)): dried and ground samples were sieved (1.2 mm) and extraction was done in a hot water bath at t = 45 ℃±5 ℃ for 60 min, samples were centrifuged at speed 5000 rpm^{-1}, 20 min, temperature +20 ℃ (Sigma 4-16KS*,* Germany) (Seo *et al*., 2005; Fikselová *et al.*, 2008).

Analyses of sample material

Extracted supernatants were analysed for total content of carotenoids (TCC), ß-carotene, lycopene, lutein, zeaxanthin and pigments – chlorophyll a (Chl a) and chlorophyll b (Chl b) and antiradical scavenging activity (AA, %) (DPPH assay) with M501 Single beam Scanning UV/Visible Spectrophotometer (Camspec UV, United Kingdom).

Extracts from by-products were analysed to identify components – pigments and carotenoids with acetone/ hexane (2:3), acetone, cyclohexane and ethanol (Merk, Sigma Aldrich®, Germany) with methods described (Braniša *et al*., 2014*;* Kļava *et al*., 2018; Kampuse & Ozola, 2015; Delia, 2004).

1±0.01g of each sample was separately homogenized with 10 mL of solvent acetone; hexane/ acetone (2:3); cyclohexane or ethanol for 2 minutes, the samples were sonicated for 3 minutes (Ultrasound processor *Hielscher Ultrasound Technology, Ultrasonic Processor UP200S*), vortexed. The spectrum of absorbance for each supernatant was measured and the absorption maxima at different wavelength was read.

The absorption coefficients ($A_{1cm}^{1\%}$) of common food carotenoids were used for calculations.

1) For determination of carotenoids and chlorophyll's acetone/ hexane mixture (2:3) was used and absorption was read at λ = 453; 505; 663 and 645 nm (Braniša *et al*., 2014).

Chl a, Chl b, β-carotene and lycopene content was calculated according to measurements using the following equations (1-4):

$$\text{Chl a } (\mu g\ mL^{-1}) = C\ (0.999A_{663} - 0.0989\ A_{645}) \quad (1)$$

$$\text{Chl b } (\mu g\ mL^{-1}) = C\ (-0.328\ A_{663} + 1.77\ A_{645}) \quad (2)$$

$$\text{Lycopene } (\mu g\ mL^{-1}) = C\ (-0.0485A_{663} + 0.204\ A_{645} + 0.372\ A_{505} - 0.0806\ A_{453}) \quad (3)$$

$$\text{ß- carotene } (\mu g\ mL^{-1}) = C\ (0.216\ A_{663} - 1.22\ A_{645} - 0.304\ A_{505} + 0.452\ A_{453}) \quad (4)$$

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

After that, calculation results were expressed as μg mL^{-1} to be compared.

2) The acetone was used as a solvent to describe content of chlorophylls a and b (Chl a; Chl b) and total carotenoids (TCC).

The absorbance maxima was read at λ = 663.6 nm for Chl a; λ = at 646.6 nm for Chl b; λ = 470.0 nm for TCC (Braniša *et al*., 2014).

Total content of carotenoids (TCC), green pigments (Chl a and Chl b) were calculated (equitation 5-7):

$$\text{Chl a } (\mu g\ mL^{-1}) = C\ (122.5\ A_{663.6} - 22.5\ A_{646.6}) \quad (5)$$

Chl b (μg mL^{-1}) = C (203.1 $A_{646.6}$ – 49.1 $A_{663.6}$) (6)

TCC (μg mL^{-1}) = C ((10000 A_{470} – 2.27* Chl a – – 81.4* Chl b) 270^{-1}) (7)

3) Cyclohexane was used as solvent to determine total content of carotenoids (TCC) at the absorbance λ = 456 nm. TCC was calculated from formulation (8).

TCC= , μg mL^{-1} (8)

TCC – Total content of carotenoids
2505 – coefficient of extinction ($E^{1\%}$)
A – absorbance at 456 nm
C – Concentration of sample solution, mg mL^{-1}.

4) Ethanol was used for determination of lutein at λ = 445 nm, ß-carotene and zeaxanthin at λ = 450 nm,
$A_{1cm}^{1\%}$ = 2620 for ß-carotene
$A_{1cm}^{1\%}$ = 2480 for zeaxanthin (Mustafa, Trevino, & Turner, 2012)about 25% of the annual production is regarded as by-products due to strict market policies. The aim of this study was to extract carotenoids from those by-products. Conventional carotenoid extraction methods require the use of organic solvents, which are costly, environmentally hazardous, and require expensive disposal procedures. Pressurized liquid extraction (PLE.

5) The antiradical activity of the samples was determined with 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·) solution in ethanol (0.1 mM DPPH) (Merk, Sigma Aldrich®, Germany). The results were expressed as the percentage of antiradical activity (AA, %) (Eskicioglu, Kamiloglu, & Nilufer-Erdil, 2015; Yavuz & Emen, 2008). The extract of sample was diluted with ethanol (0.1g mL^{-1}), 2 mL of sample solution and 2 mL of DPPH ethanol solution (0.1 mM) was added, 30 min of reaction in a dark place was allowed, and the absorbance at 517 nm was measured (Priecina & Karklina, 2014; Tirzitis & Bartosz, 2010; Soengas *et al*., 2011).

The antioxidant activity calculation according to the following formula (9):

AA, %= 100, (9)

where A1 – the absorbance before reaction, A2 – the absorbance after reaction has taken place, AA – antiradical activity of samples (%) was done.

All measurements were carried out for three independent samples (n=3), and the 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 protruding hypotheses have been tested with a p-value method and the factors have been evaluated as relevant if $p<\alpha=0.05$. In the analysis of variance, the Tukey and Friedman test was used to justify the differences in the results between the studied samples.

Results and Discussion

Oils were extracted from pumpkin by-products with subcritical CO_2 ($SCCO_2$) and Soxhlet (with n-hexane or ethanol) extraction. The yield of recovered oils from two different varieties of *Cucurbita spp* is attached in Figure 1.

The data are presented as mean values (n=3)

Oil content varied from 25.95±2.2% to 32.23±1.18% in pumpkin by-products – hulled seeds with $SCCO_2$ and Soxhlet extraction (Figure 1), the differences were not significant ($p>0.05$) in the yield of oil from pumpkin seeds with such extractions. Typically, the industrial production of pumpkin seed oil takes place from special pumpkin varieties

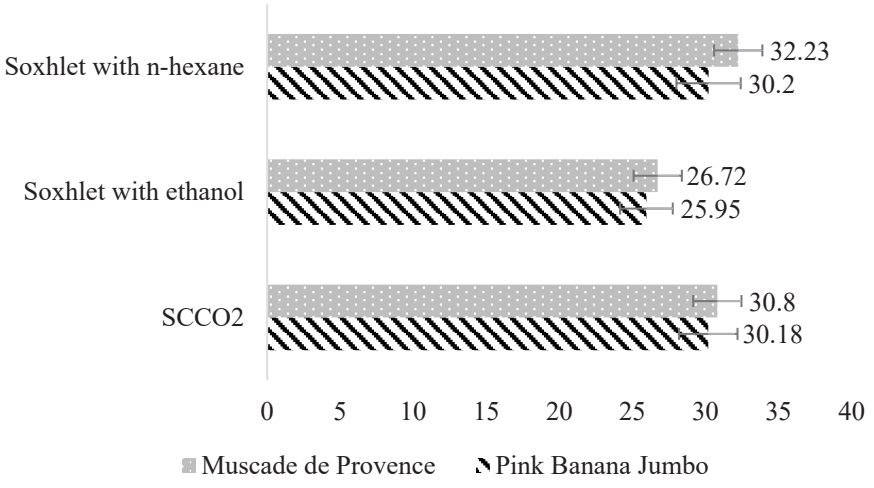

Figure 1. Oil yield (%) of pumpkin (*Cucurbita pepo* L, Pink Banana Jumbo and *Cucurbita* Moshata, Muscade de Provence) by-products with different extractions.

Table 1

Carotenoid content in edible oils used for extraction of pumpkin by-products

Oil	Solvent acetone	Solvent cyclohexane	Carotenes, µg mL^{-1}			
	TCC, µg mL^{-1}		Lycopene	ß carotene	Lutein	Zeaxanthin
Coconut oil	0.09±0.01	0.65±0.01	n.d	1.06±0.01	n.d.	1.13±0.01
Grapeseed oil	3.67±0.04	3.20±0.03	1.21±0.01	2.3±0.01	0.74±0.01	2.1±0.01
Rapeseed oil	3.45±0.08	4.17±1.08	0.24±0.01	1.5±0.01	2.91±0.09	0.16±0.01
Olive oil	6.84±0.07	8.48±0.09	1.01±0.01	5.15±0.04	2.48±0.03	5.48±0.03

n.d. – not detected, the data reported as average (n=3); ± Standard Deviation.

Table 2

Chlorophylls in edible oils for extraction of pumpkin by-products

Oil	With acetone		With acetone/hexane (2:3)	
	Chl a, µg mL^{-1}	Chl b, µg mL^{-1}	Chl a, µg mL^{-1}	Chl b, µg mL^{-1}
Coconut oil	n.d.	n.d.	n.d.	n.d.
Grapeseed oil	0.57±0.02	0.38±0.01	0.51±0.01	2.01±0.02
Rapeseed oil	0.38±0.01	0.47±0.04	0.18±0.01	1.39±0.01
Olive oil	1.75±0.03	0.43±0.03	n.d.	1.63±0.01

n.d. – not detected, the data reported as average (n=3); ± Standard Deviation.

(*Cucurbita* pepo subsp. pepo var *Styriaca*) that are already botanical naked or hull-less seeds, the content of lignin and cellulose is very low and outer hull is very thin (Fruhwirth *et al*., 2003). Studies of twelve pumpkin (*Cucurbita* maxima D) cultivars showed that the oil content ranged from 10.9 to 30.9% (Stevenson *et al*., 2007). Differences of oil content in two different cultivars in research should be of harvest time, variety, and ratio between seed kernel and shell (Redrouthu, Sundramurthy, & Zergu, 2020).

Main carotenoids in vegetables and plants are α- and β-carotene, lutein, neoxanthin, violaxanthin, and they have been studied very widely because of the beneficial effects on human health. The stability of carotenoids is very sensitive, and they can form isomers (Murković *et al*., 1996). Extraction of carotenoids and pigments were carried with $SCCO_2$ and edible vegetable oils. Carotenoid and chlorophyll profile of oils for further by-products extraction with different solvents was analysed (Table 1, Table 2). Highest content of total carotenoids (TCC) was determined in olive oil 6.84-8.48 µg mL^{-1}, TCC in coconut oil was determined 0.652 µg mL^{-1}, (Table 1). Different results were obtained with cyclohexane and acetone as solvents. Acetone as solvent for detection of chlorophylls showed better results in comparison with hexane/ acetone. TCC determination with cyclohexane gave higher extractions for analyses.

The main pigments-four carotenoids (lycopene, lutein, β-carotene, zeaxanthin) and two chlorophyll derivatives (a and b) were quantified by the near UV-visible spectrum.

For determination of chlorophyll's acetone/ hexane gave higher contents of Chl b, while acetone was better for detection of Chl a in edible oil samples.

The choice for solvent is necessary to find out what solvent can be used for detection of pigments and carotenes as some solvents gave negative data in determination of chlorophylls, lutein and lycopene (Niewiadomski, Bratkowska, & Mossakowska, 1965). It is described that vegetable oil refining also affect the carotenoid content in oils; therefore, it should be considered for further experiments for using oils as solvents for extraction (Kuncewicz, 2008).

Two varieties of pumpkin hulled seeds were analysed for pigments profile (Table 4). Solvents for analyses also are important for prediction of bioactive compounds as, for example, ethanol gave negative results for ß-carotene while lycopene was detected in all extracted samples of pumpkin seed oil. In oils extracted with ethanol and hexane ß-carotene, lutein and zeaxanthin were not detected, though (Juknevi, Judita, & Kulaitien, 2013) lutein in seeds from pumpkin cultivars Golosemiannaja, Herakles and Miranda from 25.26 to 162.70 µg g^{-1} and ß-carotene about 5.15 µg g^{-1} was detected.

Pumpkin seed extracts have high content of carotenoids (Table 3), the highest amount with $SCCO_2$ extraction. Higher carotene profile (Table 3, 4) of oils from two varieties of PS is in oil from Pink Banana

Table 3

Composition of oils from two varieties of pumpkin (Cv Muscade de Provence and Cv Pink Banana Jumbo) hulled seeds by different extraction methods

Samples	ß-carotene, µg ^{mL}-1	Lutein, µg mL^{-1}	Zeaxanthin, µg mL^{-1}	Lycopene, µg mL^{-1}	TCC, µg mL^{-1}
$_{OKET}$OH	4.28±0.02	11.22±1.01	1.12±0.09	n.d.	24.04±1.18
O_{Khe}x	7.25±0.03	11.81±2.02	0.77±0.02	0.69±0.21	13.75±2.06
O_{KCO}2	4.36±0.01	12.26±1.02	0.46±0.11	0.75±0.03	23.98±2.45
OR_{ETOH}	n.d.	n.d.	n.d.	0.48±0.06	n.d.
OR_{hex}	n.d.	n.d.	n.d.	0.37±0.08	33.30±3.18
OR_{CO2}	2.27± 0.04	18.25±1.18	0.24±0.05	0.7±0.01	47.67±2.09
O_{Rf}r	5.30±0.01	17.74±2.04	0.56±0.03	0.76±0.20	32.62±1.44

n.d. – not detected, the data reported as average (n=3); ± Standard Deviation, sample abbreviations: OK – oil from pumpkin seeds (PS) (variety Muscade de Provence), (OK_{ETOH} – oil from pumpkin seeds with ethanol; OK_{hex} – oil from PS with n-hexane; OK_{CO2} – oil from PS with ScCO2; OK_{CO2} – oil from PS with ethanol); OR – oil from PS (variety Pink Banana Jumbo), OR_{ETOH} – oil from pumpkin seeds with ethanol; OR_{hex} – oil from PS with n-hexane;OR_{CO2} – oil from PS with $SCCO_2$; OR_{fr} – oil from PS with oil press/ extruder.

Jumbo – the highest TCC was determined in oils with $SCCO_2$ extraction – 47.67±2.09 µg mL^{-1}. Temperature of extraction is decisive factor for carotenoids and bioactives that explains lower results for extracts with ethanol (TCC=24.04±1.18 µg mL^{-1}) as the temperature for Soxhlet extraction with ethanol was +70 °C. Lutein was predominantly present, its content determined from 11.22±1.01 to 18.25±1.18 µg mL^{-1}. There was a significant difference (p>0.05) between samples with different extractions in carotene profile, highest results of lutein were in $SCCO_2$ extracts of oils. There was no significant difference (p>0.05) in determination of chlorophylls with acetone and acetone/ hexane ($F>F_{crit}$, p=5.28). Predominantly chlorophylls were Chl b (1.59-13.0 µg mL^{-1}).

$SCCO_2$ and edible oils were used as solvent for recovering bioactive compounds – carotenoids (Table 6). From previous studies TCC content in dried pumpkin by-products was 91.28 mg 100 g^{-1} (Konrade *et al*., 2016). ß-carotene 0.06 to 7.4 mg 100 g^{-1}, from 0 to 7.5 mg 100 g^{-1} for α-carotene and from 0 to 17 mg 100 g^{-1} for lutein (Murkovic & Mu, 2002).Therefore, it can be considered that the highest extractions of carotenes are obtained with coconut oil as the content of TCC in oil as solvent was determined 0.09-0.65 µg mL^{-1} after recovering of carotenoids with solvents. It was observed that extraction with coconut oil with PP TCC reached 17.95 µg mL^{-1}. Coconut oil is a promising solvent for the recovery of bioactive compounds, flavours and vitamins as coconut oil is the highest natural source of lauric acid and its derivative monolaurin (Papadaki, Kyriakopoulou, & Krokida, 2017).

Under the influence of sunlight, temperature, extraction temperatures and oxidation, the pigments can be destroyed (Soengas *et al*., 2011). Studies and

Table 4

Chlorophylls in oils from pumpkin cultivars Cv Muscade de Provence and Cv Pink Banana Jumbo

Sample	With acetone		With acetone/hexane (2:3)	
	Chl a, µg mL^{-1}	Chl b, µg mL^{-1}	Chl a, µg mL^{-1}	Chl b, µg mL^{-1}
OK_{ETOH}	2.09±0.12	5.30±1.2	1.61±0.13	1.59±0.02
OK_{hex}	3.54±0.18	7.09±0.90	2.23±0.07	8.3±0.007
OK_{CO2}	0.45±0.01	2.58±0.02	2.52±0.01	0.9±0.002
OR_{ETOH}	2.68±0.02	5.05±0.08	6.50±0.01	3.89±0.14
OR_{hex}	4.96±0.01	7.95±0.09	0.80±0.01	13.0±0.02
OR_{CO2}	2.84±0.01	6.74±1.12	4.0±0.020	6.4±0.013

The data reported as average (n=3); ± Standard Deviation; Sample abbreviations: OK_{ETOH} – oil from pumpkin seeds with ethanol; OK_{hex} – oil from pumpkin seeds with n-hexane; OK_{CO2} – oil from pumpkin seeds with SCCO2; OK_{ETOH} – oil from pumpkin seeds with ethanol; OR_{hex} – oil from pumpkin seeds with n-hexane; OR_{CO2} – oil from pumpkin seeds with $SCCO_2$.

Table 5

Assay of chlorophylls, carotenoids in extracts of pumpkin peel (variety Muscade de Provence)

	Acetone			Hexane		Lutein µg mL^{-1}	ß-carotene, µg ^{mL}-1	Zeaxanthin, µg mL^{-1}
	Chl a, µg mL^{-1}	Chl b, µg mL^{-1}	TCC, µg mL^{-1}	Chl a, µg mL^{-1}	Chl b µg mL^{-1}			
$Kscco_2$	1.53±0.02	1.17±0.01	32.43±2.09	2.08±0.01	2.4±0.01	6.72±0.01	5.37±1.12	5.72±0.08
KR1	1.01±0.01	0.36±0.02	27.17±1.18	1.81±0.02	1.6±0.02	8.85±0.02	6.81±0.02	7.25±0.06
KC1	0.91±0.04	1.71±0.05	26.03±2.09	1.01±0.01	1.2±0.01	7.71±0.01	8.48±0.09	9.03±0.03
KO1	1.84±0.01	1.66±0.02	17.95±1.14	n.d.	1.44±0.04	6.0±0.06	3.79±0.03	6.61±0.01
KG1	1.00±0.03	1.09±0.01	16.32±1.11	1.4±0.01	0.08±0.02	7.01±0.02	5.37±0.03	5.72±0.04

The data reported as average (n=3); ± Standard Deviation, n.d.-not detected, Sample abbreviations: K_{SCCo2} – extracts from pumpkin by-products peel (PP) with $SCCO_2$ extraction, KR1 – extracts from PP with rapeseed oil, KC1 – extracts from PP with coconut oil, KO1 – extracts from PP with olive oil, KG1 – extracts from PP with grapeseed oil.

Table 6

Carotenoids in edible vegetable oil extracts with pumpkin by-products (hulled seeds)

	SG1	SR2	SC1	SO1
TCC, µg mL^{-1}	32.08±1.18	29.63±1.16	18.38±1.14	22.55±0.37
Lutein, µg mL^{-1}	20.71±0.23	7.87±0.18	n.d.	8.04±0.12
ß-carotene, µg m^{L}-1	7.67±0.31	12.61±0.13	7.57±2.08	4.08±1.18
Lycopene, µg mL^{-1}	n.d.	n.d.	1.04±0.01	n.d.
Chl a µg mL^{-1}	2.30±0.2	2.02±0.06	0.2±0.01	2.4±0.02
Chl b µg mL^{-1}	1.21±0.11	1.24±0.02	1.12±0.01	3.23±0.01

The data reported as average (n=3); ± Standard Deviation, n.d.-not detected. Sample abbreviations: SG1 – pumpkin seed (PS) extracts with grapeseed oil; SR2 – PS extracts with rapeseed oil; SC1 – PS extracts with coconut oil; SO1 – PS extracts with olive oil;

experiments, analyses should be done immediately after extractions.

Although the mixture of acetone-hexane is considered to be effective in the extraction of carotenoids, our results (Table 1), obtained in some extracts, appear to be problematic in determining the lycopene, chlorophyll content, as regards some determination which gives negative values (added as not detected – n.d.)

Antiradical scavenging activity (AA, %) in coconut oil – 41.3±2.04, rapeseed oil – 30.0±1.28 and in grapeseed oil – 25.1±1.18 %. From DPPH assay

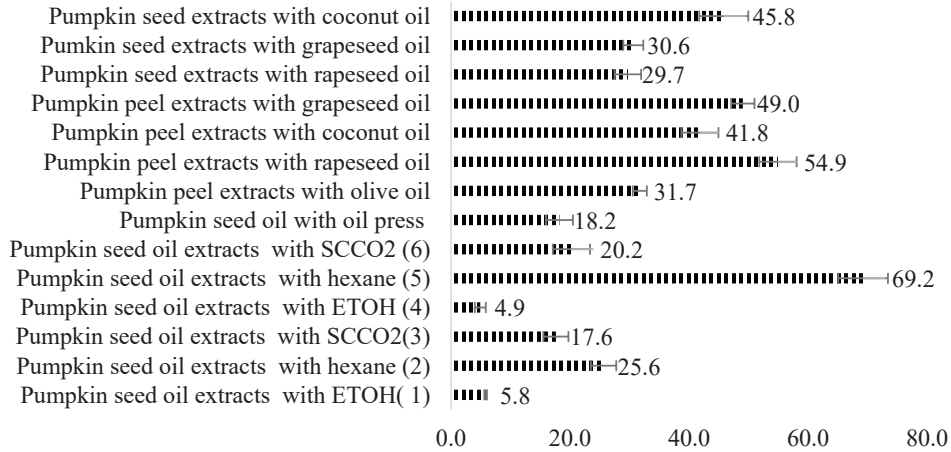

Figure 2. Antiradical scavenging activity (DPPH assay) of extracts from pumpkin by-products
The data reported as average (n=3); Samples: Cv Muscade de Provence (1-3) and Cv Pink Banana Jumbo (4-6).

(Figure 2) it is found that the ability of scavenging free radicals is high in all extracts except those with ethanol extraction. AA has been found in seed extracts with oil press even 69.2%. A very strong correlation of antiradical scavenging activity with the chl a a and chl b content in pumpkin seed extracts with edible oils ($r=0.99$ and $r=0.91$, $p>0.05$) has been found.

Conclusions

High-value bioactive compounds can be extracted with different methods – supercritical CO_2 ($SCCO_2$), Soxhlet extraction with n- hexane, ethanol and vegetable oils. Rapeseed, grapeseed and coconut oils are effective green solvents to be used for carotenoid and chlorophyll extractions from pumpkin by-products – seeds and peel. Vegetable oils with pumpkin seeds and peel gave high content of total carotenes – 47 $\mu g g^{-1}$ in oils from pumpkin seeds with hull, TCC level in vegetable oils received 32 $\mu g\ g^{-1}$ in grapeseed oil and 18.3 $\mu g\ g^{-1}$ in coconut oil with pumpkin by-products. Analyses of chlorophylls a and b profile with different solvents (acetone, hexane, ethanol) showed differences in determination – determination of chlorophyll acetone/ hexane gave higher contents of Chl b, while acetone was better for detection of Chl a in samples. Antiradical scavenging activity of biologically active compounds in extracts from pumpkin by-products with different methods was high in all experimental samples.

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