OAT HULLS AND SEA BUCKTHORN POMACE – A POTENTIAL SOURCE OF ANTIOXIDANTS FOR HEMPSEED OIL

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Abstract

Hempseed oil, as well as other oils containing polyunsaturated fatty acids, is subjected to oxidation processes caused by air, heat or light. Usually these processes are suppressed by addition of various synthetic phenol type antioxidants. The aim of current research was to increase oxidative stability of vegetable oils with natural antioxidants. Herein, we demonstrate that the oxidative stability can be increased with natural antioxidants present in oat hulls and sea buckthorn pomace – by-products of food processing. Extracts from pomace and hulls were prepared by maceration of ground plant material in the hempseed oil. The extraction was accelerated by ultrasound. It was established that the highest amount of polyphenols in extracts of both plant materials can be achieved within 30 min; further increase of extraction time sometimes even reduced the total amount of polyphenols. The highest amount of polyphenols in sea buckthorn extracts was 7.73±0.29 mg GAE 100 g^{-1}, but in oat hull extracts – 4.63±0.21 mg GAE 100 g^{-1}, when extracts were prepared by ultrasonification of hempseed oil containing 5% wt of plant material. Various amounts of plant material additives were used for extraction under optimized conditions. The highest antioxidant activity (expressed as ratio of time when peroxide value of sample and blank reaches 48 meq O_2 kg^{-1}) – 1.51±0.05 and 1.40±0.06 to 1.44±0.02, respectively – had extracts obtained from 1% wt additive of sea buckthorn pomace or 2.5–5.0% wt additive of oat hulls. The prepared hempseed oil extracts demonstrated higher oxidative stability than hempseed oil containing 0.02% additive of synthetic antioxidant BHT.

Keywords: hempseed oil, oat hull, sea buckthorn pomace, oxidative stability.

Introduction

Oats (*Avena sativa* L.) demonstrate antiatherogenic properties in *in vitro* assays, animal experiments and human studies, exhibit anti-inflammatory and antioxidative action (Andersson, Hellstrand, 2012; Pazyar et al., 2012). Oats reduce coronary heart disease (Harris, Kris-Etherthon, 2010; Truswell, 2002) *via* different mechanisms – decreasing serum low-density lipoprotein cholesterol, blood pressure and improving glucose and insulin response (Harris, Kris-Etherthon, 2010). Due to the skin protecting effect against ultraviolet rays, oats are used in cosmetic formulations (Pazyar et al., 2012; Kurtz, Wallo, 2007). Unique oat polyphenols – avenanthramides – have strong antioxidant activity *in vivo* and *in vitro*, exhibit anti-inflammatory, antiproliferative and anti-itching activity, which may provide additional protection against coronary heart disease, colon cancer and skin irritation (Meydani, 2009; Guo et al., 2009).

Sea buckthorn (*Hippophae rhamnoides* L.) is widely used due to its biological activity: oil (Kumar et al., 2011), fruits and leaves (Suryakumar, Gupta, 2011; Xu et al., 2011) are used to treat cancer and disorders of skin, as well as cerebral-cardiovascular and immune systems. Sea buckthorn is applied to lower cholesterol, platelet aggregation, blood pressure and blood sugar (Xu et al., 2011). The oil absorbs ultraviolet light and promotes healthy skin (Stobdan et al., 2013; Vernet, 2006).

Due to the great biological properties of oats we studied oat grains as a source of natural antioxidants to enhance oxidative stability of various vegetable oils. Previously we have clarified, that additives of oat grains improve the oxidative stability of vegetable oils more than 3.5 times (Jure et al., 2010); other authors (Xing, White, 1997) have established that methanolic extracts of oat hulls dramatically reduced the formation of peroxides during storage of the soybean oil. Our current studies are devoted to the examination of oat hulls as an alternative source of antioxidants for stabilization of hempseed oil; the impact of natural antioxidants extracted from oat hulls and pomace of sea buckthorn is compared. The usage of these sources of natural antioxidants is sustainable and economically beneficial, as both of them are by-products of food industry.

Materials and Methods

Materials

Hempseed oil (HO) was obtained by cold-pressing method at *DUO AG* Ltd. Sea buckthorn pomace (SB) was supplied by farm *Puntiņi* as the by-product of juice manufacturing. Oat hulls (OH) were purchased from Rigas Dzirnavnieks JSC.

Preparation of hempseed oil extracts

Ground sea buckthorn pomace or oat hulls were mixed with hempseed oil [ratio of plant material and hempseed oil was 1:99, 1:39 or 1:19 (wt:wt)]. The mixture was treated with ultrasound (for the time of ultrasonification see Table 1). The temperature was 16 to 29 °C in the sonification bath. The plant material after the extraction was separated by centrifugation at 3000 rpm. Each extract was prepared triplicate.

Preparation of the extracts of polyphenols

The hempseed oil extract (4 g) was shaken with 80% ethanol (10 mL) at room temperature in dark for 24 h by orbital shaker *Biosan OS-10*. The ethanol extract was used for further analysis of polyphenols according to the Folin-Ciocalteu method (Mierina et al., 2011). The total amount of phenolic compounds (TAP) was calculated from calibration curve (R^2=0.9936) and was expressed as mg of gallic acid equivalents per 100 g of oil (mg GAE 100 g^{-1} oil); calibration curve consisted

from 5 points and was linear in the range of obtained results. The absorption of all solutions was measured with single beam scanning UV/Visible spectrometer *Camspec M501*.

Antioxidant activity

The hempseed oil extracts of oat hulls and sea buckthorn pomace were kept under accelerated oxidation conditions in thermostat at 40 °C in dark. The samples (30 g) were filled in Petri dishes with diameter 10 cm. The oxidation process was characterized with peroxide value. Antioxidant activity (AA) was expressed according to the equation:

$$AA = \frac{t_{extract}}{t_{blank}} \quad (1)$$

where

$t_{extract}$ and t_{blank} is the time when peroxide value reaches 48 meq O_2 kg^{-1} for hempseed oil extract of plant material and hempseed oil without additive, respectively.

Analogously the impact of 0.02% butylated hydroxytoluene (BHT) additive on oxidative stability of hempseed oil was detected.

Antiradical activity

2,2-Diphenyl-1-picrylhydrazyl DPPH (2 mL, 200 μM solution in isooctane) and hempseed oil extract of sea buckthorn or oat hulls (2 mL, 40, 30, 20 or 10 mg mL^{-1} solution in isooctane) were mixed; after 30 min absorption ($Abs_{extract}$) of solution was measured at 515 nm. The blank (Abs_{blank}) was prepared as above mentioned, pure isooctane was used instead of the solution of hempseed oil extract. The inhibition of DPPH (INH, %) was expressed according to the equation:

$$INH = \frac{Abs_{blank} - Abs_{extract}}{Abs_{blank}} \times 100\% \quad (2)$$

In order to find out the concentration that inhibits 50% of DPPH, the curves were constructed between the concentration of hempseed oil extract in isooctane and inhibition of DPPH (%).

Peroxide and acid values

Peroxide and acid values were determined according to standard LVS EN ISO 3960 and LVS EN ISO 660, respectively.

Statistical analysis

Statistical analysis was carried out using Microsoft Excel software package. Standard deviation was calculated by linear least squares regression. All measurements were carried out at least triplicate.

Results and Discussion

Hempseed oil is characterized with high amount of polyunsaturated (especially, linoleic) fatty acids. The ratio of *n-6* and *n-3* fatty acids is 3:1; therefore implementation of hempseed oil into the diet would shift the ratio to the recommendations of various nutrition societies (Matthäus, Brühl, 2008). The oil is widely used in cosmetic formulations due to the presence of *γ*-linolenic acid (Da Porto et al., 2012). Besides that, the oil contains high amount of tocopherol – even higher than in flax and canola seed oil (Teh, Birch, 2013). Unfortunately it has to be taken into consideration that the linoleic and linolenic acids are highly susceptible to oxidation. Also the presence of high amounts of chlorophyll promotes the oxidation (Matthäus, Brühl, 2008).

Both sea buckthorn and oat are rich sources of antioxidants. Sea buckthorn oil (Kumar et al., 2011), fruits and leaves (Suryakumar, Gupta, 2011) are well known sources of tocopherols, tocotrienols, carotenoids, phytosterols, vitamin C, phenolic compounds and trace elements. Main oat antioxidants are vitamins, carotenoids, phenolic compounds including phenolic acids and flavonoids, phytoestrogens, phytosterols, inositol phosphates, glutathione and melatonin (Zieliński et al., 2012; Peterson, 2001). Oat is almost exclusive source of *N*-cinnamoylanthranilates called avenanthramides (Peterson, 2001). Oat hulls and sea buckthorn pomace can increase oxidative stability of polyunsaturated hempseed oil, as well as make it even more advantageous for cosmetic purposes.

Usually extracts of oat and sea buckthorn antioxidants are prepared using various organic solvents. Such extraction procedure has several disadvantages – flammability of organic solvents and necessity to evaporate solvents require special equipment, but solubility of such extracts in vegetable oils may be problematic. Besides that toxicity and harmfulness of organic solvents must be kept in mind when the extract is envisaged for food or cosmetic industry. We already described preparation of "ready to use" extracts having increased oxidative stability obtained from sea buckthorn seeds and pomace (Seržane et al., 2012) and grains of oats (Jure et al., 2010) by extraction with vegetable oils. Usually the extraction was carried out at room temperature for 24 h or the extract was prepared using various cold-pressing techniques.

It is well known that antiradical activity of hempseed oil can be increased if the seeds before the oil extraction are treated with ultrasound for 20 to 40 min (Da Porto et al., 2013). In order to enhance the extraction as well as to use conditions that can increase the oxidative stability of hempseed oil we examined ultrasound accelerated process. Ground plant material and hempseed oil were mixed in ratio 1:19 (in the previous studies we clarified that the optimal ratio for various plant materials, including oat grains and sea buckthorn pomace was 5% additive to the vegetable oil) and treated with ultrasound for various time from 10 min to 1 h (see Table 1). Ultrasonification had small impact on the quality of hempseed oil – immediately after the extraction peroxide and acid values of extracts were similar in most of the cases; ultrasonification slightly increased peroxide value of extracts in comparison with samples prepared by cold-pressing (HO-2, SB-11). Both the amount of plant material

additive and the duration of ultrasonification had negligible effect on the peroxide value. The effectiveness of extraction was characterized by the total amount of polyphenols (see Table 2) – ultrasonification raised TAP value from 4.8 to 7.7 mg GAE 100 g^{-1} oil in case of sea buckthorn extracts (within 30 min) and to 5.5 mg GAE 100 g^{-1} oil for extracts of oat hulls (within 20 min).

Table 1

Hempseed oil extracts of sea buckthorn pomace and oat hulls

Abbr.	AmA	TU	PV	AV
OH-1	5.0	10	5.24±0.36	5.05±0.32
OH-2	5.0	20	6.06±0.20	4.51±0.09
OH-3	5.0	30	5.59±0.59	4.44±0.05
OH-4	5.0	40	6.47±0.61	4.25±0.04
OH-5	5.0	50	3.88±1.31	4.48±0.14
OH-6	5.0	60	4.37±0.96	4.35±0.07
OH-7	1.0	30	5.20±0.63	4.26±0.01
OH-8	2.5	30	3.07±0.13	4.48±0.12
SB-1	5.0	10	4.59±0.42	4.64±0.07
SB-2	5.0	20	4.05±0.27	4.43±0.17
SB-3	5.0	30	4.21±0.41	4.62±0.41
SB-4	5.0	40	4.48±0.88	4.46±0.01
SB-5	5.0	50	4.42±0.62	4.28±0.04
SB-6	5.0	60	4.09±0.27	4.43±0.05
SB-7	1.0	30	5.82±0.73	4.39±0.16
SB-8	2.5	30	4.52±0.32	4.45±0.04
HO-1	0	30	5.74±0.94	4.40±0.40
HO-2	0	0	4.32±1.54	4.79±0.88
SB-11	5.0	0*	2.38±0.53	3.45±0.85

AmA – Amount of additive in the mixture of plant material and hempseed oil, %. TU – Time of the ultrasonification, min. * – The extract was prepared according to the known cold-pressing method (Poiss, 2004).

When irradiation was carried out just for 10 min (OH-1, SB-1), TAP did not change in comparison with sonicated hempseed oil without any additive (HO-1), most probably due to the partial degradation of antioxidants both in the plant material and hempseed oil. The continued (more than 30 min) ultrasonification of the samples generally slightly decreased the total amount of polyphenols: e. g., the difference between cold-pressed hempseed oil and sonicated (for 30 min) oil was 0.6 mg GAE 100 g^{-1} of oil. The increase of TAP during the short ultrasonification can be explained both with more complete extraction process and formation of endogenous antioxidants, the nature and amount of which may depend on sonication time (Da Porto et al., 2013). TAP increased with the amount of plant material used for the extraction; the difference of the TAP was about 2.4 mg GAE 100 g^{-1} of oil for the extracts obtained from 1 and 5% additive of sea buckthorn (SB-7 and SB-3). The amount of oat hulls additive did not show strong regularity and had less impact on TAP – the maximal value was reached with 5% additive by 60 min ultrasonification, but similar TAP had extract obtained only within 20 min.

Table 2

TAP and antioxidant activity of extracts of sea buckthorn pomace and oat hulls

Abbreviation	TAP	AA
OH-1	4.85±0.37	1.50±0.03
OH-2	5.47±0.10	1.30±0.02
OH-3	4.63±0.21	1.44±0.02
OH-4	4.65±0.04	1.42±0.17
OH-5	4.74±0.09	1.37±0.04
OH-6	5.58±0.28	1.36±0.04
OH-7	5.32±0.32	1.32±0.02
OH-8	4.60±0.24	1.40±0.06
SB-1	4.86±0.17	1.26±0.03
SB-2	7.07±0.19	1.27±0.06
SB-3	7.73±0.29	1.32±0.02
SB-4	6.68±0.20	1.31±0.04
SB-5	6.63±0.26	1.37±0.04
SB-6	6.73±0.15	1.35±0.04
SB-7	5.33±0.24	1.51±0.05
SB-8	6.08±0.88	1.42±0.02
HO-1	4.86±0.15	–
HO-2	5.51±0.05	1.00
SB-11	4.78±0.44	0.75±0.02

In order to evaluate the impact of extraction conditions and the amount of oat hulls and sea buckthorn pomace on the oxidative stability of hempseed oil, all samples were kept under accelerated oxidation conditions in Petri dishes at 40 °C. The accelerated oxidation was continued until the peroxide value of the sample reached at least 48 meq O_2 kg^{-1}. The curves of peroxide values were used to estimate antioxidant activity of the prepared extracts – in most of the cases antioxidant activity varied from 1.3 to 1.5 (see Table 2); such activity is considered as low or very low (Ramamoorthy, Bono, 2007). Nevertheless the effect of sea buckthorn and oat hulls is comparable to the activity of synthetic antioxidant BHT – 0.02% additive of BHT increases oxidative stability of hempseed oil 1.5 times. The oxidative stability of hempseed oil extracts did not increase proportionally to TAP value – extracts of sea buckthorn pomace demonstrated strong antioxidant-prooxidant effect; any correlation between TAP and AA was not observed for the extracts of oat hulls. The acid values of all samples at the end of experiments did not exceed 5 mg KOH g^{-1}. The extracts that were prepared under ultrasound accelerated conditions demonstrated better AA and higher TAP than the extract SB-11 obtained by cold-pressing (method described by Poiss (2004)) of mixture of hempseeds and sea buckthorn pomace.

Antiradical activity against DPPH was measured for the extracts obtained by 30 min ultrasonification (Table 3). The most common solvent for DPPH analysis is alcohol (mainly ethanol or methanol). Nevertheless extensive studies of the mechanism of antioxidant interaction with free radicals have revealed that this mechanism strongly depends on the properties of the solvent. It is established that the reaction of phenols with DPPH in alcoholic media goes as sequential proton loss and electron transfer (SPLET) (Litwinienko, Ingold, 2003; Foti et al., 2004). It is well known that oxidation of vegetable oils proceeds *via* homolytic cleavage of C-H bond mainly at *bis*-allylic and allylic positions of fatty acid moiety (Belitz et al., 2004). In order to avoid SPLET process during DPPH analysis we carried out our experiments in isooctane – a solvent that supports hydrogen atom transfer (HAT) mechanism (Litwinienko, Ingold, 2007). Hempseed oil extracts of oat hulls demonstrated better antiradical activity against DPPH in comparison to extracts of sea buckthorn pomace (Table 3).

Moderate correlation existed between inhibition of DPPH and total amount of polyphenols in extracts of sea buckthorn pomace – the antiradical properties of these extracts generally rose with the increase of TAP. Such correlation was not observed in case of extracts of oat hulls. Medium linear correlation was found both for hempseed oil extracts of sea buckthorn pomace and oat hulls between inhibition of isolectronic peroxyl and DPPH radicals; the first one was expressed as antioxidant activity and the second – as concentration (mg mL^{-1}) of oil extract in isooctane that inhibits 50% of DPPH with initial concentration 100 μM (abbreviated as IC_{50}).

Table 3

Antiradical activity of hempseed oil extracts

Hempseed oil extracts of oat hulls		Hempseed oil extracts of sea buckthorn pomace	
Extract	**IC_{50}, mg mL^{-1}**	**Extract**	**IC_{50}, mg mL^{-1}**
OH-3	8.4±0.2	SB-3	7.8±0.4
OH-7	8.0±0.1	SB-7	9.0±0.0
OH-8	7.7±0.0	SB-8	9.1±0.1

Conclusions

Our studies demonstrate that ultrasound is an effective mean to accelerate extraction of sea buckthorn pomace and oat hulls with hempseed oil. Even within 30 min the maximal total amount of polyphenols can be reached and such extracts demonstrate acceptable oxidative stability. Luck of direct correlation between total amount of polyphenols and antioxidant activity, as well as between TAP and antiradical activity of extracts let us suggest that beside polyphenols other compounds present in extracts have a strong influence on oxidative stability of hempseed oil.

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