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ANTIOXIDANT ACTIVITY OF BULGARIAN COTTON SEED OIL AND SOAPSTOCK AGAINST DPPH RADICAL

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Abstract

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In the present study the antioxidant activities of oil from the seeds of a Bulgarian cotton variety and soapstock, obtained after the neutralization of the raw oil were investigated. As neutralizing agent were used 105, 117 and 129 $g.dm^{-3}$ NaOH solutions. After the treatment with 117 and 129 $g.dm^{-3}$ NaOH gossypol was found only in the soapstock. This was proven by the absence of antiradical activity against the DPPH radicals.

Gossypol exhibited stronger antiradical activity against the DPPH radical, based on IC_{50}, in comparison with BHA, ascorbic acid and BHT.

Key words: antioxidant activity, cottonseed oil, soapstock

Introduction

Gossypol is a specific pigment, which can only be found in cotton seeds and is situated in the gossypol or pigment glands (Orthoefer, 1995; Kirk and Higginbotham, 1999). It is toxic to humans and animals and its content in food and feed products is regulated. During the production of cotton seed oil it is extracted with the oil.

The amount of gossypol in the cotton seeds is critical for the selection of a technological scheme for both extraction and refining. Various technological schemes were developed for gossypol elimination and the most important of them are based on:

• Maximal binding of gossypol to the protein component

This process allows for the obtaining of pressed oils with low gossypol content and expeller with inactivated gossypol (Koneva et al., 1987).

During the heating of the cotton seed flour two competitive processes occur: interaction of gossypol with the amino groups from the polypeptide chain of the protein molecules and interaction of the aldehyde groups of the reducing compounds with the same amino acids (Iliev et al., 1969).

• Maximal passing of gossypol to the pressed oils

In these technological schemes such parameters for conditioning are chosen that maximal quantity of gossypol passes in the pressed oil in native form. Later it is eliminated during the process of refining (neutralization).

During the neutralization of the raw oil a waste product called soapstock is obtained. Part of the gossypol passes on to the soapstock as native form or as a complex with concomitant substances form the oil (phosphatides, etc.) (Dowd, 1996; Kuk and Ballew, 1999.).

There are some reports in reference literature regarding antioxidant activity of gossypol obtained from raw cotton oil (Cai et al., 2004; Mukhamediev et al., 1986; Sotelo et al., 2005; Wang et al., 2005).

The aim of this study is to determine the antioxidant activity of the cotton seed oil and soapstock from the Bulgarian cotton variety Chirpan 539 and to compare their antiradical activity against the DPPH to the activities of some synthetic and natural antioxidants.

Materials and Methods

The cotton seed oil was obtained form seeds of the Bulgarian cotton variety Chirpan 539. This variety is accepted as a reference for fiber yield and is used for comparison of all selected varieties with economic significance in Bulgaria.

The cotton seeds were subjected to milling, conditioning and pressing. The milling was performed with two-roll mill

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and the conditioning – in a one level conditioner for 30 min. The pressing was carried out with a final pressing Andromeda DK press (Rasma 01U) at 2026.5 Pa for 15 min., 100°C treatment temperature and initial cotton seed flour moisture content 5.68 $g.kg^{-1}$. In previous studies (Uzunova and Perifanova-Nemska, 2008) was determined that from a technological point of view (high glyceride oil yield combined with minimal content of gossypol with high tocopherol content in it) this is the most suitable scheme for cotton seed oil production.

The obtained cotton seed oil was neutralized under laboratory conditions by three methods (method 1, method 2 and method 3) using different concentrations of neutralizing agent – 105, 117 and 129 $g.dm^{-3}$ NaOH solutions, respectively.

The antioxidant activity was analyzed for: the obtained raw pressed oil; the oil neutralized by the three methods; commercially produced refined sunflower and cotton seed oil, and the soapstock obtained after the neutralization of oil produced by all three methods (Hadjiski, 1974).

The cotton oils were diluted with 95 $g.kg^{-1}$ ethanol until the desired concentrations were obtained. To the solutions was added 1 cm^3 0,3 mM ethanol solution of 2,2-diphenyl-1-picryl hydrazyl (DPPH). Blank and control samples were prepared (1 cm^3 0.3 mM ethanol solution of 2,2- diphenyl-1-picryl hydrazyl + 2.5 cm^3 ethanol). After 30 min at room temperature in the dark, the absorption of the samples and the blank were measured at 518 nm against ethanol (Mensor et al., 2001).

The antioxidant activity (AA) was calculated using the following equation:

$$AA\%=100- \{[(Abs._{sample}-Abs._{blank})x100]/Abs._{control}\} ,$$

where: $Abs._{sample}$ – absorption of the sample;
$Abs._{blank}$ – absorption of the blank;
$Abs._{control}$ – absorption of the control.

The activity of the tested cotton seed oils was compared to that of the synthetic butylhydroxytolene (BHT) and butylhydroxyanisole (BHA) and the natural antioxidants rutin and ascorbic acid through the comparison of the concentration ($\mu g.cm^{-3}$) for 50% inhibition of DPPH (IC_{50}).

The results were determined as the mean value of 5 replicates. For statistical data processing were used Sigma Plot 2002 and Microsoft Excel 2003 at confidence level $\alpha=0.05$ (Alekseev and Pahomov, 1987).

Results and Discussion

The antioxidant activity of the produced pressed and neutralized cotton seed oils was studied through the investigation of the inhibition of the DPPH radical.

Commercially produced refined sunflower and cotton seed oils were used as controls.

The data depicted on Figure 1 shows, that the highest percentage of free DPPH radical inhibition was obtained by the raw pressed cotton seed oil, where 81.25% inhibition was reached by 0.007 $\mu g.cm^{-3}$ oil concentration. The oil neutralized by method 1 exhibited lower activity. It allowed for 62.90% inhibition of the DPPH radical at 0.007 $\mu g.cm^{-3}$ concentration.

The high antioxidant activity of the raw oil and the neutralized by method 1 oil was probably due to the presence of the pigment gossypol. The rest of the studied oils (neutralized by methods 2 and 3) did not show antiradical activity. The absence of antioxidant activity proved that gossypol was removed completely from these oils.

The oils neutralized by methods 2 and 3 and the controls (refined sunflower and cotton seed oils) did not show antiradical activity against DPPH.

The antioxidant activity of the raw pressed cotton seed oil, containing gossypol, was compared to the activity of other antioxidants (Figure 2). The results show that the concentra-

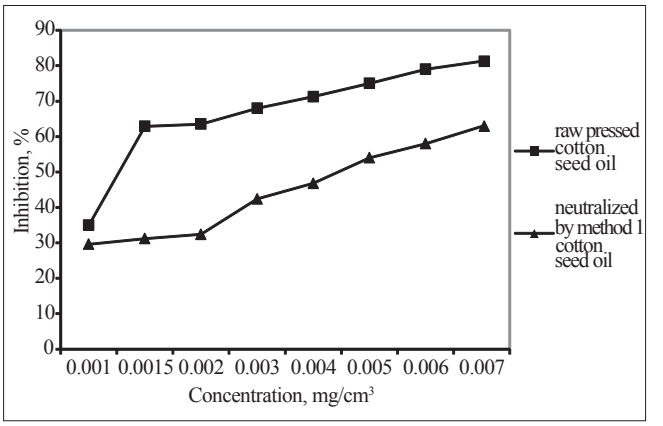

Fig. 1. Antioxidant activity of cotton seed oils against DPPH radical

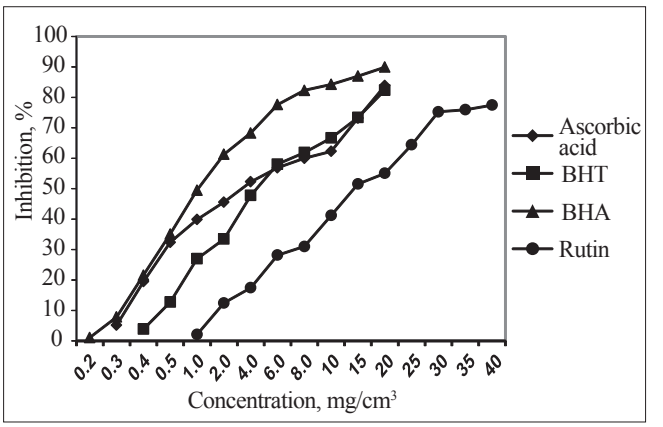

Fig. 2. Antioxidant activity of ascorbic acid, BHT, BHA and rutin against the DPPH radical

tion necessary for 80% inhibition of DPPH by ascorbic acid, BHT and BHA was 20 μg.cm^{-3}, and 40 μg.cm^{-3} rutin elicited 77.4 % inhibition. In comparison, the raw pressed cotton seed oil caused more than 80% inhibition of the DPPH radical at 0,006 μg.cm^{-3} concentration, which are several orders lower than the concentration of the antioxidants mentioned above. This proves that the gossypol in the raw pressed cotton seed oils is a significantly stronger antioxidant than the tested controls.

The high antiradical activity of the neutralized by method 1 oil clearly shows that the neutralization conditions did not eliminate the antioxidant gossypol completely (Figure 1)

The concentration, needed for 50% scavenging of the radical – IC_{50} is commonly used for comparison of the antioxidant activity of different biologically active substances. The lower its value, the stronger the antioxidant properties of the investigated substance are. The calculated IC_{50} for the tested cotton seed oils and four of the most common antioxidants are presented in Tables 1 and 2.

The data shows, that the raw pressed cotton seed oil had 3.28 times higher antioxidant activity than the neutralized by method 1 oil, i.e. partial removal of gossypol was achieved.

In comparison with the natural and synthetic antioxidants – rutin, ascorbic acid, BHT and BHA, the investigated oils (i.e. the gossypol in them) exhibited antioxidant activity which was several orders higher than that of the controls (refined sunflower and cotton seed oils). The raw pressed oil had antioxidant activity against the DPPH radical, which presented as IC_{50} concentration was 3159 times higher than the one exhibited by BHT, 3000 times higher than ascorbic acid, 800 times than BHA and more than 10000 times than rutin's activity.

The neutralized by method 1 oil had 958.69, 913.04 and 243.47 times higher activity based on IC_{50} concentration than BHT, ascorbic acid and BHA, respectively. Its activity was more than 3000 stronger than that of rutin.

The neutralized by methods 2 and 3 oils and the controls – refined sunflower and cotton seed oils did not show antioxidant activity.

During the neutralization of raw cotton seed oil gossypol and its derivatives pass in the soapstock, which was the reason to investigate its antioxidant activity against the DPPH radical. The corresponding IC_{50} is presented in Figure 3 and Table 3. The soapstock had lower antioxidant activity than the oil. The soapstock (produced during the neutralization by methods 2 and 3) showed maximal antiradical activity of 90.2 and 98.9%. The soapstock, obtained during

Table 1
Activity of the investigated cotton seed oils against the DPPH radical

Cotton seed oils	IC_{50}*	R^{2**}
Raw press cotton seed oil	$0.14.10^{-2}$	0.998
Neutralized by method 1 cotton seed oil	$0.46\ 10^{-2}$	0.997
Neutralized by method 2 cotton seed oil	0	
Neutralized by method 3 cotton seed oil	0	
Refined sunflower oil - control	0	
Refined cotton seed oil - control	0	

*concentration (μg.cm^{-3}) necessary for 50% inhibition of DPPH;
**R^2 – correlation coefficient

Table 2
Activity of natural and synthetic antioxidants against the DPPH radical

Antioxidants	IC_{50}*	R^{2**}
BHA	1.12	0.996
Ascorbic acid	4.20	0.994
BHT	4.41	0.998
Rutin	14.65	0.991

*concentration (μg.cm^{-3}) necessary for 50% inhibition of DPPH;
**R^2 – correlation coefficient

Table 3
Activity of cotton seed oil soapstock against the DPPH radical

Soapstock	IC_{50}*	R^{2**}
Soapstok from method 1	7.02	0.998
Soapstok from method 2	0.24	0.997
Soapstok from method 3	0.21	0.996

*concentration (μg.cm^{-3}) necessary for 50% inhibition of DPPH;
**R^2 – correlation coefficient

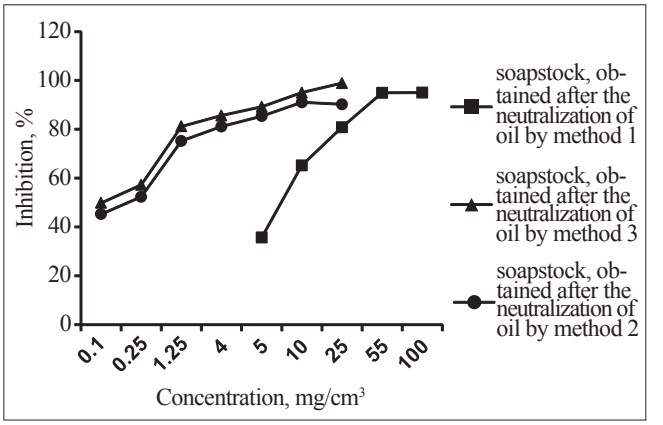

Fig. 3. Antioxidant activity of soapstock, obtained by neutralization of raw pressed cotton seed oil

method 1 neutralization, had maximal activity of 95.02%. IC_{50} for soapstock from methods 1, 2 and 3 was 7, 0.24 and 0.21 μg.cm^{-3}, respectively, i.e. the activity of soapstocks from method 2 and 3 was many times higher. The results clearly show that during neutralization methods 2 and 3 gossypol passed in the soapstock almost completely. The soapstock, obtained through method 1 neutralization exhibited lower antiradical activity than those produced by methods 2 and 3, i.e. remained in the oil.

Based on IC_{50}, the soapstock, obtained by methods 2 and 3 exhibited stronger antiradical activity against the DPPH radical than BHA, ascorbic acid and BHT.

The results from the investigation of the antioxidant activity of the raw cotton seed oil, the neutralized by the three methods oils and the soapstock obtained by the three methods, could be used as a criterion for gossypol removal from unprocessed cotton seed oil.

Conclusions

The raw pressed cotton seed oil possesses very high antioxidant activity, which is demonstrated by the value of IC_{50} – 0.14.10^{-2} g.dm^{-3}. The antioxidant activity of the raw oil, resulting from the presence of gossypol, is multiple times higher than that of rutin, ascorbic acid, BHT and BHA, based on their IC_{50}.

The method for oil neutralization influences the removal of gossypol and its derivatives. The most appropriate neutralization methods are 2 and 3 (neutralizing agent - 117 and 129 g.dm^{-3} Na OH, respectively). Gossypol is not present in the oils neutralized by these methods, which was proved by the absence of antiradical activity for these oils.

The neutralization of oil by method 1 (neutralizing agent - 105 g.dm^{-3} Na OH) does not lead to complete removal of gossypol, and due to this it exhibits a significant antioxidant activity - IC_{50} is 0.46.10^{-2} μg.cm^{-3}.

The application of methods 2 and 3 (neutralizing agent - 117 and 129 g.dm^{-3} Na OH, respectively) for oil neutralization causes the pigment gossypol to pass in the soapstock. This confirmed by the much higher antioxidant activity of the soapstock obtained by method 1.

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