Determination of the antioxidant activity of *Ginkgo biloba* leaves extract

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Summary
Nowadays, there is a growing interest in using natural antioxidants rather than synthetic ones. In this study, the antioxidant activity of *Ginkgo biloba*'s active compounds was investigated by addition of its extracts in vegetable oils. The rate of oxidation was determined by Oxidograph apparatus at the temperature of 110 °C. The collected leaves of *Ginkgo biloba* were extracted with ethanol and their antioxidant activity was compared with the commercial extract of *Ginkgo biloba* and also with the synthetic antioxidant BHT. It was found out, that both of ginkgo extracts demonstrated an antioxidant activity, but it was lower than the antioxidant activity of BHT.

Keywords
Ginkgo biloba; antioxidants; flavonoids; oils; extracts

Recent research in the field of antioxidants is aimed to improve the existing substances or to develop the new types of active molecules. Plants are supposed to be an alternative to the traditional chemical agents since they are known to represent a rich source of bioactive substances metabolized to nontoxic products. However, they are less effective compared with the commercial antioxidants and therefore their practical use is rather limited.

Antioxidants are found in various plant products such as fruits, vegetable, cereals, spices, teas and oils, which contain flavonoids, tannins, phenols, terpenoids and many others [1, 2]. In recent years, *Ginkgo biloba* is coming to the attention.

Especially the leaves of *Ginkgo biloba* contain compounds possessing an antioxidant character. There have been isolated three main compounds from *Ginkgo biloba* with an antioxidant activity – kaempferol, quercetin and isoharmnetin [3]. The antioxidant activity of a ginkgo extract is determined mainly by flavonoids, which scavenge and destroy free radicals and the reactive forms of oxygen [4]. The activated oxygen forms such as peroxide, hydrogen peroxide, hydroxyl radical and singlet oxygen may cause various diseases such as carcinogenesis, inflammation, atherogenesis, as well as food deterioration, for which the naturally occurring antioxidants may be effective [1].

Ginkgo biloba (*Ginkgoaceae*) is probably the oldest species of tree known, dating back to 300 million years and it is often called the "living fossil". The female trees produce a fruit with an orange or yellow flesh surrounding a hard, tan shell containing the kernel of the seed, which is edible [2]. More than twenty ginkgo variations exist that differ in tree habitat and in shape, colour and size of leaves, the latter being the raw material for the pharmaceutical industry [4].

Ginkgo biloba is a tree with a long history in the traditional Chinese herbal medicine [5]. It was first mentioned in the major Chinese medical texts dating back to 1436 AD during the Ming dynasty. Nowadays, ginkgo is marketed under the two standardized extracts. The extracts are prepared in multiple steps, which may vary from producer to producer. During the process, some compounds are enriched and others are removed. Ginkgolic acids are claimed to be allergenic and therefore should be removed from phytopreparations. In the standardized extract of *Ginkgo biloba* the concentration of ginkgolic acids is lower than 10 $mg.kg^{-1}$. The final extract contains a large number of constituents from various classes. The firstly developed was EGb 761 (Tanakan®, Ipsen, France) containing 24% of flavonoid glycosides and 6% of terpene lactones. The second extract is labelled LI

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1370 (Lichtwer Pharma, Köln, Germany), which contains 27% flavonoid glycosides and 7% terpene lactones. The other compounds present in *Ginkgo biloba* extracts are proanthocyanidins (7%), carboxylic acids (13%), catechins (2%), non-flavonol glycosides (20%) and others [6].

The primary active constituents of its leaves include flavonoids and unique diterpenes well known as ginkgolides, the latter are considered inhibitors of the platelet-activating factor [5]. By antagonizing these platelet-activating factors, *Ginkgo biloba* may help to protect brain tissue against damage during trauma. Flavonoid glycosides are thought to be responsible for its free radical scavenging abilities. Free radicals are metabolites that are released as a result of tissue trauma and other physiological reactions. These metabolites have been implicated in the degeneration of nerve tissue [6].

Clinical studies showed that ginkgo extracts exhibit therapeutic effects in a variety of diseases including Alzheimer's disease, memory loss, age-related dementia, cerebral and ocular blood flow occlusion, premenstrual problems and altitude sickness. Because of its potent antioxidant properties and ability to enhance a peripheral and cerebral circulation, ginkgo shows a prospective value in the treatment of cerebrovascular dysfunctions and peripheral vascular disorders [5]. It also helps during the treatment of glaucoma [7]. Several studies assume its neuroprotective properties. *Ginkgo biloba* can enhance concentration, improve memory, but there was no effect observed on short memory [6]. There have been no studies aimed at the application of *Ginkgo biloba* extract as a potential natural antioxidant used in food industry. The goal of this research was to investigate the antioxidant capacity of *Ginkgo biloba* leaves extract in comparison with a synthetic antioxidant and its possibility to increase the oxidative stability of our common oils.

MATERIAL AND METHODS

Reagents and standards

Butylated hydroxytoluene (BHT) was purchased from Sigma-Aldrich Chemi (Deisenhofen, Germany). The commercial extract of *Ginkgo biloba* (EGB2) was purchased from AHD International (Atlanta, Georgia, USA).

Sunflower and rapeseed oils were processed from Slovakian oilseed cultivars by expeller pressing and solvent extraction, crude oil was refined on an industrial scale and deodorized in Palma-Tumys (Bratislava, Slovak Republic). The leaves of *Ginkgo biloba* (EGB1), collected in Bratislava during August, were extracted with ethanol under laboratory conditions. The preparation of the extract from *Ginkgo biloba* leaves is given below.

Preparation of ethanol extract from *Ginkgo biloba* leaves

Ginkgo biloba extract was prepared as follows: The leaves were cut and dried into 2–5 mm pieces. After that, leaves (100 g) were mixed with a solvent (900 ml; 96% v/v aqueous ethanol), refluxed gradually for 30 min in Soxhlet extractor and left aside to cool overnight at an ambient temperature. The suspension was filtered over Büchner funnel and the residue was washed twice, each time with 100 ml of the same solvent. The filtrates were combined and the solvent was evaporated under reduced pressure. The extract of a dark green greasy consistency was transferred in a conical flask filled with argon and stored in the dark in a refrigerator at –20 °C. During the extraction and sample preparation, the laboratory glass was covered with aluminium foil to protect the extract against the light.

Specification of basic parameters

The basic chemical parameters (acid value, peroxide value and iodine value) of the oils were determined according to procedures mentioned in the Slovak norms STN [8-10]. Estimated chemical parameters are given in Tab. 1. All measurements were repeated three times; the mean values and the respective standard deviations were reported.

The fatty acid composition was determined by a gas capillary chromatography. Fatty acids were first converted into the respective methyl esters [11] and then separated on a wide - bore capillary column Supelcowax 10 (30 m length, 0.75 mm i.d., and 1.0 μm film thickness) in a Hewlett-Packard 5890 series II gas chromatograph (Hewlett-Packard, Palo Alto, California, USA) equipped with a flame ionization detector at the temperature of 240 °C [12].

Determination of antioxidant activity

To monitor the oxidation of sunflower and rapeseed oil and to determine the antioxidant activity of the extract of *Ginkgo biloba*, Oxidograph apparatus (Mikrolab Aarhus, Aarhus, Denmark) was used.

The Oxidograph was designed to monitor the oxidation of oil and fats measured by the consumption of oxygen by means of pressure transducers. Oxidation results in pressure change that leads to the increase of vacuum. The extent of oxidation is recorded on a six-channel recorder and PC where signals are shown as a function of time. Conditions of the measurement were as follows: the tempera-

Tab. 1. Basic chemical parameters of sunflower and rapeseed oil.

Type of vegetable oil	Parameter		
	Iodine value (g I_2/100 g)	Peroxide value (mmol $^1/_2O_2$/kg)	Acid value (mg KOH/g)
Sunflower oil	132.44 ± 0.73	$1.05 \pm 1.8 \times 10^{-2}$	$0.18 \pm 5.1 \times 10^{-3}$
Rapeseed oil	109.22 ± 0.61	$0.78 \pm 1.3 \times 10^{-2}$	$0.12 \pm 6.6 \times 10^{-3}$

Tab. 2. Fatty acid composition [% of area] of sunflower and rapeseed oil.

Type of vegetable oil	Fatty acid [area %]					
	16:0	18:0	18:1	18:2	18:3	others
Sunflower oil	5.38	4.02	26.51	60.85	0.74	2.50
Rapeseed oil	4.46	2.01	61.84	19.48	8.43	3.78

16:0 - palmitic acid, 18:0 - stearic acid, 18:1 - oleic acid, 18:2 - linoleic acid, 18:3 - linolenic acid.

ture of oxidation was 110 °C, the weight of oil 5 g and the initial flush of oxygen 2–4 $l.min^{-1}$.

The Oxidograph is measuring up to overreach of the induction period. After that the value of induction period (*IP*) is deducted by the method of tangents applied to two parts of the oxidation curve. Each analysis was repeated in triplicate; mean values and standard deviations were calculated. Oils without any additives were analysed as blanks and the antioxidant efficiency was expressed as the protection factor: $PF = IP/IP_0$, where *IP* is an induction period of oil with addition of an antioxidant and IP_0 is an induction period of oil without the addition of the antioxidant [13].

Stabilizing oils by *Ginkgo biloba* extract

To stabilize a sunflower (SO) and rapeseed oil (RO), the ethanol extract of *Ginkgo biloba* leaves (EGB1) was used. To compare its antioxidant activity, the commercial extract of *Ginkgo biloba* (EGB2) and the addition of BHT were also used. The concentration of the extracts in oils was 0.2; 0.5; 0.7; 1% by weight and the concentration of BHT in oils was 100 $mg.kg^{-1}$.

Statistical analysis

The statistical analysis was carried out with the program Statgraphics Plus, Version 1.4 for Windows (Manugistic, Rockville, USA). The significance of differences between mean values was determined at the $p = 0.05$ (5%) level, using a one way analysis of the variance and the t-test.

RESULTS AND DISCUSSION

Common chemical characteristics and the fatty acid composition of industrial sunflower and rapeseed oils are shown in Tab. 1 and Tab. 2. The unsaturated fatty acid contents corresponded to values currently observed among the respective oils grown in Central Europe.

The oxidative stability of the oils was gauged by the results measured using the Oxidograph apparatus. The antioxidant activity of the extracts was expressed as the protection factor *PF*. The values of $PF > 1$ indicate an antioxidant activity, the value of $PF = 1$ corresponds to no antioxidant activity and the values of $PF < 1$ mean prooxidative activity. The values of a protection factor for a sunflower and rapeseed oil without antioxidants and with addition of extract from *Ginkgo biloba* leaves (EGB1) and commercial extract of *Ginkgo biloba* (EGB2) are given in Tab. 3 and Tab. 4. The protection factor for BHT (at the concentration of 100 $mg.kg^{-1}$) added into a sunflower oil was $1.24 \pm 3.1 \times 10^{-3}$ and for a rapeseed oil it was $1.19 \pm 4.3 \times 10^{-3}$.

Analyzing the results that are given in Tab. 3 and 4 it was found out, that the both extracts showed antioxidant activity and increased oxidative stability of sunflower and rapeseed oil. As can be seen from Tab. 3, a better stabilizing effect in the rapeseed oil was achieved by the commercial extract of *Ginkgo biloba*. At the concentration of 0.2% by weight there was a growth of the oxidative stability by about 22% with addition of the commercial extract (EGB2), while for the ethanol extract (EGB1) there was a growth of the oxidative stability by only 6%. The highest investigated concentration caused (addition of 1% by weight) a 34% growth of the oxidative stability in the case of the commercial extract, while for the ethanol extract it was lower by 21%. Similar results were obtained for sunflower oil. The commercial extract of *Ginkgo biloba* had a better stabilizing ef-

fect than the ethanol extract of *Ginkgo biloba* leaves, even though the difference between the values of the protection factor was smaller. At the concentration of 0.2% by weight the difference between the protection factors of both extracts was only 0.02, at the concentration of 1% by weight there was a 34% growth of oxidative stability for the commercial extract (EGB2), while the ethanol extract (EGB1) gave about 6% lower growth in than the commercial extract. Both extracts were then compared with the synthetic antioxidant - butylated hydroxytoluene (BHT) used as a food additive. Even if BHT was added into oils only at the concentration 100 $mg.kg^{-1}$, its stabilizing effect was comparable with protection factors given by both extracts at the lowest tested concentration (0.2% concentration by weight). As mentioned earlier, the protection factor for BHT in the rapeseed oil was 1.19, while for the commercial extract of *Ginkgo biloba* it was 1.22 and for the ethanol extract of *Ginkgo biloba* leaves it was mere 1.06. In sunflower oil the value of the protection factor for BHT was 1.24 and similar results were obtained for both extracts (EGB1 = 1.20 and EGB2 = 1.22 at the concentration 0.2% by weight). The results can thus be summarized by stating that BHT has a better antioxidant activity in the both vegetable oils than the extracts from *Ginkgo biloba*. The next investigation should be aimed at increasing the antioxidant activity by optimization of the extraction method and isolation or fractionation of the antioxidant active compounds from *Ginkgo biloba*

According to the results given in Tab. 3 and Tab. 4, *Ginkgo biloba* extracts did not stabilize the rapeseed and the sunflower oil in the same way. Both extracts showed higher antioxidant activity in the more polyunsaturated sunflower oil. The reason for the different antioxidant activity may be the fatty acid content of oils, the different scale of non-lipid compounds such as tocopherols (gama-tocopherol in rapeseed oil, alfa-tocopherol in sunflower oil), sterols and others and the interactions between them and the further compounds found in the extracts. The same stabilization effect of natural antioxidants in vegetable oils, resulting in the higher antioxidant activity of antioxidants in sunflower oil, was also examined in the study of VAJDÁK et al. [14], where natural lycopene was added to the oils.

Tab. 3. Values of induction period and protection factor for rapeseed oil with and without addition of antioxidants.

Concentration [% by weight]	Type of antioxidant			
	EGB1		EGB2	
	IP [h]	*PF*	*IP* [h]	*PF*
0	$4.59 \pm 2.4 \times 10^{-2}$	1.00	$4.59 \pm 2.4 \times 10^{-2}$	1.00
0.2	$4.87 \pm 2.5 \times 10^{-2}$	$1.06 \pm 4.9 \times 10^{-3}$	$5.60 \pm 1.4 \times 10^{-2}$	$1.22 \pm 3.1 \times 10^{-3}$
0.5	$4.96 \pm 1.7 \times 10^{-2}$	$1.08 \pm 3.5 \times 10^{-3}$	$5.88 \pm 1.7 \times 10^{-2}$	$1.28 \pm 3.7 \times 10^{-3}$
0.7	$5.09 \pm 2.2 \times 10^{-2}$	$1.11 \pm 4.7 \times 10^{-3}$	$5.97 \pm 1.5 \times 10^{-2}$	$1.30 \pm 3.2 \times 10^{-3}$
1	$5.19 \pm 1.2 \times 10^{-2}$	$1.13 \pm 2.6 \times 10^{-3}$	$6.20 \pm 2.2 \times 10^{-2}$	$1.34 \pm 4.8 \times 10^{-3}$

IP - induction period, *PF* - protection factor, EGB1 - ethanol extract of *Ginkgo biloba*, EGB2 - commercial extract of *Ginkgo biloba*.

Tab. 4. Values of induction period and protection factor for sunflower oil with and without addition of antioxidants.

Concentration [% by weight]	Type of antioxidant			
	EGB1		EGB2	
	IP [h]	*PF*	*IP* [h]	*PF*
0	$2.38 \pm 1.3 \times 10^{-2}$	1.00	$2.38 \pm 1.3 \times 10^{-2}$	1.00
0.2	$2.86 \pm 1.5 \times 10^{-2}$	$1.20 \pm 6.1 \times 10^{-3}$	$2.90 \pm 0.86 \times 10^{-3}$	$1.22 \pm 3.6 \times 10^{-3}$
0.5	$2.90 \pm 1.0 \times 10^{-2}$	$1.22 \pm 4.3 \times 10^{-3}$	$2.95 \pm 0.98 \times 10^{-3}$	$1.24 \pm 4.1 \times 10^{-3}$
0.7	$2.98 \pm 1.3 \times 10^{-2}$	$1.25 \pm 5.4 \times 10^{-3}$	$3.14 \pm 1.3 \times 10^{-2}$	$1.32 \pm 5.4 \times 10^{-3}$
1	$3.02 \pm 0.90 \times 10^{-2}$	$1.27 \pm 3.8 \times 10^{-3}$	$3.17 \pm 0.69 \times 10^{-3}$	$1.33 \pm 2.9 \times 10^{-3}$

IP - induction period, *PF* - protection factor, EGB1 - ethanol extract of *Ginkgo biloba*, EGB2 - commercial extract of *Ginkgo biloba*.

CONCLUSION

The goal of this paper was to analyse the effects of *Ginkgo biloba* extracts on the antioxidant status of the sunflower and rapeseed oil with the Oxidograph device. According to the results, we have found that both extracts showed antioxidant activity. Under our test conditions the commercial extract of *Ginkgo biloba* seems to be more effective in stabilizing oils than the laboratory samples of ethanol extracts of *Ginkgo biloba*. The lower antioxidant activity of a laboratory prepared extract of *Ginkgo biloba* can be caused by the mean of storage of the extract, the used extraction method and the influence of other factors. Nonetheless important reason is that during processing of commercial extract some compounds are removed and the amount of others is changed [15] so that the final antioxidant activity could be quite different from the *Ginkgo biloba* extract prepared in laboratory. Optimization of extraction conditions, isolation or fractionation of the active antioxidant compounds will be investigated in due course.

REFERENCES

1. Rhee, D. - Myers, J.: Complementary and alternative medicine for glaucoma. Survey of Ophthalmology, *46*, 2001, 1, pp. 43-55.
2. Máriássyová, M.: Antioxidant activity of some herbal extracts in rapeseed and sunflower oils. Journal of Food and Nutrition Research, *45*, 2006, 3, pp. 104-109.
3. Spence, K. E. - Jane, J.: Chemical and physical properties of ginkgo starch. Carbohydrate Polymers, *40*, 1999, pp. 261-269.
4. Ellain Wojtaszek, M. - Krucynski, Z. - Kasprzak, J.: Variations in free radical scavenging activity of *Ginkgo biloba* leaves in the period of complete development of green leaves to fall of yellow ones. Food Chemistry, *79*, 2002, pp. 79-84.
5. Moulton, P. L. - Boyko, L. N.: The effect of *Ginkgo biloba* on memory in healthy male volunteers. Physiology and Behaviour, *73*, 2001, pp. 659-665.
6. Byeoung-Soo, P. - Sung-Eun, L.: Antioxidative activity of *Ginkgo biloba* leaves - derived components on free radicals and active oxygen species. Food Science and Biotechnology, *9*, 2000, pp. 317-321.
7. Ndjoko, K. - Wolfender, J. - Hostettmann, K.: Determination of trace amounts of ginkgolic acids in *Ginkgo biloba* leaf extracts and phytopharmaceuticals by liquid chromatography - electro spray mass spectrometry. Journal of Chromatography B, *744*, 2000, pp. 249-255.
8. STN EN ISO 660:2001-08. Animal and vegetables fats and oils. Determination of acidity and of acid value and of acidity. Bratislava : Slovak Standards Institute SUTN, 2001. 12 pp.
9. STN EN ISO 3960:2005-11. Methods of testing for fats and oils. Determination of peroxide value. Bratislava : Slovak Standards Institute SUTN, 2005. 12 pp.
10. STN 58 0101/Z6. Methods of test for fats and oils. Bratislava : Slovak Standards Institute SUTN, 2001. 12 pp.
11. Christopherson, S. W. - Glass, R.: Preparation of milk fat methyl esters by alcoholysis in an essentially non-alcoholic solution. Journal of Dairy Science, *52*, 1969, pp. 1289-1290.
12. Niklová, I. - Schmidt, Š. - Habalová, K. - Sekretár, S.: Effect of evening primrose extracts on oxidative stability of sunflower and rapeseed oils. European Journal of Lipid Science and Technology, *103*, 2001, pp. 299-306.
13. Davídek, J. - Pokorný, J. - Janíček, J.: Chemie potravin. 1st. ed. Praha : SNTL/Alfa, 1983. 632 pp.
14. Vajdák, M. - Schmidt, Š. - Ližičárová, I. - Zahradníková, L. - Sekretár, S.: Vplyv lykopénu a antioxidantov na oxidačnú stabilitu slnečnicového a repkového oleja. Bulletin potravinárskeho výskumu, *43*, 2004, pp. 219-228.
15. Liu, C. S. - Cheng, Y. - Zhang, W. - Chen, N. H. - Zhang, J. T.: Comparison of antioxidant activities between salvianolic acid B and *Ginkgo biloba* extract (EGb 761). Acta Pharmacologica Sinica, *27*, 2006, pp. 1137-1145.

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