COMPARATIVE STUDY ON LIPID COMPOSITION OF FALSE INDIGO (*AMORPHA FRUTICOSA* L.) SEEDS AFTER THEIR HYDROTHERMAL TREATING

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False indigo (*Amorpha fruticosa* L*).* fruits were steam distilled for recovery of essential oil. The content and composition of biological active substances – phospholipids, sterols and tocopherols and their changes after hydrothermal treating were studied. Phosphatidylinositol (30.6%), phosphatidylcholine (29.7%) and phosphatidylethanolamine (25.5%) were found to be the main components in the phospholipid fraction. β-sitosterol (69%) predominated in the sterol fraction. γ-tocopherol (37.4%) and α-tocopherol (27.0%) were the main components in the tocopherol fraction. The decrease of amounts of phospholipids and tocopherols after treating was established. Gas liquid and high performance liquid chromatographic analysis of sterols and tocopherols, obtained from the treated seeds, indicate the occurrence of degradation processes.

false indigo; *Amorpha fruticosa* L.; phospholipids; sterols; tocopherols

INTRODUCTION

False indigo *(Amorpha fruticosa* L.), fam. Fabaceae is a perennial bush rich in essential oil (mono- and sesquiterpene hydrocarbons) which is applied in cosmetics and perfumery. Besides essential oil the seeds contain glyceride oil whose amount ranges from 8.7% to 11.0%. Mouhamedova et al. (1987) and Lee, Shin (1977) established 1.3–3.0% phospholipids (mainly phosphatidylcholine). Sterols were identified in free form (4.4%) and esterified form (14.4%) in the oil. Hydrodistillation is one of the most commonly used procedures for production of essential oils. The glyceride oil is extracted from the seeds by nonpolar solvent as n-hexane after distillation. During this deep hydrothermal process the oil is continuously heated at high temperatures in the presence of moisture and atmospheric oxygen. Under these conditions oxidation, polymerization and degradation occur. These reactions lead to the changes in the content and composition of the treated oil and its components. There is a little information concerning the effect of hydrothermal treating of the content and composition of the biological active substances of the glyceride oil such as phospholipids, sterols and tocopherols.

The main object in the present work is to study the content and composition of Bulgarian false indigo (*Amorpha fruticosa* L). seed glyceride oil and the changes of the phospholipids, sterols and tocopherols after their hydrothermal treating.

MATERIALS AND METHODS

Fruit material. The investigated seeds were provided from the Plovdiv region in South Bulgaria, crop 1998. The investigations were carried out on air dried seeds in technical ripeness.

Essential oil isolation. Fresh ground seeds were steam distilled for 3 h 30 min using a laboratory glass apparatus of British Pharmacopeia, modified by Balinova and Djakov (1974) and the residue was dried under room temperature.

Glyceride oil isolation. The oils were extracted in Soxhlet apparatus with n-hexan for 8 h. After rotation vacuum distillation of the solvent the extracted oils were weighed.

Phospholipid composition. Lipids were extracted from the seeds by procedure of Folch et al. (1957). Polar lipids were divided from unpolar lipids by column chromatography (Kates, 1972). The phospholipid constituents were separated by two-directional thin-layer chromatography on Silica gel 60 G "Merck", impregnated with 1 g per 100 g $(NH_4)_2SO_4$ water solution (Beshkov, Ivanova, 1972). The first direction was carried out in chloroform : methanol : ammonia 65 : 25 : 5 v/v/v and second in chloroform : methanol : ammonia : acetic acid : water 50 : 20 : 10 : 10 : 5 v/v/v/v/v. The spots of the separated individual phospholipids were identified by spraying with specific reagents (Kates, 1972). In addition, R_F and standard spots were used for definitive identification. The quantitative evaluation was carried out spectrophotometrically at 700 nm (Beshkov, Ivanova, 1972).

Sterol composition. The free and esterified sterols were separated from the other oil constituents by preparative TLC on Silica gel 60 G "Merck" and mobile phase n-hexan : diethyl ether 1 : 1. The esterified sterols were saponified with ethanolic KOH, extracted and purified by TLC. The quantitative evaluation was carried out spectrophotometrically (Ivanov et al., 1972). Individual composition was identified by gas chromatography, using HP 5890 A unit with FID, 25 m capillar column impregnated with OV-17 and conditions as follows:

– column temperature 260–300 °C, 6 °C/min
– detector temperature 320 °C, injector temperature 300 °C
– gas carrier – nitrogen.

Identification was confirmed by retention time comparison of the individual constituents with those of authentic samples.

Tocopherol composition. Tocopherols and tocotrienols were analyzed directly in the oils by HPLC with fluorescence detection (ISO, 1989; Ivanov, Aitzetmüller, 1995). "Merck-Hitachi" unit fitted with column "Nucleosil" Si 50-5 250 x 4 mm and fluorescent detector "Merck-Hitachi" F 1000 was used. The operating conditions were as follows: excitation 295 nm, emmision 330 nm, mobile phase n-hexan : dioxan 94 : 4, rate of mobile phase 1 cm^3/min. The peaks were identified using authentic individual tocopherols.

RESULTS AND DISCUSSION

The data about general composition of investigated samples have been presented in Table I. Compared to the quantities reported earlier by Lee and Shin (1977) a lower amount of oil was established. The quantities of phospholipids and tocopherols in the oils after hydrothermal treating is significantly lower in comparison with those in the oil from raw seeds. The content of sterols did not change significantly.

Phospholipid composition is presented in Table II. The obtained results show that there is no difference in qualitative composition. All major phospholipid classes are presented. Phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol were the main constituents in the phospholipid fraction recovered from the raw seeds. High levels of lysophosphatidylcholine (22.8%) and phosphatidic acids (10.7%) were estimated in the oil from hydrothermal treated oil as a result of hydrolyse processes.

Total content of sterol in both samples of oils (Table III) was found to be 0.9%. This percentage is significantly lower than the data reported by Lee and Shin (1977) – 18.8% total content. The greatest part of the sterols are presented as sterol esters (68.7% in oil from raw seeds and 59.6% in oil from treated seeds). The obtained ratio free : esterified sterols differ from sterol distribution in the majority of vegetable oils where free sterols predominated. Sterol fraction of *Amorpha fruticosa* L. boil consisted mainly of β-sitosterol, followed by campesterol, stigmasterol and minute amounts of cholesterol, Δ^5-avenasterol, Δ^7-avenasterol and Δ^7-stigmasterol. Higher amounts of stigmasterol in both sterol fractions were established in free form (17.0% and 13.5% in raw and treated seeds, respectively), then in sterol esters (9.6% and 4.2%, respectively) as a result of hydrolyse processes. The content of the more unsaturated (with two double bonds) stigmasterol derivatives decreased from 19.9% to 15.4% in free form and from 11.0% to 7.2% in sterol esters. This decreasing was balanced mainly by a higher percentage of β-sitosterol from 69.0% to 75.2% in free form and 74.2% to 79.0% in sterol esters. In the other sterol constituents a marked differences were not observed.

Table IV illustrates the variation of tocopherol composition. All tocopherols (T) and tocotrienols (T-3) were identified in the oils. Tocopherols were found to be the major part in both oils gin raw and treated seeds – the

I. General composition of *Amorpha fruticosa* L. seeds

Compounds	Content in raw seeds (% wt)	Content in seeds after hydrothermal treating (% wt)
Oil in seeds	6.8	3.1
Phospholipids in oil	4.0	0.5
Sterols in oil	1.0	0.9
Tocopherols in oil (mg/kg)	2670.0	1120.0

II. Phospholipid composition of *Amorpha fruticosa* L. seed oil (mean of three replicates)

Phospholipids	In raw seeds (% wt)	In treated seeds (% wt)
Phosphatidylcholine	29.7	20.7
Phosphatidylinositol	30.6	17.5
Phosphatidylethanolamine	23.4	13.2
Phosphatidic acids	3.6	6.7
Lysophosphatidylcholine	1.6	22.8
Lysophosphatidylethanolamine	2.1	6.2
Monophosphatidylinositol	2.3	2.2
Diphosphatidylinositol	6.7	6.7

III. Sterol composition of *Amorpha fruticosa* L. seed oil (mean of three replicates)

Sterols	Raw seed content (% wt)		Treated seeds content (% wt)	
	free sterols	esterified	free sterols	esterified
Cholesterol	2.4	3.8	1.9	3.4
Campesterol	4.8	5.2	5.4	5.8
Stigmasterol	17.0	9.6	13.5	4.2
β-sitosterol	69.0	74.2	75.2	79.0
Δ^5-avenasterol	1.5	3.1	1.1	1.4
Δ^7-avenasterol	2.3	2.6	1.0	3.2
Δ^7-stigmasterol	2.9	1.4	1.9	3.0
Ratio free : esterified sterols	67.8	32.2	59.6	40.4
Ratio sterols with 1 : 2 double bonds	80.1 : 19.9	87.8 : 12.2	84.6 : 15.4	92.8 : 7.2

ratio T : T-3 was 76.9 : 23.1. γ-tocopherol and α-tocopherol were the predominant components in raw and treated seed oils. The oil of treated seeds contains higher levels of saturated derivatives – the ratio T : T-3 was found to be 96.4 : 3.6 as γ-tocopherol and α-tocopherol increased to 43.2 and 31.8%, respectively, while β-tocotrienol decreases from 10.4% to 1.0%.

IV. Tocopherol composition of *Amorpha fruticosa* L. seed oil (mean of three replicates)

Tocopherols	Raw seeds content (%)	Treated seeds content (%)
α-tocopherol	27.0	31.8
α-tocotrienol	7.3	1.3
β-tocopherol	9.8	14.6
β-tocotrienol	10.4	1.0
γ-tocopherol	37.4	43.2
γ-tocotrienol	4.9	2.0
δ-tocopherol	2.7	5.7
δ-tocotrienol	0.5	0.3
Ratio tocopherols : tocotrienols	76.9 : 23.1	96.4 : 3.6

CONCLUSION

As a result of the hydrothermal treating of false indigo (*Amorpha fruticosa* L). seeds, the content of the biological active substances, such as phospholipids, sterols and tocopherols, was significantly changed. Phospholipids were thermically unstable and upon action of the heat and water vapours were hydrolyzed to phosphatidic acids, lysophospha-tidylcholine and lysophosphatidylethanolamine. In the sterol and tocopherol fractions the hydrothermal treating leads to decrease of the content of sterols and tocopherols and particularly of unsaturated derivatives as stigmasterol and tocotrienols.

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ZLATANOV, M. – STOJANOVA, A. – GEORGIEV, E. (University of Plovdiv, Plovdiv, Bulgaria; Higher Institute of Food and Flavor Industries, Plovdiv, Bulgaria):

Porovnávací studie o složení lipidů semen netvařce křovitého (*Amorpha fruticosa* L.) po jejich hydrotermálním moření.

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Plody netvařce křovitého (*Amorpha fruticosa* L.) jsme destilovali v páře, aby se regenerovala silice. Studovali jsme obsah a složení biologicky aktivních látek – fosfolipidů, sterolů a tokoferolů – a jejich změny po hydrotermálním moření. Zjistili jsme, že fosfatidylinostol (30,6 %), fosfatidylcholin (29,7 %) a fosfatidyletanolamin (25,5 %) jsou hlavními složkami fosfolipidní frakce. β-sitosterol (69 %) převládal ve frakci sterolu, γ-tokoferol (37,4 %) a α-tokoferol (27,0 %) byly hlavními složkami v tokoferolové frakci. Dále jsme zjistili pokles množství fosfolipidů a tokoferolů po moření. Analýza sterolů a tokoferolů pomocí plynové chromatografie a vysokoúčinné kapalinové chromatografie sterolů a tokoferolů z mořeného osiva naznačuje výskyt degradačních procesů.

netvařec křovitý; *Amorpha fructicosa* L.; fosfolipidy; steroly; tokoferoly

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