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THE YIELD AND QUALITY OF MILK THISTLE [*SILYBUM MARIANUM* (L). GAERTN.] SEED OIL FROM THE PERSPECTIVE OF ENVIRONMENT AND GENOTYPE – A PILOT STUDY

VÝNOS A KVALITA OLEJE PLODŮ OSTROPESTŘCE MARIÁNSKÉHO [*SILYBUM MARIANUM* (L). GAERTN.] Z POHLEDU PROSTŘEDÍ A GENOTYPU – PILOTNÍ STUDIE

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During the years 2007 – 2008, samples of Milk thistle [*Silybum marianum* (L.) Gaertn.] variety Silyb (silybine type, CZE) from five Czech growing regions were assessed for seed oil content (%) and neutralization number. The second part included six different genotypes with the evaluation of oil content (%), the spectrum of main fatty acids, neutralization number (NN). The analyses showed highly significant differences in the seed oil content for environment and year conditions. The highest average oil content had the sample from cereal region 2 (21.28 %), the lowest value was in potato region 2 (18.97 %). In 2007, the oil content was 20.30 %, in 2008 – 19.59 %. The NN was significantly affected also by both factors. Regarding the genotypes, the highest oil content had variety Silyb – silybine type (21.6 %), followed by the variety Mirel (21.0 %). The lowest content of oil was recorded in Rosa Canina and Leros samples (17.5 % and 18.1 %). The neutralization number (NN) varied highly in the samples (4.3 – 33.0). For all samples dominant fatty acid (FA) was linoleic, followed by oleic, palmitic and stearic acid and another minor FA (0.03 till 0.7 %). The highest amount of linoleic acid was recorded in variety Mirel (66.4 %), the lowest content had Serbian sample (50.6 %).

Key words: milk thistle, *Silybum marianum*, seed oil, fatty acids, environment

Milk thistle [*Silybum marianum* (L.) Gaertn.], a medicinal plant from Apiaceae (*Umbelliferae*) family, is cultivated for the seeds, achenes, which contain flavonolignans, known as silymarin complex. This substance is used in therapy for its hepatoprotective activity (Habán et al., 2009). Milk thistle is also an alternative oil crop while the side product of seed pressing is fatty oil with high content of polyunsaturated fatty acids. Among them, linoleic acid prevails (Buchta et al., 2010). The oil is used in animal nutrition, cosmetology, dermatology for its specific properties (Součková, 2011). It is suitable for the production of environmental friendly organic coatings together with the interesting price (Středa, 2003).

The area of milk thistle varies from 700 to 4500 ha. There are two varieties in the Czech Republic, one is registered with the Plant Variety Rights (PVR), second has the PVR only. Variety Silyb was registered for the isolation of silymarin complex in pharmaceutical industry. Mirel variety was applied to PVR for seed oil content and fatty acids composition but was not spread last year (Součková, 2011; ÚKZÚZ, 2011). Milk thistle production is interested for a lot of farmers and agricultural companies but the certified seed material is not available on the market. The aim of the work was to assess oil content and neutralization number of milk thistle fruits from different localities in the Czech Republic during two years. In the second part, we have tested the oil content and fatty acids spectrum in different genotypes or provenances.

Material and methods

In the first part of the experiments, the samples from five growing regions in the years 2007 – 2008 were assessed for seed oil content (%) and neutralization number. These samples from silybine type seeds of variety Silyb were sampled from the production plots in amount of 1.5 kg. Localities covered two potatoes growing regions (P1, P2), two cereals growing regions (C1, C2) and one sugar beet growing region (S). All analyses were performed six months after the harvest.

The second part of the research covered six samples of different genotypes of Milk thistle obtained from companies, gene banks and from the food supplement market. The samples were from the years 2009 – 2010, but the year was not a factor of the interest in this part of assessment. Varieties Silyb (silybine and silydianine type) and Mirel were grown in the Czech Republic. The samples from Czech Republic (CZE1 and CZE2) were from the market. As we found out by the questionnaire, they were Czech origin, but without the description of the variety and locality. Serbian genotype had no description. The aim of this part was to evaluate the oil content (%), the spectrum of main fatty acids in the oil (% ratio) and neutralization number (NN).

The total oil content was evaluated by the gravimetric method in %. 20.0 g of oil seed and was mixed with 500.0 mL of petroleum ether in evaporation flask. This mixture was stirred in water bath (40 ^{o}C) for 2 hours. The mixture was filtered and the filtrate was distilled under vacuum. After distillation of majority of the mixture, petroleum ether was spilled over and the distillation under vacuum was continued. The flask with the rest of seed oil was weighted. The neutralization number was tested by the method of Ph. Eur. VI. (2008). The sample were subjected to titration by the alcoholic solution of potassium hydroxide on phenolphthalein till to mild pink colour.

In the extracted oil samples fatty acids profiles were evaluated by gas chromatography with flame- ionization detector (GC/FID). Extracted oil (40 – 60 mg) was dissolved in isooctane and homogenized by ultrasound. After that sodium methanolate was added and the mixture was boiled under reverse condenser. BF3 was added, after that, isooctane and saturated solution of NaCl was mixed with hot liquid. Methyl esters of fatty acids (FAMEs) were shortly and intensively shaken out into isooctane. Organic and water phase were separated and FAMEs were analyzed by GC on HP4890 with capillary column DB-23, in temperature program from 100 °C * 3 min * 10 °C/min * 170 °C * o min * 4 °C/min * 230 °C * 8 min * 5 °C/min * 250 °C * 15 min, temperature of the injector 270 °C, temperature of the detector 280 °C. The injection volume was 2 ml, the carrier gas was nitrogen. The flame-ionization detector was connected to the column output. Final data were processed in CSW Station software (version 1.7, Data Apex).

The results in the first part were evaluated by the analyses of variance for two factors (year, locality) in STATISTICA CZ 8.0 software, on the level of significance α = 0.95. The parameters in the second experiment related to the genotypes were the average values from two assessments. Because of the lack of the data, we could not provide any further statistical analyses except the descriptive statistics.

Results and discussion

The effect of growing locality and the year on the oil content and neutralization number

Analyzed data showed statistically highly significant differences in the seed oil content among the growing localities. The average values of oil content are presented in the Figure 1. The highest average oil content was recorded in the sample from C2 region (21.28 %) and the lowest value was in P2 region (18.97 %). The differences between the years 2007 (20.30 %) and 2008 (19.59 %) were also statistically highly significant. The oil content is affected by the genotype, the year and the environment mainly and also by their interactions (Baranyk et al., 2007). We confirmed this common known fact. The reaction to the conditions in both potato regions and both cereal regions were similar in both years, but the effect of the year was dominant as it can be seen from the Figure 1. The contents of the oil in the samples from all of localities and from both years were of 20 % lower in average that is mentioned in Czech experiments or literary sources. According to Buchta et al. (2010), the average oil content in the achenes varied between 25 – 35 % in the Czech Republic. Baranyk et al. (1995) found out the average value 24.5 % for the locality of central Bohemia. This region includes cereal growing region (C) and sugar beet growing region (S) Their results were very variable and were caused by the heterogeneity in the ripeness of the achenes, which is strongly influenced by the weather conditions during that period.

The neutralization number (NN) is statistically significantly affected by both factors, environment and year conditions. The highest NN was found out in the sample from C2 region (5.2), the lowest value was in S region (4.11). The NN in 2007 was 4.86, in 2008, it was 4.82. The values are shown in Figure 2. Surprisingly, the same values of NN were found in the different environment. The sample from sugar beet region differed from the others of the highest value. All the samples corresponded with the limit given by the Specification of the product (2008), where the NN should be lower than 10 mg KOH per g. The assessment was performed till 6 months after the harvest, so even we know that the production was processed in one company, we can not confirm the quality of oil from the point of view of the storage conditions in this organization. The values could be affected by the growing environment or storing by agricultural producers. Generally, the quality of oil seed is influenced by the period of the storage, the temperature and also there are the differences among the varieties (Ghasemnezhad and Honermeier, 2009).

The effect of genotype on the oil content, neutralization number and fatty acids profile

Seed oil contents in the assessed genotypes are shown in the Figure 3. The highest oil content had sample of variety Silyb – silybine type from the holder of PVR (21.6 %), followed by the variety Mirel, also from the holder of PVR (21.0 %). According to the DUS tests, Mirel has declared 21.6 % of oil in average (ÚKZÚZ, 2011). The lowest content of oil was recorded in two samples from pharmaceutical and food companies (17.5 %, 18.1 %). The level of oil content in our genotypes was lower than is mentioned in literature, for example in Hosnedl et al. (1998) and in Buchta et al. (2010). Generally, the content of oil in our genotypes was lower than for example in the samples from Iran – 25.0 % (Hasanloo et al., 2008) or 26 – 31 % (Fathi-Achachlouei and Azadmard-Damirchi, 2009). Because we have limited number of the results in one year and low

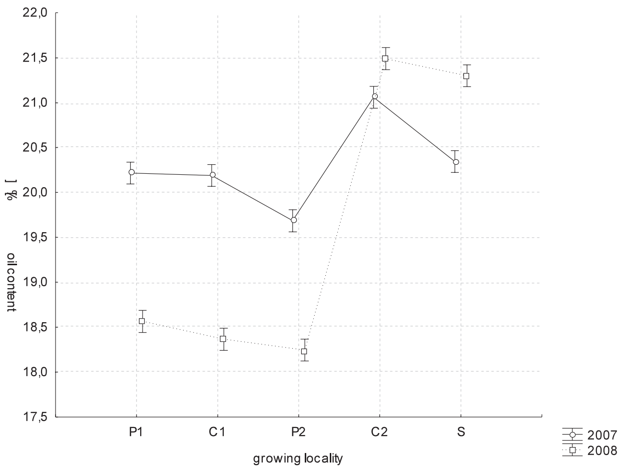

Figure 1 Effect of the locality and the year on oil content
Obrázok 1 Efekt lokality a ročníka na obsah oleja

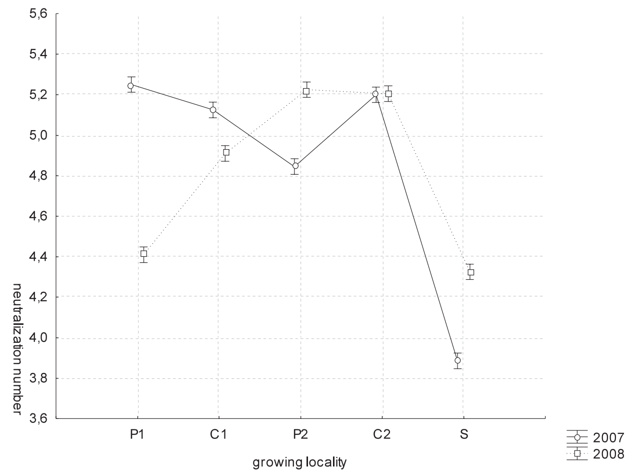

Figure 2 Effect of the locality and the year on the neutralization number
Obrázok 2 Efekt lokality a ročníka na číslo neutralizácie

Table 1 Fatty acid ratio (%) in the oils of various genotypes. SAT total saturated FA, MUFA total monounsaturated FA, PUFA total polyunsaturated FA

Fatty acids	Czech Republic 1 (CZE)	Czech Republic 2 (CZE)	Mirel (CZE)	Silyb (silydianine, CZE)	Silyb (silybine, CZE)	Serbia
C14:0	0.075	0.067	0.072	0.076	0.071	0.081
C15:0 IS	0.023	0.018	0.021	0.022	0.019	0.019
C16:0	7.826	7.561	7.249	8.480	7.697	9.209
C16:1n7c	0.054	0.056	0.049	0.071	0.056	0.070
C18:0	4.677	4.843	3.568	5.285	5.329	5.925
C18:1n9c	19.191	21.201	16.263	21.339	22.887	25.447
C18:1n7c	0.440	0.438	0.393	0.520	0.443	0.497
C18:2n6c	60.527	58.920	66.401	56.955	55.906	50.589
C18:3n3c	0.290	0.200	0.266	0.278	0.188	0.226
C20:0	2.730	2.790	2.090	2.978	3.269	3.450
C20:1n9c	0.990	0.935	1.066	0.929	0.916	0.915
C22:0	2.253	2.239	1.894	2.335	2.454	2.760
C22:1n9c	0.216	0.038	0.049	0.045	0.036	0.082
C24:0	0.653	0.657	0.555	0.648	0.695	0.700
C24:1n9c	0.055	0.037	0.064	0.039	0.034	0.030
SAT	18.237	18.175	15.449	19.824	19.480	22.144
MUFA	20.946	22.705	17.884	22.943	24.372	27.041
PUFA	60.817	59.120	66.667	57.233	56.148	50.815

Tabuľka 1 Obsah mastných kyselín v oleji, rôznych genotypov v %. SAT celkové nasýtené mastné kyseliny, MUFA celkové nenasýtené mastné kyseliny, PUFA celkové polo nasýtené mastné kyseliny

number of the genotypes, we can not strictly judge why the oil content in Czech samples were lower. It was probably caused by the influence of the year condition and genotype, as stated by Černý et al. (2010). Even the Milk thistle oil is chemically similar to sunflower oil; it is not suitable to compare the influence of sunflower genotypes on oil content and quality because of lack of the literature and serious scientific backgrounds in Milk thistle genotypes chemical description.

The neutralization number (NN) varied highly in the samples, the highest value was at variety Mirel (33), and the lowest NN was at variety Silyb – silybine type (4.3). When the maximal limit for this parameter is 10 mg of KOH.g^{-1}

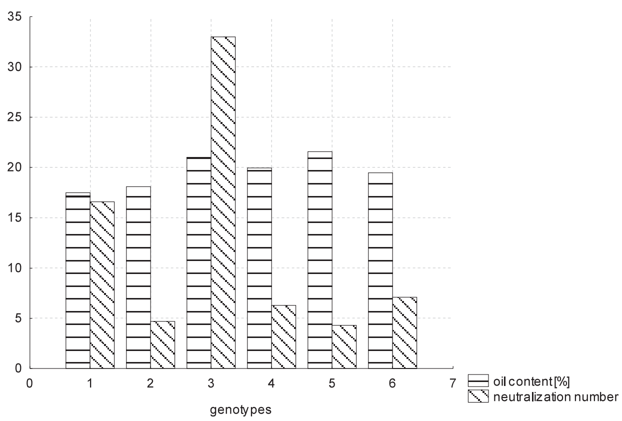

Figure 3 Content of oil in Milk thistle genotypes and their neutralization numbers
1 – Czech Republic 1, 2 – Czech Republic 2, 3 – Mirel, 4 – Silyb (silydianine type), 5 – Silyb (silybine type), 6 – Serbia

Obrázok 3 Obsah oleja a číslo neutralizácie v genotypoch pestreca mariánskeho
1 – Czech Republic 1, 2 – Czech Republic 2, 3 – Mirel, 4 – pestrec (silydianine typ), 5 – pestrec (silybine typ), 6 – Srbsko

(Specification of the product, 2008), also the sample from food producers (CZE1) was out of the limit (16.6). High values of this parameter can be caused by totally inadequate way of storing of the seeds. The other samples corresponded with the standard requirements (Figure 3).

The Table 1 shows the ratios of fatty acids detected in the samples. Dominant fatty acid (FA) is linoleic for all the samples, followed by oleic acid, palmitic and stearic acid. Beside these main fatty acids, another minor FA was detected in the oil samples in amounts from 0.03 till 0.7 %. The highest amount of linoleic acid was recorded in the oil from variety Mirel (66.4 %), the lowest content had the sample from Serbia (50.6 %, without the description of variety). According to the DUS tests of the Central Institute of testing and Controlling in Agriculture, for Mirel, it is declared 8.5 % of palmitic acid, 22.5 % of oleic acid and 57.9 % of linoleic acid (ÚKZÚZ, 2009). Buchta et al. (2010) have evaluated the main FA in Milk thistle oil, linoleic acid ranged from 55.0 % to 72.0 %, 15.0 – 20.0 % of oleic acid and 8.0 – 14.0 % of saturated FA. Even there is lack of the literature about the individual FA profiles in the different genotypes of Milk thistle of the globe; there is some information about the seed oil quality from the other countries. Pakistani samples had 26 – 39 % of oil, with 23 – 47 % of oleic acid, 9 – 58 % of linoleic acid, 12 – 34 % of palmitic acid and 6 – 15 % of stearic acid. Higher contents of oleic (26.38 %) and linoleic acids (64.4 %) were also found (Khan et al., 2007). On the other hand, Iranian samples showed lower content of linoleic acid (45.36 %), but higher content of oleic acid (31.58 %) (Hasanloo et al., 2008). Regarding the absence of relevant literature, an interesting composition had ground defatted Milk thistle fruit, declared as Milk thistle flour (Botanic Oil Innovations, USA), where the content of the oil was 7.5 %, linoleic acid 4.6 %, oleic acid 37.1 %, palmitic acid 27.4 %, stearic acid 17.7 % (Parry et al., 2008). This product has surprisingly high content of saturated FA. Iranian authors have performed the experiments regarding influence of the water conditions on the fatty acids composition in the oil. They included two foreign varieties – Budakalaszi (Hungary) and CN-seed (England), and two native varieties (Khoreslo, Babak Castle) using dryland and irrigated farming conditions. The type of farming did not influence the fatty acids composition. Native varieties had different levels of saturated and monounsaturated FA, but no differences in polyunsaturated FA were observed (Fathi-Achachlouei and Azadmard-Damirchi, 2009).

Conclusions

The seed oil content in Milk thistle is affected by the environment in the frame of the dominant effect of the year conditions. It is called for better regionalization of this crop to the suitable regions. Another important factor influencing the seed oil content is a genotype, so there is the evidence of the interactions of three important factors. Nutrition quality of the oil is given mainly by the spectrum of unsaturated fatty acids. The samples of Milk thistle of various origins had predominant content of linoleic acid, followed by oleic acid. The oil of Milk thistle can be considered as nutritionally valuable, similar to sunflower oil as it was confirmed in previous published results. According to our search, majority of the processing companies are not interested in the origin of the raw material. They have no information about the variety, the region of production. The processing companies have to determine the origin of Milk thistle variety and implement appropriate technology in given environment.

Súhrn

Vzorky nažek ostropestřce mariánského [*Silybum marianum* (L.) Gaertn.], odrůdy Silyb (silybinový typ, ČR) z 5 výrobních oblastí České republiky byly během let 2007 – 2008 testovány na obsah oleje (%) a číslo kyselosti. Ve druhé části bylo hodnoceno 6 genotypů z hlediska obsahu oleje (%), spektra mastných kyselin a čísla kyselosti. Výsledky prokázaly statisticky průkazný vliv lokality a ročníku na obsah oleje. Nejvyšší obsah oleje měl vzorek z obilnářské výrobní oblasti 2 (21.28 %), nejnižší hodnoty byly zjištěny v bramborářské výrobní oblasti 2 (18,97 %). Obsah oleje byl v roce 2007 20,30 %, v roce 2008 19,59 %. Číslo kyselosti bylo rovněž statisticky průkazně ovlivněno oběma faktory. Co se týká genotypů, nejvyšší obsah oleje měla odrůda Silyb – silybinový typ (21,6 %), následovaný odrůdou Mirel (21,0 %). Nejnižší obsah měly vzorky z produkce CZE1 a CZE2 (17,5 % a 18,1 %). Číslo kyselosti bylo velmi variabilní, dosahovalo hodnot od 4,3 do 33,0. Dominantní mastnou kyselinou ve všech vzorcích byla linolová, dále olejová, palmitová a stearová a také některé minoritní MK (0,03 – 0,7 %). Nejvyšší obsah k. linolové měla odrůda Mirel (66,4 %), nejnižší obsah byl stanoven u srbského genotypu (50,6 %).

Klíčová slova: ostropestřec mariánský, *Silybum marianum*, olej, mastné kyseliny, prostředí

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