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Original Paper

Evaluation of the oil content of Silybi mariani fructus cultivated in a warm climatic region (Dolná Malanta, district Nitra)

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Milk thistle [*Silybum marianum* (L.) Gaertn.] is a medicinal plant from the Asteraceae family that is grown for its silymarin content. In the production of the silymarin complex, oil is obtained as a secondary product that must be removed from the seeds before extraction of silymarin. The oil contains a favorable ratio of fatty acids and essential phospholipids and has a high vitamin E concent. The objective of this study was to determine the quality of the harvest and the oil content in the dry matter of milkthistle fruit in three varieties (Silyb, Silma, Mirel) during growing seasons 2019 and 2020. The field experiment was conducted in a warm climatic region of western Slovakia (at the experimental site, Dolná Malanta locality). The results showed that the average yield of milk thistle fruit was 452.72 ±61.71 kg ha^{-1}. The oiliness results wereat the level of 28.62 ±1.12%. The maximum and minimum oil content values in the dry matter were determined in the variety Silma 2019. The differences between the individual varieties were not statistically significant. The factor of the growing season had a statistically significant effect on the oil content. Based on two-year fertility and oil content results, it is recommended to continue to monitor the quantitative and qualitative potentials of the milk thistle in the following growing season.

Keywords: milk thistle, fruit, yield, oiliness, Soxhlet extraction

1 Introduction

Milk thistle [*Silybum marianum* (L.) Gaertn.] is an annual to a biannual medicinal plant of the largest and most widespread family Asteraceae. The Mediterranean plant is now common worldwide and has been cultivated for centuries throughout Europe, Africa, China, India and Australia (Polyak et al., 2013). Milk thistle seeds contain silymarin (Engelberth et al., 2008). It is a group of flavonolignans and the main active compound in milk thistle. Silymarin has both anti-inflammatory and antioxidant properties (Khalili et al., 2009). It has a positive effect in the treatment of liver diseases and cancer by inhibiting the proliferation of cancer cells (Vaknin et al., 2008). The effects of silymarin include regeneration, detoxification during cancer treatment, and prevention of hepatotoxicity during chemotherapy and radiotherapy. In folk medicine, it is used for the treatment of liver disorders. Moreover, it normalizes digestion, stimulates lactation, and reduces inflammation of the upper respiratory tract and lungs (Abascal and Yarnell, 2004). It is also used to treat prostate, skin, and breast cancers, cirrhosis, and kidney disease (Migahid et al., 2019). Freedman et al. (2010) reported that silymarin is the most commonly used plant product for chronic liver disease. Although milk thistle is grown commercially to produce silymarin (Alemardan et al., 2013), milk thistle seeds also contain small amounts of flavonoids (taxifolin) and about 20–35% of fatty acids and other polyphenolic compounds (Ramasamy and Agarwal, 2008). In addition, they contain saponins, proteins, polysaccharides, vitamin K, resins, and mucilage, as well as macroelements and microelements (Abascal and Yarnell, 2004). In the

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production of the silymarin complex, the oil is obtained as a secondary product (Růžičková et al., 2011; Vagnerova et al., 2016) that must be removed from the seeds before the extraction of silymarin (Hadolin et al., 2001). *S. marianum* oil is rich in fatty acids in the following proportions: linoleic acid with the highest percentage (45.7–43.1%) followed by oleic (29.2–35.1%), palmitic (9.61–7.7%), stearic (5.8–8.5%), linolenic (2.4–4.3%), and arachidonic (1.8–1.9%) acids (Estaji and Niknam, 2020). Other fatty acids were also detected as reported by Růžičková et al. (2011). The oil contains essential phospholipids and has a relatively high vitamin E content (Hadolin et al., 2001; Fathi and Azadmard, 2009). The determination of the oil is traditionally based on leaching the ground seeds with an organic solvent and weighing the residue after the evaporation of the solvent from the extract. The most used procedure to remove fat from the seed matrix is a conventional Soxhlet extraction (Luque-Garcia and Castro, 2004). Fruit and silymarin yields vary depending on environmental conditions, genotypes, sowing and harvest dates, gaps and rows, and agricultural practices (Karkanis et al., 2011). Recently, the cultivation of milk thistle has increased, globally due to the high level of unsaturated fatty acids and silymarin. These compounds are essential for the food and pharmaceutical industry. Although milk thistle is relativity resistant to drought stress, severe drought, and reduced precipitation during the critical period of plant growth can adversely affect the seed yield and quality (Estaji and Niknam, 2020). The present study was conducted to evaluate the oil content in different varieties of Silybi mariani fructus. To the best of our knowledge, no study has been published comparing the oiliness of more than two different varieties of milk thistle. Each variety of this plant represents a different chemotype. Therefore, we can assume that the amount of oil will vary between varieties.

2 Material and methods

2.1 Plant material

Plant material of milk thistle fruit was obtained from three variants (Silyb, Silma, Mirel). The variety Silyb was registered in the Czech Republic in 1988. This variety is a silibinin type variety, containing approximately 2.5% of silibinin. Silychristin is present in an amount of 1.5%, whereas silydianin is absent (Indrák and Chytilová, 1992; Andrzejewska et al., 2011). Silma was bred and registered in Poland in 1990. Its average harvest is 2,000 kg ha^{-1}, and the average yield of silymarin is 26.5 kg ha^{-1} (Andrzejewska et al., 2011). The variety Mirel has been legally protected since 2010. It is characterized by high oil content with a specific spectrum of fatty acids, primarily linolenic acid (66%) (Růžičková et al., 2011). It is a fertile variety suitable for deep and nutrient-supplied soil.

2.2 Field experiment

The field experiment was established at the experimental site of the Slovak University of Agriculture (Dolná Malanta) in the Nitra Region of Slovakia. This area is located in a warm climatic region, in a warm and dry climatic zone on a silt loam Haplic Luvisol. Data on average temperatures and precipitation during the experiment were provided by the Department of Biometeorology and Hydrology of the Faculty of Horticulture and Landscape Engineering of the Slovak University of Agriculture in Nitra (Čimo, 2020 short communication), and are presented in Table 1. Phosphorus (20.0 kg ha^{-1}) and potassium (80.0 kg ha^{-1}) were plowed into the soil in the autumn, and nitrogen (20 kg ha^{-1}) was applied during the spring pre-sowing soil preparation. Irrigation was not used in the experiment. Sowing was performed on 22 March 2019 and 26 March 2020 in strips of 120 m^2, separately for each variety, to a depth of 40 mm, while maintaining a row spacing of 125 mm. The harvest was performed at the full maturity of the seeds on 16 July 2019 and 27 July 2020. The fruit yield was analyzed from a growing area of 30 m^2 in three repetitions from each treatment, expressed in kg ha^{-1}. Harvesting was performed mechanically using a small-plot harvester in the growth phenophase of full fruit ripeness. The fruits were weighed and dried naturally to maintain the quality indicator – oil content.

2.3 Oil extraction

Chemical analysis of samples was conducted at the Mendel University in Brno, Faculty of Agronomy, Department of Crop Science, Breeding and Plant

Table 1 Average temperatures and average precipitation in individual months of the growing season in 2019 and 2020

Physical indicators	Year	March	April	May	June	July	Mean
Average temperature (°C)	2019	8.1	9.7	9.3	18.7	21.9	13.5
	2020	6.6	11.3	13.8	19.2	20.8	14.3
Average precipitation (mm)	2019	15.6	12.3	114.7	32.6	21.0	39.2
	2020	63.8	5.4	38.5	81.2	21.8	42.1

Medicine. Soxhlet extraction was used for the extraction of oil from achenes. Petroleum ether was used as the extraction solvent. Representative samples were ground on a grinder. Three grams were weighed and crushed in a mortar. Then they were poured into an extraction thimble, placed in an oven to dry, and inserted into the apparatus. The extraction solvent was added to the apparatus in the amount of 50 ml and heated to a boiling point (120 °C). The extraction was performed in three steps. The first step, called the boiling step, in which the sample was extracted in the extraction solvent, lasted 15 minutes. During the second step, the rinsing step, the sample was extracted for 45 minutes in solvent vapors. In the last step, the solvent recovery phase, the extraction solvent was restored for 10 minutes. At the end of the extraction, the samples were removed from the apparatus and cooled under a fume hood and then placed in an oven where the remaining solvent was evaporated at 85 °C. The extraction of each sample was repeated three times. After the first and second extractions, the sample was removed from the apparatus, crushed again in a mortar to disrupt the structure of the sample further to maximize the oil yield, and the process was repeated. The rinsing step lasted only 30 minutes for the second and third extractions. The beakers of extracted oil were cooled in desiccators for a minimum of 1 hour and weighed to the nearest 1 mg. The oiliness was expressed in the weight percentage by Formula (1):

$$w_o = m_2/m_1 \times 100 \qquad (1)$$

where:
w_o – the percentage of oil content in weight; m_1 – the weight of the sample; m_2 – the weight of the dried extract

The dry matter was determined from the same samples as the oil content. The weighed samples were dried for 3 hours at 103 ±3 °C. After drying, the beakers were covered with lids, placed in a desiccator to cool and weighed. The samples were placed back in the oven for 1 hour, and the whole procedure of drying, cooling in a desiccator, and weighing was repeated. The results of three parallel analyses from each sample were averaged and expressed as a percentage of the dry matter of the sample according to Formula (2):

$$w_d = (m_1 - m_2)/(m_1 - m_0) \times 100 \qquad (2)$$

where:
w_d – the percentage of the dry matter; m_0 – the weight of the beaker; m_1 – the weight of the beaker with the sample before drying; m_2 – the weight of the beaker with the sample after drying

The results presented in the paper are expressed as a percentage of oil in dry matter (*w*) according to Formula (3):

$$w = w_o/w_d \times 100 \qquad /3)$$

2.4 Statistical analysis

The program MS Office Excel 2007 and statistical software STATISTICA CZ version 10 were used for all data analyses. Multi-factor analysis of variance (ANOVA) and Fisher´s LSD test ($p = 0.05$) was used to evaluate the data. The significance of relationships was tested at $p < 0.05$.

3 Results and discussion

The fruit yield of milk thistle ranged from 327 to 546 kg ha^{-1}. Comparing the years in our experiment, the yield was higher in 2020 when the average precipitation at the time of seed filling (June) was higher. The difference is not statistically significant. Fertility by growth years and variety is shown in Table 2. Afshar et al. (2014) reported that the highest seed yield (1,171 kg ha^{-1} in 2012 and 1,358 kg ha^{-1} in 2013) was obtained from an irrigation experiment. The irrigation regime and soil amendments significantly affect seed yield and its components. Andrzejewska and Sadowska (2008) found a yield of 940–1,580 kg ha^{-1} in the Polish cultivar Silma during the growing season 2003–2005.

Table 2 Average yields of milk thistle ± standard deviation expressed in kg ha^{-1} at the standard humidity level (14%) with their statistical analysis

Variety		*p*-value
Silyb	498.50 ±31.05^{a}	0.062
Silma	432.33 ±63.59^{a}	
Mirel	427.33 ±57.10^{a}	
Year		
2019	447.44 ±63.26^{a}	0.602
2020	458.00 ±59.65^{a}	

p-value – the effect of the chemotype on the yield and effect of the growing season on the yield; a – values in the column with the same superscript do not differ significantly at $p < 0.05$

The lowest yield of the *S. marianum* was found in the Silma variety in 2019 and the highest in the Silyb variety in 2019. The Silyb variety was also dominant in 2020. The average fruit yield in individual years is presented in Table 3. The yield potential of milk thistle in agroecological conditions of the experimental station Dolná Malanta has been continuously studied since 2002. Pilot results were published by Habán et al. (2009), where the yields of the Silyb variety are given within the range from 232.9 to 1,832.0 kg ha^{-1} in the vegetation years 2004–2007. The results confirm the highly significant yield variability and significant biological plasticity of plant materials.

Table 3 Yields of milk thistle expressed in kg ha^{-1} ± standard deviation of the correlation between the factors observed in the experiment

Year	Variety	Yield of milk thistle	*p*-value
2019	Silyb	507.00 ±27.82[a]	0.054
	Silma	380.33 ±39.40[a]	
	Mirel	455.00 ±39.65[a]	
2020	Silyb	490.00 ±31.79[a]	
	Silma	484.33 ±33.58[a]	
	Mirel	399.66 ±58.46[a]	

p-value – interactions between a growing season and the effect of a variety on the yield; a – values in the column with the same superscript do not differ significantly at $p < 0.05$

The results of oiliness obtained from the research are similar to the results of other studies (Ahmad et al., 2012; Koláčková et al., 2014). Takase et al. (2014) showed oil content in seeds of up to 46%. Li et al. (2012) reported an oil yield on a dry basis of up to 45.70% using enzymatic pre-treatment. Hadolin et al. (2001) obtained an oil yield of 22.5% in a Soxhlet apparatus with petroleum ether. The moisture content of the light yellow lipophilic extracts was from 0.09 to 0.12%. We measured the dry matter content of the crushed seeds, and the moisture ranged from 5.91 to 6.56%. We analyzed a total of 18 samples of achenes in three varieties of milk thistle. The lowest and highest measured value of the oil content in the dry matter was 26.26% and 30.31% in the variety Silma 2019. In 2020, the minimum content was 28.58% in Mirel and the maximum 29.99% in Silma. Zheljazkov et al. (2006) found that the content of seed oil in the Silma variety ranged from 18.4 to 26.2%, with the highest content being when no herbicides were used in both studied years. Pendimethalin or metribuzin alone reduced the seed oil content compared to untreated growth in both years, while trifluralin plus bentazone reduced the seed oil content in 1995 but not in 1996. The oil content of seeds in 2011 ranged between 28% and 30% on a dry-weight basis (Fadhil et al., 2017). The average values of oiliness between the varieties differed in descending order of Silma, Mirel, and Silyb (Table 4). Differences between varieties were observed but were not significant. There was a statistically significant difference of 1.48% between the values in 2019 and 2020 when considering all three examined varieties.

Table 4 Oiliness in dry matter expressed in percentage as average ± standard deviation with their statistical analysis

Variety		*p*-value
Silyb	28.41 ±0.98[a]	0.745
Silma	28.86 ±1.38[a]	
Mirel	28.60 ±0.77[a]	
Year		
2019	27.88 ±1.05[a]	0.032
2020	29.36 ±0.41[b]	

p-value – the effect of the chemotype on the oiliness and the effect of the growing season on the oiliness; a, b – means within a column bearing different superscript differ significantly at $p < 0.05$

The highest average oil content in the dry matter was found to be for the variety Silma 2020. The Silma variety also dominated in the previous year when its average amount was 1.33% lower than in 2020. The lowest average oil content in the dry matter was reported in the variety Silyb 2019 (Table 5). Our results of average oil yields are different from the results of studies by Růžičková et al. (2011), in which the Silyb variety had an oil content of 21.6% and Mirel of 21.0%.

Table 5 Oiliness in the dry matter expressed in percentage as mean ± standard deviation of the correlation between the factors observed in this experiment

Year	Variety	Oiliness	p-value
2019	Silyb	27.45 ±0.11[a]	0.796
	Silma	28.19 ±1.66[a]	
	Mirel	28.02 ±0.51[a]	
2020	Silyb	29.37 ±0.25[a]	
	Silma	29.52 ±0.38[a]	
	Mirel	29.19 ±0.50[a]	

p-value – interactions between the year and the effect variety on the oiliness; a – values in the column with the same superscript do not differ significantly at $p < 0.05$

Correlation analysis showed that with the increasing yield of milk thistle, the oil content in the dry matter increased, but the correlation dependence was not confirmed (Figure 1). The linear regression analysis of the dependence of oil production on the crop yield is characterized by the following equation $y = 27.8894 + 0.0016x$ with a determination index of 9.17% ($R^2 = 0.0917$).

According to the results of a study conducted by Estaji and Niknam (2020), the oil content in the seeds of milk thistle decreased with increased stress due to the lack of water. Linoleic acid, linolenic acid, stearic acid, and total unsaturated fatty acids were significantly affected. The amount of stearic acid increased significantly with the

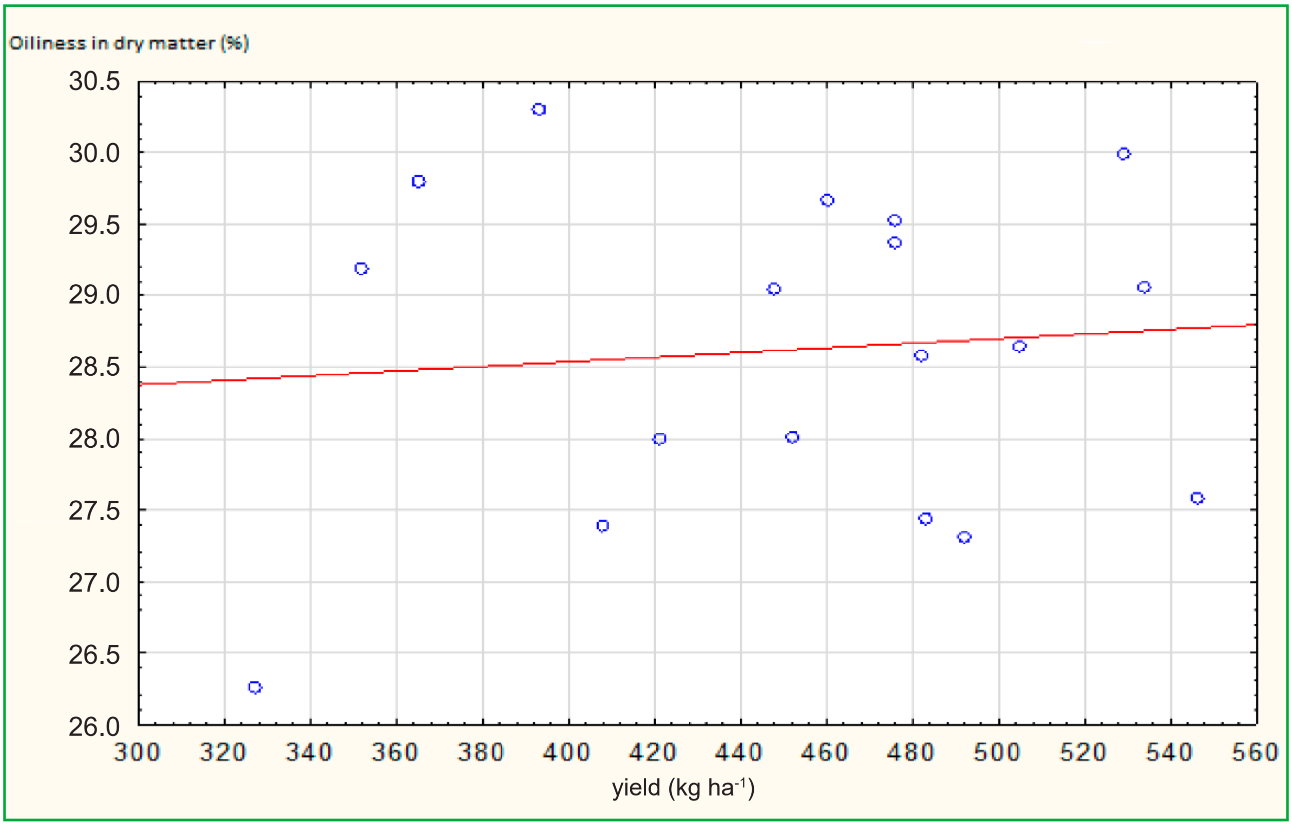

Figure 1 Linear regression analysis of the dependence of oil content on the yield of Silybi mariani fructus

increasing level of irrigation. Andrzejewska et al. (2011) studied the fruit yield and the content of flavonolignans in the milk thistle variety Silma, grown on light soil in a moderate climate. They found that the oil content of the fruit varied significantly at 45 g kg^{-1} between 2004 when the air temperature in the last month of seed filling (July) was 16.6 °C and 2006 when the relevant temperature was 22.4 °C. The oil content of the fruit depended on weather conditions. Sadowska et al. (2011) also found that weather conditions more significantly determinedthe fat content of the fruit of milk thistle in variety Silma in the research years than by the experimental factors. In the year that

Table 6 Physicochemical parameters of Silybi mariani oil in studies

Property	Takase et al., 2014	Fadhil et al., 2016	Fadhil et al., 2017	Anum et al., 2018
Density (g ml^{-1})	0.9214*	0.925**	0.9141***	
Kinematic viscosity 40 °C (mm^2 s^{-1})	37.5	45.20	42.0	
Flashpoint (°C)		236	230	
Acid value (mg KOHg^{-1})		20	13.60	5.049
Saponification value (mg KOHg^{-1})	191.54	204	195	126.2
Iodine number (mg I_2 g^{-1})		0.982	0.99	2.79
Could point (°C)		-3	-2	
Pour point (°C)		-7	-8	
Refractive index 20 °C		1.4619	1.470	
Mean molecular weight (g mol^{-1})	887.9		857	
Peroxide value (meq peroxide g^{-1})				7.056
Wax value (mg g^{-1})				6.89
pH value				8.09
Water content (%)	0.089			

Density detected at a temperature *16 °C, **15.5 °C, ***15 °C showed the highest average temperatures throughout the fruit ripening, the achenes contained the most fat, and in the coldest year, the fruits accumulated the lowest amount of fat. The difference in fat oil content between extreme years was up to 45 g kg^{-1}. The use of supercritical CO_2 extraction reached a maximum oil content of 31.83% with unsaturated fatty acids ranging up to 70–85%. Linoleic acid was the most abundant acid (47.64–66.70%) (Rahal et al., 2015). It is an essential fatty acid, which forms a part of the omega-3 acids and is involved in several physiological functions (Denis et al., 2013). *S. marianum* oil can attenuate lipid peroxidation, restore antioxidant enzyme activities, modulate the expression of related factors, and alleviate mitochondrial damage, which has significant effects on radicals. This antioxidant activity reflects the composition of the bioactive molecules in the oil extracted from *S. marianum*. Oleic acid, which is also present, is of great importance because thistle oil can be mixed with other oils or used alone to prepare meals (Zhu et al., 2014). For use in the food and pharmaceutical industries and other branches of industrial production, it is necessary to know the physicochemical parameters of the oil (Table 6). The rapid depletion of conventional oil resources as well as the environmental concerns posed by global warming call for renewable energy sources and have attracted considerable interest worldwide (Fadhil et al., 2016). Therefore, scientists and researchers are trying to produce biodiesel from renewable sources, such as plants. For this purpose, the oil obtained from the milk thistle is suitable. It is a by-product of plant processing in the pharmaceutical industry and has a composition that can be adjusted to a suitable level by chemical processes. Furthermore, it is renewable, non-toxic, ecological, biodegradable, and healthy (Ramadhas et al., 2005; Fadhil et al., 2016; Zhu et al., 2018).

4 Conclusions

The average yield of milk thistle was 452.72 ±61.71 kg ha^{-1}. The obtained yields of Silybi mariani fructus differed depending on the variety, in ascending order: Mirel – Silma – Silyb. We found that the average oil content was 28.62 ±1.12%. The oil content of the fruit was determined by chemical analyzes and differed depending on the variety, in ascending order: Silyb – Mirel – Silma. Insignificant differences between the varieties confirmed the fact that individual genotypes were bred to influence the components of the silymarin complex but not the oil content. There was a significant difference between results from 2019 and 2020, which can be caused by climatic factors. The year 2020 was more productive in terms of fruit production and oil content. Based on the results, we can say that milk thistle can be used not only for medicinal properties but also in the food industry due to the oil content of the seeds. This is owing to the favorable physicochemical composition of the oil forother branches of industrial production. In future research, it would be appropriate to analyze the content and proportion of fatty acids contained in the extract obtained in our study for use in food.

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