Acta Sci. Pol. Hortorum Cultus, 19(6) 2020, 71–78

https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 DOI: 10.24326/asphc.2020.6.6

ORIGINAL PAPER

Accepted: 27.01.2020

# PLANT MORPHOLOGY, VEGETATIVE BIOMASS COMPOSITION AND ENERGY CONTENT OF THREE DIFFERENT *Silybum marianum* ACCESSIONS

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**ABSTRACT**

*Silybum marianum* (L.) Gaertn. (milk thistle) is plant species that has been utilized principally for medicinal purposes for more than 2000 years. Recently it was proposed for biomass production in marginal environments, but vegetative biomass compositional analyses had not been available so far. The study of plant morphology and biomass composition was conducted on three different *S. marianum* accessions grown under open field conditions. The results indicate that plant morphological traits show major differences between accessions: this suggests that the available natural variability can be further utilized in order to develop improved *S. marianum* cultivars. Biomass compositional analysis shows that extractives, ash, lignin and cellulose content are comparable to other herbaceous bioenergy crops and that these traits display only limited variability in the studied accessions. Hemicellulose fraction is composed only by xylans and its content appears averagely lower in comparison to other herbaceous biomasses. Interestingly, in *S. marianum* biomass total nitrogen content is lower if compared to other herbaceous species. The possible involvement of this specific biomass trait in *S. marianum* nitrogen utilization efficiency has to be further investigated.

**Key words:** milk thistle, cellulose, hemicellulose, lignin, nitrogen concentration, lignocellulose

## INTRODUCTION

*Silybum marianum* (L.) Gaertn. (Asteraceae, common name: milk thistle) is an annual or biannual species native to southern Europe, Asia Minor and northern Africa [Morazzoni and Bombardelli 1995]. The species has been utilized mainly for medicinal purposes for more than 2000 years due to the medicinal properties of the bioactive flavonolignans (referred to as silymarin) contained in the fruit [Morazzoni and Bombardelli 1995]. At present, *S. marianum* is still grown as a medicinal plant in Europe and Asia [Estaji et al. 2011, 2016, Andrzejewska et al. 2015] and it is between the most marketed officinal species in different countries [ISMEA report 2013, Smith et al. 2016].

From an agronomic perspective, the species is characterized by significant fruit and vegetative biomass yield under both Mediterranean and semiarid conditions [Sulas et al. 2008, Andrzejewska et al. 2011, Ledda et al. 2013, Afshar et al. 2015, Domínguez et al. 2017a], also the possible utilization of the different feedstock obtainable from this species has recently been reviewed [Andrzejewska et al. 2015].

In *S. marianum,* areal biomass productivity can greatly vary depending on applied growing conditions. Domínguez et al. [2017a] reported a 1.4 Mg ha$^{-1}$ productivity in a heavy metals contaminated site during an extremely dry growing season. Nevertheless, under

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these limiting growth conditions, *S. marianum* showed the higher biomass productivity in comparison to the other tested specie. Afshar et al. [2015] studied *S. marianum* biomass productivity under different irrigations regimes and reported a productivity of 6.8–8 Mg ha$^{-1}$: they concluded that this species might be considered for biomass production in semiarid regions. Under more favourable agronomic conditions, a vegetative biomass productivity ranging between 13.7 and 20 Mg ha$^{-1}$ was described [Sulas et al. 2008, Ledda et al. 2013].

In the next future, the diversification of available bioenergy crops will likely represent a prime aspect to allow the increase of world bioenergy production thereby minimizing the competition with food and feed productions [Somerville et al. 2010]. Due to its adaptability to marginal environments, *S. marianum* lignocellulosic biomass was tested for biogas and bioenergy production with interesting results [Kalamaras and Kotsopoulos 2014, Domínguez et al. 2017a, b].

Despite the increasing interest in *S. marianum* as a multipurpose crop suitable for lignocellulose production, a detailed biomass compositional analysis of this species is still not available.

In the present study, the plant morphology and the compositional analysis of the vegetative biomass obtained from three different *S. marianum* accessions will be described. This in order to provide valuable information about this novel bioenergy crop and to inform about the naturally occurring variability present within the species for biomass traits.

## MATERIALS AND METHODS

**Plant material.** The milk thistle accessions G15 and G20 were collected in Germany and obtained from the Institute for Agrobotany (Nébih, Tápiószele, Hungary; FAO code HUN003). Accession G23 was a wild genotype sampled in locality Pelago (Florence, Italy).

Plants were sown under open field condition at Budrio (Bologna, Italy) experimental farm at the beginning of October 2014. The three accessions were sown following a randomized complete block design with three repetitions. Each repetition was composed of a 2 m single row of 10 plants (along the row distance 20 cm; between row distance 3 m). Plants were fertilized with 50 kg ha$^{-1}$ of nitrogen (applied as urea) in February at vegetative stage – BBCH growth stage 39 [Martinelli et al. 2015].

Morphologic measurements and areal biomass harvest were performed at the end of the growth cycle (BBCH growth stage 89; June 2015) on three central plants of each plot. The measurements were performed as follows: plant height refers to the maximum plant height; central head height refers to the height of central head, and this is usually shorter than flower heads on side branches; lower branch height refers to the distance from the ground of the insertion point of the lower second order branch on the main stem; number of side branches refers to the total number of second order side branches; main stem diameter was measured 2 cm above ground level; heads number refers to the total number of flower heads on a single plant; fruit harvest index was calculated as the fraction of fruit dry mass over total areal dry biomass. As for biomass sampling, defoliated dry stems were sampled and stored at room temperature before analyses.

**Biomass analysis.** The compositional analysis of vegetative biomass was performed according to the method proposed by the National Renewable Energy Laboratory (NREL) [Sluiter et al. 2010] with some modifications [Martinelli 2019]. In particular, Klason lignin and structural carbohydrates determination was downscaled according to Ibáñez and Bauer [2014] for 50 mg samples and extractives determination was modified as proposed by Kuchelmeister and Bauer [2015]. After biomass acid hydrolysis with sulphuric acid, HPLC determination of soluble sugars was performed according to Sluiter et al. [2010] exception made for the utilization of a Low-Temperature Evaporative Light-Scattering Detector (LT-ELSD; Sedex 85, Sedere, Alfortville, France) for sugars quantification. In order to avoid the interference of the sulphuric acid with the LT-ELSD detector, sample desulphatation was carried out before the analyses according to Wathelet et al. [2004]. Briefly, 0.6 cm$^3$ DEAE Sephadex A-25 resin was loaded into a microcolumn (8 mm internal diameter). The resin was equilibrated at pH 5.6 with 30 cm$^3$ of 25 mmol acetate buffer. Thereafter, 0.2 cm$^3$ of hydrolyzate was loaded. The desulphated sample was recovered by adding 1 cm$^3$ of deionized water to the microcolumn for two consecutive times (sample dilution into the column 1/10). Just before HPLC analysis, raffinose was added to the sample as internal standard in order to control LT-ELSD signal stability between subsequent analyses. Total C, N and H determinations were performed with a Truspec CHN Analyzer (Leco, Saint Joseph, Michigan. U.S.) on completely dry plant material. Protein content was calculated multiplying total nitrogen content by 6.25 [Merrill and Watt 1973]. Soluble lignin content was measured at 240 nm utilizing an absorptivity of 25 $dm^3$ $g^{-1}$ $cm^{-1}$ [Sluiter et al. 2010]. The entire biomass analytical procedure was further validated utilizing wheat straw analytical standard (National Institute of Standards and Technology. U.S., prod. n° 8494).

**Calorimetric analysis.** Gross calorific value (GCV) was measured with the aid of a C 2000 basic calorimeter (IKA-Werke, Germany) using the isoperibolic 25°C method according to manufacturer instructions. As a reference substrate for instrument calibration, benzoic acid tablets were utilized. Measurements were performed using 0.5 g of dry *S. marianum* biomass contained in C 12 combustion bags (IKA-Werke, Germany) with known GCV.

**Statistical analysis.** Analysis of variance was performed using SPSS 17.0 software (IBM, USA) and Tukey's HSD post hoc test was performed in order to identify significant differences between accessions. The coefficient of variation (CV) was calculated as the standard deviation divided by the mean.

## RESULTS

**Plant morphology.** Statistically significant differences were recorded for all the observed morphological traits except for main stem diameter and heads number (Tab. 1). The average main stem diameter was 36.25 mm and heads number per plant was 21.19 under the applied growing conditions. Plant height measurements highlighted that accession G15 and G20 showed taller plants if compared to accession G23. On the contrary, central head and lower branch height was higher in accession G20 if compared with accessions G20 and G15. In *S. marianum,* the average plant height, central head height and lower branch height were 228.17, 186.89 and 87.33 cm, respectively. Accession G15 showed a higher number of side branches when compared with accession G23. On average 8.61 side branches per plant were measured. Fruit harvest

**Table 1.** Plant morphology of three different *Silybum marianum* accessions

| *S. marianum* accessions | Plant height (cm) | Central head height (cm) | Lower branch height (cm) | Main stem diameter (mm) | N° side branches (n° plant$^{-1}$) | Heads number (n° plant$^{-1}$) | Fruit harvest index (g $g^{-1}$) |
|---|---|---|---|---|---|---|---|
| Accession G15 | 238.83 a$^{\alpha}$ | 179.33 b | 82.50 b | 40.11 | 9.50 a | 22.44 | 0.14 b |
| ±SD | ±10.10 | ±15.01 | ±4.44 | ±4.32 | ±0.71 | ±5.21 | ±0.03 |
| Accession G20 | 244.17 a | 203.50 a | 100.83 a | 35.28 | 8.67 ab | 19.22 | 0.21 a |
| ±SD | ±9.75 | ±6.06 | ±8.81 | ±4.01 | ±0.76 | ±4.35 | ±0.01 |
| Accession G23 | 201.50 b | 177.83 b | 78.67 b | 33.36 | 7.67 b | 21.89 | 0.19 ab |
| ±SD | ±7.57 | ±4.07 | ±1.61 | ±4.19 | ±0.29 | ±2.22 | ±0.02 |
| P value | < 0.01 | < 0.05 | < 0.01 | ns | < 0.05 | ns | < 0.05 |
| Average *S. marianum* | 228.17 | 186.89 | 87.33 | 36.25 | 8.61 | 21.19 | 0.18 |
| ±SD | ±23.25 | ±14.41 | ±11.85 | ±3.48 | ±0.92 | ±1.72 | ±0.03 |
| CV | 10.19% | 7.71% | 13.57% | 9.59% | 10.66% | 8.13% | 18.93% |

SD – standard deviation; CV – coefficient of variation; ns – not significant; $^{\alpha}$ letters indicate statistically significant differences between accessions ($p < 0.05$, $n = 3$)

index highlights the proportion of areal biomass allocated to fruit production. Interestingly, fruit harvest index ranged between 0.21 and 0.14 showing the higher value in accession G20 and the lower one in accession G15 (Tab. 1).

The observed CV ranged between 7.71 and 18.93% for the different traits. The higher CVs were observed for fruit harvest index and branch height; on the contrary, the less variable trait was the central head height (Tab. 1).

**Biomass composition.** In *S. marianum*, the biomass compositional analysis highlighted an average content of 140.0 ±15.1, 66.2 ±5.6, 20.6 ±4.1 g $kg^{-1}$ DW (±SD) for extractives, ash and protein, respectively. Insoluble lignin averagely represents 175.1 ±7.9 g $kg^{-1}$ DW of total *S. marianum* biomass and the average soluble lignin content was 37.8 ±1.3 g $kg^{-1}$ DW (±SD). On average, glucose units represent the 334.8 ±4.4 g $kg^{-1}$ DW (±SD) of the total biomass. Xylose is the only sugar present in hemicellulose fraction representing the 120.4 ±4.9 g $kg^{-1}$ DW (±SD) of the total *S. marianum* biomass.

As for the differences between accessions, statistically significant differences were measured for extractives, proteins, soluble lignin and insoluble lignin (Fig. 1). Accession G15 showed the higher extractives content and, together with accession G23, the higher protein content. Accession G23 also presents the lower extractives and higher insoluble lignin content. On the contrary, accession G20 showed the lower insoluble lignin content that is counterbalanced by a higher soluble lignin content (Fig. 1).

Elemental analysis indicates that *S. marianum* biomass contains 3.3, 462.8 and 54.8 g $kg^{-1}$ DW of nitrogen, carbon and hydrogen, respectively (Tab. 2). Significant differences between accessions were observed for all the analysed elements.

As for biomass compositional traits variability, nitrogen and extractives showed the higher CV with 201.2 and 108.2 g $kg^{-1}$ DW, respectively. On the con-

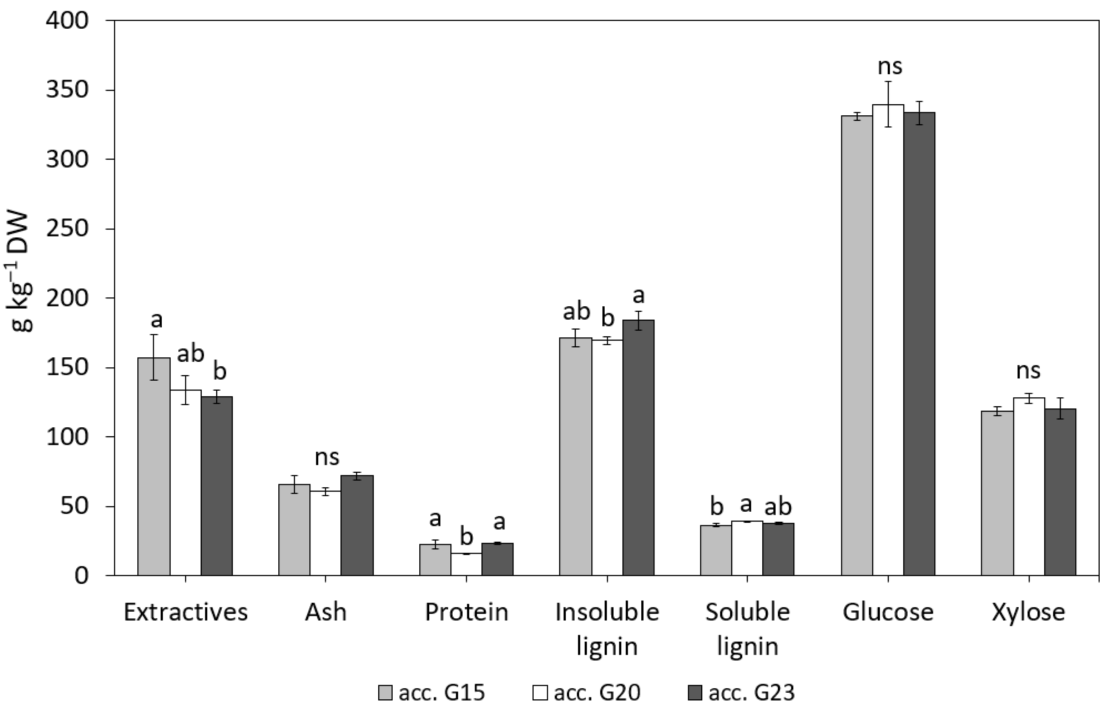


**Fig. 1.** *Silybum marianum* biomass compositional analyses of three different accessions (light grey, accession G15; white, accession G20; dark grey, accession G23). All the results are presented as g $kg^{-1}$ of total biomass dry weight. When visible error bars represent standard deviation (n = 3). For each analysed trait, letters indicate statistically significant differences between accessions ($p < 0.05$) according to Tukey's HSD test; ns – not significant

**Table 2.** Elemental analyses and gross calorific value of biomass from three *Silybum marianum* accessions

| *S. marianum* accessions | Total nitrogen (g kg$^{-1}$ DW) | Total carbon (g kg$^{-1}$ DW) | Total hydrogen (g kg$^{-1}$ DW) | Gross calorific value (kJ g$^{-1}$ DW) |
|---|---|---|---|---|
| Accession G15 | 3.6 a$^{\alpha}$ | 457.8 b | 53.9 b | 17.83 |
| ±SD | ±0.6 | ±4.8 | ±0.4 | ±0.10 |
| Accession G20 | 2.5 b | 467.7 a | 55.3 a | 17.96 |
| ±SD | ±0.1 | ±3.1 | ±0.4 | ±0.22 |
| Accession G23 | 3.7 a | 463.0 ab | 55.1 a | 18.24 |
| ±SD | ±0.2 | ±2.6 | ±0.3 | ±0.25 |
| P value | < 0.01 | < 0.05 | < 0.01 | ns |
| Average *S. marianum* | 3.3 | 462.8 | 54.8 | 18.01 |
| ±SD | ±0.7 | ±4.9 | ±0.7 | ±0.21 |
| CV | 20.12% | 1.07% | 1.29% | 1.17% |

SD – standard deviation; CV – coefficient of variation; ns – not significant; $^{\alpha}$ letters indicate statistically significant differences between accessions ($p < 0.05$, n = 3)

trary, the most stable trait was total carbon content that shows a CV of only 10.7 g kg$^{-1}$ DW.

GCV presents no differences between accessions and is 18.01 kJ g$^{-1}$ DW on average for *S. marianum* biomass (Tab. 2).

## DISCUSSION

Accessions G15, G20 and G23 were previously evaluated for a wide set of fruit phenotypic traits and were shown to belong to different phylogenetic groups [Martinelli et al. 2016]. Therefore, these three *S. marianum* accessions have been selected in order to initially explore the natural variability present in this species also for biomass traits.

Regarding morphology, the observed dissimilarities indicate major differences in plant architecture between the three accessions. In specific, the observed variation in agronomically important traits, such as harvest index, lower branch insertion height and plant height, is of interest for the design *S. marianum* ideotypes suitable either for biomass or fruit production and more adaptable to crop mechanization procedures. As for previously reported morphometric measurements of *S. marianum* plants, Ledda et al. [2013] reported comparable average results for plant height, number of side branches/stalks, and number of heads in a Sardinian ecotype. On the contrary, Ram et al. [2005] and Shokrpour et al. [2011] detected significantly lower values indicating a wide phenotypic variability related to both plant genotype and environmental conditions.

The knowledge of biomass structure is important information in order to evaluate its possible utilization for biofuel and chemicals production [Sorek et al. 2014, Williams et al. 2017]. Ultimate and proximate analysis of *S. marianum* biomass was previously performed [Sulas et al. 2008, Ledda et al. 2013, Domínguez et al. 2017b], but at present biomass compositional analysis according to NREL analytical procedure is not available for *S. marianum*.

In agreement with Martinelli [2019], *S. marianum* stem biomass is mainly composed of cellulose which represents ca 33.5% of total biomass. This data is comparable to the average cellulose content of biomasses originating from herbaceous crops like corn stover, switchgrass, sugar cane bagasse and from the phylogenetically related species *Cynara cardunculus* [Williams et al. 2017, Gominho et al. 2018]. Hemicellulose content is apparently lower in *S. marianum* than in other herbaceous crops [Williams et al. 2017, Gominho et al. 2018]. In specific, *C. cardunculus* stalks were described to have a xylose content ranging between 12.1 and 27% with an average of 16.8% [Gominho et al. 2018]. This comparatively low hemicellulose content might be of interest in biomass fermentation processes because not all the organisms commercially utilized for this process are able to utilize pentose sugars [Sorek et al. 2014]. *S. marianum* ash content is similar to corn stover, *Sorghum* and mixed grasses but higher that *Miscanthus* and sugar cane bagasse [Williams et al. 2017]. Biomass ash content can significantly change as a consequence of fertilization strategy and due to possible soil contamination during harvest procedures. In a previous report, Ledda et al. [2013] reported an ash content of 9.6% for *S. marianum* stems therefore showing a higher content in comparison to the here reported *S. marianum* average (6.62%).

Despite the significant differences highlighted between the analysed *S. marianum* genotypes, the observed low CVs suggest only limited possibility for genetic improvement of biomass compositional traits based on conventional breeding. The most variable trait is protein/nitrogen content that therefore represents an aspect that could possibly be taken into consideration for further genetic improvement of this species [Estaji et al. 2016]. As for the biomass nitrogen content, Ledda et al. [2013] described stem nitrogen content of 0.5%, with a value apparently lower than the other tested species. On the contrary, Domínguez et al. [2017b] described a 1.32% nitrogen content taking into consideration both stems and leaves. The here reported 0.33% average N content for *S. marianum* biomass is lower that the data reported for other herbaceous species and similar to *Miscanthus* biomass N content [Williams et al. 2017]. Also *C. cardunculus* displays higher stem N content with values ranging between 0.7 and 1.5% (average content 0.98%) [Gominho et al. 2018]. The here observed comparatively low biomass *S. marianum* N concentration together with the previously described significant total biomass productivity [Sulas et al. 2008, Andrzejewska et al. 2011, Ledda et al. 2013, Afshar et al. 2015, Domínguez et al. 2017a] might result in comparatively high nitrogen utilization efficiency in this species with a possible positive impact on biomass productivity under minimal nutrient inputs [Jakubowski et al. 2017].

*Silybum marianum* biomass displays a lignin content similar to other herbaceous bioenergy crops. Nevertheless, a slightly higher insoluble lignin content was observed in comparison to *C. cardunculus* stalks, this means a content ranging between 13.3 and 17.1% with an average of 15.35% [Gominho et al. 2018].

The here reported average GCV of 18.01 kJ g $DW^{-1}$ is higher than previously reported results which described values ranging between 14.8 and 16.2 kJ g $DW^{-1}$ for total biomass and of 16.7 kJ g $DW^{-1}$ for stems only [Sulas et al. 2008, Ledda et al. 2013, Domínguez et al. 2017a]. Biomass GCV is positively correlated with total C content [Toscano and Foppa Pedretti 2009]. Therefore, the comparatively high GCV is probably related to the reported total C biomass content that is higher if compared to previously published results [Ledda et al. 2013].

## CONCLUSIONS

Plants morphological traits highlight major differences between accessions indicating that the observed differences might be exploited in order to design *S. marianum* ideotypes that are both more suited for fruit or vegetative biomass production and more adaptable to mechanization.

Biomass compositional analysis shows that extractives, ash, lignin and cellulose content are comparable to average values reported for other herbaceous bioenergy crops and that these traits display only limited variability between the studied accessions. As for hemicellulose fraction, this is composed only by xylans and its content appears lower in comparison to other herbaceous biomasses.

Interestingly, *S. marianum* biomass N concentration is comparatively low and shows variability between the studied accessions highlighting possibility for the further genetic improvement of this trait. The possible contribution of this specific biomass trait to the overall *S. marianum* nitrogen utilization efficiency has to be further investigated.

## ACKNOWLEDGEMENTS

Thanks are due to Dr. R. Iori for the precious suggestions concerning the desulfatation procedure of the extracts. Thanks are due to A. Di Candilo, V. Milito, M. Iannucci, F. Brunetti and F. Govoni for the fieldwork at Budrio (Bologna, IT) experimental farm. Thanks to A. Moschella for her support in calorimetric analysis. Many thanks to C. Carletti for proofreading the manuscript.

## SOURCE OF FUNDING

This work was supported by the Italian Ministry of Agriculture (MiPAAF) under the AGROENER project (D.D. n. 26329, 1 April 2016).

## REFERENCES

Afshar, R.K., Chaichi, M.R., Alipour, A., Jovini, M.A., Dashtaki, M., Hashemi, M. (2015). Potential of milk thistle for biomass production in semiarid regions. Crop Sci., 55, 1295–1301. DOI: 10.2135/cropsci2014.10.0678

Andrzejewska, J., Sadowska, K., Mielcarek, S. (2011). Effect of sowing date and rate on the yield and flavonolignan content of the fruits of milk thistle (*Silybum marianum* L. Gaertn.) grown on light soil in a moderate climate. Ind. Crop. Prod., 33, 462–468. DOI: 10.1016/j.indcrop.2010.10.027

Andrzejewska, J., Martinelli, T., Sadowska, K. (2015). *Silybum marianum*: non-medical exploitation of the species. Ann. Appl. Biol., 167, 285–297. DOI: 10.1111/aab.12232

Domínguez, M.T., Montiel-Rozas, M.M., Madejon, P., Diaz, M.J., Madejon, E. (2017a). The potential of native species as bioenergy crops on trace-element contaminated Mediterranean lands. Sci. Total Environ., 590–591, 29–39. DOI: 10.1016/j.scitotenv.2017.03.018

Domínguez, M.T., Madejon, P., Madejon, E., Bianco, M.J.D. (2017b). Novel energy crops for Mediterranean contaminated lands: valorization of *Districhia viscosa* and *Silybum marianum* biomass by pyrolysis. Chemosphere, 186, 968–976. DOI: 10.1016/j.chemosphere.2017.08.063

Estaji, A., Souri, M.K., Omidbaigi, R. (2011). Evaluation of different levels of nitrogen and flower pruning on milk thistle (*Silybum marianum* L.) yield and fatty acids. Z. Arznei-Gewurzpfla, 16, 170–175.

Estaji, A., Souri, M.K., Omidbaigi, R. (2016). Evaluation of Nitrogen and Flower Pruning Effects on Growth, Seed Yield and Active Substances of Milk Thistle. J. Essent. Oil-Bear. Plants, 19, 678–685. DOI: 10.1080/0972060X.2014.981592

Gominho, J., Curt, M.D., Lourenço, A., Fernández, J., Pereira, H. (2018). *Cynara cardunculus* L. as a biomass and multi-purpose crop: a review of 30 years of research. Biomass Bioenerg., 109, 257–275. DOI: 10.1016/j.biombioe.2018.01.001

Ibáñez, A.B., Bauer, S. (2014). Downscaled method using glass microfiber filters for the determination of Klason lignin and structural carbohydrates. Biomass Bioenerg., 68, 75–81. DOI: 10.1016/j.biombioe.2014.06.013

ISMEA report (2013). "Piante officinali in Italia: un'istantanea della filiera e dei rapporti tra i diversi attori". Available: https://www.politicheagricole.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/6678 [date of access: 25.11.2020].

Jakubowski, A.R., Jackson, R.D., Casler, M.D. (2017). Can biomass yield of switchgrass be increased without increasing nitrogen requirements? Crop Sci., 57, 2024–2031. DOI: 10.2135/cropsci2017.03.0193

Kalamaras, S.D., Kotsopoulos, T.A. (2014). Anaerobic co-digestion of cattle manure and alternative crops for the substitution of maize in South Europe. Bioresour. Technol., 172, 68–75. DOI: 10.1016/j.biortech.2014.09.005

Kuchelmeister, C., Bauer, S. (2015). Rapid Small-Scale Determination of Extractives in Biomass. Bioenerg. Res., 8, 68–76. DOI: 10.1007/s12155-014-9493-x

Ledda, L., Deligios, P., Farci, R., Sulas, L. (2013). Biomass supply for energetic purposes from some Carduae species grown in a Mediterranean rainfed low input cropping system. Ind. Crop. Prod., 47, 218–226. DOI: 10.1016/j.indcrop.2013.03.013

Martinelli, T., Andrzejewska, J., Salis, M., Sulas, L. (2015). Phenological growth stages of *Silybum marianum* according to the extended BBCH scale. Ann Appl. Biol., 166, 53–66. DOI: 10.1111/aab.12163

Martinelli, T., Potenza, E., Moschella, A., Zaccheria, F., Benedettelli, S., Andrzejewska, J. (2016). Phenotypic evaluation of a milk thistle germplasm collection: fruit morphology and chemical composition. Crop Sci., 56, 3160–3172. DOI: 10.2135/cropsci2016.03.0162

Martinelli, T. (2019). Identification of milk thistle shatter resistant mutant lines with altered lignocellulosic profile for the complete domestication of the species. Crop Sci., 59, 2119–2127. DOI: 10.2135/cropsci2019.02.0103

Merrill, A.L., Watt, B.K. (1973). Energy Value of Foods: Basis and Derivation. In: Agriculture Handbook No. 74. ARS United States Department of Agriculture, Washington DC.

Morazzoni, P., Bombardelli, E. (1995). *Silybum marianum* (*Carduus marianus*). Fitoterapia, 66, 3–42.

Ram, G., Bhan, M.K., Gupta, K.K., Thaker, B., Jamwal, U., Pal, S. (2005). Variability pattern and correlation studies in *Silybum marianum* Gaertn. Fitoterapia, 76, 143–147. DOI: 10.1016/j.fitote.2004.10.006

Shokrpour, M., Gigloo, M.T., Asghari, A., Bahrampour, S. (2011). Study of some agronomic attributes in milk thistle (*Silybum marianum* Gaertn.) ecotypes from Iran. J. Med. Plant. Res., 5, 2169–2174.

Sluiter, J.B., Ruiz, R.O., Scarlata, C.J., Sluiter, A.D., Templeton, D.W. (2010). Compositional Analysis of Lignocellulosic Feedstocks. 1. Review and Description of Methods. J. Agric. Food Chem., 58, 9043–9053. DOI: 10.1021/jf1008023

Smith, T., Kawa, K., Eckl, V., Johnson, J. (2016). Sales of Herbal Dietary Supplements in US Increased 7.5% in 2015 Consumers spent $6.92 billion on herbal supplements in 2015, marking the 12th consecutive year of growth. HerbalGram, 111, 67–73.

Somerville, C., Youngs, H., Taylor, C., Davis, S.C., Long, S.P. (2010). Feedstocks for lignocellulosic biofuels. Science, 329, 790–792. DOI: 10.1126/science.1189268

Sorek, N., Yeats, T.H., Szemenyei, H., Youngs, H., Somerville, C.R. (2014). The implications of lignocellulosic biomass chemical composition for the production of advanced biofuels. BioScience, 64, 192–201. DOI: 10.1093/biosci/bit037

Sulas, L., Murgia, L., Ventura, A. (2008) Phytomass production from *Silybum marianum* for bioenergy. Opt. Méd., 79, 487–490.

Toscano, G., Foppa Pedretti, E. (2009). Calorific value determination of solid biomass fuel by simplified method. J. Agric. Eng., 40, 1–6. DOI: 10.4081/jae.2009.3.1

Wathelet, J.P., Iori, R., Leoni, O., Quinsac, A., Palmieri, S. (2004). Guidelines for glucosinolate analysis in green tissues used for biofumigation. Agroindustria, 3, 257–266.

Williams, C.L., Emerson, R.M., Tumuluru J.S. (2017). Biomass compositional analysis for conversion to renewable fuels and chemicals. In: Biomass volume estimation and valorisation for energy, Tumuluru, J.S. (ed.). InTechOpen, London. DOI: 10.5772/65777