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NUTRIENT COMPOSITION OF PARTIALLY DEFATTED MILK THISTLE SEEDS

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Abstract

Milk thistle (Silybum marianum) is an annual or biennial plant of the Asteraceae family that and usually grows in dry, sunny areas in Romania, but throughout in the world.The milk thistle seeds have been used since ancient times to treat a large variety of liver and gallbladder disorders. Theoprastus (IV century B.D.) and Plinius (1st century A.D.) were the first to report the medicinal benefits of this plant. All parts of the plant can be used, but the milk thistle seeds are considered to be the most medicinally potent for therapeutic use. The seeds and extracts of the milk thistle plant are a well established herbal food for protecting, detoxifying and regenerating the liver, one of the most important organs of the human body.
We explored the physico-chemical properties as well the amino acids content of the milk thistle partially defatted seeds, The obtained results revealed that partially defatted milk thistle seeds are a good source of protein (20.35%), lipids (11,69%), total carbohydrates (38.16%) from which crude fiber (27.24%).
This by-product presents a high mineral content (mg/100g): calcium (912), magnesium (433), iron (80,5), zinc (7,38) and copper (2,69).
The partially defatted milk thistle seeds protein contained markedly amounts of essential amino acids such as arginine, leucine valine and lysine.

***Key words**: Milk thistle, Milk thistle seed oil, Protein, Amino Acids, Crude fiber,*

INTRODUCTION

The milk thistle plant, *Silybum marianum* (family: *Astraceae*) is an annual or biennial plant, native to the Mediterranean area and some parts of the United States, which has now spread to other warm and dry regions (Hadolin M, et al. 2001).
The Asteraceae are one of the largest plant families, with more than 1600 genera and about 23,000 species, most of them existing in temperate regions (Jeffrey, 2007).
The herbalist John Gerard (1545–1612), author of the herbal Generall Historie of Plantes wrote, "My opinion is that milk thistle is the best remedy that grows against all melancholy diseases."
Several studies reveal the important role played by unconventional species, as excellent sources of macro and micronutrients in contributing to human dietary requirements.
Extracts from the mature milk thistle seeds are used as medical remedies for liver disease, liver cirrhosis and to prevent liver cancer (Angeles et al, 2005; Ramasamy K et al., 2008).

Fig. 1. Milk thistle flower

The milk thistle contains silymarin, which is composed of the flavanolignans silybin,

silydianin and silychristine, with silybin being the most biologically active. Silymarin is found in the highest concentrations in the fruit portion of the plant but is also found in the leaves and seeds. The seeds also contain betaine, trimethylglycine and essential fatty acids, which may contribute to silymarin's hepatoprotective and anti-inflammatory effects (Ramasamy K et al., 2008).
The seeds have numerous health beneficial components such as protein, with valuable content of essential amino acids, carbohydrates (especially crude fibres), minerals and some phytochemicals that have antioxidants or antimicrobial properties (Parry et al., 2008).
Therefore, the relevance of studying this traditionally consumed plant is an important strategy to improve the diversity of available foods, which today is receiving the focus of renewed attention.
The aim of the present study was to determine the nutritional composition of partially defatted milk thistle seeds, a byproduct during the manufacturing of the milk thistle seeds oil.
The milk thistle seeds' proteins have a good potential to be used as a valuable source of protein in nutrition.
In their study, Mahmoud A. El-haak et al. (2015) concluded that the whole milk thistle seeds contain high amounts of protein, lipids and total carbohydrates including crude fibers which could be used as a novel source of plant protein, oil and crude fibers. Also, it could be utilized as a suitable food ingredient in low fiber containing food.
Currently there are few works that reveal valuable potential of partially defatted milk thistle seed flour.
Therefore, the main objective of this study was to evaluate the content of valuable compounds from partially defatted milk thistle seeds flour, for use in food industry.
Adding partially defatted milk thistle seeds flour in food products improves the dietary intake of most micronutrients and fiber.
Daily consumption of these products is recommended in order to help preventing major non-communicable diseases such as cardio-vascular diseases and certain cancers (OMS, 2003). Therefore, evaluation of the minerals and other chemical elements contained in food are important.

MATERIALS AND METHODS

Partially defatted milk thistle seeds, a byproduct during manufacture of the milk thistle seeds oil, was kindly supplied by SC Hofigal Export Import SA, (Bucharest, Romania).
This meal has been obtained from milk thistle (*Silybum marianum*) seeds on a large scale through dehulling, grinding and degreasing at low temperatures of less than 40 ^{0} C. The degree of damage to the components of this material may be considered to be low because all steps were performed at low temperature.
Moisture was determined at 103 ^{0}C (±2 ^{0}C) until constant weight (ICC Standard No. 110/1).
Total fat was determined by extracting 10 g of sample with petroleum ether 40-65^{0}C, using a Soxhlet apparatus.

Fig. 2. Milk thistle seeds

Total nitrogen was analysed following Kjeldahl method (official method no 950.36). Ash content (official method no 930.22) in muffle furnace at 450 - 500°C. Crude protein content was calculated by multiplying total nitrogen content by the factor 6.25. Crude fibers of samples were determined using a Fibretherm-Gerhardt apparatus.
Carbohydrate contents were calculated as the difference of 100 - (ash + protein + fat + moisture).
The extracted oil used for fatty acid profile evaluation, was determined according to Mahmoud A. El-haak et al. (2015). Ground seeds (approx. 10 g) were processed with 30 ml hexane/isopropanol (3:2, v/v) at room temperature under vigorous shaking for 1 hour in steel tubes containing four steel balls to facilitate homogenization. Ten tubes, each of them containing 10 g ground seeds, were used to extract oil from 100 g ground seeds. After 1

h shaking, the extracts were filtered through defatted filter papers with a Buchner funnel under vacuum, the remained defatted cake was also washed twice with 20 ml of the same solvent to extract all the possible residual oil content. Thereafter 35 ml of 6.7% sodium sulfate was added to the oil-containing solvent and thoroughly mixed. The upper organic solvent layers containing the oil were then separated and rotary-evaporated under reduced pressure at 35 ^{0}C. The extracted oil was stored at -20 ^{0}C for further analysis.

Fatty Acid profile

Using ^{1}H-NMR spectral technique, the fatty acids composition was determined, especially the concentrations of short-chain saturated fatty acids (C4-C8), di-unsaturated fatty acids, mono-unsaturated fatty acids and long-chain saturated fatty acids (>C8). 1H-NMR spectra were recorded on a Bruker Ascend 400 MHz spectrometer, operating at 9.4 Tesla corresponding to the resonance frequency of 400.13 MHz for the 1H nucleus. Samples were analyzed in 5 mm NMR tubes (Wilmad 507). The NMR samples were prepared by dissolving 0.2 mL oil in 0.8 mL CDCl3. The chemical shifts are reported in ppm, using the TMS as internal standard.

The mineral contents were determined with inductively coupled plasma-mass spectrometer equipment (ICP-MS; Perkin Elmer NexION 300Q).

Quantity was performed using external standards (Merck, multi element standard solution) and all the standard curves were obtained at 6 different concentrations. Total mineral content was measured using their most abundant isotopes.

The dried samples were digested in a mixture of concentrated HCl. For the analysis of the amino acids content, samples were hydrolyzed at 100–120 ºC in 6N hydrochloric acid for 22–24 hours under vaccuum.

After evaporation to dryness of hydrochloric acid, dry residue was diluted using 4 mM stock solution of Norleucine (diluted 500 x with a deionized water solution containing 20 mg/L NaN_3). For the separation of amino acids by gradient anion exchange with pulsed electrochemical detection (PED) was used an ICS300 (Dionex-USA) equipment with the following eluents: deionized water, 0.250 M Sodium hydroxide and 1M Sodium Acetate.

Statistical analysis

All the measurements were performed at least in triplicate. The values of different parameters were expressed as the mean ± standard deviation (s_r).

RESULTS AND DISCUSSION

Chemical composition of partially defatted milk thistle seeds flour is shown in table 1 and display that this byproduct is a rich source of protein, lipids and crude fibers, since protein, lipids and crude fibers contents are 20.35%, 11.69%, and 27.24%, respectively. Ash is 10.79% and total carbohydrate is 57.17%.

Fig. 3. Partially defatted milk thistle seeds flour

These data confirm that partially defatted milk thistle seed flour is a good source of bio-compounds, especially crude fibers (27.24%, d.m.).

Table 1. Chemical composition of Partially defatted Milk thistle seeds (g/100 g based on dry weight bases)

Constitutes	Defatted milk thistle seed flour
Total lipids	11,69 ± 0.24
Ash	10.79 ±0.11
Crude fibers	27.24± 0.45
Total protein (N x 6.25)	20.35 ± 0.24
Total carbohydrates	57,17 ± 0.05

M ± SD = means and standard division of triplicate trails.

Partially defatted milk thistle seed should be considered a source of interesting added value carbohydrate compounds with potential known prebiotic properties, useful to formulate functional foods as well as nutraceuticals.

In the present study, the contents of four biologically essential mineral elements were analysed: calcium (Ca), magnesium (Mg), iron (Fe) and manganese (Mg) and two essential trace elements: zinc (Zn) and copper (Cu).

Table 2. Minerals contents of defatted milk thistle seed flour (mg /100 g)

Constitutes	Defatted milk thistle seed flour	RDI (FDA 2011)
Calcium	912 ± 2.19	1000
Potassium	790 ± 1.65	4700
Magnesium	433 ± 2,20	400
Sodium	11.2 ± 2.6	2400
Iron	80.5 ± 1.85	18
Manganese	7.97 ± 1.91	400
Zinc	7.38 ± 1.70	15
Copper	2.69 ± 0.54	2

From performed analyses regarding minerals content it can be observed that partially defatted milk thistle seeds represent a material having important minerals content, 100 g assuring the daily intake for some of these elements according to The Reference Daily Intake (RDI) of macronutrients and micronutrients recommended by the FDA (2011).

The fatty acids profile of samples is presented in table 3.

Table 3. Fatty acids content of defatted milk thistle seed flour (g/100 g)

Constitutes	Defatted milk thistle seed flour
Short-chain saturated fatty acids	26,53
Mono-unsaturated fatty acids	33,47
Di-unsaturated	40,08
Poli-unsaturated	0

The amino acids composition of partially defatted milk thistle seeds is given in table 4.

The results show that partially defatted milk thistle seeds contained high amounts of all essential amino acids as arginine (12.59%), leucine (9.84%), valine (7.97%), lisine (7.38%).

However, partially defatted milk thistle protein has a slightly smaller source of methionine (2.46%) and cysteine (2.16%).

Essential amino acids are very important for health since they are building blocks of proteins, which carried functions of the human body (Zho, 2007).

Leucine has beneficial effects for skin, bone and tissue wound healing and promotes growth hormone synthesis.

Table 4. Amino acids composition of partially defatted milk thistle seeds flour (as g amino acid/100 g protein).

Type	Amino acids	Partially defatted milk thistle seed
Indispensable amino acids	Arginine	12.59
	Leucine	9.84
	Valine	7.97
	Lysine	7.38
	Phenylalanine	6.10
	Isoleucine	5.41
	Threonine	5.12
	Histidine	3.35
Dispensable amino acids	Glutamic	29.62
	Glycine	8.27
	Serine	7.67
	Proline	7.28
	Aspartic	6.91
	Alanine	6.69
	Tyrosin	5.41
	Methionine	2.46
	Cysteine	2.16

Lysine and valine are essential for muscle proteins. Tyrosine is of foremost importance for dopamine, norepinephrine and adrenalin synthesis. Isoleucine is necessary for the synthesis of hemoglobin in red blood cells. Phenylalanine may be useful against depression and suppressing appetite.

Regarding the nonessential amino acids content there are large amounts of glutamic acid

(29.62%) glycine (8.27%), serine (7.67%) and proline (7.28%).

In short, studies of the food potential of partially defatted milk thistle seeds are not yet available in the scientific literature. Taking into account that consumers are more and more aware about the food quality, especially from the nutritive point of view, the new food resources rich in bioactive compounds are necessary to be found. In this respect, partially defatted milk thistle seeds meet the expectations of such consumers.

CONCLUSIONS

The aim of this study was to evaluate the functional potential of partially defatted milk thistle seeds flour in order to be used for getting food rich in valuable biocompounds.

The chemical composition of the partially defatted milk thistle seeds flour using both classical and spectral methods was analyzed.

Increasing the content of partially defatted milk thistle flour in various food products lead to the increase in dietary fibers, minerals, protein, ash, amino acids and total fat content.

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