

ALFALFA CONCENTRATE – A RICH SOURCE OF NUTRIENTS FOR USE IN FOOD PRODUCTS

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Abstract

Alfalfa (Medicago sativa L.) is primarily grown for and used in animal feed, but in recent years it started to be used more often for human nutrition, as it is a rich source of easily assimilated proteins, minerals (calcium, phosphorus, iron, magnesium, potassium, zinc, copper, selenium, organic silicon, manganese), vitamins (C, K, D, E, U, provitamin A, B1, B2, B6, B12, folic acid/B9, biotin, niacin), as well as β -carotene and eight essential amino acids (alanine, lysine, arginine, histidine, cysteine, proline, methionine, tyrosine).

The aim of the study herein was to evaluate the chemical composition of the alfalfa concentrate, a by-product obtained at the end of the production process for the dietary supplement "Alfalfa Complex". Alfalfa concentrate was found to be a rich source of dietary minerals (K, Ca, Mg, Fe) and dietary fibers. The amino acid composition recorded was: Threonine (0,66%); Lysine (0,71%); Leucine (1,26%); Isoleucine (0,67%); Valine (0,89%); Methionine (0,26%); Phenylalanine (0,84%); Histidine (0,30%); Aspartic acid (1,17%); Tyrosine (0,46%); Cysteine (0,11%); Alanine (0,89%); Glycine (0,80%); Proline (0,95%); Arginine (0,73%); Serine (0,69%); Glutamic acid (1,51%).

The chemical characterization performed in this study is evidence that the alfalfa concentrate can serve as a valuable source of nutritional components for the food industry.

Key words: alfalfa, amino acids, mineral content, dietary fiber

1. INTRODUCTION

Alfalfa (*Medicago sativa* L.) is a legume that has been cultivated for about 2000 years and mainly used in animal nutrition due to its high protein and fiber contents, ranging from 170 to 220 g of protein and about 40–50% of cellulose and lignin per kilogram of shoot dry weight (Gawel & Grzelak 2014).

The latest trends for inexpensive sources of proteins generated an increased interest in plant proteins, as a supplement or replacement of more expensive animal proteins.

Although primarily used in animal feed, alfalfa has been introduced recently into human diet due to its richness in easily assimilated proteins, minerals, vitamins, and essential amino acids. Furthermore, as a nutritious food, the herb has a mild flavor and works well in salads, tortillas, croquettes, puddings, soups and casseroles. In Europe, it is mainly consumed as sprouts, which are added to salads and sandwiches either raw or cooked (Mielmann 2013).

Alfalfa is usually available as a nutritional supplement (capsules, tablets, powder) consisting of plant protein isolate or powdered aerial part, as well as in the form of infusions from leaves and seeds.

Due to its rich content in proteins, vitamins, minerals, isoflavones and other substances with estrogenic activity, anti-inflammatory properties and antioxidant activity (carotens, chlorophyll), alfalfa can be used as an efficient functional ingredient in the dietary prevention and treatment of several metabolic conditions, especially the metabolic syndrome (Lamsal et al. 2007, Bora & Sharma 2011, Sadeghi et al. 2016)

Leaves of alfalfa, in particular, have significant potential for human use due to their nutritive value and their high yields of protein per hectare (Pirie 1972).

Based on their solubility, leaf proteins of alfalfa are classified into two categories. The insoluble green fraction is associated with lipids, chlorophyll and carotenoids and it consists mainly of chloroplastidial lamellar proteins. The white fraction (soluble), has 65% RuBisCO (Ribulose-1,5-diphosphate carboxylase/oxygenase), which is the protein considered most widespread in the world (Firdaous et al. 2017).

Both fractions have a good balance in their essential amino acid composition according to FAO (Food and Agriculture Organization) and the WHO (World Health Organization) recommendations. Their high functionality make alfalfa proteins an important source for both food and pharmaceutical industries (Lamsal et al. 2007).

Furthermore, alfalfa proteins have great functional properties and therefore many applications in the pharmaceutical sector: for example, their antioxidant properties have been reported to be beneficial in diabetes by acting on damaged cells (Sadeghi et al. 2016), atherosclerosis cardiac disorders, hypercholesterolemia (Reilly 1989) and a variety of central nervous system (CNS) disorders such as anxiety (Bora & Sharma 2011).

The objective of the present study was to evaluate the nutritional composition of some by-products obtained at the end of the production process of the dietary supplement "Alfalfa Complex", with the future prospect of using these by-products in the food industry.

2. MATERIALS AND METHODS

2.1. Materials

Alfalfa concentrate flour, a by-product resulted from the production process of the dietary supplement "Alfalfa Complex", was kindly supplied by *Hofigal Export – Import SA* (Bucharest, Romania).



Fig 1. Alfalfa concentrate flour

2.2. Chemical analysis

Moisture content was determined at 103 °C (± 2 °C) (2 g test samples) until constant weight was attained (ICC Standard No. 110/1). The ash content was determined by incineration at 525 ± 25 °C (ICC No. 104/1). Total fat content was determined by extracting 10 g of sample with petroleum ether at 40-65 °C, using a semi-automatic Soxhlet Foss Extraction System 2055 (Foss, Sweden). Total nitrogen (N) and crude protein content ($N \cdot 6.25$, conversion factor) was determined by the Macro Kjeldahl Method (Kjeltec System, FOSS, Sweden). The carbohydrate content was calculated by difference: $100 - (\text{ash content} + \text{protein content} + \text{fat content} + \text{moisture content})$. All experiments were performed in triplicate.

2.3. Crude fiber content analysis

Crude fibers include cellulose, hemicellulose, and lignin. The crude fiber content of the samples was determined using a Fibretherm-Gerhardt apparatus.

The method for determination of crude fibers begins with treating the sample with an acid detergent solution (20 g *N*-cetyl-*N,N,N*-trimethylammonium bromide dissolved in 1 L sulfuric acid 0,5 M). In this solution, cellulose and lignin from the analysed material are insoluble, unlike all other components. Using special FibreBags, the dilution and filtration steps are simplified. The most important aspects of this method of analysis of fibers are adherence to strict boiling times and to weighing procedures.

After treatment with the acid detergent solution, the insoluble residue is dried, weighed and then calcinated. The acid detergent fiber (ADF) content represents the insoluble part of the sample that is left after boiling in acid detergent solution from which the ash obtained upon calcination is subtracted:

$$\%ADF = \frac{((\chi - \alpha) - (\delta - \xi)) * 100}{\beta}$$
$$Blank\ value\ (\xi) = \gamma - \psi$$

where:

α = mass of FibreBag (g)

β = sample mass (g)

χ = mass of crucible and dried FibreBag, after digestion (g)

δ = mass of crucible and and ash (g)

ζ = blank value of empty FibreBag (g)

γ = mass of crucible and ash of the empty FibreBag (g)

Ψ = mass of crucible (g)



Fig 2. Equipment for crude fiber determination

2.4. Mineral content analysis

Mineral content was determined using an atomic absorption spectrophotometer (ContrAA 700; Analytikjena). Total ash was determined by incineration at 550°C, in an oven. Analysis was performed using an external standard (Merck, multi element standard solution) and calibration curves for all minerals were obtained using 6 different concentrations. Dried samples were digested in concentrated HCl.

2.5. Amino acid content analysis

For the analysis of amino acid content, samples were hydrolyzed at 100–120 °C in 6N hydrochloric acid for 22–24 hours under vacuum.

After evaporation to dryness of hydrochloric acid, the dry residue was diluted using 4 mM stock solution of Norleucine.

The chemical score of alfalfa concentrate flour was calculated according to FAO/WHO (1985) as follows:

$$\text{Chemical Score} = \frac{\text{mg/g of essential amino acid in test protein}}{\text{mg/g of essential amino acid reference protein}} \times 100$$



Fig 3. Equipment for amino acid content determination

2.6. Statistical analysis

All analyses were performed in triplicate and the mean values with the standard deviations were reported. Microsoft Excel 2003 Program was employed for statistical analysis of the data with the level of significance set at 95%. Analysis of variance (ANOVA) followed by Tukey's test was used to assess statistical differences between samples. Differences were considered significant for a value of $p < 0.05$.

3. RESULTS AND DISCUSSION

The chemical composition of alfalfa concentrate flour is shown in table 1 and shows that this by-product is a rich source of protein and crude fiber, as their contents are 34.24% and 21.38%, respectively.

Table 1. Chemical composition of alfalfa concentrate flour (g/100 g, based on dry weight)

Constituents	Alfalfa concentrate flour*
Total protein (N x 6.25)	34.24 ± 0.26
Ash	11.65 ± 0.11
Crude fibers	21.38 ± 0.32
Total lipids	1.39 ± 0.11
Total carbohydrates	57.17 ± 0.05

* Results given as: M ± SD (mean ± standard deviation) of triplicate trials.

Alfalfa concentrate flour can be regarded as a source of interesting added value carbohydrate compounds with potential prebiotic properties, useful in the formulation of functional foods as well as nutraceuticals.

In the present study, the contents of six biologically essential mineral elements were analysed: potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), sodium (Na) and manganese (Mg), as well as an additional one essential trace element: zinc (Zn).

Table 2. Mineral contents of alfalfa concentrate flour (mg /100 g)

Constituents	Alfalfa concentrate flour (mg/100g)	RDI (FDA 2011)
Potassium	2300 ± 2.05	4700
Calcium	2100 ± 0.89	1000
Magnesium	790 ± 1,20	400
Iron	17 ± 1.85	18
Sodium	9 ± 1.6	2400
Manganese	3.8 ± 1.91	400
Zinc	2.5 ± 1.70	15

From the performed analyses, it can be observed that the alfalfa concentrate flour represents a material with important mineral contents, 100 g of this material providing the daily intake for some of these elements according to The Reference Daily Intake (RDI) of macronutrients and micronutrients recommended by the FDA (2011). Specifically, 100 g of this by-product contain the necessary daily intake of iron and half of the daily demands of potassium, calcium, and magnesium.

The amino acid composition of alfalfa concentrate flour is given in table 3. The results show that alfalfa concentrate flour contains high amounts of all essential amino acids, particularly leucine (6.42%), valine (4.25%), and phenylalanine (4.12%). However, alfalfa concentrate flour protein is a fairly low source of methionine (1.42%) and cysteine (0.53%).

Essential amino acids are very important for human health as they cannot be biosynthesized *de novo*, but serve as building blocks of certain proteins with major roles in the human body (Zho 2007).

Table 3. Amino acids composition of alfalfa concentrate flour (as g amino acid/100 g protein).

Type	Amino acids	Alfalfa concentrate flour
Indispensable amino acids	Methionine	1.42
	Leucine	6.42
	Valine	4.25
	Lysine	3.46
	Phenylalanine	4.12
	Isoleucine	3.23
	Threonine	3.59
	Histidine	1.51

Dispensable amino acids	Glutamic ac.	7.49
	Glycine	4.34
	Serine	3.54
	Proline	4.74
	Aspartic ac.	5.71
	Alanine	4.83
	Tyrosine	2.35
	Arginine	3.94
	Cysteine	0.53

Whitaker & Tannenbaum (1977) developed the chemical score procedure as a direct evaluation of the ability of a protein source to meet human amino acid requirements. The procedure consists in calculating the percent that each amino acid in the tested protein represents out of the amount of the respective amino acid in the standard protein, where egg protein was initially set as standard for evaluation of food proteins.

Figure 4 shows the amino acid contents of alfalfa concentrate in comparison with those of the standard protein set by FAO/WHO/UNU (1985).

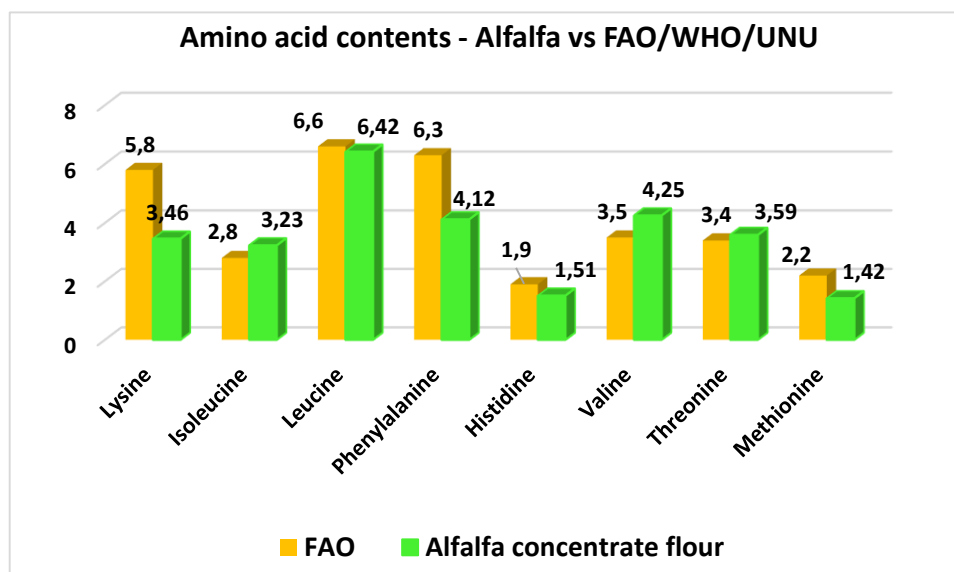


Fig 4. The percentages of amino acids in the alfalfa concentrate protein in comparison with the percentages of amino acids in the standard protein.

Table 4 presents the chemical scores of selected amino acids from the alfalfa concentrate flour calculated as defined in section 2.5.

Table 4. Chemical scores of amino acids in the alfalfa concentrate flour

	FAO/WHO/UNU	Alfalfa concentrate flour	% Amino Acid Scores
Lysine	5.80	3.46	59.66
Isoleucine	2.80	3.23	115.36
Leucine	6.60	6.42	97.27
Phenylalanine	6.30	4.12	65.40
Histidine	1.90	1.51	79.47
Valine	3.50	4.25	121.43
Threonine	3.40	3.59	105.59
Methionine	2.20	1.42	64.55

A strong correlation was reported between chemical scores determined in this manner and values obtained through biological assays for protein quality (Hegsted 1974). Data from table 4 indicates that the alfalfa concentrate protein is a rich source of valine with a chemical score of 121.43, isoleucine with 115.36 and threonine with 105.59. There are also sizeable scores for each of leucine and histidine, at 97.27 and 79.47, respectively. Furthermore, it can be noticed that lysine is the most limited amino acid, followed by methionine and phenylalanine.

4. CONCLUSIONS

The aim of this study was to evaluate the functional potential of alfalfa concentrate flour for use in preparation of foods rich in valuable biocompounds.

The chemical composition of the alfalfa concentrate flour was assessed using both classical and spectral methods.

The characterization performed in this study proved that the alfalfa concentrate flour is a valuable source of minerals, especially potassium, calcium, magnesium, and iron.

Increasing the content of alfalfa concentrate flour in various food products can lead to an increase in content of dietary fibers, minerals, proteins, and amino acids.

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