JAP&T
TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2020, *26*(4), 294-298

Journal of Agroalimentary Processes and Technologies

The influence of some technological operations on the concentrations of proteins from *Cannabis Sativa*

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Abstract

A significant number of valuable bio compounds are lost during food processing. In the research related to the design of a dietary supplement from the wastes of hemp cakes (from which the oil was extracted) and Jerusalem artichoke fibers, the influence of technological operations on the concentration and viability of hemp proteins was analyzed. In the new food supplement concept, were used only organic raw materials and by-products which resulting from the partial processing of other raw materials (e.g. hemp pomace).

It is very important to monitor the influence of certain technological treatments on some protein compounds in Cannabis Sativa.

With the help of these valuable organic compounds, can be built a range of functional foods characterized by a higher nutritional density.

Keywords: hemp pomaces, protein extraction, functional foods

1. Introduction

A significant number of valuable bio compounds are lost during food processing. Therefore, the food processing technologies must be improved to ensures the preservation of ingredient properties, thus offering sustainable high-quality healthy nutrition to the consumers. The main goal of our research project is to obtain high value functional food from organic raw materials and organic sub products which have potential, but are not currently exploited [1]. Very important in obtaining good yields in organic farming is the use of precision measuring instruments - supported by the satellite coordinated system [2].

The applied technologies are not so cheap, but the system can develop the functional food, in the frame of the sustainable development [3]. Today, in the design and construction of these food supplements, some resources are used (such as hemp seeds), thus contributing to maintaining and even increasing food security and reducing the food waste for a growing human population [4].

The feeding a growing world population is a major global challenge, which consists to increase agricultural productivity, guarantee access to food for all individuals, and at the same time develop the food security for consumers [5].

The paper presents some of the results of an important research project that give as objectives using active bio-compounds from Cannabis Sativa (hemp cakes left after removing the oil) and Jerusalem artichoke residues. These leftovers are used to build a food supplement with high nutritional density and a functional food, that addresses consumers with severe anemia. From Jerusalem artichoke were transferred in innovative products, certain compounds (such inulin) but also a number of minerals (especially iron).

Protein, oil, and carbohydrates content of Cannabis sativa L. seeds, as well as fatty acids composition were mainly affected by genotype; whereas, the growing year had a major effect on phytochemical components and on antioxidant activity [6].

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In order to be able to grow hemp in Romania is a special regime of approvals, certifications and approvals from the Police, Health Minister and Agriculture Minister. Not only the cultivation of hemp is almost impossible in Romania, but also, it's processing for medicinal purposes. Thus, Order 244/2005 includes cannabis on the list of dangerous plants. In this Order of the Minister of Agriculture, no distinction is made between the plant with 50 thousand utilities and the one with the same ownership.

The hemp seed contains: 36% oil, 28% protein, 14-27% non-extractive extract, 17.8-26.3% cellulose and 2.5-6.8% ash. Due to this composition, hemp seeds can be used for extracting oil directly used in food and for margarine production. Unrefined oil is used to obtain varnishes, paints, linoleum, soap and waxed cloths.

2.Materials and methods

The first author is involved (as a Project Responsible) in a scientific research program which addresses the possibility of recovery, separation - concentration of active principles from hemp seed residue (results from pressing and extraction of hemp oil) and from *Helianthus tuberosus* leaves [7]. First studies have been carried out in this scope - to know the nutritional potential of these parts of the plant, which will be used in the design and construction of a very valuable food supplement for people with a deficient immune system, with digestive problems, anemia or diabetes [8].

For the production of special dietary supplements, one species of hemp seeds is used in Romania: Secuieni 1, Irene, Denise, Zenit, Diana, Dacia-Secuieni, Secuieni-Jubileu and Ratza - developed and cultivated in the Secuieni Agricultural Development Research Center, Neamt County, Romania. In this research and production unit, been acclimatized and developed award-winning hemp varieties in Europe [9].

The experimental variants were:
C0 – the peeled hemp seeds Variant
C1 – the pressed of peeled hemp seeds Variant
C2 – the dry and ground Hemp pomaces Variant
C3 – the Hemp protein extract Variant
V1 – *Helianthus tuberosus*, Dacic variety
V2 – *Helianthus tuberosus*, Rustic variety
V3 – *Helianthus tuberosus*, Olimp variety
V4 – *Helianthus tuberosus*, local population Dabuleni variety
V5 – *Helianthus tuberosus*, organic agricultural system from Dabuleni

The V1-V5 Variants of *Helianthus tuberosus* were from the Development Research Unit Dabuleni, Dolj County, Romania.

These experimental variants were carefully prepared, according the standardized working methods.

The agricultural technology of these plants (from which some edible parts for the development of valuable food supplements are used) was one free of pesticide and any growth hormones.

Additional measures have also been provided to monitoring the heavy metal content of these plants (used to design and build appropriate food supplements). It knows the high plant capacity of absorption these plants for heavy metals from soil, water, air. Use the Atomic Absorption Spectrometry techniques, got normal values of Mercury, Lead, Iron, Cadmium, Arsenic, Cobalt and Nickel from plant.

Molecular Absorption Spectroscopy and Single Added Method were used in quantification of the main bio compounds of these products. The quantitative determination used Pure Analysis standards of hemeproteins (oxidized and reduced forms), flavoproteins and riboflavin.

The main bio-compounds were extracted from hemp pomaces, respective from Jerusalem artichoke using Supercritical Fluid Extraction (SFE). For this processing it was used one of best knowing SFE System Helix Nature Product from Applied Separation (USA). The operative conditions were 150-650 bars (pressure) and 32-50C degree (temperature), was used SC-CO_2 as extraction solvent and the CO_2 flow rate was 2.5 ml/min.

3.Results and Discussion

In these average samples of *Helianthus tuberoses* leaves were recorded nitrogen values (mg per 100 grams) between 1.263 (in the sample from the organic farming system) and 1.940 (in the local population Dabuleni), phosphorus (between 2.95 in the population from the ecological plot and 3.68 from the Olimp variety), potassium (between 24.33 in the population in the ecological plot and 31.34 in the local population Dabuleni), Calciu (between 1.45 in the population in the ecological plot and 5.47 in the population local Dabuleni), Magnesiu

(between 0.95 in the population of the ecological plot and 2.36 in the local population Dabuleni) [10]

Other elements were recorded at ppm level: Copper (between 5.37 ppm in the population of the ecological plot and 7.94 ppm in the local population Dabuleni), Zinc (between 19.86 ppm in the population of the ecological plot and 28.16 ppm in the local population Dabuleni), Iron (between 95.47 ppm in the population of the ecological plot and 147.42 ppm in the local population Dabuleni), Manganese (between 25.44 ppm in the population of the ecological plot and 33.26 ppm in the local population Dabuleni), Molybdenum (between 3.86 ppm in the population from the ecological plot and 4.25 ppm in the local population Dabuleni).

There were no traces of Lead, Cadmium, Mercury, Arsenium. All these elements were recorded on the leaves of some plants where no treatments were performed and no stimulants were applied. Also, from *Helianthus tuberosus* leaves were detected (percentage of dry matter) proteins (24.2 - 26, 8), simple carbohydrate content (0.6-1.8), cellulose (5.8-6.4).

The maximal level of proteins was registered at C0 and C3 experimental variants. The mild food processing was started by pressing the hemp seeds and remove of the specific oil. This experimental variant (C1) show the decreased level of protein content. This decrease was stopped at 94% from the original hemp seeds. The level of content of hemp oil was 6-8% in this case. Between this percent and the percent of tocopherols from hemp is a direct corresponding and this can show the existing link between the hemp oil level and the active forms concentration of proteins.

Removal of hemp oil causes a certain reduction in the reaction medium. As a result of the grinding and drying processes, the cells viability decreases a lot, so that the C2 variant registers the lowest value (figure 1).

The concentrations of active forms of reduced hemoproteins are recorded in the experimental variant C3 and represents about 30% of the concentration of total hemp proteins (The C0 Variant from figure 1).

The level of hemp processing can influence the level of main protein compounds. One of best indicators for level of processing in this case was the active protein compounds level.

The mild hemp processing can lead to the best final products and assure good level of nutritional density in the final food supplements.

As shown in Figure 2, the concentrations of oxidized forms are higher than the concentrations of reduced hemoprotein forms and this difference is registered in the case of flavoproteins (Figure 3) and riboflavins (Figure 4).

Also, from figures 1 and 2 it results that the lowest redox potential (calculated using Nernst's equation) was found in variant C1 (after oil extraction), where the ratio of the concentrations of oxidized and reduced forms is 1.064. The redox potential of variant C3 (protein extract) was medium and influenced by the ratio of concentrations of oxidized and reduced forms of hemoproteins, at this variant, equal with 1.039.

Using plasma field influences, a transfer of bivalent iron from Jerusalem artichoke extract to Cannabis Sativa protein extracts can be observed. Thus, it could increase the concentration of reduced hemoproteins and increases the antioxidant character of the resulting functional foods.

From figure 3, it can be seen that, following the separation-concentration operation (by migration of proteins in the ionic field), the concentration of active forms of flavoproteins is about 30.7% - in Variant C3, of the total hemp flavoproteins before processing (C0 Variant).

From figure 4, it can be seen that, following the separation-concentration operation (by migration of proteins in the ionic field), the concentration of active forms of riboflavin (especially Flavin Mono Nucleotide) was about 30.25% - in Variant C3, of the total hemp riboflavin concentrations before processing (C0 Variant).

The remaining forms are oxidized and will increase the redox potential of media. It is undesirable way of processing which can blocked the antioxidants form. In case of hemp protein extract can be finding less 32% than from original content.

The mild food processing domain of these converting or even organic products was very poorly developed. The hemp seeds mild processing and fiber hemp processing can be improved in the near future. These processes can produce the high nutritional product and food supplements [7].

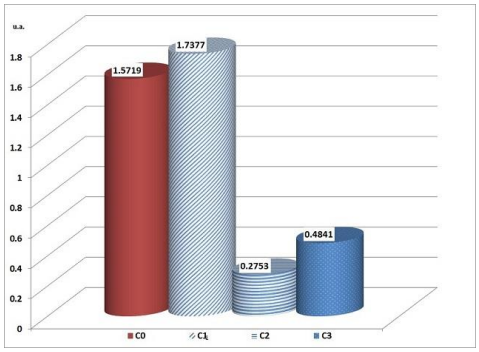

Figure 1. The concentrations of Hemeproteins from *Cannabis Sativa* experimental variants - ***reduced forms of Hemeproteins***

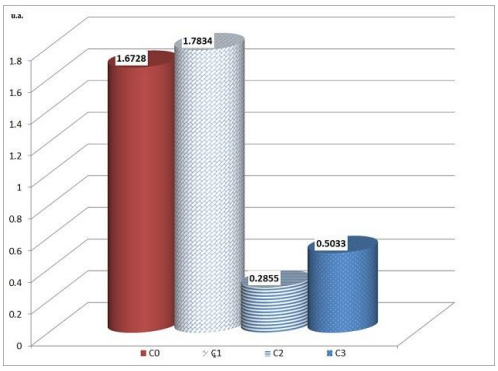

Figure 2. The concentrations of Hemeproteins from Cannabis Sativa experimental variants - ***oxidized forms of Hemeproteins***

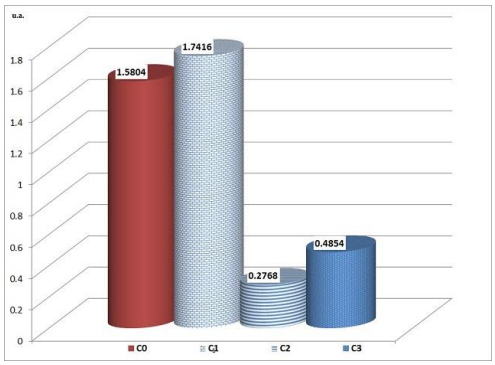

Figure 3. The concentrations of Flavoproteins from *Cannabis Sativa* experimental variants

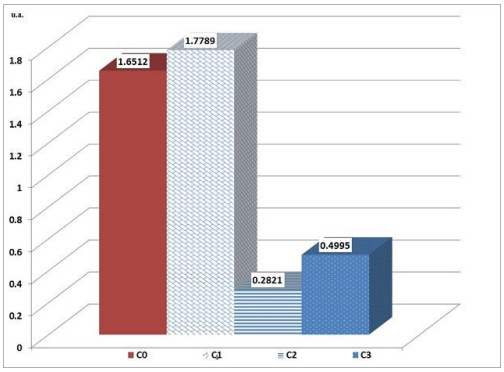

Figure 4. The concentrations of Riboflavin (4) from *Cannabis Sativa* experimental variants

The modern agriculture and technological improvements have sharply increased yields from cultivation, but at the same time have caused widespread ecological damage and negative human health effects [11]. Very important in the development of higher valuable and non-narcotic and hallucinogenic food supplements are Dacia-Secuieni varieties (with a fiber content of 31-33% and 0.013% THC content) and Jubilee Secuieni (with fiber content 20-24% and 0.002% THC content). On the food side, hemp seeds are much richer in protein, essential amino acids, Omega 3 and 6, compared to soybean, pumpkin or sesame seems [12]. And in the pharmaceutical sector, hemp's intake is recognized even in cancer treatments. Also, hemp can extract the healthiest oil as well as environmentally friendly fuel. The Romanian hemp has THC content (delta-9-tetrahydrocannabinol) far below 0.2%, the level required by the EU for industrial crops. On the other hand, compared to the other EU states, in our country, a law adopted in 2000, hemp is called generic "cannabis". There is no difference between THC-free cultivar varieties and THC-rich plant [13].

The Jerusalem artichoke variant V4 contains a higher concentration of iron, but it is less active. Combining the C3 protein extract variant with the Jerusalem artichoke extract V4, an activation of this iron was observed. Protein activity increases, extraction efficiency increases when protective bio-membranes are used for extraction, and a laminar flow regime of supercritical extraction fluid (CO_2) [14].

4. Conclusions

One of best indicators for level of processing in this case was the protein compounds level.

The mild hemp processing can lead to the best final products and assure good level of nutritional density in the final food supplements.

Follow the un-thermic processing, the remove of hemp oil creates one of reduced environment in the hemp pomace.

After the separation-concentration operation (by migration of proteins in the ionic field), the concentrations of active forms of flavoproteins were about 30.7% (at C3), from the total hemp flavoproteins concentrations, before processing (C0).

The level of hemp processing can influence the level of main protein compounds.

Protein activity increases, extraction efficiency increases when protective bio-membranes are used for extraction, and a laminar flow regime of supercritical extraction fluid (CO_2).

The use of plasma technologies can facilitate the transfer of active bivalent iron from Jerusalem artichoke extracts to the new food supplement.

FMN is a basic coenzyme of flavoproteins and is very important in the transfer of oxygen at the solid / air interface of the functional food. Depending on this activity, could be chosen the functional food capsule and the inner and outer packaging.

The organic farming system can be an important source of raw materials for the design and development of extremely valuable food supplements for certain categories of people.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

Acknowledgement: This work was supported by a grant of the ROMANIAN NATIONAL AUTHORITY FOR SCIENTIFIC RESEARCH AND INNOVATION, CNCS/CCCDI – UEFISCDI, PROJECT NUMBER PN-III-P1-1.2-PCCDI-2017- 0566 (CONTR. NR. 9 PCCDI/2018/P4 - *Complex system of full capitalization of some agricultural species with energy and food potential*). *"Valuing the food potential of selected agricultural crops (artichoke tubers, sorghum seeds, hemp pomace - resulting from cold pressed hemp oil)"*

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