

## PHYSICO-CHEMICAL AND MICROBIOLOGICAL CHARACTERIZATION OF *FAGOPYRUM ESCULENTUM* MOENCH. FATTY OIL OBTAINED BY COLD PRESSURE

SAMI KHALASS<sup>1#</sup>, CARMEN POPESCU<sup>2,3#\*</sup>, IOANA ANDREEA POPESCU<sup>1#</sup>, ANDREEA LETIȚIA ARSENE<sup>1#</sup>, DENISA IOANA UDEANU<sup>1#</sup>, DUMITRU LUPULIASA<sup>1#</sup>

<sup>1</sup>“Carol Davila” University of Medicine and Pharmacy, Faculty of Pharmacy, Bucharest, Romania

<sup>2</sup>S.C. HOFIGAL Export Import S.A., Bucharest, Romania

<sup>3</sup>“Vasile Goldiș” Western University, Faculty of Pharmacy, Arad, Romania

\*corresponding author: [popescu\\_carmen88@yahoo.com](mailto:popescu_carmen88@yahoo.com)

#All authors have equal contribution

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### Abstract

Due to the application of ecological principles of life, organic and biologically grown plants (without the use of pesticides) have a large share in the diet as well as in medicine. Lately, in the pharmaceutical and cosmetics industry, vegetal raw material from rustic, unpolluted areas, which are numerous in Romania, is increasingly being used. The habit of cultivating and using *Fagopyrum esculentum* Moench. has systematically and significantly declined throughout the 20<sup>th</sup> century, being disadvantaged by the emergence and spread of nitrogen-based fertilizers, which have facilitated the growth of cereal production. However, *Fagopyrum esculentum* Moench. is very rich in nutrients and antioxidants, including rutine, tannins and catechins. In this study we analysed the physico-chemical parameters (organoleptic, relative density, refractive index, acidity and peroxide index), the fatty acids content (oleic, linoleic, palmitic and stearic acid) and evaluated the microbial contamination of the fatty oil obtained from the seeds of *Fagopyrum esculentum* grown in Romania, in view of further development of a nutraceutical product.

### Rezumat

Datorită aplicării principiilor de viață ecologică, o pondere mare în alimentație precum și în medicină o au plantele cultivate ecologic sau biologic (fără aplicarea unor pesticide). În ultimul timp, în industria farmaceutică și cosmetică, se utilizează tot mai mult materie primă vegetală provenită din zone rustice, nepoluate, care sunt numeroase și în țara noastră. Obiceiul de a cultiva și folosi *Fagopyrum esculentum* Moench. a decăzut sistematic și semnificativ de-a lungul secolului XX, datorită apariției și răspândirii fertilizatorilor pe bază de azot, care au facilitat creșterea producției de cereale. Totuși, *Fagopyrum esculentum* Moench. este o plantă foarte bogată în nutrienți și antioxidanți, printre care rutin, taninuri și catechine. În acest studiu am urmărit analiza parametrilor fizico-chimici (organoleptic, densitatea relativă, indicele de refracție, indicele de aciditate și cel de peroxid), conținutul în acizi grași (acid oleic, linoleic, palmitic și stearic), și am evaluat contaminarea microbiană a uleiului obținut din semințele de *Fagopyrum esculentum* cultivat în România, în vederea dezvoltării ulterioare a unui produs de tip nutraceutic.

**Keywords:** *Fagopyrum esculentum* Moench. seeds, cold press, fatty oils, refractive index, acidity index, peroxide index, microbiological contamination

### Introduction

*Fagopyrum esculentum* Moench. (*Polygonaceae*), also known under the popular name of common buckwheat is considered a crop of secondary importance in many countries, and the main producers are the Russian Federation, China, Ukraine and Kazakhstan [1]. The plant became famous in the last years, in spite of a traditional use in food products, and now is available for sale such as buckwheat flour, bread, tea or sprouts [1, 2, 5].

*Fagopyrum esculentum* Moench. is often included in the vegetarian and low gluten diets due to a complex composition of amino-acids, unsaturated fatty acids, vitamins and antioxidants which sustain the valuable biological effects for a therapeutic use.

The most investigated parts of the plant are flowers, seeds, leaves and rhizome because are rich in active principles.

Recent studies have revealed that the main beneficial effects of the buckwheat consumption were correlated with its antioxidant, anti-inflammatory, antimicrobial, neuroprotective, antidiabetic and hypocholesterolemic properties [3, 4, 9-11].

The cold press is a reliable method for obtaining high quality vegetal extracts with preserved composition close to the natural product and is preferred for obtaining health beneficial products [2].

The paper aims to investigate the chemical composition of the fatty oil extracted by cold press from the

buckwheat seeds for future use in nutraceutical products.

## Materials and Methods

### *The vegetal samples*

We used three fatty oil samples obtained by cold pressure technique from *Fagopyrum esculentum* Moench. seeds harvested from three different parcels and denoted as U1, U2 and U3.

### *The physico-chemical characterisation of the cold pressed fatty oil extracted from buckwheat seeds (Fagopyrum esculentum Moench.)*

The following analyses were performed: organoleptic, relative density, refractive index, acidity index and peroxide value, together with the content of the main fatty acids, namely oleic, linoleic, palmitic and stearic acids.

### *Relative density ( $d_{20}^{20}$ )*

The assay complies to the European Pharmacopoeia 9<sup>th</sup> Ed. recommendations [8]. Briefly, the relative density ( $d_{20}^{20}$ ) was calculated as the ratio between the weights of the pycnometer filled with water and afterwards with fatty oil, at 20°C.

### *Refractive index*

The assay complies to the Eur. Ph. 9<sup>th</sup> Ed. [8]. *Equipment*: refractometer (Thermo Scientific, USA). Briefly, a few drops of the fatty oil samples were analysed with the refractometer at  $20 \pm 0.5^\circ\text{C}$  and the wavelength of the sodium D radiation was  $\lambda = 589.3 \text{ nm}$ .

### *Acidity index*

*Reagents* (Sigma Aldrich, USA): ethylic alcohol (96%, v/v), diethyl ether, light petroleum ether R3, potassium hydroxide, phenolphthalein.

5.0 g sample of fatty oil extracted by cold press from buckwheat seeds were diluted with equal volumes of ethylic alcohol and light petroleum ether previously neutralized with 0.1 M potassium hydroxide solution using phenolphthalein as indicator. The mixture was heated at 90°C to dissolve faster the samples and then the mixture was titrated with 0.1 M potassium hydroxide [8].

### *Peroxide index*

*Reagents* (Sigma Aldrich, USA): chloroform, acetic acid, potassium iodide, mercuric iodide, sodium thiosulfate, starch.

5.0 g of fatty oil sample were dissolved in chloroform and acetic acid. Saturated solution of potassium iodide and water were added and mixed for one minute and then titrated with 0.01M sodium thiosulphate in the presence of a starch suspension. A control sample was analysed respecting the same procedure and conditions [8].

### *The assay of the fatty acids content*

Gas chromatographic analyses of the chemical compounds were implied, according to the Eur. Ph. 9<sup>th</sup> Ed. [8].

*Reagents* suitable for gas chromatography were purchased from Sigma Aldrich (USA): petroleum ether,

hydrochloric acid in methanol, isooctane, heptane, anhydrous sodium sulphate.

### *Equipment*

Gas chromatograph with mass spectrometer detector (GC-MS tip Focus GP-DSQ-2, Finnigan Thermo-Scientific, USA), split injection, automatic system for integrating peak area with the following characteristics: macrogol 20000 GC column ( $l = 30 \text{ m}$ ;  $\varnothing = 0,25 \text{ mm}$ ). *Chromatographic conditions*: temperature program: the initial temperature was 160°C (time 0 - 5 min), the temperature was increased with 10°C/min to 200°C (time 6 - 10 min), and then increased to 225°C and maintained constant for 10 min; injection port temperature: 220°C; detector temperature 220°C; split ratio: 1/60; carrier gas: helium, at a flow rate of 1.5 mL/min; sample injected volume 1  $\mu\text{L}$  for each solution.

### *Samples preparation*

The sample of *Fagopyrum esculentum* Moench. seed fatty oil was dissolved in petroleum ether and hydrochloric acid and was maintained at 65°C for 1 h under reflux. The sample was cooled and washed several times with water and then isooctane solution. The isooctane extract was separated and mixed with anhydrous sodium sulphate for water removal and then filtered.

*Standard solution*: the standard calibration solution was prepared according to the Eur. Ph. 9<sup>th</sup> Ed. using heptane solvent. The chemical composition of the standard solution was the following: methyl laureate, methyl myristate, methyl palmitate, methyl stearate, methyl arachidate and methyl oleate.

### *GC-MS method*

1  $\mu\text{L}$  of standard solution and 1  $\mu\text{L}$  of *Fagopyrum esculentum* Moench. seed fatty oil sample were injected in the GC/MS system and the retention times were determined. The minimum resolution determined by methyl oleate and methyl stearate in the standard solution chromatogram was minimum 1.8 between peaks. The seed fatty oil composition was identified based on MS spectrum library with M/Z values: 40 - 450. The normalizing procedure was applied in order to determine the percent concentration of the components.

### *The evaluation of the microbial contamination of the Fagopyrum esculentum Moench seed oil [8]*

*Equipment*: Venticell 111 oven, Incucell Incubator 404, Friocell Incubator 404, microbiological box-laminar air flow Telstar AV 100 and BSC-EN I-IV.

*Reagents, culture mediums and solutions* used for (i) the determination of the total number of aerobic microorganisms (TAMCs): casein soya bean digest agar, buffered sodium chloride broth pH = 7.0; (ii) the determination of the total number of yeasts and filamentous fungi (TYMC): Sabouraud agar with dextrose, buffered sodium chloride broth pH = 7.0; (iii) the isolation and identification of gram-negative bacteria-tolerant bile salts: *Enterobacteria enrichment* broth (Mossel), violet red bile glucose agar, casein

soya bean digest broth; (iv) the control of specific microorganisms, namely *E. coli* and *Salmonella sp.*: MacConkey broth, MacConkey agar, casein soya bean digest broth, triple sugar iron agar (TSI), Rappaport Vassiliadis *Salmonella* enrichment broth, xylose, lysine, deoxycholate agar.

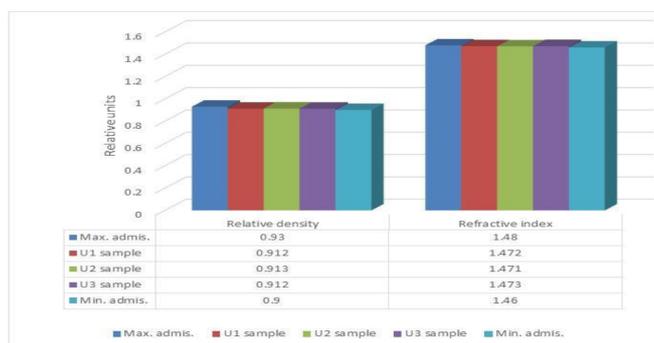
## Results and Discussion

*The physico-chemical characterisation of the cold pressed fatty oil extracted from buckwheat seeds (Fagopyrum esculentum Moench.)*

The three samples of fatty oil obtained from *Fagopyrum esculentum* seeds appeared as clear

yellow-gold oily liquids with characteristic flavour and smell. Regarding the relative density, the samples showed uniform results on this parameter (U1 = 0.912, U2 = 0.913, U3 = 0.912) and are in accordance with the analytical regulations in force ( $d_{20}^{20}$  ranging between 0.900 - 0.930, Figure 1) [8].

The refractive index assay established values that are in accordance with the actual Eur. Ph. (between 1.460 and 1.480), for all the three samples considered, namely for U1 fatty oil sample the refractive index was 1.472, for U2 sample its value was 1.471 and 1.473 for U3 *Fagopyrum esculentum* fatty oil (Figure 1).



**Figure 1.**

The results obtained for the relative density and refractive index assays, for the studied *Fagopyrum esculentum* seeds fatty oil samples

Regarding the acidity index, the Pharmacopoeia establishes a maximum value of 2.0. The assay of this parameter on our three vegetal samples developed accurate results, namely a value of 0.20 for the U1 sample, 0.21 for the U2 sample, while for U3 the acidity index was 0.18 (Figure 2).

According to the official regulations, the peroxide index should not exceed 5.0 for any product submitted to the evaluation. Our samples showed results of 0.80 (U1), 0.81 (U2) and 0.78 (U3), which comply with the above mentioned limitations (Figure 2).

**Figure 2.**

The results obtained for the acidity and peroxide indexes assays, for the studied *Fagopyrum esculentum* seeds fatty oil samples

### *The assay of the fatty acids content*

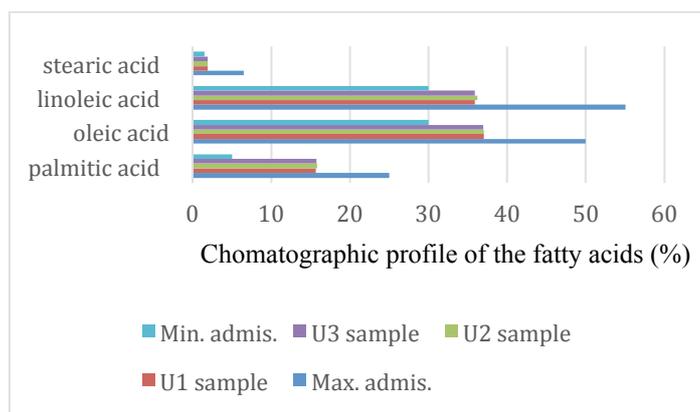
From the evaluation of the chromatographic profiles of the three fatty oil samples obtained by the cold pressing of the buckwheat seeds, the following results were obtained: a very good content in oleic acid and linoleic acid together with the presence of

certain amounts of palmitic and stearic acids to the lower limit of admissibility (Figure 3).

Namely, for the oleic acid, the limits of acceptance should range between 30% - 50% (w/w). The obtained oleic acid content was 37.05% for U1, 37.36% for U2, and 36.98% for U3 respectively (Figure 3). The linoleic acid content's range officially accepted are

between 30% and 55% (w/w) and our samples developed concordant results, as follows: a linoleic

acid concentration of 35.88% for U1, 36.22% for U2 and 35.91% for U3, respectively (Figure 3).



**Figure 3.**

The concentrations of oleic, linoleic, stearic and palmitic acids determined in the studied *Fagopyrum esculentum* seeds fatty oil samples

*The evaluation of the microbial contamination of the Fagopyrum esculentum Moench seed oil*

The results obtained from the microbiological analysis of fatty oil samples of *Fagopyrum esculentum* Moench. are presented in Table I. Our data showed

that there was no microbial contamination developed during the technological process (cold pressing) of the buckwheat seeds fatty oil preparations, for all the soil samples studied.

**Table I**

The results obtained from the microbiological assays of fatty oil samples of *Fagopyrum esculentum* Moench.

Microbial contamination assays	Limits	U1 sample	U2 sample	U2 sample
Total aerobic microorganisms (TAMC), CFU/mL, max.	$1 \times 10^4$	< 10	< 10	< 10
Combined total yeast and filamentous fungi (TYMC), CFU/mL, max.	$1 \times 10^2$	< 10	< 10	< 10
Gram-negative bile salts tolerant bacteria, CFU/mL, max.	$1 \times 10^2$	< 10	< 10	< 10
<i>Escherichia coli</i> /mL	absent	absent	absent	absent
<i>Salmonella spp.</i> /25 mL	absent	absent	absent	absent

In this regard, the total aerobic microorganisms (TAMC), the combined total yeast and filamentous fungi (TYMC) and the gram-negative bile salts tolerant bacteria were below 10 CFU/mL, very much below the acceptance limits (Table I). Also, the samples were tested for the presence of *Escherichia coli* and *Salmonella spp.*, according to the official rules in force. Our results showed the absence of the mentioned strains, for all the studied fatty oil samples.

Our study considered the preparation of *Fagopyrum esculentum* seeds fatty oil through the cold pressure method, and its analysis from the physico-chemical point of view, together with the assay of the vegetal product's microbial contamination. We evaluated three buckwheat seeds fatty oil samples, obtained from plant species cultivated on different parcels. The parameters assayed were requested by the European regulations in force, namely the macroscopic characteristics (aspect, colour, smell, taste) and also specific ones, the refractive, acidic and peroxide indexes. Our results showed that all the *Fagopyrum esculentum* fatty oil samples complied with the

official limitations' ranges, for each of the parameters considered.

We also determined the sample's content in regard to the main fatty acids requested, the oleic, linoleic, palmitic and stearic acids. Our results showed concordant and relevant concentrations of oleic and linoleic acids, together with low amounts of palmitic and stearic fatty acids. Similar data were also reported in the literature, although differences may arise due to the different geographical areas the plant was cultivated [6, 7].

The assay of the microbial contamination attested the fact that the processing technology of the vegetal material did not allow the presence, nor the growth of any microorganism with harmful potential.

Our future studies will try to establish the biological potential of this product, together with its toxicological profile.

**Conclusions**

*Fagopyrum esculentum* Moench. (buckwheat) has gained great attractiveness in time and attempts are

being made to develop useful formulations based on the different parts of this plant.

In this context, we obtained fatty oils out of the plant's seeds through the cold pressure technique and we evaluated the physico-chemical parameters and also, the microbial contamination of the vegetal products. In this context, we report encouraging results for a future development of a potential buckwheat fatty oil nutraceutical.

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