

RUTIN CONTENT IN BUCKWHEAT (*FAGOPYRUM ESCULENTUM* MOENCH, *F. TATARICUM* (L.) GAERTN. AND *F. CYMOSUM* MEISSN.) GROWTH IN THE FAR EAST OF RUSSIA

VSEBNOST RUTINA V AJDI (*FAGOPYRUM ESCULENTUM* MOENCH, *F. TATARICUM* (L.) GAERTN. IN *F. CYMOSUM* MEISSN.) PRIDELANI NA DALJNEM VZHODU RUSIJE

Alexey KLYKOV^{1*}, Elena CHAIKINA², Michail ANISIMOV², Svetlana BOROVAYA¹, Elena BARSUKOVA¹

<http://dx.doi.org/10.3986/fbg0068>

ABSTRACT

Rutin content in buckwheat (*Fagopyrum esculentum* Moench, *F. tataricum* (L.) Gaertn. and *F. cymosum* Meissn.) growth in the Far East of Russia

The paper presents results of the complex research of different species of *Fagopyrum* (*F. esculentum* Moench, *F. tataricum* (L.) Gaertn., *F. cymosum* Meissn.) on rutin content and their usage prospects as a resource of flavonoids. Relation between rutin content in the overground mass and the plant colour was found. Biological significance of rutin, prospects of its usage as a diagnostic trait in selection, are also shown in the article. Bio-chemical and technological traits of *F. esculentum* varieties cultivated in the Far East Russia were also studied and reflected in the paper. We therefore examined rutin content in the overground phytomass of the three species of *Fagopyrum* on the phase of mass flowering and discovered that high indices belong to: *F. esculentum* (Izumrud variety) - 3.8 %, *F. tataricum* (sample k-62 from Canada) - 4.4 % and *F. cymosum* (k-4231 from India) - 4.1 %.

Rutin content in the hull of common buckwheat ranged from 0.08 till 0.20 %. Maximum rutin quantity was determined in the hull of Ussurochka (35.7 kg/ha), and minimum (17.8 kg/ha) in the hull of Pri 7. The studies show that the ash content, obtained after burning the hull of *F. esculentum* (600°C) is in average 2 %. The following elements were found in the ash: potassium, sodium, copper, silver, calcium, magnesium, zinc, aluminum, manganese, iron, nickel, chromium, phosphorus, and their concentration depend on variety and type of raw material of *F. esculentum*.

Key words: *Fagopyrum esculentum*, *F. tataricum*, *F. cymosum*, overground mass, hull, rutin.

IZVLEČEK

Vsebnost rutina v ajdi (*Fagopyrum esculentum* Moench, *F. tataricum* (L.) Gaertn. in *F. cymosum* Meissn.) pridelani na Dalnjem vzhodu Rusije

V prispevku so predstavljeni rezultati kompleksne raziskave različnih vrst rodu *Fagopyrum* (*F. esculentum* Moench, *F. tataricum* (L.) Gaertn., *F. cymosum* Meissn.) o vsebnosti rutina in možnosti njihove uporabe kot vira flavonoidov. Ugotovljeno je bilo razmerje med vsebnostjo rutina v nadzemni masi in barvo rastlin. V članku je prikazan tudi biološki pomen rutina, možnosti njegove diagnostične uporabe pri selekciji oziroma njegov pomen pri selekciji. V delu so bile proučene tudi biokemijske in tehnološke lastnosti sort *F. esculentum*, ki jih gojijo na Dalnjem vzhodu Rusije. Zato smo določali vsebnost rutina v nadzemnih delih treh vrst rodu *Fagopyrum* v fazi cvetenja in ugotovili, da visoki indeksi pripadajo: *F. esculentum* (sorta Izumrud) - 3,8 %, *F. tataricum* (vzorec k-62 iz Kanade) - 4,4 % in *F. cymosum* (k-4231 iz Indije) - 4,1 %. Vsebnost rutina v luščinah navadne ajde je znašala od 0,08 do 0,20 %. Največja količina rutina je bila določena v luščinah Ussurochka (35,7 kg/ha) in najmanjša (17,8 kg/ha) v luščinah Pri 7. Študije kažejo, da je vsebnost pepela, pridobljenega po upočeljenju luščin *F. esculentum* (600°C), v povprečju 2 %. V pepelu so bili določeni naslednji elementi: kalij, natrij, baker, srebro, kalcij, magnezij, cink, aluminij, mangan, železo, nikelj, krom, fosfor, njihova koncentracija pa je odvisna od sorte in rastlinskega dela vrste *F. esculentum*.

Ključne besede: *Fagopyrum esculentum*, *F. tataricum*, *F. cymosum*, nadzemna masa, luščine, rutin.

¹ FSBSI “FSC of agribiotechnology in the Far East named after A.K. Chaika”, Russia

² G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Far Eastern Branch of the Russian Academy of Sciences, Russia

* E-mail address of corresponding author: alex.klykov@mail.ru

INTRODUCTION

Species of *Fagopyrum* Mill. genus have valuable edible and medicine traits. *Fagopyrum esculentum* Moench is a cereal and melliferous crop which is widely cultivated in many countries of the world. The main producers of buckwheat are China, Russia and Ukraine. In some countries of the South-Eastern Asia (China, India) *F. tataricum* (L.) Gaertn., and *F. cymosum* Meissn. are used as an edible and medicine crop. Plants of species of *F. esculentum* are widely used in popular medicine. As a medicinal raw they use leaves and tops of shoots in blooming stage (KREFT et al. 2006, HINNEBURG & REINHARD 2005). Representatives of *Fagopyrum* genus are prospective resources of flavonoids. The main flavonoids is 3-O-rutinozid quercetin (rutin or vitamin P), which has antioxidant, angioprotective, antibacterial and hepatoprotective traits (ODETTI et al. 1990, GRINBERG et al. 1994, HE et al. 1995, GUARDIA et al. 2001, HOLASOVA et al. 2002, MASHKOVSKY 2004, SRINIVASAN et al. 2005).

In some countries (Russia, Canada, Ukraine, Japan) there were developed special buckwheat varieties for

rutin production with its high content. As a result of the chemical analysis of buckwheat plants selected for colour, it was found that plants with red coloring of stems contain more rutin in comparison with green plants, green-red and red-green. Buckwheat plants are selected according to the stalk coloring in a fruit formation stage, choosing plants with dark red (anthocyanin) colour (KLYKOV & MOISEYENKO 2005, ANISIMOVA 2011). Our research has shown that intra-variety changes of the plants colour have a wide spectrum (red, red-green, green-red and green) and are affected not only by the variety genotype but largely by variability, which effect depends on various factors: sowing date, mineral fertilizers, seeding rate and method of sowing (KLYKOV 2000, KLYKOV & MOISEYENKO 2010).

In our view, anthocyanin colour of the stalk is a good diagnostic indicator that can be used for the goal selection of buckwheat plants with high rutin content in the overground part. In connection with this fact, *Fagopyrum esculentum* is a promising domestic source of rutin for the pharmaceutical industry.

MATERIALS AND METHODS

Plant materials: The field experiments were carried out at FSC of agribiotechnology in the Far East named after A.K. Chaika (44.34°N, 131.58°E), Ussuriysk district, Primorsky krai, Russia and in the Pacific Institute of Bioorganic Chemistry named after G.B. Elyakov the Far Eastern Branch of Russian Academy of Sciences. As the research object there were used representatives of the family *Polygonaceae* Juss: cultivated species of the genus *Fagopyrum* Mill. (*Fagopyrum esculentum* Moench, *Fagopyrum tataricum* (L.) Gaertn., *Fagopyrum cymosum* Meissn.).

Soil of the experimental field was meadow-brown, bleached. Power of the arable layer is 24 cm, humus content is 3.8 %, P_2O_5 – 17.1 mg/100 g of soil, K_2O – 14.2 mg/100 g of soil, pH – 5.7.

Representatives of *Fagopyrum* Mill. genus of domestic and foreign origin were received from the Gene Bank of the All-Russia Institute of Plant Growing named after N.I. Vavilov (St. Petersburg, Russia). There was evaluated collection of 4 Tartary buckwheat (κ-6, Germany; κ-8, France; κ-17, China; κ-62, Canada) accessions with different place of origin.

Common buckwheat accessions in tested collection were originated from the states of the former Russia (Primorskaya local, Pri 7, Ussurochka, Pri 10 and

Izumrud). The research objects were sample of *F. cymosum* (κ-4231, India).

Preparation of aqueous plant extracts: Max. 30 plants of each genotype were used for the analysis. Dry matter content and so called total rutin were determined in whole plants. After drying the plants parts were ground finely. Three analytical methods for rutin determination were tested: high-performance liquid chromatography (HPLC) and spectrophotometric method (VYSOCHINA et al. 1987). The absorption of the extract solution was measured at 360 nm on spectrophotometer Shimadzu UVmini-1240 (Japan) and compared to that of a standard rutin curve.

Rutin determination by HPLC (KREFT et al. 2002). An amount of 0.1-0.5 g of ground plant material was extracted with 10 ml of a solution (methanol-acetic acid-water 100:2:100) for 1 hour on a shaker at laboratory temperature. 2 ml of the extract were centrifuged for 10 min. at 9 000 rot/min. A clear supernatant was filtered through a microfilter with a regenerated cellulose membrane, and it was analysed on a Waters Alliance 2690 liquid chromatograph. The filtrate was applied to a Lichrospher 100RP-18 column and eluted by gradient elution with a mixture of methanol-water: 0-2 min 20 % methanol 2-4 min 60 % methanol. Detection with a

UV detector was carried out at 360 nm. Rutin was eluted at the 6th min. and the peak area was compared with standard solutions of pure rutin. Rutin concentration in samples was determined by a calibration curve.

For rutin identification there were used NMP ¹H spectrums, that were registered on spectrometer AC-250 in CDCl₃ and d₆. Then they compared the spectrums with the pure rutin ("Chemopol", Czech Republic). Mass-spectrums were produced on the equipment LKB-9000S (Sweden) with straight input under the energy of ionizing electrons equal to 18 and 70 eV(electron-volt).

Polysaccharides were analyzed with the help of X-ray Diffractometer Shimadzu Lab XRD 6000. IR

spectroscopy method high-efficient size-exclusion chromatography were used for studying structural peculiarities of the found polysaccharides in Institute of Chemistry of Far East Branch of the Russian academy of Sciences (ZEMNUKHOVA et al. 2004 a, ZEMNUKHOVA et al. 2004 b).

Statistical analysis: The means and standard deviations were calculated using Microsoft Office Excel 2003. Significant differences of these data were calculated using analysis of variance (ANOVA-Duncan's multiple test, SIGMASTAT 9.0). The reliability of the results between the control and experimental samples was evaluated using Student's t-test.

RESULTS AND DISCUSSION

Rutin content depends on genus, species and variety (KLYKOV et al. 2003, JIANG et al. 2007, JINFENG GAO et al. 2007, YAN CHAI et al. 2007). We investigated rutin content and productivity of overground part of different samples of three *Fagopyrum* species plants.

The data show that high rutin content was present in overground part of *F. esculentum* (Izumrud variety) - 3.8 %, *F. tataricum* (sample k-62 from Canada) - 4.4 %, *F. cymosum* (k-4231 from India) - 4.1 % (Table 1).

Table 1: Productivity of the overground part in the mass flowering stage and rutin content in three distinguished samples of *Fagopyrum* species plants

Preglednica 1: Pridelok nadzemnih delov v polnem cvetenju in vsebnost rutina v rastlinah treh vrst *Fagopyrum*

| Variety, number in the catalogue ARSRIPG, origin | Green mass, ton /ha | | Dry matter, ton /ha | | Rutin, % | | Rutin, kg /ha | |
|--|---------------------|----------|---------------------|---------|----------|---------|---------------|-----------|
| | lim | ȐX±SȐ | lim | ȐX±SȐ | lim | ȐX±SȐ | lim | ȐX±SȐ |
| <i>Fagopyrum esculentum</i> | | | | | | | | |
| Izumrud (Russia) | 21.1-28.4 | 25.3±0.2 | 2.8-3.5 | 3.2±0.1 | 3.4-4.2 | 3.8±0.1 | 95.1-147.3 | 121.2±71 |
| Primorskaya local (Russia) | 18.3-24.5 | 22.4±0.2 | 2.5-3.2 | 2.8±0.1 | 2.9-3.5 | 3.2±0.1 | 72.5-112.3 | 92.5±4.7 |
| Pri7 (Russia) | 19.2-25.3 | 23.7±0.2 | 2.7-3.4 | 3.0±0.1 | 2.8-3.4 | 3.1±0.1 | 76.3-116.2 | 96.2±39 |
| <i>Fagopyrum tataricum</i> | | | | | | | | |
| k-6 (Germany) | 17.1-26.2 | 20.1±0.2 | 2.5-3.0 | 2.8±0.1 | 3.7-4.4 | 4.1±0.2 | 92.1-132.3 | 112.1±6.0 |
| k-8 (France) | 20.4-24.3 | 22.3±0.2 | 2.2-2.8 | 2.5±0.1 | 3.9-4.6 | 4.1±0.2 | 86.1-129.6 | 107.4±5.1 |
| k-17 (China) | 25.0-28.5 | 26.2±0.2 | 2.8-3.4 | 3.1±0.1 | 3.2-4.8 | 4.2±0.2 | 89.4-163.3 | 126.3±7.2 |
| k-62 (Canada) | 24.1-32.2 | 27.1±0.2 | 2.6-3.3 | 2.9±0.1 | 3.6-5.0 | 4.4±0.1 | 94.2-165.4 | 129.1±7.9 |
| <i>Fagopyrum cymosum</i> | | | | | | | | |
| k-4231 (India) | 18.4-24.3 | 20.3±0.2 | 1.9-2.4 | 2.2±0.1 | 3.2-4.4 | 4.1±0.1 | 61.3-106.7 | 83.2±4.4 |

Productivity of overground mass and dry matter differed between samples of *F. tataricum* k-17 (China), k-62 (Canada), *F. esculentum* – variety Izumrud (Russia, Primorskiy Krai) and *F. cymosum* k-4231 (India). Procurement of raw materials is the most efficient when the maximum rutin concentration coincides with peak growth of the plant phytomass.

Studied species of *Fagopyrum* genus undergo a complete cycle of development: vegetative (shoots-flowering), generative (flowering – seed filling). Total

duration of the vegetation period of *Fagopyrum* is 72-90 days. Due to phenological observations it was noted that vegetation period was longer for samples of *F. tataricum* and *F. cymosum* (from 33 to 52 days) than those of *F. esculentum* (24-26 days). The generative period duration for samples of *F. tataricum* ranged from 32 to 46 days, for *F. cymosum* – 38 days, for *F. esculentum* – 48-49 days. The longest vegetation period was observed for *F. cymosum* (k-4231 from India – 90 days), and the shortest period number 63

days for sample of *F. tataricum* (k-6 from Germany). For the varieties of *F. esculentum* it ranged from 72 to 75 days.

Rutin content in the vegetative and generative parts, roots of *F. esculentum* (Izumrud variety, Russia), *F. tataricum* (sample k-62, Canada) and *F. cymosum* (sample k-4231, India) during vegetation varies from the early stages of plant development. The highest rutin content in all studied samples was observed in flowers (4.7-6.3 %), much less in the stems (0.6-1.4 %) and the lowest rutin content was found in the roots (0.3-0.8 %).

The maximum rutin content was observed in leaves of *F. esculentum* in the budding stage (4.6 %), in *F. tataricum* - at the beginning of flowering (4.8 %), and minimum rutin content in the seed filling phase (2.8 %), as well as at the beginning of vegetation and seed filling phase (*F. esculentum* – 3.0 and *F. tataricum* – 3.1 %, respectively). To determine the optimal harvesting time for the raw materials, when the most rutin from the overground phytomass of the studied species can be got, there was calculated rutin quantity derived from 1 ha of the experimental plot with the density of 120 plants per 1 m² (Table 2).

Table 2. Yield of overground part and rutin quantity of three *Fagopyrum* species in different development phases of the plants

Preglednica 2: Pridelek nadzemnih delov in vsebnost rutina pri rastlinah treh vrst *Fagopyrum* glede na faze razvoja rastlin

| Species | Development phase | Overground mass yield, ton /ha | Rutin, kg /ha |
|-----------------------------|----------------------|--------------------------------|---------------|
| <i>Fagopyrum esculentum</i> | Vegetation beginning | 0.48±0.3 | 12.0±1.8 |
| | Budding | 0.84±0.4 | 25.2±2.6 |
| | Flowering beginning | 1.68±0.5 | 62.2±5.0 |
| | Mass flowering | 2.88±0.7 | 109.4±7.1 |
| | Seed filling | 3.82±0.8 | 84.6±5.4 |
| <i>Fagopyrum tataricum</i> | Vegetation beginning | 0.36±0.2 | 8.3±0.9 |
| | Budding | 1.44±0.4 | 46.1±4.2 |
| | Flowering beginning | 1.80±0.6 | 73.8±5.4 |
| | Mass flowering | 3.00±0.7 | 117.0±8.3 |
| | Seed filling | 4.00±0.8 | 104.0±8.1 |
| <i>Fagopyrum cymosum</i> | Vegetation beginning | 0.24±0.2 | 4.6±0.6 |
| | Budding | 2.28±0.4 | 66.1±5.8 |
| | Flowering beginning | 2.88±0.7 | 100.8±7.3 |
| | Mass flowering | 3.12±0.8 | 115.4±8.3 |
| | Seed filling | 4.92±0.9 | 108.2±7.9 |

Maximum rutin quantity (117 kg/ha) is possible to be derived from overground phytomass of *F. tataricum* in the mass flowering stage. Rutin content in dry raw materials in this stage is high enough (4.4 %), and yield of dry phytomass in this period was 3.00 ton/ha. We consider it to be important for gathering rutin per hectare.

Close to them there were rutin output indices of *F. cymosum* (1154.7 g) and *F. esculentum* (109.4 kg) obtained from 1 ha of the experimental plot in the same stage. During the seed filling phase there was also observed high rutin output in the overground phytomass of *F. tataricum* (104 kg) and *F. cymosum* (108.2 kg).

Preparation of raw materials (overground phytomass) of studied *Fagopyrum* species is reasonable to perform in the stage of the plants mass flowering, because during this period, the amount of rutin was the highest. At present time rutin content in the roots of *Fagopyrum* Mill. species is studied scarcely as well as

its role in selection on lodging resistance. Therefore, we studied growth dynamics of overground and root mass, the root system maintenance, rutin content in roots of *F. esculentum* and *F. tataricum*.

As a result of the research there were determined significant correlations of *F. tataricum* between rutin content in the roots with aboveground part ($r = 0.92$) and root mass ($r = 0.93$), and the root system maintenance ($r = 0.89$). Intensive growth of *F. esculentum* root mass takes place before the budding stage. Then deceleration in growth is observed and reduction on seed filling phase. It is connected with ageing and decay of the roots. Increase of *F. tataricum* root mass was observed throughout the whole vegetation period.

It was found that *F. esculentum* plants, resistant to lodging, contain 0.68 % of rutin in the roots (bright coloring of the root system), and sensitive plants contain – 0.31 % (dark brown coloring of roots). Apparently, resistance to lodging of plants on a seed filling

phase, is connected to physiologically active root system (viable), which directly influences the intense accumulation of rutin, in comparison with sensitive ones. The identified relation between rutin content, the roots coloring and the root mass became the basis for development of selection method of buckwheat plants on lodging resistance (KLYKOV & MOISEYENKO 2003).

This method provides selection of buckwheat forms on three indicators:

- 1) the root system colouring;
- 2) maintenance of the root system;
- 3) rutin content in the roots.

At the starting stage of breeding visual selection was taking into account. These indicators will contribute to increase efficiency in the samples selection with high resistance to lodging.

The study showed that rutin content in seed of buckwheat plant depend on genotype varies from 0.07 to 2.4 % of the dry matter (Table 3). The seed of Tatarian buckwheat contains higher amounts of rutin (about 0.8-1.7 % dry weight) than that of common buckwheat (0.01 %) and is rich in vitamins (RAINA & GUPTA 2015). It was observed that buckwheat groats were coloured in light brown, light green and green. The particular interest presents possibility of rutin content effect upon depend on colouring of buckwheat

groats (green color implies increased rutin content). Varieties of *F. esculentum* with green colouring of buckwheat groats had the highest rutin content (0.10-0.15%). Rutin content was significantly lower in *F. esculentum* than that of *F. tataricum* and *F. cymosum*. Wild species *F. tataricum* and *F. cymosum* are valuable in breeding as genetic rutin source. The studied species differed on colouring of buckwheat groats. Thus buckwheat groats of *F. esculentum* variety Pri 7 was light brown, variety Ussurochka - light green, variety Izumrud - green, *F. tataricum* - yellow-green, and *F. cymosum* - bright yellow-green. Colouring of buckwheat groats can serve as a diagnostic sign of visual selection of forms with high rutin content.

Our results show a relationship between rutin content and colouring of the plant different parts (stem, flower, root system, buckwheat groats) of *F. esculentum*, which allows making some adjustments in existing methods of selection of forms with high rutin content, more adapted to abiotic and biotic stresses. There were identified very important diagnostic characteristics that should be used in selection in order to create new varieties with high rutin content in buckwheat groats for production functional foods. The above ground part of *F. esculentum* plant is reasonable to be used as prospective domestic source of rutin for pharmaceutical industry.

Table 3: Rutin content in fruits of three *Fagopyrum* species
Preglednica 3: Vsebnost rutina v plodovih pri treh vrstah *Fagopyrum*

| Species | Variety, catalogue number of ARSRIPG, origin | Rutin content in seed, % | Buckwheat groats colouring |
|-----------------------------|--|--------------------------|----------------------------|
| <i>Fagopyrum esculentum</i> | Pri 7 (Russia) | 0.07±0.01 | light-brown |
| | Ussurochka (Russia) | 0.10±0.01 | light-green |
| | Izumrud (Russia) | 0.15±0.01 | green (salad paint) |
| <i>Fagopyrum tataricum</i> | к-62 (Canada) | 2.4±0.1 | yellow-green |
| <i>Fagopyrum cymosum</i> | к-4231 (India) | 1.1±0.1 | bright yellow-green |

In common buckwheat (*F. esculentum*) production there are formed significant amounts of waste (secondary resources) such as straw and fruit shells (hull), which so far have not been effectively implemented. Proportion of total overground weight of the plant straw depends on the variety and is 40-60 %. As for hull, it is 20-30 % from the grain weight. Straw is usually crushed and remains in the fields or burned.

Rutin content in hull of common buckwheat ranged from 0.08 till 0.20 %. Maximum rutin quantity

one can get from the hull of Ussurochka (35.7 kg/ha), minimum (17.8 kg/ha) in hull of Pri 7 (Table 4). The cereal processing plant use hull as carburant. Recently it is used as a filling for pillows. It seems that the most promising would be their use as the secondary resources involvement as an additional source of raw materials for pharmaceutical industry to obtain rutin and micro-fertilizers, as well as to solve problem of environmental pollution.

Table 4: Rutin content in fruit shells (hull) of common buckwheat**Preglednica 4: Vsebnost rutina v luščinah navadne ajde**

| Variety | Grain yield, ton/ha | Filmness, % | Rutin, % | Number of hull, kg/ha | Rutin, kg/ha |
|------------|---------------------|-------------|-----------|-----------------------|--------------|
| Pri 7 | 1.12 | 20.3±0.2 | 0.08±0.01 | 223.3±2.3 | 17.8±0.1 |
| Izumrud | 1.34 | 23.8±0.2 | 0.10±0.01 | 309.4±2.9 | 27.8±0.1 |
| Pri 10 | 1.63 | 19.5±0.1 | 0.15±0.01 | 312.0±3.0 | 31.2±0.2 |
| Ussurochka | 1.78 | 21.0±0.2 | 0.20±0.01 | 357.0±3.1 | 35.7±0.2 |

Buckwheat nowadays is widely used in various industries. The most promising in the near future seems to be involvement in the processing of fruit shells (hull), which accumulates in large volume at cereal processing plants. Chemistry Institute the Far Eastern Branch of Russian Academy of Sciences jointly with FSC of agribiotechnology in the Far East named after A. K. Chaika studied content and composition of polysaccharides of the fruit coating and straw of varieties Izumrud and Pri 7. For this purposes they used sequential extraction with water, solutions of ammonium

oxalate and sodium hydroxide (ZEMNUKHOVA et al. 2004 a, ZEMNUKHOVA et al. 2004 b).

Our study showed that content of polysaccharides depends on the type of raw material (fruit outer coating or straw) and variety. The largest output of polysaccharides derived from the wastes can be received in all the cases using the first (water) extraction. The colour of dry product depends on the source of raw materials and ways of getting the polysaccharides and has white, light brown or almost black coloring (Table 5).

Table 5: Characteristics of polysaccharides from extracts of *F. esculentum* fruit shells (hull)**Preglednica 5: Polisaharidi v ekstraktih luščin *F. esculentum***

| Variety | Extragent (concentration) | Soluble substances in raw materials, % | Characteristics of polysaccharides | | |
|---------|---|--|------------------------------------|-------------|-----------|
| | | | Output of raw materials, % | Colour | Structure |
| Pri 7 | H ₂ O | 14.7 | 2.76 | light brown | amorphous |
| | (NH ₄) ₂ C ₂ O ₄ (0.5 h) | 2.8 | 1.52 | light brown | crystal |
| | NaOH (0.5 h) | 23.7 | 1.31 | black | amorphous |
| Izumrud | H ₂ O | 14.3 | 2.63 | light brown | amorphous |
| | (NH ₄) ₂ C ₂ O ₄ (0.5 h) | 2.6 | 1.40 | light brown | crystal |
| | NaOH (0.5 h) | 19.2 | 4.40 | black | amorphous |

Polysaccharides of water and oxalate extractions of all samples are characterized by a high content of glucose.

Polysaccharides of alkaline extraction have a more complex monosaccharide structure and contain residues of rhamnose, arabinose, xylose, mannose, glucose and galactose (Table 6).

Inositol was found in trace amounts. Uronic acids are presented by D-Galakturonic and glucuronic acids.

While extracting raw materials, metals which were in the plant, were extracted into the solution along with organic substances. Obtained data prove that polysaccharides, allocated from the extracts in solid state,

absorb metals from the solution. The rest of raw material, which was not dissolved in water, practically doesn't form ashes.

The studies show that the ash content, obtained after burning the hull of *F. esculentum* (600°C) is in average 2 %. The following elements were found in the ash: potassium, sodium, copper, silver, calcium, magnesium, zinc, aluminum, manganese, iron, nickel, chromium, phosphorus, and their concentration depends on variety and type of raw material of *F. esculentum*.

It is important from the point of view of usage of *F. esculentum* fruit shells (hull) as secondary raw material as a source of macro- and micronutrients.

Table 6: Monosaccharide composition of polysaccharides from extracts of *F. esculentum* fruit shells (hull)
Preglednica 6: Monosaharidna sestava polisaharidov v ekstrakti luščin *F. esculentum*

| Variety | Extraction medium | Monosaccharide composition, molar % | | | | | | | The presence of uronic acids | |
|---------|---|-------------------------------------|-----|-----|-----|-----|-----|-----|------------------------------|------|
| | | Rha | Ara | Xyl | Man | Glc | Gal | Int | GalA | GlcA |
| Pri7 | H ₂ O | сл | сл | сл | 13 | 72 | 15 | сл | сл | – |
| | (NH ₄) ₂ C ₂ O ₄ | – | 18 | сл | сл | 61 | 21 | сл | + | сл |
| | NaOH | 8 | 13 | 17 | 10 | 33 | 19 | – | + | + |
| Izumrud | H ₂ O | 3 | 4 | сл | 8 | 64 | 21 | сл | + | сл |
| | (NH ₄) ₂ C ₂ O ₄ | 8 | 21 | 5 | 9 | 39 | 18 | сл | + | – |
| | NaOH | 10 | 18 | 25 | 7 | 22 | 18 | – | + | + |

Notes: Rha – rhamnose, Ara – arabinose, Xyl – xylose, Man – mannose, Glc – glucose, Gal –galactose, Int – inositol; uronic acids: GalA – D-Galacturonic acid, GlcA – glucuronic acid – absent, сл – traces, + presents in small amounts.

Pre-sowing preparation of *Fagopyrum esculentum* seeds is important for productivity increase. We found that treatment of the seeds with the hull ash of *F. esculentum* in quantities of 100 kg/ ton had the greatest harvest increase (0.22 ton/ha).

The literature analysis and our research on testing the possibility of usage of *F. esculentum* waste as

raw material in order to obtaining valuable chemical products, shows that the list of the proposed usage goals of the waste is great and, the waste has not yet been fully utilized. The integrated recycling of *F. esculentum* production waste may prove to be economically favorable.

CONCLUSION

The research defined that rutin content in over-ground part of *F. esculentum* depends on genus, species and variety. As a promising source of rutin we recommend: *F. esculentum* (variety Izumrud, Russia – 3.8 %), *F. tataricum* (sample k-62 from Canada – 4.4 and sample k-17 from China – 4.2 %). There was defined the relation between forms of the plant and amount of rutin, and morphological and economically valuable traits.

Results of *F. esculentum* study as raw material for rutin as well as data on the production maintenance with raw materials can be used in practical application in pharmaceutical industry. There were obtained new

data that may become basic for understanding the functional role of rutin in buckwheat, as well as for identifying mechanisms of rutin accumulation in plants depending on various factors, that will allow to define the most promising sources and to develop practical recommendations for their usage in agriculture, food industry and medicine. The above ground part of *F. esculentum* plant can be used as prospective domestic source of rutin for pharmaceutical industry.

The work is done in the framework of the complex program of fundamental research of the FEB RAS “Far East” in 2018-2020 project No. 18-5-025.

REFERENCES

ANISIMOVA, M.M., 2011: Pharmacological research of *Fagopyrum sagittatum* Gilib. Synopsis of a thesis on competition of a scientific degree of the candidate of pharmaceutical sciences. Samara. (Russia).

GAO, JINFENG, SHOUQIANG AN, XIAOLI GAO, DONGLII GAO, YAN CHAI & BAILI FENG, 2007: Effects of sowing at different time on flavonoids content of buckwheat. Proc 10th Int Symp. on Buckwheat. Shaanxi. China: 338-342.

GRINBERG, L.N., E.A. RACHMILEWITZ & H. NEWMARK, 1994: Protective effects of rutin against hemoglobin oxidation. Biochem. Pharmacol. 48: 643-649.

GUARDIA, T., A.E. ROTELLI, A.O. JUAREZ & L.E. PELZER, 2001: Anti-inflammatory properties of rutin, quercetin and hesperidin on adjuvant arthritis in rat. Farmaco. 56: 683-687.

HE, J., M.J. KLAG, P.K. WHELTON, J.P. Mo, J.Y. CHEN, M.C. QIAN, P.S. Mo & G.Q. HE, 1995: Oats and Buckwheat intake and cardiovascular disease risk factors in an ethnic minority of China. Am. J. Clin. Nutr. 6: 366-372.

HINNEBURG, I. & NEUBERT R.H.H. 2005: Influence of extraction parameters on the phytochemical characteristics of extracts from buckwheat (*Fagopyrum esculentum*) herb. *J. Agric. Food Chem.* 53: 3-7.

HOLASOVA, M., V. FIEDLEROVA, H. SMRCINOVA, M. ORSAK, J. LACHMAN & S. VAVRENOVA, 2002: Buckwheat – the source of antioxidant activity in functional foods. *Food Res. Intern.* 35: 207-211.

JIANG, P., F. BURCZYNSKI, C. CAMPBELL, G. PIERCE, J.A. AUSTRIA & C.J. BRIGGS, 2007: Rutin and flavonoid contents in three buckwheat species *Fagopyrum esculentum*, *F. tataricum* and *F. homotropicum* and their protective effects against lipid peroxidation. *Food Res. Int.* 40: 356-364.

KLYKOV, A.G. & L.M. MOISEYENKO, 2005: Patent 2255466 RU: IPC7 A 01 H 1/04. Method of choice of *F. esculentum* Plants with high Rutin Content in the Overground Mass (Russia).

KLYKOV, A.G., 2000: Study of *F. esculentum* Initial material in order to develop varieties with high rutin content: dissertation. ...cand.agr. sci. Blagoveshchensk. Russia.

KLYKOV, A.G. & L.M. MOISEYENKO, 2010: Influence of buckwheat cultivation conditions upon rutin content. *Advances in Buckwheat Research: proc. 11th Internat. Symposium of Buckwheat*. Orel. Russia, pp.475-483.

KLYKOV, A.G., L.M. MOISEYENKO & P.G. GOROVY, 2003: Season dynamics of rutin content and productivity of the over ground phytomass of three species of *Fagopyrum* Mill., cultivated in Primorsky krai. *Plant Resources (Russia)* 39(3): 77-82.

KLYKOV, A.G. & L.M. MOISEYENKO, 2003: Patent 2201075 RU; IPC7 A 01 H 1/04. The Choice Method for *F. esculentum* Plants with Lodging Resistance (Russia).

KREFT, I., N. FABJAN & K. YASUMOTO, 2006: Rutin content in buckwheat (*Fagopyrum esculentum* Moench) food materials and products. *Food Chem.* 98 (3): 508-512.

KREFT, S., STRUKELJ, B., GABERSCIK, A., & I. KREFT, 2002: Rutin in buckwheat herbs grown at different UV-B radiation levels: comparison of two UV spectrophotometric and an HPLC method. *J. Exp. Bot.* 53: 1801-1804.

MASHKOVSKY, M.D., 2004: Medicines: Manual for Physicians. Moscow.

ODETTI, P.R., A. BORGOLIO, A.D. PASCALE, R. ROLANDI & L. ADEZAD, 1990: Prevention of diabetes increased aging effect on rat collagen-linked fluorescence by aminoguanidine and rutin. *Diabetes*. 39: 796-801.

RAINAS, A. & V. GUPTA, 2015: Evaluation of buckwheat (*Fagopyrum* species) germplasm for rutin content in seeds. *Indian J. Plant Physiol.* 20 (2): 167-171.

SRINIVASAN, K., C.L. KAUL & P. RAMARAO, 2005: Partial protective effect of rutin on multiple low dose streptozotocin-induced diabetes in mice. *Indian J. Pharmacol.* 37: 327-328.

VYSOCHINA, G.I., T.G. KULPINA & T.N. BEREZOVSKAYA, 1987: Flavonoids Content in Some Species of *Polygonum* L. section *Persicaria* (Mill) D.S. Flora of Siberia. *Plant Resources* 23(2): 229-234.

YAN, CHAI, DONGLI GAO, BAILI FENG, XIAOLI GAO, JINFENG GAO & SHOUQIANG AN, 2007: Flavonoides content of buckwheat in different development stage. *Proc. 10th Int. Symp. on Buckwheat*. Shaanxi. China: 417-421.

ZEMNUKHOVA, L.A., E.D. SHKORINA, G.A. FEDORITHEVA, S.V. TOMSHICH & A.G. KLYKOV, 2004 a: Buckwheat processing waste as a raw material for chemical industry. *Advance in Buckwheat Research: Proceed. of the 9th Internat. Symposium of Buckwheat*. Suchdol. Prague, pp. 646-649.

ZEMNUKHOVA, L.A., S.V. TOMSHICH, E.D. SHKORINA & A.G. KLYKOV, 2004 b: Polysaccharides from buckwheat production waste. *Russian Journal of Applied Chemistry* 77(7): 1178-1181.