1. INTRODUCTION

Vegetarianism and other alternative diets based on the consumption of vegetable, fruits, legumes, cereals and nuts were considered as a healthful and nutritionally balanced. It reduces the risk of ischemic heart disease, type 2 diabetes, hypertension, certain type of cancers, and obesity (Melina et al., 2016). According to European Vegetarian Union, there are no reliable statistics on the vegetarian lifestyle choice and products in European Union (http://www.euroveg.eu/public-affairs/statistics-on-vegetarian-lifestyles-and-products/). Such surveys were only conducted in national level or within specific group of consumers. For example, vegetarianism varied from 5.7 to 10.7 % among 16–18 years old girls during years 1999–2013 in Finland (Parviainen et al., 2017). A certain type of vegan diet is strictly limited to consumption of raw (uncooked) plant-based material. A raw food vegan diet is believed to provide a better disease protection, faster healing or easier weight control (Hobbs, 2005). According to a popular book of Ruthan Russo, a raw food propagator, thermal treatment decreases the content of nutrients and enzymes, which could otherwise be helpful for human health (Russo, 2009). Since shelf-life of vegetables and fruits is very limited, drying up to 46 °C was accepted among raw food adherents. Actually, a wide range of drying temperatures from 40 °C to 48 °C was found in various recipes during the search in Internet. They believe that the nutrient contents remained almost unchanged up to these temperatures. It was well described in research studies that drying might decrease the content of health-related substances such as vitamins and polyphenolics (Lemus-Mondaca et al., 2016; Rodríguez et al., 2016). The latter are presented in vegan diet in abundance, which contributes to its positive effect on the human health. The detrimental effect of drying temperature on the nutrient content is affected by many factors such as type of food, temperature, drying time, flow rate of drying air and geometry of the sample. Therefore, both decrease and increase trends could be observed with the increase in drying temperature in literature. Recently, we have provided a meta-analysis of data with relation to the effect of drying temperature on the content of ascorbic acid, total phenolic and flavonoid contents (Červenka et al., 2017). Comparing the levels of ascorbic acid in fresh and dried plant-based food derived from various studies, we found that the drying at 40 °C had detrimental effect. On the other hand, significant decline in phenolic and flavonoid contents were observed upon drying at higher temperatures (60 °C and 70 °C). Even though drying may increase the content of phenolic constituents (Lemus-Mondaca et al., 2016; Santos et al., 2014), the chemical reactions between free amino groups of amino acid and carbonyl groups in reducing sugars has

occurred at higher temperatures forming Maillard compounds (Birlouez-Aragon et al., 2001; Zhang et al., 2014; Przygodska et al., 2015; Huang et al., 2017).

Drying of food products at mild temperature in the range from 40 °C to 60 °C poses a hygienic risk in relation to the growth and survival of microorganisms. In our previous works, we proved that "raw food" meals dried at 40 °C and 50 °C for 20 h had high level of total viable count, spore-forming bacteria and total coliforms (Brožková et al., 2016). The latter has long been recognized to indicate faecal contamination in food processing plants; however, recent discoveries found that majority are environmental contaminants, and only a small fraction is faecal in origin (Martin et al., 2016). In order to ensure the safety of plant-based food products, inactivation of *Escherichia coli* strains was essential during the preparation of spinach and soybean sprouts (Dikici et al., 2015), fresh-cut kale (Kang and Song, 2017) and radish seeds (Song et al., 2016).

Most recipes for the preparation of "raw" meal use buckwheat groats as the main ingredient. Buckwheat is an alternative crop that is consumed as groats and flour for centuries (Zhu, 2016; Quin et al., 2013). It is rich in gluten-free protein, carbohydrate, minerals, vitamins, and phytochemicals. Among phytochemicals, the polyphenolic compounds in buckwheat products are extensively examined due to its health-promoting effects. It is known that its content is subjected to changes during various thermal operations such as boiling, roasting or baking (Quin et al., 2013; Lukšič et al., 2016; Terpinc et al., 2016). Steeping buckwheat groats in water overnight followed by mixing with other constituents (nuts, dried fruits etc.) and subsequent drying at mild temperatures is the common practice among "raw" food devotees. The soaking process of crops is usually associated with the decline of anti-nutrients and increase of content of polyphenolic compounds (Quin et al., 2013; Kumari et al., 2015). On the other hand, this process may lead to proliferation of spoilage and pathogenic microorganisms, particularly at ambient temperature. For instance, soaking of cassava in water at 30 °C was identified as a serious hazard with respect to the growth and survival of coliform bacteria, *Staphylococcus aureus* and *Bacillus cereus* (Obadina et al., 2008). Significant increase in total coliform, yeasts and fungi was observed during soaking of rice at ambient temperature for 2–4 h (Wang et al., 2016).

The aim of this research is to examine various type of soaking procedures with respect to the growth of coliform bacteria and antioxidant properties of buckwheat-based products. Two levels of temperature (5 °C and 20 °C) with and without changing the steeping water were examined followed by drying at mild temperatures in the range from 40 °C to 70 °C. This

study provides a comprehensive view of procedure for "raw" food vegan meal, which can be used in domestic environment; and thus, may has direct impact on consumer´s health.

2. MATERIALS AND METHODS

2.1 Materials

Acetonitrile (gradient grade), formic acid, quercetin hydrate and rutin hydrate used for liquid chromatography analysis were purchased from Sigma (Sigma-Aldrich Chemical Co., St. Louis, MO, USA). The solvents obtained from Lach-Ner, s.r.o. (Neratovice, Czech Republic) and other chemical compounds (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) were of analytical grade.

2.2 Preparation of sample

De-hulled buckwheat groats (*Fagopyrum esculentum*), hazelnuts and prunes were purchased in local market in Czech Republic and were manufactured with the agreement of Council regulation no. 834/2007 on organic production and labelling of organic products. All the ingredients were separately soaked in sterile tap water in ratio of 1: 3–4 (ingredient: water). Soaking process obviously takes place at ambient temperature when prepared "raw" food meal at home (i.e. 20 °C). Low temperature soaking (5 °C) was used as a safer way for preparation of buckwheat-based products. For both temperature treatments, the soaking water was removed after 6 h, the ingredients were washed by sterile tap water, and a fresh portion of water was added for subsequent soaking (samples referred as ChW regime). Concurrently, soaking procedure without washing and changing the soaking water was performed (NChW regime). The total soaking time was 20 h for all the samples. After soaking, the excess of water was removed and a mixture of buckwheat groats (140 g), hazelnuts (15 g) and prunes (15 g) was thoroughly mixed for 2 min at 15.000 min^{-1} in a Sterilmixer 12 rotary blender (International P. B. I., Milan, Italy). Then, cylinder-shaped products were aseptically formed using stainless steel mould (6.0 cm in diameter, 1.0 cm in height). Drying was performed in air-forced oven at 40 °C, 50 °C, 60 °C and 70 °C for 20 h. Microbial analysis and determination of moisture content were performed immediately after soaking and drying, otherwise the samples were stored at -80 °C for further analyses.

2.3 Preparation of sample extract

A portion of the sample was milled for 20 s at 2000 min^{-1} in a knife mill Grindomix GM 200 (Retsch GmbH, Haan, Germany) prior to extraction. Sample extract was prepared using

ultrasound-assisted extraction to 50% (v/v) ethanol as was used in our previous study (Brožková, et al., 2016). These extracts were further used for the determination of total phenolic content (TPC), total flavonoid content (TFC), Trolox equivalent antioxidant capacity (TEAC) assays and LC analysis of rutin and quercetin. Two extracts were prepared for each measurement.

2.4 Determination of dry matter

Moisture content of the sample was determinated gravimetrically at 102 °C using moisture analyser MLB50–3 (Kern & Sohn, Balingen, Germany). Homogenized sample was placed on scale (sensitivity ±0.02 g) and dried by two halogen lamps placed above the sample until the weight constancy.

2.5 Determination of antioxidant activity

Total phenolic content (TPC) was determined using Folin-Ciocalteu reagent and the mixture was measured at 765 nm (Lemus-Mondaca et al., 2016). TPC values were obtained using gallic acid as a standard for calibration. The results were expressed in mg gallic acid equivalents per kg of dry matter (mg GAE/kg d. m.). TFC was determined upon reaction of flavonoids in sample with $AlCl_3$, subsequent measurement of the product at 415 nm. Quercetin was used as standard for calibration, and results were expressed as mg quercetin equivalent per kg of dry matter (mg QE/kg d. m.).

Antioxidant capacity was measured using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'- azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) stable radicals according to the procedures described in our previous work (Brožková et al., 2016). The results were expressed as mg Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) equivalent per kg of dry matter (mg TE/kg d. m.). All the colorimetric assays were performed in UV/VIS Spectrophotometer DU 530 (Beckman Coulter Inc., Brea, CA, USA)

2.6 Liquid chromatography analysis

It was well described in the literature that buckwheat seeds and related products are rich in phenolic compounds, dominated by rutin (quercetin-3-rutinoside) (Guo et al., 2017; Kiprovski et al., 2015; Lee et al., 2016). Therefore, both rutin and quercetin were analysed in this study using Agilent 1100 Series (Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary pump, a degasser, an auto sampler, a thermostatted column compartment, a UV and MS detector Agilent 1100 Series LC/MSD Trap SL. A Gemini 5um C18 (150 × 3.0 mm,

5.0 μm) column was used (Phenomenex®, Torrance, CA, USA). Mixture of deionized water acidified with formic acid to pH 3.05 (solution A) and acetonitrile (solution B) was used as mobile phase at gradient flow rate 0.7 mL/min (formic acid: acetonitrile from 900: 100 mL: mL to 500: 500 mL: mL for 0–15 min). The analysis was performed at 40 °C and peaks of rutin and quercetin were detected at 360 nm. Quantification was based on the separation of standard solutions of quercetin and rutin dissolved in ethanol (50%, v/v) at concentrations from 1.0 to 100.0 μg/mL. Peak area (Y) plotted against the concentration (c) of rutin and quercetin gave the calibration equation $Y=2.26\times c+4.72$ ($R^2=0.998$) and $Y=4.79\times c-2.88$ ($R^2=0.999$), respectively. An ion trap mass spectrometry detector with an ESI source was used to confirm the presence of both flavonoids. The concentration of both flavonoids was expressed in mg per kg of dry matter (mg/kg d. m.).

2.7 Determination of advanced Maillard products

FAST method was used for the determination of free fluorescent advanced Millard products. It has derived from the reaction of reducing sugars and tryptophan as was described by Birlouez-Aragon et al. (2001). FAST index was calculated using equation: $FAST=100xF_{AMP}/F_{Trp}$, where F_{AMP} is fluorescence of advanced Maillard products (excitation at 353 nm/emission at 438 nm) and F_{Trp} is fluorescence of tryptophan at 290/340 nm. Acryl cuvettes and fluorimeter Fluorat® 02 Panorama (Lumex Instruments, Mission, Canada) were used.

2.8 Determination of total coliforms and *Escherichia coli*

Initial microbiological analysis revealed that *E. coli* was not naturally present in our sample. Therefore we artificially inoculated common buckwheat groats before soaking process as followed: a suspension of freshly grown culture strain of *E. coli* CCM 4517 (Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic) was prepared in physiological saline in density of 6.0 log cfu/g using McFarland turbidimetric standard. Appropriate dilution was added to buckwheat groats yielding initial count of 2.43 log cfu/g. Total coliforms and *E. coli* counts were determined prior and after soaking process, as well as after each drying treatment. Sample (10.0 g) was homogenized in a plastic bag with 90 mL of physiological saline using peristaltic masticator (ÍUL Instr., Barcelona, Spain). Aliquots (0.1 mL) of the appropriate dilution was transferred on the surface of violet red bile agar (VRBA, HiMedia Laboratories, Mumbay, India) and Trypton bile X-glucuronide agar (Chromocult TBX, Merck, Darmstadt, Germany) for total coliforms and *E. coli*, respectively. Red-to-violet

colonies were enumerated on VRBA after incubation at 30 °C for 24–48 h, and blue coloured colonies were enumerated on Chromocult TBX after incubation at 37 °C for 24–48 h. The results were expressed as logarithmic colony forming unit per gram of sample (log cfu/g).

2.9 Statistical analysis

The results of chemical analysis represent the mean of four replicates (N=4). An order statistic is more suitable for the small sample size, therefore Horn´s procedure was used for the estimation of the mean and the deviation, where pivot half sum (P_L) and 95% confidence interval (95% CI) were calculated, respectively (Horn, 1983). Non-parametric methods were applied for further statistical treatment of data. Tukey´s multiply comparison method was used to find differences between means. The effect of soaking temperature and soaking regime (changing/not changing soaking water after 6 h) on the selected variables was tested using Kruskal-Wallis analysis of variance (ANOVA) for raw matter. Drying temperature alone was used as a single factor in Kruskal-Wallis ANOVA for determination of the effect on the selected variables of dried buckwheat-based products. Spearman correlation coefficients (*r*) were calculated to describe the mutual associations between variables. All the statistical methods were done at the probability level of P = 0.05 (Statistica CZ, StatSoft CR s.r.o., Prague).

3. RESULTS AND DISCUSSION

3.1 The effect of soaking and drying temperature on the total phenolic and flavonoid contents

The effect of soaking process and subsequent drying at 40 °C–70 °C on total phenolic content in buckwheat-based product was depicted in Fig. 1A. TPC values ranged from 546.7 mg GAE/kg to 702.2 mg GAE/kg d. m. in soaked matter containing 140 g of buckwheat groats, hazelnuts and prunes (both in 15 g). TFC values were determined in the range of 132.5–144.1 mg QE/kg d. m. for all the soaking processes used in our experiment (Fig. 1B). According to the review of literature, different effects of soaking process on the content of phenolic substances were observed. Terpinc et al. (2016) found that soaking of common buckwheat grains in water for 8 h at 20 °C did not result in significant changes of TPC. Both negative and positive effects of soaking were described in literature; an increase in TPC after soaking of Tartary buckwheat seeds was reported (Quin et al., 2013). A decrease in phenolic content occurred when two soybean varieties were soaked for 12 h at ambient temperature (Kumari et al., 2015). Since the analysis of buckwheat, hazelnuts and prunes before soaking was not performed in our study, we only elucidated the effect of soaking temperature and soaking

regime. Significantly higher TPC values (590.3–702.4 mg GAE/kg d. m.) of raw matter were obtained when soaked at 5 °C ($P < 0.01$) in comparison to those at 20 °C (546.7–563.9 mg GAE/kg d. m.) as revealed by ANOVA procedure. No effect of soaking temperature has been recorded for TFC values. The changing steeping water after 6 h did not resulted in significant changes in TPC and TFC values of raw matter. In our study, the higher amount of TPC in raw buckwheat matter soaked at 5 °C can be explained by the inhibition of polyphenol oxidase at low temperature. This statement is supported by study of Li et al. (2017), where decrease of the expression level of polyphenol oxidase genes was observed when fruits were conditioned at 10 °C for 3 days. As can be seen from Fig. 1A, higher TPC values were recorded in dried buckwheat-based products in comparison with the raw matter ($P < 0.01$). The increasing in the quantity of phenolic compounds after drying could be due to structural changes in the matrices, which may allow a greater extraction of phenolics (Santos et al., 2014). In this work, drying process at 40 °C reached the highest level of TPC followed by the decrease after drying at 50 °C, 60 °C and 70 °C (Supplementary material 1A). Although there were no differences in TPC values when dried above 50 °C, the effect of drying temperature was significant ($P < 0.001$). Drying process at 40 °C was also favourable to the content of total flavonoids showing 2–3 times higher content in comparison with raw matter, with exception of drying buckwheat cookies soaked at 5 °C in NChW regime. Higher temperatures of drying decreased TFC to almost the same levels observed in raw matter (see Fig. 1B). Drying temperature had significant effect on TFC values as was determined by ANOVA procedure ($P< 0.001$). Supplementary material 1B also showed that drying above 50 °C brought the same levels of TFC. The increase of TPC and TFC values after drying at low temperatures were also reported for *Stevia rebaudiana* leaves (Lemus-Mondaca et al., 2016). The formation of new phenolic compounds via non-enzymatic inter-conversion reaction from available precursors presented during low temperature drying was attributed to that increase.

3.2 The effect of soaking and drying temperature on antioxidant capacity

Antioxidant capacity of Tartary buckwheat raw matter after soaking procedure was measured in terms of DPPH and ABTS radical scavenging activities. While soaking temperature had no significant effect of the antioxidant capacity of raw matter, ChW regime resulted in significantly lower values ($P < 0.01$) of $TEAC_{DPPH}$ (332.8–400.2 mg TE/kg d. m.) when compared to NChW regime (532.3–564.5 mg TE/kg d. m.). The effect of soaking procedure on DPPH radical scavenging capacity was shown in Fig. 1C. It may imply that some substances with antioxidant activity leaked into the soaking water. Similar behaviour was

evident for $TEAC_{ABTS}$ values where higher antioxidant capacity was found in samples processed by NChW regime (2535.1–4262.7 mg TE/kg d. m.) than by ChW regime (2404.2–2455.4 mg TE/kg d. m.) as shown in Fig. 1D. As can be seen from Fig. 1C and 1D, higher antioxidant capacities were observed in samples dried at 40 °C than in raw matter, which reflects higher content of phenolic and flavonoid contents. This is in agreement with previous studies where drying of quinoa seeds (Miranda et al., 2010) or plums (Michalska et al., 2006) at 40° C resulted in higher antioxidant properties in comparison with raw material. Drying temperature significantly affected the values of $TEAC_{DPPH}$ ($P < 0.001$) and $TEAC_{ABTS}$ ($P < 0.001$). A gradual decrease of antioxidant capacity with the increase of drying temperature was observed for both $TEAC_{DPPH}$ (from 917.8 to 664.0 mg TE/kg d. m.) and $TEAC_{ABTS}$ (from 3808.4 to 3396.3 mg TE/kg d. m.) (Supplementary material 1C and 1D, respectively). The multiply comparison showed that antioxidant capacity of buckwheat-based dried samples was similar at 40 °C and 50 °C for both TEAC assays. A strong and positive correlations were found between $TEAC_{DPPH}$ and TPC ($r = 0.893$, $P < 0.001$) and TFC ($r = 0.814$, $P < 0.001$). $TEAC_{ABTS}$ was weakly but significantly correlated with TPC and TFC giving $r = 0.649$ ($P < 0.01$) and $r = 0.577$ ($P < 0.05$), respectively.

3.3 The effect of soaking and drying temperature on the content of rutin and quercetin

Comparing Fig. 2A and Fig. 2B, higher levels of rutin (8.9–84.6 mg/kg d. m.) than quercetin (2.5–21.8 mg/kg d. m.) were determined in common buckwheat samples without respect to the processing. This finding is in agreement with other studies dealing with Tartary buckwheat products (Guo et al., 2017; Zhu, 2016; Lukšič et al., 2016). For instance, rutin content in alcohol extracts of whole grain tea was 5-fold higher in comparison with quercetin (Guo et al., 2017). Luksič et al. (2016) found rutin in Tartary buckwheat flour at the level of 8105 mg/kg d. m. while quercetin remained at 876 mg/kg d. m. after extraction procedure lasted for 8 h. While changing the steeping water did not affect the contents of rutin and quercetin ($P > 0.05$), a slightly higher amount of quercetin was observed after soaking at 5 °C ($P < 0.05$) than at 20 °C. Soaking process is required for softening of the groats and germination during which the amount of phenolic substances has increased. It was previously found that the level of rutin in germinated buckwheat sprouts (20 h, 23 °C) was 1.5 higher than in non-germinated buckwheat seeds sample (Koyama et al., 2013).

The content of rutin and quercetin usually showed a decreasing trend during various technology processes. The preparation of spaghetti form whole buckwheat flour and its subsequent cooking resulted in 44.7% loss of rutin and disappearance of rutin under limit of

detection (Verardo et al., 2011). Roasting or steaming also caused significant decrease of those flavonols (Zielinski et al., 2009; Keriene et al., 2016). As can be seen in Fig. 2A, drying of buckwheat sample resulted in higher content of rutin in comparison with raw matter, particularly at 70 °C. Quercetin content also elevated in dried buckwheat cookies, however no clear pattern was observed (Fig. 2B). Quin et al. (2013) reported the change of rutin and quercetin contents during the thermal treatment of Tartary buckwheat seed. They hypothesised that steaming activated intermolecular conversion of quercetin to rutin that further slightly decreased after drying at 150–200 °C for 5 min. As was shown in Supplementary material 2A, rutin content exhibited growing trend with the increase of temperature, whereas quercetin levels showed random values with respect to the increasing drying temperature (Supplementary material 2B). This behaviour may reflect the fact that rutin but not quercetin was readily extracted from the buckwheat sample dried at higher temperatures as was reported for hydrothermally treated Tartary buckwheat flour (Lukšič et al., 2016). The content of rutin/quercetin did not correlate with antioxidant capacity and only weak association was found between rutin content and TFC values ($r = 0.588$, $P < 0.05$). This finding indicates that other phenolic compounds contributed to the antioxidant characteristics of buckwheat samples in our study. Except rutin, epigallocatechin and orientin also contributed considerably to the total antioxidant capacity of common and Tartary buckwheat groats and hulls (Lee et al., 2016).

3.4 The effect of soaking and drying temperature on Maillard products

FAST index was determined as the ratio between the fluorescence of advanced Maillard products (AMP) and the fluorescence of tryptophan in the soluble protein fraction of buckwheat cookie samples. While the soaking process did not alter FAST index, the drying process exhibited slight but gradual increase of FAST index when dried at 40 °C, 50 °C and 60 °C. The average increase of FAST index was up to 46 % at 60 °C in comparison with raw matter. Drying at 70 °C resulted in a steep increase of FAST index in all buckwheat samples in our study (Fig. 3). Michalska et al. (2016) found considerable increase in the FAST index values in plums dried at 40–60 °C with further decrease at 85 °C. They proved that this behaviour was dependent on the content of available lysine and seemed to be product-specific. It is evident from Figure 3 that the soaking process had an impact on the development of AMP during drying at 70 °C. Lower values of FAST index were determined in samples in ChW regime (82.5 % and 104.0 % for 5 °C and 20 °C, respectively). We assume that compounds forming AMP during heating previously leaked into the soaking

water as was described in the study of Yuan et al. (2014) where 8–40% reduction of acrylamide formation was observed after microwaving of potato slices. It is not clear why there were higher FAST index values in buckwheat samples soaked at 20 °C before drying at 70 °C. It is likely, that low soaking temperature hinder the solubility of proteins/amino acids or reducing sugars, which are capable to form Maillard products during drying. Correlation coefficients between FAST index and antioxidant capacity, TPC and TFC were negative but non-significant in our study. The negative correlation may indicate the inhibitory activity of antioxidant compounds against the formation of AMP during the drying of buckwheat cookies. In a study of Zhang et al. (2014), quercetin was responsible for the inhibition of development of fluorescent glycation products after baking of cookies at 200 °C for 10 min. Rutin was also determined as an inhibitor of furosine formation during baking of rye-buckwheat cakes fortified with various spices (Przygodska et al., 2015). To our surprise, strong negative influence of rutin on FAST index was obtained in our study ($r = 0.853$, $P < 0.001$). Rutin seems to act as a compound with pro-oxidant effect under our experimental conditions. It was previously reported that antioxidant/pro-oxidant effect of phenolic compounds is dose-responded. For example, Huang et al. (2017) observed that inhibition of acrylamide formation increased by addition of flavanols within the range of 1–100 μmol/L but declined when addition level surpassed 100 μmol/L.

3.5 The effect of soaking and drying temperature on total coliforms and *Escherichia coli*

The soaking temperature and soaking regime were evaluated with respect to the total coliform naturally occurred in buckwheat matter. The initial content of coliform bacteria was 3.77 ± 0.45 log cfu/g followed by 0.65–1.44 log cfu/g increase after soaking in sterile tap water (Fig. 4A). Both soaking temperature and soaking regime have no influence on the content of coliform bacteria ($P > 0.05$). *E. coli* was artificially inoculated into the buckwheat matter before soaking yielding 2.73 ± 0.23 log cfu/g of initial count. Soaking temperature significantly affected the proliferation of *E. coli* in buckwheat matter after 20 h, as can be seen from Fig. 4B. Soaking at 20 °C resulted in the increase of *E. coli* counts to 4.18–5.15 log cfu/g while soaking at low temperature (5 °C) caused 0.73 log cfu/g reduction of *E. coli* counts. However, drying of Tartary buckwheat samples at 40 °C increased both total coliform and *E. coli* to unacceptable levels > 6.0 log cfu/g. Although drying at 40 °C for 20 h significantly reduced the moisture content (from 156.2–214.7 to 67.9–94.1 g/kg), the remaining moisture content was favourable for the growth of coliform bacteria. Water activity is more appropriate parameter for predicting the growth and survival of microorganisms;

however, it was not determined in our study. The effect of mild temperature on the reduction of *E. coli* was studied by several authors. Dikici et al. (2015) found that reducing the count of *E. coli* O157:H7 and non-0157 STEC strains at 40 °C and 50 °C in soybean sprout and spinach was very ineffective. The optimal temperature for the reduction of *E. coli* was determined at 55 °C during washing of kale leaves (Kang and Song, 2017) or at 64.5 °C, for radish seeds (Song et al., 2016). In our experiment, both coliform and *E. coli* counts decreased below a limit of detection (< 1.0 log cfu/g) after drying at 50 °C for 20 h.

4. CONCLUSIONS

The criteria for the preparation of "raw" vegan food included soaking of plant-based material and drying at moderate temperature not exceeded 46 °C. So-prepared meal should remain high level of nutrients and it is believed to promote the health status. It is usually called "live" food. Based on the results of this study, soaking buckwheat groats and other ingredients at 5 °C increased phenolic content and reduced *E. coli* counts. The change of soaking water during the process was not essential for the quality of the product. Nevertheless, we suggest changing the soaking water during the procedure due to the removing of anti-nutrients (not tested in this study). Although drying of pre-soaked material at 40 °C for 20 h has led to the development of meal with significantly higher antioxidant properties, it has also increased coliforms and *E. coli* counts to level that is not acceptable in foods. Drying buckwheat-based products at 50 °C and 60 °C decreased antioxidant activity and the content of phenolics and flavonoids, but it ensures microbial safety with lower level of advanced Maillard products.

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CONFLICT OF INTEREST

The authors confirm that they do not have conflict of interest

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